Systematics of *Vriesea* (Bromeliaceae): phylogenetic relationships based on nuclear gene and partial plastome sequences

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Vriesea is the second largest genus in Tillandsioideae, the most diverse subfamily of Bromeliaceae. Although recent studies focusing on Tillandsioideae have improved the systematics of *Vriesea*, no consensus has been reached regarding the circumscription of the genus. Here, we present a phylogenetic analysis of core Tillandsioideae using the nuclear gene *phyC* and plastid data obtained from genome skimming. We investigate evolutionary relationships at the intergeneric level in Vrieseeae and at the intrageneric level in *Vriesea s.s.* We sampled a comprehensive dataset, including 11 genera of Tillandsioideae and nearly 50% of all known *Vriesea* spp. Using a genome skimming approach, we obtained a 78 483-bp plastome alignment containing 35 complete and 55 partial protein-coding genes. Phylogenetic trees were reconstructed using maximum-likelihood based on three datasets: (1) the 78 483 bp plastome alignment; (2) the nuclear gene *phyC* and (3) a concatenated alignment of 18 subselected plastid genes + *phyC*. Additionally, a Bayesian inference was performed on the second and third datasets. These analyses revealed that *Vriesea s.s.* forms a well-supported clade encompassing most of the species of the genus. However, our results also identified several remaining issues in the systematics of *Vriesea*, including a few species nested in *Tillandsia* and *Stigmatodon*. Finally, we recognize some putative groups within *Vriesea s.s.*, which we discuss in the light of their morphological and ecological characteristics.

ADDITIONAL KEYWORDS: Atlantic Forest – chloroplast – epiphytes – genome skimming – monocotyledons – Neotropics – next-generation sequencing – Tillandsioideae.

INTRODUCTION

Bromeliaceae are a large, nearly entirely Neotropical plant family with a wide geographical distribution ranging from the southern United States to northern Patagonia in Argentina; a single species occurs in Africa (Smith & Downs, 1974). Diversification in the family has been suggested to be the result of rapid speciation and adaptive radiations mainly triggered by the evolution of key innovations that enabled species to colonize new adaptive zones (Givnish *et al.*, 2011, 2014; Silvestro, Zizka & Schulte, 2014). Morphological features, such as tank-forming leaves, epiphytism, water- and nutrient-absorbing leaf trichomes and CAM photosynthesis, have allowed bromeliads to colonize highly heterogeneous environments (Benzing,

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2000; Silvestro *et al.*, 2014). Consequently, bromeliads occur from mesophytic forests to xerophytic rock outcrops, from sea level (e.g. restingas in Atlantic Forest) to the top of mountains in the Andes and the Brazilian Shield (Gilmartin, 1973; Krömer, Kessler & Herzog, 2006; BFG, 2015, 2018). The wide variation in morphological characters and the low rates of molecular evolution observed in Bromeliaceae (Smith & Donoghue, 2008; Maia *et al.*, 2012) make this family of *c*. 3587 species in 75 genera (Butcher & Gouda, cont. updated) a challenging group for taxonomists. Because of these characteristics, the delimitation of species, or sometimes even genera, has proved difficult (Palma-Silva *et al.*, 2016).

Based on molecular phylogenetic analyses, eight subfamilies of Bromeliaceae, the circumscription of which was usually based on only a few plastid markers, are currently recognized in the literature (Terry, Brown & Olmstead, 1997a, b; Horres *et al.*, 2000; Crayn, Winter & Smith, 2004; Barfuss *et al.*, 2005; Givnish *et al.*, 2007). In the last decades, many phylogenetic studies have improved our understanding of the evolutionary history of these subfamilies, but species representation has been highly heterogeneous among studies (Escobedo-Sarti *et al.*, 2013; Palma-Silva *et al.*, 2016). For instance, among the subfamilies with the highest number of published phylogenetic studies (Bromelioideae and Tillandsioideae), Tillandsioideae are also the subfamily with the most scattered and least connected sampling because most publications analysed only small clades (Escobedo-Sarti *et al.*, 2013). As a result of the low rates of substitution in plastid regions, the tree topologies are often poorly resolved not helping in the circumscription of some genera in Bromeliaceae (Smith & Donoghue, 2008; Maia *et al.*, 2012; Palma-Silva *et al.*, 2016); non-natural genera are frequently reported in Bromeliaceae (Barfuss *et al.*, 2005; Evans *et al.*, 2015; Schütz *et al.*, 2016; Maciel *et al.*, 2018) and only 30% of the currently recognized genera are monophyletic (Escobedo-Sarti *et al.*, 2013). To overcome these problems, recent studies have increased the amount of analysed data either by increasing the number of molecular markers and their phylogenetic informativeness (e.g. more quickly evolving nuclear regions or even AFLP) or increasing the numbers of terminals (Krapp *et al.*, 2014; Heller *et al.*, 2015; Barfuss *et al.*, 2016; Pinangé *et al.*, 2016; Schütz *et al.*, 2016; Goetze *et al.*, 2017; Gomes-da-Silva & Souza-Chies, 2017; Leme *et al.*, 2017; Maciel *et al.*, 2018; Matuszak-Renger *et al.*, 2018). Nevertheless, large and complex to delimit genera such as *Tillandsia* L. (741 species), *Pitcairnia* L'Heritier (408 species), *Vriesea* Lindl. (226 species), *Puya* Molina (226 species) and *Guzmania* Ruiz & Pav. (218 species) remain vastly under-sampled (Gouda, Butcher & Gouda, cont. updated; Palma-Silva *et al.*, 2016; Schütz *et al.*, 2016).

Vriesea, the fourth largest genus in Bromeliaceae (Gouda *et al.*, cont. updated), highlights well the problems faced by current phylogenetic studies. In the genus, 88% of the species in the genus occur in Brazil, and 84% of all *Vriesea* spp. are endemic to the country (BFG, 2015, 2018). They are distributed from xeric to mesic environments (Versieux & Wendt, 2007; Versieux, 2008; Costa, Gomes-da-Silva & Wanderley, 2014; Machado, Forzza & Stehmann, 2016) and are a conspicuous element of the Atlantic Forest (Martinelli *et al.*, 2008). Some species reach a western inland distribution, growing as epiphytic, terrestrial or rupicolous plants on inselbergs and in rocky savanna-like habitats (Versieux & Wendt, 2007; Versieux *et al.*, 2008; Costa *et al.*, 2014). The geographical distribution of species varies from wide ranges covering different habitats to microendemics that are particularly frequent in mountainous environments (Versieux & Wendt, 2007; Costa *et al.*, 2014; Machado, Forzza & Stehmann, 2016). Due to the restricted distributions of many *Vriesea* spp. and the continuous loss of habitat, the genus is the second most endangered in Brazil when considering the absolute number of endangered plant species (Martinelli *et al.*, 2013). The wide climatic tolerance of *Vriesea* is coupled with morphological variation (Costa *et al.*, 2014; Costa, Gomes-da-Silva & Wanderley, 2015). Phenotypic variability occurs also at the intraspecific level, making the delimitation of taxa complicated, and leading to the recognition of several species complexes (Almeida *et al.*, 2009; Gomes-da-Silva & Costa, 2011; Versieux, 2011; Neves *et al.*, 2018).

The polyphyletic condition of *Vriesea* and the difficulty in recognizing unique morphological characters to circumscribe the genus have been known for over two decades (Terry *et al.*, 1997a; Barfuss *et al.*, 2005; Givnish *et al.*, 2011; Gomes-da-Silva *et al.*, 2012; Costa *et al.*, 2015; Gomes-da-Silva & Souza-Chies, 2017). Recent studies using morphological characters and molecular markers have elucidated the intergeneric relations in Tillandsioideae and helped to improve the circumscription of *Vriesea* (Barfuss *et al.*, 2016; Gomes-da-Silva & Souza-Chies, 2017). However, conflicting results are found in the literature, potentially due to the low species sampling of *Vriesea*, which remains poorly representative of the whole genus. To achieve a robust classification, the last taxonomic revision of Tillandsioideae proposed a series of rearrangements dividing large polyphyletic genera into small, monophyletic, well-circumscribed morphological groups (Barfuss *et al.*, 2016). Based on the stigma morphology, *Vriesea s.l.* was split into five new genera (*Goudaea* W.Till & Barfuss, *Jagrantia* Barfuss & W.Till, *Lutheria* Barfuss & W.Till, *Zizkaea*

W.Till & Barfuss, *Stigmatodon* Leme, G.K.Br. & Barfuss) and *Vriesea s.s.* (the Brazilian lineage) was recognized as monophyletic. Finally, *Cipuropsis* Ule was resurrected to accommodate the mesomorphic northern Andean '*Vriesea*' spp. (Barfuss *et al.*, 2016). However, the phylogenetic analysis conducted by Gomes-da-Silva & Souza-Chies (2017) based on a total evidence approach (morphological data and four plastid markers) did not support the rearrangement of *Vriesea s.l.* proposed by Barfuss *et al.* (2016). Instead, Gomes-da-Silva & Souza-Chies (2017) recognized only two lineages: (1) *Vriesea s.s.* encompassing all Brazilian species including *Stigmatodon*, a new genus described by Barfuss *et al.* (2016); and (2) *Vriesea* clade ß composed partly by the *Cipuropsis–Mezobromelia* L.B.Sm. complex and by *Josemania*, *Goudaea*, *Lutheria* and *Jagrantia*, new genera described in Barfuss *et al.* (2016). Thus, a consensus on genus delimitation in Vrieseeae, particularly in *Vriesea s.l.*, has not yet been reached, calling for additional systematics studies of this group.

Here, we use the opportunity offered by next-generation sequencing to produce a plastid dataset obtained using the genome skimming approach. We combined this plastid dataset with sequences from the nuclear gene *phyC* to reconstruct the phylogenetic tree of core Tillandsioideae. We test the monophyly of the genera in Vrieseeae, investigate phylogenetic relationships at the intergeneric level and within *Vriesea s.s.* and discuss the efficiency of genome skimming for shallow-level phylogenetic reconstruction in bromeliads.

MATERIAL AND METHODS

Taxon sampling

Our dataset included 206 specimens (148 species) for plastid data and 171 specimens (134 species) for nuclear data, totalling 227 specimens representing 151 species and three infraspecific taxa from 11 genera of Tillandsioideae. Of these, 110 species belonged to *Vriesea s.l.*, representing 49% of the total number of described species according to Gouda *et al.* (cont. updated). Besides *Vriesea*, the number of species sampled in this study and the number of species per genus according to Gouda *et al.* (cont. updated) were as follows: *Alcantarea* (E.Morren ex Mez) Harms (11/42), *Goudaea* (2/2), *Guzmania* (4/218), *Lutheria* (2/4), *Mezobromelia* (1/5), *Racinaea* M.A.Spencer & L.B.Sm. (1/78), *Stigmatodon* (5/18), *Tillandsia* (8/741), *Werauhia* J.R.Grant (3/93) and *Zizkaea* (1/1) (Supporting Information, Table S1).

Most samples were collected during fieldwork in Brazil. We tried to cover the greatest possible morphological and geographical variation of the species and type localities. To make the sampling as complete as possible, we also included specimens from the living collections of the Marie Selby Botanical Garden (SEL, United States), the Botanical Garden of the University of Vienna (WU-HBV, Austria), the Rio de Janeiro Botanical Garden (RBvb, Brazil) and the Jardin des Serres d'Auteuil (P, France). Details of the studied species and accession numbers are given in the Supporting Information (Table S1).

Ananas comosus (L.) Merr. was included as an outgroup for the different analyses. For the *phyC* nuclear gene, sequences from additional six species (including *Ananas*) where obtained from GenBank: *Ananas comosus* (NCBI number KU095956.1), *Guzmania wittmackii* (André) André ex Mez (NCBI number KX753898.1), *Lutheria splendens* (Brongn.) Barfuss & W.Till (NCBI number KX753915.1), *Tillandsia heubergeri* Ehlers (NCBI number KX753920), *Tillandsia stricta* Sol. KX753950.1) and *Tillandsia tenuifolia* L. (NCBI number KX753909.1). For the genome skimming, raw reads from a whole genome sequencing of *Ananas comosus* 'N67-10' (NCBI number DRX020985) were added to the analyses together with the reads obtained in this study.

DNA extraction

Total genomic DNA was isolated from silicagel-dried leaves collected in the field or taken from living collections using the Quiagen DNAeasy Plant Mini Kit (Qiagen, USA). We adjusted the manufacturer's protocol to optimize the DNA extraction from the thick and fibrous leaves of bromeliads (contact authors for more details). Total DNA samples were evaluated with agarose gels and a NanoDrop Spectrophotometer (Thermo Scientific, Waltham, MA, USA). The DNA content was quantified with a Qubit Fluorometer v.2.2 (Thermo Fisher Scientific, Waltham, MA, USA).

Nuclear *phyC* gene amplification, assembly and alignment

The nuclear *phyC* region amplification was based on primers and protocols described by Louzada *et al.* (2014) and Barfuss *et al.* (2016). Sequencing reactions were carried out with the same amplification primers using the sequencing service from Microsynth (Switzerland). Forward and reverse sequences were trimmed, edited and assembled using Geneious v.6.1.8 (Kearse *et al.*, 2012). Consensus sequences were aligned using ClustalW implemented in Geneious v.6.1.8 and individual gap positions were treated as missing data.

Library preparation and genome skimming sequencing

For library preparation, we quantified the DNA and diluted the samples in a 100 µl solution containing 500 ng of DNA and fragmented with a Bioruptor sonicator (Diagenode) to obtain fragments of 300–700 bp. For samples with lower DNA content, we used 100 µl of available aliquot without any dilution.

Library preparation was performed following de La Harpe *et al.* (2018). DNA clean-up, size selection, end-repair and the A-tailing steps were achieved with a KAPA LTP library preparation kit (Roche, Basel, Switzerland). Adaptor ligation and adaptor fill-in steps were based on Meyer & Kircher (2010). For the majority of samples, an aliquot of 4 µl of the ligated fragment solution was amplified for eight cycles using the KAPA HiFi DNA Polymerase (Roche, Basel, Switzerland) and the set of 60 dual-index primers designed by (Loiseau *et al.*, 2019). Dual-indexed primers were chosen to avoid inaccuracies in multiplex sequencing (Kircher, Sawyer & Meyer, 2012). Samples with a low amount of DNA were amplified for 12 cycles using 11 µl of the ligated fragment solution. The products of library amplification were quantified using a Qubit Fluorometer v.2.2 (Thermo Fisher Scientific, Waltham, MA, USA). Genomic DNA libraries were pooled equimolarly and sequenced in an Illumina HiSeq 3000 Genome Analyzer (Illumina, San Diego, California, USA) in 1.5 lanes using 2 × 150 bp paired-end reads at the University of Bern.

DNA quality, assembly and alignment

Quality control, quality score per base, sequence duplication level and overrepresented sequences were checked with FASTQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). To remove sequencing errors, the reads were trimmed with CONDETRI v.2.2 (Smeds & Künstner, 2011) using 20 as high-quality threshold parameter.

For each sample, all reads were mapped to the *Tillandsia adpressiflora* Mez pseudo-reference genome built in de La Harpe *et al.* (2018) using BOWTIE2 v.2.2.5 (Langmead & Salzberg, 2012) and the '--very-sensitive-local' option. This pseudo-reference genome was build using the high-quality and annotated *Ananas comosus* reference genome (Ming *et al.*, 2015) as a start point. The method consisted of incorporating specific variation of *T. adpressiflora* into the *Ananas* genome to improve the mapping efficiency of samples of Tillandsioideae. This pseudo-reference contains the plastid genome.

To ensure the data quality, reads were realigned around indels, and the base quality of reads was re-calibrated using GATK v.3.6 (McKenna *et al.*, 2010). SNP calling was performed for the plastid genome using UnifiedGenotyper of GATK v.3.6 (McKenna *et al.*, 2010) and the EMIT_ALL_SITES option to recover both variant and invariant sites. SNP calling was not performed for the nuclear genome as the sequencing depth of the genome skimming methods is not high enough to recover this genomic region. Only sites with a quality Q > 20, < 50% missing data and with a minimum depth of 3× were retained using vcftools v.0.1.13 (Danecek *et al.*, 2011). Fasta files were generated using vcf-tab-to-fasta (https://github.com/JinfengChen/vcf-tab-to-fasta). Sequences were aligned using ClustalW implemented in Geneious 6.1.8 (Kearse *et al.*, 2012). The samples that had > 50% missing data were removed from the alignment.

Subsampling of plastid genes

Most phylogenetic studies in Bromeliaceae are based on a few plastid genes and/or nuclear genes (Louzada *et al.*, 2014; Evans *et al.*, 2015; Aguirre-Santoro, Michelangeli & Stevenson, 2016; Barfuss *et al.*, 2016; Gomes-da-Silva & Souza-Chies, 2017; Kessous *et al.*, 2019), resulting in poor resolution in some genera. Increasing the molecular sampling effort would help in elucidating some of the hypotheses or resolving discordances (Kessous *et al.*, 2019). Next-generation sequencing (NGS), and especially genome skimming that allows the sequencing of the whole plastid genome, represent great opportunities to overcome molecular data limitations in phylogenetic studies (Coissac *et al.*, 2016). However, NGS methods have high cost and different challenges at the library preparation, sequencing and bioinformatics steps. For this reason, we tested if the use of a limited number of plastid genes would be sufficient to obtain high phylogenetic support within *Vriesea*. For this purpose, we performed the annotation of the partial plastome and selected the genes with a length > 900 bp (similar to the length usually obtained using Sanger sequencing) from the genome skimming alignment. Annotation for the partial plastome alignment obtained for one species (*Vriesea marceloi*) was performed in Geneious v.6.1.8 (Kearse *et al.*, 2012) using *Tillandsia usneoides* L. as a reference genome (NCBI number KY293680.1, Poczai & Hyvönen, 2017). Then, a set of 18 high-quality genes with sequence length greater than 900 bp was selected and extracted from the annotated sequence. This selection included genes normally used in phylogenetic studies with Bromeliaceae and also described as variables in Poczai & Hyvönen (2017).

To ensure that each region extracted from the annotated sequence of *V. marceloi* Versieux & T.Machado corresponded to the selected genes,

sequences were checked using nucleotide BLAST (Altschul *et al.*, 1990) available at NCBI (https://www.ncbi.nlm.nih.gov/). After a quality check, each selected gene sequence was used as a reference to extract the corresponding region from the plastid alignment of all our samples. Then, the alignments extracted for each gene were checked again using nucleotide BLAST (Altschul *et al.*, 1990), available at NCBI (https://www.ncbi.nlm.nih.gov/), to confirm the equivalence with the selected genes.

Phylogenetic analyses

We performed phylogenetic inference using three datasets: (1) a concatenated unpartitioned alignment of the plastome alignment generated by the genome skimming approach; (2) the nuclear gene *phyC* and (3) a concatenated alignment of 18 selected plastid genes and the nuclear *phyC* (18 plastid genes + *phyC*) to evaluate whether a reduced set of genes would have success in recovering well-supported trees. The latter was partitioned by genes and the best nucleotide substitution model for each gene was selected using JModelTest v.2.1.7 (Darriba *et al.*, 2012) based on the Bayesian information criterion (BIC) (Table 1). The numbers of variable sites, conserved sites and potentially parsimony-informative sites were calculated in MEGA 7 (Kumar, Stecher & Tamura, 2016) for all alignments (Table 1).

Maximum-likelihood (ML) analyses were performed using RAxML v.8.2.10 (Stamatakis, 2014) with 1000 rapid bootstrap replicate searches (Stamatakis, Hoover & Rougemont, 2008). The partial plastome alignment, nuclear *phyC* and the concatenated 18 plastid genes + *phyC* were analysed using GTRGAMMA as a nucleotide substitution model.

Additionally, we applied a Bayesian inference (BI) approach to *phyC* and to the concatenated alignment of 18 plastid genes + *phyC*, using MrBayes v.3.2.6 (Ronquist *et al.*, 2012). As the size of the dataset (> 200 species and > 70 000 bp) exceeds the computational limits of MrBayes, it was not possible to conclude the Bayesian analysis for the partial plastome. For each gene, we applied the best model of substitution selected by JModelTest (Table 1). Two independent runs of four Markov chains and 25 million generations were performed with sampling every 1000 generations. All phylogenetic analyses were performed on the CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2011). Convergence among the two runs was assessed in Tracer v.1.6.0 (Rambaut & Drummond, 2003). The consensus phylogenetic tree and node posterior probabilities were computed from the posterior distribution of trees after removal of a burn-in of 25%.

RESULTS

Genome skimming

The genome skimming approach generated 358 443 406 high-quality reads (Q > 20; including mitochondrial, nuclear and plastid reads) across the 206 samples successfully sequenced in this study. After mapping, SNP calling and quality filtering, an alignment of 77 836 high-quality plastid bases was obtain for the 206 samples. Only 3.6% of missing data were observed in the alignment and 75 654 bp were obtained on average per sample. The final alignment (including gaps after adding outgroups) was 78 483 bp and contained 6212 variable characters of which 2849 were potentially parsimony-informative. To avoid ambiguity about our plastid genome data set, we will refer to it as 'partial plastome' from here on.

Subsampling of plastid genes and nuclear *phyC* gene

The partial plastome annotated of *V. marceloi* featured the four typical plastid regions: one large single copy (LSC 56 433 bp), inverted repeat A (IRa 3197 bp), one small single copy (SSC 13 259 bp) and inverted repeat B (IRb 4947 bp). The IRa and IRb were different sizes since the percentage of coverage and the recovered genes in each of these parts were not the same. Ninety genes were recovered for *V. marceloi*, 35 totally and 55 partially. Eighteen of these were selected and extracted from the partial plastome alignment and they are shown in Table 1.

The *phyC* alignment for the 171 samples was 1006 bp long with 297 variable characters of which 159 were potentially parsimony-informative. The concatenated alignment of the 18 plastid genes + *phyC* had a total length of 31 241 bp, 2323 of which were variable and 1205 potentially parsimony-informative (Table 1). For the calculation of conserved and variable sites, gaps were not considered. All sequences generated in this work were included in a free and online repository (NCBI, National Center for Biotechnology Information) number SUB6280204 for the raw read data and see Supporting Information, Table S1 for *phyC* sequences.

Phylogenetic relationships

The phylogenetic tree based on the partial plastome dataset (Fig. 2) had higher bootstrap support (BS) and recovered more clades than the one obtained from the *phyC* alone (Supporting Information, Fig. S1) and from 18 plastid genes + *phyC* (Supporting Information, Fig. S2). The main clades (tribes Tillandsieae and Vrieseeae, subtribes Cipuropsidinae and Vrieseinae) were recovered with high support on trees obtained from partial plastome and 18 plastid

Table 1. Matrix allignment statistics. Recovery percentage is calculated in comparison with the genome used as reference for plastid annotation *T. usneoides* (Poczai & Hyvönen, 2017). For the calculation of conserved and variable sites, gaps were not considered.

	Length of alignment	Recovery %	Number of conserved sites (%)	Nunber of variable sites (%)	Number of potentially parsimony-informative sites (%)	Substitution model
atpA	1527	100	1461 (96)	66 (4)	32 (2)	HKY+I
atpB	1086	75	1047 (96)	39 (4)	16 (1)	HKY+I
atpF	1395	100	1286 (92)	109 (8)	54 (4)	F81+I+G
clpP	951	46	868 (91)	80 (8)	37 (4)	HKY+I
ndhA	2060	96	1886 (92)	168 (8)	94 (5)	HKY+I
ndhD	1230	81	1142 (93)	73 (6)	32 (3)	HKY+G
psaA	1945	87	1889 (97)	56 (3)	23 (1)	HKY+I
psaB	1016	46	954 (94)	54 (5)	41 (4)	HKY+I+G
psbA	1008	95	980 (97)	28 (3)	15 (1)	HKY+I
psbB	1246	81	1190 (96)	40 (3)	26 (2)	HKY+I
rpl16	1221	82	1139 (93)	77 (6)	44 (4)	HKY+I+G
rpoB	2153	67	2038 (95)	105 (5)	58 (3)	GTR+I+G
rpoC1	2204	79	2053 (93)	134 (6)	75 (3)	HKY+I+G
rpoC2	2172	55	2007 (92)	165 (8)	74 (3)	HKY+I
trnK-UUU	1838	68	1633 (89)	203 (11)	100 (5)	GTR+I+G
trnI-GAU	906	89	895 (99)	11 (1)	2 (0.2)	K80
ycf1	4813	85	4171 (87)	563 (12)	297 (6)	HKY+I+G
ycf3	1464	73	1386 (95)	67 (5)	30 (2)	HKY+I
phyC	1006	_	693 (69)	297 (30)	159 (15)	SYM+I+G
All genes concatenated	31241	_	28730 (92)	2323 (7)	1205 (4)	GTR+G
Partial plastome	78483	49	71623 (91)	6212 (8)	2849 (4)	GTR+G

genes + *phyC*. However, the tree obtained from *phyC* recovered Cipuropsidinae as sister to Tillandsieae (BS 65/PP 0.95) and both as a sister group of Vrieseinae (BS 92/PP 0.89). The *phyC* tree recovered extremely low support values within *Vriesea* (BS < 50 and PP < 0.50). For the tree obtained with 18 plastid genes + *phyC*, some clades were found in *Vriesea*, but in general, the groups received weak support (Supporting Information, Fig. S2). Therefore, only the results from the partial plastome are presented and discussed here (Fig. 2); the phylogenetic trees of the *phyC* and 18 plastid genes + *phyC* genes are shown in the Supporting Information (Figs S1, S2).

Tillandsieae were recovered with strong support (BS 100, Fig. 2) in a sister position to Vrieseeae, also strongly supported (BS 100, Fig. 2). *Guzmania* is monophyletic (BS 99) and sister to a clade composed of *Tillandsia*, *Racinaea* and *Vriesea lutheriana* J.R.Grant (BS 100, Fig. 2). Cipuropsidinae were strongly supported (BS 100, Fig. 2) and contained two clades also supported by a BS value of 100. The first was formed by *Werauhia* (BS 99) and *Lutheria* (BS 100). The second was composed of *Zizkaea*, the non-monophyletic *Goudaea* and the lineages of the *Cipuropsis–Mezobromelia* complex (BS 100).

Vrieseinae, containing *Alcantarea*, *Vriesea s.s.* and *Stigmatodon*, were also recovered as monophyletic with strong support (BS 99, Fig. 2). Although *Alcantarea* was monophyletic with strong support (BS 100), *Vriesea s.l.* is polyphyletic. A group of species was nested in *Stigmatodon* (BS 100) and one species (*V. lutheriana*) clustered with *Tillandsia*. The separation between *Vriesea* and *Stigmatodon* was strongly supported with a BS value of 100 (Fig. 2). *Vriesea drepanocarpa* (Baker) Mez (Fig. 1G) was recovered as sister to all other species of *Vriesea s.s.*. Within this group, 12 main clades were recovered with moderate-strong support in the partial plastome tree (BS values ranging from 80 to 100) (Fig. 2). Many *Vriesea* spp., including *V. longicaulis* (Baker) Mez, *V. itatiaiae* Wawra, *V. medusa* Versieux and *V. ensiformis* (Vell.) Beer, that were represented by more than two accessions were not recovered as monophyletic.

DISCUSSION

Genome skimming efficiency and phylogenetic utility

The genome skimming approach uses Illumina technology to obtain high-copy fractions of the genome

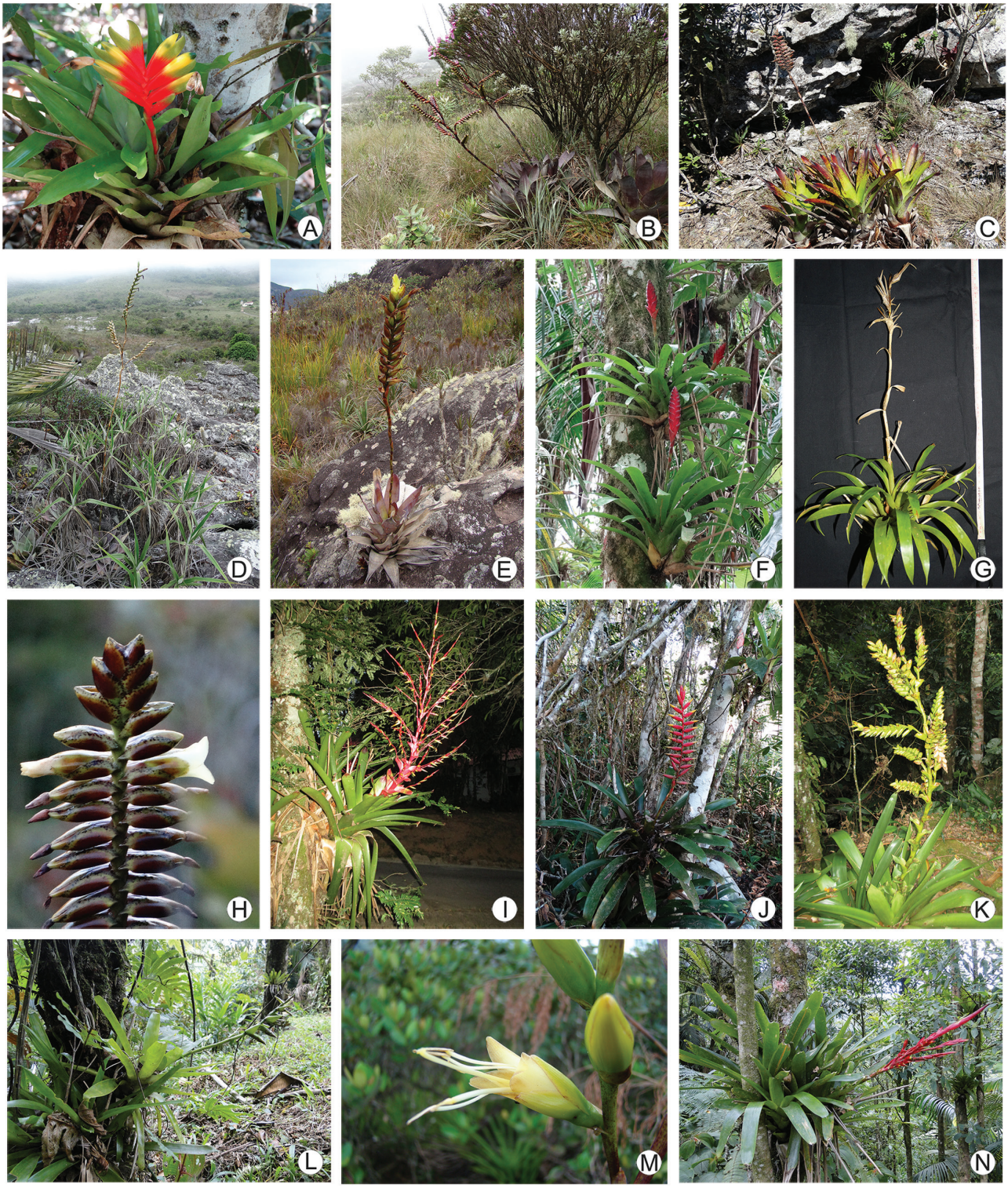
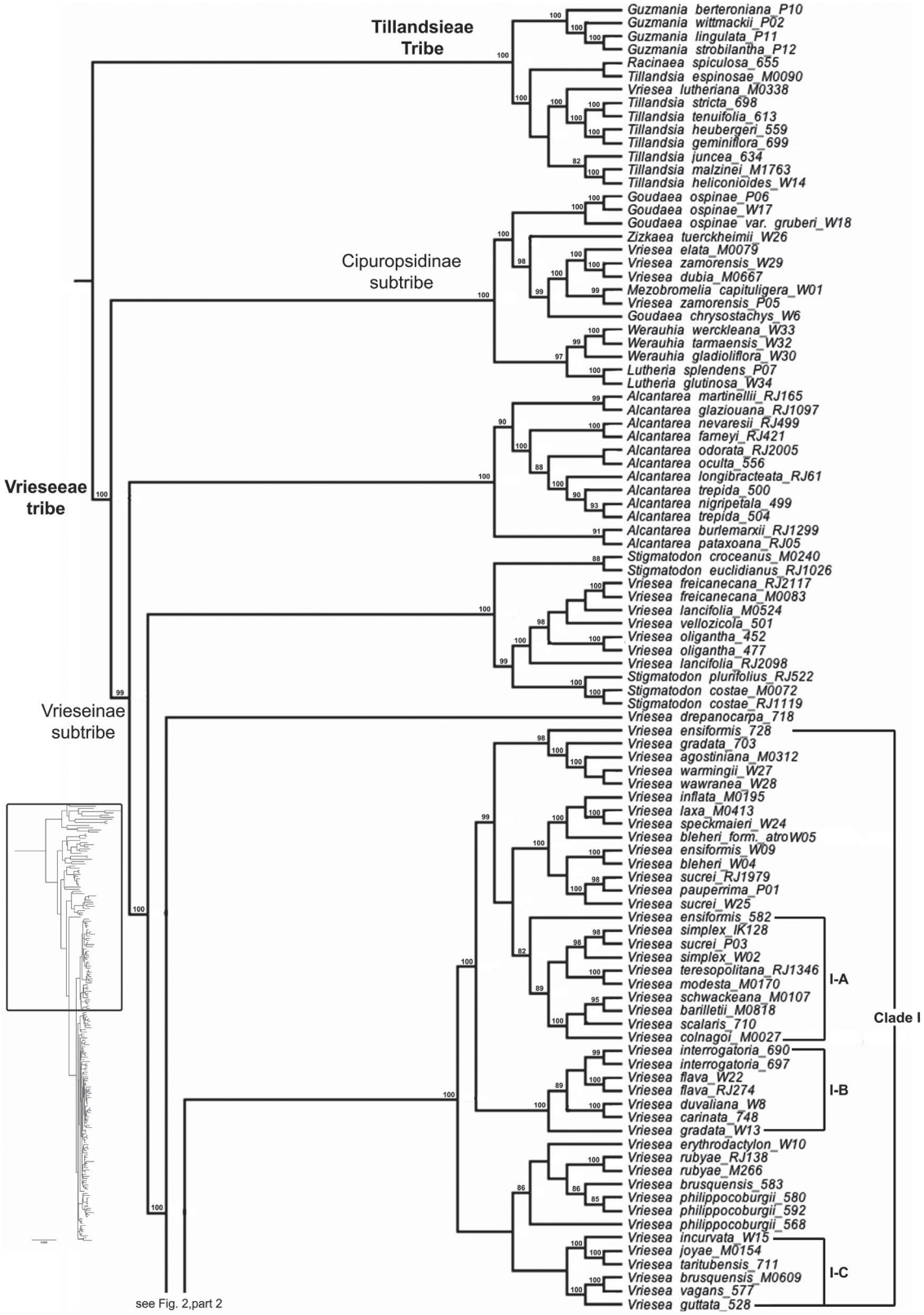


Figure 1. Species of the *Vriesea s.s.* clade showing habitat and inflorescence. A, *Vriesea carinata*. B, *Vriesea medusa*. C, *Vriesea minor*. D, *Vriesea diamantinensis*. E, *Vriesea clausseniana*. F, *Vriesea gradata*. G, *Vriesea drepanocarpa*. H, *Vriesea pseudoatra*. I, *Vriesea philippocoburgii*. J, *Vriesea ensiformis*. K, *Vriesea hydrophora*. L, *Vriesea unilateralis*. M, *Vriesea longistaminea*. N, *Vriesea brusquensis*. Photographs: T.M. Machado.



in low coverage, making it possible to retrieve sequence data for almost whole plastid genomes (Straub *et al.*, 2012). When combined with a multiplex protocol (Meyer & Kircher, 2010; Kircher *et al.*, 2012), it allows multiple samples to be sequenced in a single run and thus to obtain a large amount of genomic data at an affordable cost (Straub *et al.*, 2012; Malé *et al.*, 2014; Dodsworth, 2015). Using this approach, we were able to recover nearly half of the plastid genome for 206 species of Tillandsioideae, to our knowledge representing an unprecedented genomic dataset for an evolutionary study in Bromeliaceae. The fact that we did not recover > 49% (an average of 75 654 bp) of the plastid genome compared to the reference genome of the most closely related species, *T. usneoides* (159 657 bp; Poczai & Hyvönen, 2017) may be explained by the stringent quality filtering to retain a position in our study and by some extended deletions observed in our samples compared to the reference genome. Some authors suggest that a high level of multiplexing may reduce the sequencing capacity by up to 10–30% (Straub *et al.*, 2012). In our case, we obtain a high sequencing coverage for the plastid (36× on average per sample) and only 3.5% of missing data in our alignment of 77 836 bp. The continuous advances in Illumina sequencing technologies now providing hundreds of millions of reads per lane and the option of using more than one Illumina lane of sequencing allowed us to obtain high-quality data while multiplexing > 200 samples.

Bromeliaceae show a low substitution rate (Smith & Donoghue, 2008) and, in general, phylogenetic analyses produce trees with low resolution (Sass & Specht, 2010; Maia *et al.*, 2012; Versieux *et al.*, 2012; Evans *et al.*, 2015; Palma-Silva *et al.*, 2016). Thus, phylogenetic studies of Bromeliaceae currently have sought more robust resolution by increasing the number of plastid markers and including nuclear markers (Krapp *et al.*, 2014; Aguirre-Santoro *et al.*, 2016; Barfuss *et al.*, 2016; Palma-Silva *et al.*, 2016). The *phyC* is the most used nuclear gene in phylogenetic studies of Bromeliaceae (Silvestro *et al.*, 2014; Louzada *et al.*, 2014; Barfuss *et al.*, 2016; Castello *et al.*, 2016; Schütz *et al.*, 2016; Goetze *et al.*, 2017). In our dataset, 15% of all characters of *phyC* were potentially parsimony-informative, being the second most variable among the selected genes (Table 1). However, when analysed separately, it generated trees with strong support only for intergeneric relations, whereas within *Vriesea* support values were extremely low. A large polytomy and clades with low support were also found when analysing intrageneric relationships in Pitcairnioideae *s.l.* using *phyC*, especially in relation to *Puya* and *Dyckia* Schult.f. (Jabaily & Sytsma, 2010; Krapp *et al.*, 2014; Schütz *et al.*, 2016).

The concatenated matrix of 18 plastid genes + *phyC* resulted in trees with moderate support. Some of the major clades recovered by partial plastome were also recovered by this concatenated dataset, but the relationship between clades remained unclear due to lack of statistical support. Many of the selected genes were not fully recovered by genome skimming, but some showed a considerable number of potentially parsimony-informative and variable sites.

Comparing the results of the trees obtained with partial plastome data and the concatenated 18 plastid genes + *phyC* matrix, it is evident that the genome skimming approach is advantageous due to the large number of data obtained, in terms of both the number of base pairs and the number of species that can be multiplexed. In the case of our data, for which only partial plastomes were recovered, we were able to reconstruct phylogenetic trees that clarify intergeneric relationships with strong statistical support. However, for intrageneric relationships, the relationships between the recovered clades still lack statistical support. Other studies have shown that genome skimming may be effective in clarifying intrageneric relationships if few species are multiplexed, allowing a greater genome coverage (Parks, Cronn & Liston, 2009; Kane *et al.*, 2012; Malé *et al.*, 2014; Dodsworth, 2015). In taxa that diverged recently, as is the case for most species of Bromeliaceae (Givnish *et al.*, 2011, 2014), whole genome sequencing or target sequencing that produces hundreds of nuclear markers would be the next step to clarify intrageneric relationships.

Phylogenetic relationships in core Tillandsioideae

Petal appendages were a traditional morphological character used by Smith & Downs (1977) to differentiate *Tillandsia* from *Vriesea*. However, ontogenetic sequences revealed that petal appendages are the last external multicellular structures formed during the development (Brown & Terry, 1992). Several studies have demonstrated the fragility of this character to differentiate genera due to high levels of homoplasy (Schulte & Zizka, 2008; Barfuss *et al.*, 2016; Gomes-da-Silva & Souza-Chies, 2017). Nevertheless, the transfer

Figure 2. part 1. Cladogram from the ML analysis based on the partial plastome alignment (78 483 bp) for 206 specimens of Tillandsioideae. Names of tribes, subtribes and genera follow the classification of Tillandsioideae proposed by Barfuss *et al.* (2016). Numbers above the branches represent ML and BS values. Only values > 50% are shown. See Supporting Information for trees based on the nuclear gene *phyC* and the concatenation of 18 plastid genes + *phyC*. In detail: phylogram with proportional branch lengths from the ML tree.

of *Vriesea* spp. with petal appendages to *Tillandsia* proposed by Grant (1993, 1995, 2004) was corroborated by the phylogenetic analyses of Barfuss *et al.* (2016) and Gomes-da-Silva & Souza-Chies (2017). In our analysis, *T. heliconioides* Kunth, *T. malzinei* (E.Morren) Baker (formerly included in *Vriesea*) and *T. juncea* (Ruiz & Pav.) Poir. represent *Tillandsia* subgenus *Tillandsia*. This group is sister to a well-supported clade composed of one species representative of the *Tillandsia gardneri* Lindl. complex, two species of *Tillandsia* subgens *Anoplophytum* (Beer) Baker and *Vriesea lutheriana*. The phylogenetic proximity of *V. lutheriana* with Tillandsieae highlights that, despite recent taxonomical rearrangements, *Vriesea* as currently circumscribed is still polyphyletic. *Vriesea lutheriana* was described by Grant (1992: 199) as a species from Costa Rica with tripinnate inflorescences and conduplicate-spiral stigma type. These morphological characteristics are closely related to that of *V. duidae* (L.B.Sm.) Gouda and *V. rubrobracteata* Rauh that have never been sequenced and *V. elata* (Baker) L.B.Sm. and *V. zamorensis* (L.B.Sm.) L.B.Sm. that were both recovered in the *Cipuropsis–Mezobromelia* complex *sensu* Barfuss *et al.* (2016). In addition to *V. lutheriana,* the tripinnate inflorescence type is also known from six *Tillandsia* spp. from Peru (León & Alva, 2008). Two of these, *T. hildae* Rauh and *T. ferreyrae* L.B.Sm., were sampled in Barfuss *et al.* (2016) and formed a well-supported clade with *T. heliconioides* and *T. malzinei* in *Tillandsia* subgenus *Tillandsia*. Likewise, the position of *V. lutheriana* with *Tillandsia* spp. revealed in our analyses is statistically supported, although we cannot establish the exact phylogenetic position due to our low sampling of Tillandsieae; we suggest further investigations of the relationships among the tripinnate species (*V. lutheriana*, *T. hildae*, *T. ferreyrae*).

In Cipuropsidinae, two well-supported main clades were identified. The first is composed of *Werauhia* and *Lutheria.* The second clade includes the species of *Goudaea* and *Zizkaea*, both newly described by Barfuss *et al.* (2016), and a clade called the *Cipuropsis–Mezobromelia* complex by Barfuss *et al.* (2016) that is composed of the North-Andean species *V. dubia* (L.B.Sm.) L.B.Sm., *V. zamorensis*, *V. elata* and *Mezobromelia capituligera* (Griseb.) J.R.Grant. This group deserves further attention in order to clarify whether the type species of *Cipuropsis* (*C. subandina* Ule) will emerge among the mesomorphic northern Andean '*Vriesea*' spp. and whether they are related to *Mezobromelia* (Barfuss *et al.*, 2016; Gomes-da-Silva & Souza-Chies, 2017). Finally, the two *Goudaea* spp. do not form a monophyletic group, as *G. chrysostachys* (E.Morren) W.Till & Barfuss was found to be more closely related to the *Cipuropsis–Mezobromelia* complex than to *G. ospinae* (H.Luther) W.Till &Barfuss. Therefore, our results do not support the circumscription of Barfuss *et al.* (2016) of *Goudaea* as a monophyletic genus.

In Vrieseinae, which comprise the eastern Brazilian lineages and were the focus of our taxonomic sampling, *Alcantarea* emerges as the sister group of *Stigmatodon*, a new genus segregated from *Vriesea* (Barfuss *et al.* 2016) and *Vriesea s.s.* Intrageneric phylogenetic relationships in *Alcantarea* were generally well-supported (Fig. 2). Although our sampling of this genus is limited, evolutionary relationships seem to be linked to the geographical distribution since sister species tend to occur in geographical proximity [e.g. *A. burle-marxii* (Leme) J.R.Grant and *A. pataxoana* Versieux; *A. glaziouana* (Leme) J.R.Grant and *A. martinellii* Versieux & Wand.], a pattern already highlighted by Versieux *et al.* (2012) for the species from the Serra dos Órgãos mountain range. Related species showing grouped geographical distribution pattern may be the result of forest fragmentation leading to geographical isolation of ancestral populations during the cycles of climatic changes of the Plio-Pleistocene and has been hypothesized as a putative driver of speciation in this genus (Benzing, 2000; Versieux *et al.*, 2012). Our result indicates several *Vriesea* spp. [*V. freicanecana* J.A.Siqueira & Leme, *V. lancifolia* (Baker) L.B.Sm., *V. oligantha* (Baker) Mez and *V. vellozicola* Leme & J.A.Siqueira] cluster within the recently described genus *Stigmatodon* (Barfuss *et al.*, 2016). These species, which are epiphytes on species of *Vellozia* Vand. or rupicolous plants on inselbergs and quartzite areas of the Espinhaço mountain range differ from the other members of the *Stigmatodon* clade in several morphological characters (e.g. stigma, rosette and floral/inflorescence structure) and habitat preferences. The taxonomic revision of *Stigmatodon* was performed by Couto (2017) who named the species of *Vriesea* nested in this genus the *Vriesea limae* L.B.Sm. group. Taxonomic rearrangements will be proposed for the re-circumscription of *Stigmatodon* as a monophyletic genus (D. Couto, personal communication).

Vriesea s.s. is considered to be the 'true' *Vriesea* because *V. psittacina* (Hook.) Lindl., the type species of the genus, is found in this lineage (Barfuss *et al.*, 2016; Gomes-da-Silva & Souza-Chies, 2017). According to Gomes-da-Silva & Souza-Chies (2017), *Vriesea s.s.* contains species essentially distributed in the Paraná dominion (mostly Atlantic Forest) with a few occurrences in the Chacoan dominion. However, the increased taxonomic sampling of our analysis revealed that extra-Brazilian species such as *V. laxa* (Griseb.) Mez and *V. speckmeieri* W.Till from Venezuela, *V. macrostachya* (Bello) Mez from Puerto Rico and *V. oxapampae* Rauh from Peru, are also present in this clade, suggesting that calling this group of species the 'Brazilian lineage' is potentially misleading. Despite

their geographical disjunction, these four species share a stigma convolute-blade type (Grant, 1997; Till, 2008), a character referred to by Gomes-da-Silva & Souza-Chies (2017) as an important synapomorphy for the lineage. Thus, future research efforts in *Vriesea* should focus on the sampling of *Vriesea* spp. from the Amazon, the Guiana Shield and the Andes to investigate whether they belong to *Vriesea s.s.* or to the *Cipuropsis–Mezobromelia* complex.

In summary, in our study *Vriesea* spp. emerged in at least four evolutionary distinct lineages: (1) *Tillandsia*; (2) Cipuropsisinae; (3) *Stigmatodon* and (4) *Vriesea s.s.* Based on this result, further taxonomic rearrangements will be necessary to render *Vriesea* monophyletic.

Phylogenetic relationships in *Vriesea*

In *Vriesea s.s.*, *V. drepanocarpa* (Baker) Mez was found in a sister position to the remaining species of the clade. This result contrasts with previous phylogenetic studies based on morphological and molecular data, in which this species was nested in *Tillandsia*, in a sister position to *Racinaea* (Gomes-da-Silva & Souza-Chies, 2017). Unlike the other species of *Vriesea*, which have six to 12 columns of ovules in the locule, *V. drepanocarpa* only has four columns, similar to some species of *Tillandsia* and *Racinaea* (Kuhn *et al.*, 2016). Therefore, the close position of *V. drepanocarpa* to *Tillandsia* and *Racinea* in the study of Gomes-da-Silva & Souza-Chies (2017) might be explained by the fact that they included this morphological character in their phylogenetic analyses. In our ML phylogeny, a long branch separates *V. drepanocarpa* from the rest of the *Vriesea s.s.* clade. This suggests that the morphological differentiation of this species is underpinned by significant genetic divergence. Given that *V. drepanocarpa* also has a differentiated floral morphology with a simple-erect stigma type resembling that of *Goudaea chrysostachys* (Gomes-da-Silva & Souza-Chies, 2017), further studies are needed to confirm the exact phylogenetic position of this species.

Our study expands the sampling of *Vriesea s.s.* to species from other Brazilian biomes such as the Caatinga and the Cerrado, as well as to extra-Brazilian species. Based on our plastid phylogenetic tree, we define 12 main clades that have a good statistical support within *Vriesea s.s.,* and discuss their morphological or geographical characteristics.

Clade I includes 31 species (Fig. 2) combining nearly all sampled species with flat and simple inflorescences and tubular and distichous flowers, except for *V. rhodostachys* L.B.Sm. that was recovered as sister to clade II. Most of these species have complex morphological delimitations, often resulting in misapplied names in herbarium specimens (Costa *et al.*, 2014). Some of these problematic species have been recently revised (Costa, Rodrigues & Wanderley, 2009; Kessous, Salgueiro & Costa, 2018; Neves *et al.*, 2018). For instance, the *V. paraibica* Wawra complex was treated taxonomically by Costa *et al.* (2009) and is represented in our study by clade I-B comprising *V. duvaliana* E.Morren, *V. carinata* Wawra and *V. gradata* (Baker) Mez. However, the keels formed by two sepals, a synapomorphy for the group (Costa *et al.*, 2015), are missing in the last species. Taxa in the *V. incurvata* Gaudich. complex, recently re-circumscribed (Neves *et al.*, 2018), present simple inflorescences and suberect peduncles bearing bracts similar to the floral ones. In our phylogenetic tree, this group was recovered with high support in clade I-C, but it did not form a clade since *V. sucrei* L.B.Sm. & Read was nested instead in clade I-A. The latter clade combines the largest number of species including the *V. ensiformis* complex treated by Kessous *et al.* (2018). Although clade I is composed mostly of species with simple inflorescences, it also includes four species with composed inflorescences and tubular flowers [*V. vagans* (L.B.Sm.) L.B.Sm., *V. brusquensis* Reitz, *V. philippocoburgii* Wawra and *V. schwackeana* Mez] and one species with simple inflorescences and campanulate corolla type (*V. wawranea* Antoine). Finally, with the exception of *V. schwackeana*, which occurs in the Cerrado domain, all species in this clade are mostly epiphytes (rarely terrestrial) distributed in the Atlantic Forest.

Clade II comprises the taxa from the *V. corcovadensis* Mez complex revised by Gomes-da-Silva & Costa (2011) and Gomes-da-Silva *et al.* (2012), including *V. poenulata* (Baker) Mez, *V. flammea* L.B.Sm., *V. lubbersii* (Baker) E.Morren and *V. triangularis* Reitz. The monophyly of the group was already questioned in a cladistic analysis (Gomes-da-Silva *et al.*, 2012), but further morphological and molecular analyses (Costa *et al.*, 2015; Gomes-da-Silva & Souza-Chies, 2017) confirmed that the *V. corcovadensis* complex is monophyletic. Our analysis confirms the previous result since *V. arachnoidea* A.F.Costa was found nested in a weakly supported clade related to clades VII and VIII. Although our sample of *V. arachnoidea* was labelled as a topotype in the living collection where we sampled it, we cannot exclude a misidentification to explain this unexpected placement in the phylogenetic analysis. In addition, *V. paratiensis* E.Pereira clustered in clade II even though it does not have stolons, utriculiform rosettes or linear-triangular blades that are considered as synapomorphies for this group.

Clade III includes eight species divided into two groups. The first one is composed of *Vriesea penduliflora* L.B.Sm. and *V. thyrsoidea* Mez, two epiphytic, terrestrial or rupicolous species that grow as at elevations > 1500 m. *Vriesea penduliflora* is restricted

to the higher areas of the Mantiqueira mountain range and is included in the *Red List of the Brazilian Flora* (CNCFlora, 2012a), whereas *V. thyrsoidea* is restricted to the higher parts of the Serra dos Orgãos. Both species have compound inflorescences, primary bracts covering the pedicel of the branches and tubular flowers secund at anthesis (Smith & Downs, 1977). The second group includes *V. longicaulis*, *V. itatiaiae*, *V. stricta* L.B.Sm., *V. bituminosa* Wawra and *V. jonghei* (K.Koch) E.Morren. Several samples were included for each of these species and our result indicates that none of them are monophyletic. *Vriesea sceptrum* Mez emerged as sister of all other species in clade III and is a species restricted to the higher parts of the Mantiqueira mountain range. It is morphologically similar to *V. penduliflora* with compound inflorescences, primary bracts covering the pedicel of the branches but with tubular flowers never secund at anthesis (Machado & Menini Neto, 2010).

Clade IV includes *V. unilateralis* (Baker) Mez, *V. botafogensis* Mez, *V. oxapampae* Rauh and *V. saundersii* (Carrière) E.Morren ex Mez. *Vriesea saundersii* and *V. botafogensis* were also recovered as member of a similar clade (i.e. clade R) in Gomes-da-Costa & Souza-Chies (2017). *Vriesea botafogensis* was considered as a synonym of *V. saundersii* until a decade ago because these species have similar inflorescences, flowers and rosettes and both are endemic to inselbergs in the city of Rio de Janeiro (Smith & Downs, 1977; Leme & Costa, 1994).

Clade V includes 17 species (Fig. 2) with simple or compound inflorescences and campanulate corolla type, with the exception of *Vriesea stricta* L.B.Sm. and *V. jonesiana* Leme that have tubular flowers. The floral morphology of most species in this clade was formerly used as a criteria to delimitate *V. section Xiphion* (E.Morren) E.Morren ex Mez, a group that has been shown to be non-natural (Versieux *et al.*, 2012; Costa *et al.*, 2015; Gomes-da-Silva & Souza-Chies, 2017). Two smaller groups stand out in clade V for their geographical structure. Clade V-A comprises *V. pulchra* Leme & L.Kollmann, *V. linharesiae* Leme & J.A.Siqueira, *V. carmeniae* R.Moura & A.F.Costa and *V. minutiflora* Leme, all restricted to either the north-eastern region of Brazil or the Caatinga domain at elevations > 800 m, except for *V. pulchra* that also occurs in the Atlantic Forest. Clade V-B includes the sympatric species *V. medusa* (Fig. 1B) and *V. nanuzae* Leme, restricted to montane areas in the Diamantina Plateau region along the central portion of the Espinhaço mountain range (Versieux, 2008; Versieux *et al.*, 2010). These two species show great morphological similarity, both with compound and highly branched inflorescences as well as campanulate flowers secund at anthesis (Versieux, 2008; Leme, Trindade-Lima & Ribeiro, 2010). These species are often misidentified in herbarium collections as *V. diamantinensis* Leme and *V. simulans* Leme (clade VIII) due to their similar morphologies. Furthermore, they also exhibit a great intraspecific morphological variation that renders their taxonomic delimitation challenging (pers. obs.) (Fig. 1B, D).

Clade VI includes species with simple inflorescences, distichous flowers and campanulate corolla type. Although they are morphologically closely related to clade V, there is no support in our phylogenetic trees for a sister relationship among these two clades. *Vriesea macrostachya* (Bello) Mez, a species distributed in the Greater Antilles (Moura, 2011) was recovered as sister to all other species of the clade. Despite their disjunct distributions, *V. macrostachya* morphologically resembles *V. chapadensis* Leme, a species restricted to the rock outcrops in the northern part of the Espinhaço mountain range (Moura, 2011). One species in this clade (*V. longicaulis)* has a distinct morphology with distichous floral bracts and flowers secund at anthesis. However, the three samples of this species are placed in distinct clades, making the position of this species uncertain.

Clades VII, VIII and IX are moderately supported clades containing 12 morphologically related species. The phylogenetic relationships among these three clades are weakly supported (Fig. 2). Most species present compound inflorescences with campanulate corolla type and flowers secund at anthesis. Although they share similarities regarding the inflorescence morphology, these clades are distributed in different habitats. Although the species of clade VII are epiphytes from the Atlantic Forest, species in Clade VIII a rupicolous species of the Cerrado domain and species of Clade IX are found at the transition between this biome and the Atlantic Forest (BFG, 2018).

Clade X includes six species: *Vriesea neoglutinosa* Mez, *V. friburgensis* Mez, *V. procera* (Mart. ex Schult. & Schult.f.) Wittm., *V. rodigasiana* E.Morren and *V. densiflora* Mez. *Vriesea densiflora* has a tubular rosette and congest inflorescences with tubular yellow flowers. This species is rupicolous and is an endemic of rock outcrops in the Diamantina Plateau region along the central portion of the Espinhaço mountain range in the Cerrado domain (Versieux & Wendt, 2006; Versieux *et al.*, 2010). The placement of *V. densiflora* in clade X, outside the '*V. minarum* L.B.Sm. group', proposed by Versieux (2011), is surprising given its morphological and ecological divergence with the other species of this clade. All species except *V. densiflora* have infundibuliform rosettes, lax and compound inflorescences and usually reddish, yellow and tubular flowers with exserted stamens (except in *V. procera* in which they are included) (Smith & Downs, 1977). They are epiphytic, rupicolous or terrestrial species distributed along the Atlantic Forest in several

habitats of this domain as inselberg mats and restingas (BFG, 2018). *Vriesea procera* has a wide geographical distribution with species reaching northern South America, and is divided into four varieties (Smith & Downs, 1977). Our data show that *V. procera*, *V. neoglutinosa* and *V. friburgensis* are related, and they have been recognized as the *V. procera* complex due to the difficulty in recognizing and delimiting taxa in this group (Versieux & Wendt, 2006; Costa, Gomes-da-Silva & Wanderley, 2014; Uribbe, 2014).

Clade XI comprises *V. sincorana* Mez and *V. atropurpurea* Silveira, which share similarities in the rosette and inflorescences and have flowers with campanulate corollas and exserted stamens (Moura, 2011). Both species are restricted to the Espinhaço mountain range, but *V. atropurpurea* occurs to the south in the Cerrado domain, whereas *V. sincoranea* occurs to the north in the Caatinga domain (Moura, 2011; BFG, 2018). There is a striking phenotypic similarity between *V. atropurpurea* and *V. longstaminea* C.C.Paula & Leme that leads to frequent misidentifications in herbaria (Leme & Paula, 2004; Coffani-Nunes *et al.*, 2010; Moura, 2011). They were recovered as sister species in the study of Gomes-da-Silva & Souza-Chies (2017) and were treated as synonyms in Moura (2011). *Vriesea longistaminea* (Fig. 1M) is restricted to an area of < 8 km^2 in an ironstone outcrop region known as the Iron Quadrangle (Leme & Paula, 2004; Jacobi *et al.*, 2007) and is categorized as Critically Endangered (CR) in the *Red List of the Brazilian Flora* (CNCFlora, 2012b). *Vriesea atropurpurea* and *V. longistaminea* do not occur sympatrically but their distributions are separated by only 100 km. Despite their strong morphological similarity and geographic proximity, our analysis suggests that these species are not phylogenetically closely related since *V. longistaminea* is sister to clade VIII. Therefore, the morphological similarity between *V. atropurpurea* and *V. longistaminea* is probably a case of convergent adaptation to similar environmental conditions. In a large genus such as *Vriesea*, occupying contrasting environments including rock outcrops and ombrophilous forests, identifying occurrences of phenotypic convergence can help in elucidating the processes involved in the diversification of the group.

Clade XII includes *Vriesea minarum* L.B.Sm., *V. clausseniana* (Baker) Mez and *V. marceloi* Versieux & T.M.Machado, all rupicolous and heliophytic species growing at > 1000 m elevation along the southern portion of the Espinhaço mountain range (Versieux *et al.*, 2008). Specimens of *V. marceloi* can be found in herbaria identified as *V. clausseniana* and the two species occur sympatrically, although *V. marceloi* only grows above 1900 m of elevation where mist formation is frequent (Versieux & Machado, 2012). *Vriesea clausseniana* has infundibuliform rosettes, and strongly secund yellow flowers at anthesis with campanulate corollas and exserted stamens. This floral morphology suggests a bat pollination, whereas *V. minarum* and *V. marceloi* have tubular rosettes and yellow tubular flowers secund at anthesis that are pollinated by hummingbird, suggesting that these floral characters are labile in this group (Versieux, 2011; Versieux & Machado, 2012). These species were considered morphologically related to *V. stricta* (clades III and V) and *V. densiflora* (clade X) in taxonomic works, but our genomic data placed them in different clades.

Many *Vriesea* spp. show considerable intraspecific morphological variation, and this is often insufficiently documented in morphological descriptions and poorly represented in identification keys (Costa *et al.*, 2009; Neves *et al.*, 2018). To address these complex species delimitations, we included more than one sample per species in our phylogenetic analysis. Our results show that many of these species are not monophyletic. For instance, two samples (547 and 550) of *V. medusa* collected in the same locality were found in distant positions in the phylogenetic tree. Likewise, the samples of *V. itatiaiae* (704 and 574) from the same locality did not appear as a monophyletic lineage. These findings could be explained either by the poor resolution between clades in *Vriesea s.s.* due to the lack of informative characters at a low level of divergence. Alternatively, the non-monophyly of many species of *Vriesea* could reflect true biological processes. Indeed, species complexes and non-monophyletic species are common in plants (Naciri & Linder, 2015; Pinheiro, Dantas-Queiroz & Palma-Silva, 2018) and are thought to be the result of hybridization or incomplete lineage sorting. Assuming that post-zygotic barriers for reproduction of bromeliads are potentially weak (Palma-Silva *et al.*, 2011; Wagner *et al.*, 2015) and that the individuals often grow in mixed aggregates of species sharing the same pollinator, interspecific gene flow may occur and lead to the formation of natural hybrids (Palma-Silva *et al.*, 2011; Lexer *et al.*, 2016; Zanella *et al.*, 2016; Neri, Wendt & Palma-Silva, 2017; Mota *et al.*, 2019). Furthermore, natural hybrids of closely related *Vriesea* spp. have been identified (Zanella *et al.*, 2016; Neri *et al.*, 2017). Therefore, we hypothesize that hybridization could be one of the evolutionary process driving phenotypic variation and blurring species delimitation in the genus.

CONCLUSIONS

Our study provided the first phylogenetic hypothesis for Vrieseinae based on a comprehensive sampling of *Vriesea* and an extensive coverage of the plastid genome. The genome skimming approach used in this study allowed to recover large-scale plastid data to infer the evolutionary relationships in core Tillandsioideae with good support. Our results are congruent with

the taxonomic rearrangements proposed by Barfuss *et al.* (2016), with the exception of *Goudaea*, which was not recovered as monophyletic. We show that *Vriesea* remains polyphyletic, as suggested in previous works, a finding that calls for further taxonomic rearrangements based in phylogenetic relationships and in-depth morphological studies. More specifically, this would include: the transference of *V. lutheriana* to *Tillandsia*; the transfer of several taxa to *Stigmatodon*; and a revision of *Cipuropsis*. Our analysis does not corroborate the delimitation of *Vriesea s.s.* proposed by Gomes-da-Silva & Souza-Chies (2017), since we recovered *Stigmatodon* as a separate lineage, whereas it was nested in *Vriesea s.s.* in their study. Furthermore, we showed that *Vriesea s.s.* is not restricted to eastern Brazil; it also contains species distributed in the Andes, the Caribbean and in other southern South American countries. In *Vriesea s.s.*, 12 strongly supported clades are recognized and supported by morphological characters or geography. Therefore, our phylogenetic study of *Vriesea* contributes to overcome some of the limitations of the traditional taxonomy based solely on morphological characters where well-defined morphological groups of species may include convergent, yet unrelated, taxa. Finally, our work provides the basis for the selection of *Vriesea s.s.* species to be used in future microevolutionary studies, which will aim at a better understanding of the drivers of the evolution of this important floristic component of the Brazilian Atlantic Forest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Cladogram from the maximum-likelihood (ML) analysis based on nuclear gene *phyC* for 171 specimens of Tillandsioideae. First numbers above the branches represent bootstrap values (BS) and the second number represents posterior probabilities (PP). Only values > 50% are shown. In detail, phylogram with proportional branch lengths from the BI and ML analyses.

Figure S2. Cladogram from the Bayesian inference (BI) based on 19 concatenated genes (*atpA*, *atpB*, *atpF*, *clpP*, *ndhA*, *ndhD*, *psaA*, *psaB*, *psbA*, *psbB*, *rpl16*, *rpoB*, *rpoC1*, *rpoC2*, *trnK-UUU*, *trnl-GAU*, *ycf1*, *ycf3*, *phyC*) for 206 species of Tillandsioideae. The first number above the branches represents PP, and the second number represents BS values. Only values > 50% are shown. In detail, phylogram with proportional branch lengths from the BI and ML analyses.

Table S1. Studied material with silica number/NCBI sample names for plastid reads (SUB6280204), voucher, locality and GenBank accession numbers for nuclear gene *phyC*. Abbreviations: BHCB, Herbarium of Universidade Federal de Minas Gerais; R, Herbarium of Museu Nacional (Brazil); RBvb, Bromeliad Living Collection of the Botanical Garden of Rio de Janeiro; SEL, Marie Selby Botanical Garden; WU-HBV, Botanical Garden of the University of Vienna; P, Jardin des Serres d'Auteuil Botanical Garden.