RESEARCH ARTICLE

Target-enrichment sequencing reveals for the first time a well-resolved phylogeny of the core Bromelioideae (family Bromeliaceae)

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Abstract A phylogenomic analysis of the so far phylogenetically unresolved subfamily Bromelioideae (Bromeliaceae) was performed to infer species relationships as the basis for future taxonomic treatment, stabilization of generic concept, and further analyses of evolution and biogeography of the subfamily. A target-enrichment approach was chosen, using the Angiosperms353 v.4 kit RNA-baits and including 86 Bromelioideae species representing previously identified major evolutionary lineages. Phylogenetic analyses were based on 125 target nuclear loci, assembled off-target plastome as well as mitogenome reads. A Bromelioideae phylogeny with a mostly well-resolved backbone is provided based on nuclear (194 kbp), plastome (109 kbp), and mitogenome data (34 kbp). For the nuclear markers, a coalescent-based analysis of single-locus gene trees was performed as well as a supermatrix analysis of concatenated gene alignments. Nuclear and plastome datasets provide well-resolved trees, which showed only minor topological incongruences. The mitogenome tree is not sufficiently resolved. A total of 26 well-supported clades were identified. The genera *Aechmea*, *Canistrum*, *Hohenbergia*, *Neoregelia*, and *Quesnelia* were revealed polyphyletic. In core Bromelioideae, *Acanthostachys* is sister to the remainder. Among the 26 recognized clades, 12 correspond with currently employed taxonomic concepts. Hence, the presented phylogenetic framework will serve as an important basis for future taxonomic revisions as well as to better understand the evolutionary drivers and processes in this exciting subfamily.

Keywords Angiosperms353 kit; genus concept; hybrid enrichment; mitogenome; off-target data; phylogenetic conflict; phylogenomics; plastome; Poales

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

The angiosperm family Bromeliaceae comprises 3630 species (Gouda & al., 2018), all but one distributed in the Neotropics. The ecological range of the family spans from hyper-arid deserts (Atacama) to humid lowland and mountain forests, and from sea level (e.g., *Pitcairnia halophila* L.B.Sm.; nomenclature follows Gouda & al., 2018) to elevations of over 3000 m (*Puya raimondii* Harms), with diversity centers in Central America, the Northern Andes, the Guayana Highlands, and the Brazilian Atlantic Forest. Although highly variable and widely distributed, a recent conservation assessment according to IUCN categories revealed 81% of the assessed species as possibly threatened with extinction (Zizka & al., 2020). Moreover, with over 1770 epiphytic species it is the most diverse family among Neotropical epiphytes (Zotz, 2016). The extraordinary physiological and morphological diversity includes features like leaf succulence, Crassulacean acid metabolism (CAM), and peculiar key innovations such as highly developed absorptive hairs and water storage in the leaf rosette (tank habit) (e.g., Horres & Zizka, 1995; Benzing, 2000; Silvestro & al., 2014; Crayn & al., 2015; Males, 2016). This, together with the relatively recent diversification (crown age of the extant lineages: 19.1 ± 3.4 Ma; Givnish & al., 2011), makes the family a fascinating and scientifically informative model to study evolution in the Neotropics, including colonization and radiation in the major Neotropical biomes (e.g., Givnish & al., 2014; Cruz & al., 2017; Maciel & al., 2020).

From the systematic point of view, the family is divided into eight subfamilies (Givnish & al., 2007), with the Bromelioideae

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the most diverse in the number of genera (38) and, after Tillandsioideae, the second most diverse in species number (987, Gouda & al., 2018). Ancestral Bromelioideae are inferred to have been terrestrial with C_3 photosynthesis, without water-impounding tanks (Silvestro & al., 2014). These traits are currently present in so called "early diverging lineages" that are distributed in temperate to cool, and mesic to humid habitats (*Fascicularia* Mez, *Greigia* Regel, *Ochagavia* Phil.), and in dry tropical conditions (*Bromelia* L., *Deinacanthon* Mez, Cryptanthoid complex). However, the center of Bromelioideae species diversity is the Atlantic rainforest of Brazil (Zizka & al., 2020), where the subfamily underwent rapid speciation, and species almost exclusively are characterized by tank habit, diploidy, and CAM photosynthesis (so called "core Bromelioideae"; Gitaí & al., 2014; Silvestro & al., 2014; Crayn & al., 2015; Paule & al., 2020a).

While systematics, generic delimitation, and phylogenetic relationships in the other subfamilies are fairly resolved (e.g., Tillandsioideae: Barfuss & al., 2016, Pitcairnioideae: Schütz & al., 2016; Pinangé & al., 2017; Gomes-da-Silva & al., 2019; Moura & al., 2019), this is not the case for Bromelioideae, where the huge, clearly polyphyletic genus *Aechmea* Ruiz & Pav. and its allies pose a major challenge for efforts to understand the phylogeny, evolution, and biogeographic history within the subfamily. Several molecular systematic approaches based on Sanger-sequencing have tackled this problem, but often lacked representative sampling and comprised limited sequence information. Our ongoing work has included 434 Bromelioideae species, but sequenced loci have been limited to five markers from the nuclear genome and five markers from the plastome (Heller, 2018). These data have contributed to revisions of established Bromelioideae clades (Leme & al., 2017, 2021, 2022; Matuszak-Renger & al., 2018; Maciel & al., 2019). However, a fully resolved backbone for core Bromelioideae has not been provided up to now (Sass & Specht, 2010; Silvestro & al., 2014; Evans & al., 2015).

Next-generation sequencing (NGS) technologies can provide sequence data for hundreds of genes and even whole-genomes for large numbers of species. However, so far, only isolated clades within Bromelioideae have been studied using this approach. A recent study based on plastome and rDNA cistron sequence data focused only on the genera *Fascicularia* and *Ochagavia* (Paule & al., 2020b). Similarly, a study based on de novo as well as re-sequenced nuclear genomes focused narrowly on several *Ananas* Mill. species and varieties (Chen & al., 2019).

Increasingly, target capture (or target enrichment) combined with high-throughput sequencing became popular to obtain sequence information for hundreds of putatively single-copy protein-coding genes in large sample sets and has been effectively utilized in phylogenomic studies (e.g., Fragoso-Martínez & al., 2017; Villaverde & al., 2018; Ogutcen & al., 2021; Schneider & al., 2021a,b). The method employs RNA-probes to capture fragments of interest in an NGS library, which are subsequently sequenced. The approach requires the identification of suitable target regions a priori, which may require high financial and temporal investments upfront, especially in non-model organisms (Schneider & al., 2021a). Consequently, the identification of universal probe sets, applicable across a wide range of taxa, has garnered widespread interest. For plant phylogenomics, the Angiosperms353 probe set (Johnson & al., 2019), which targets 353 nuclear single-copy genes in all angiosperms, has gained particular popularity as it resolved relationships in several species-rich and rapidly radiating groups across the angiosperm tree of life (e.g., Larridon & al., 2020; Pérez-Escobar & al., 2021; Shah & al., 2021; Thomas & al., 2021).

Accordingly, based on representatives from major evolutionary lineages of Bromelioideae detected in former studies (Schulte & al., 2009; Sass & Specht, 2010; Silvestro & al., 2014; Evans & al., 2015; Leme & al., 2017, 2021; Heller, 2018; Matuszak-Renger & al., 2018; Maciel & al., 2019) and extensive DNA sequence information generated using the Angiosperms353 kit we aimed in this study (1) to reconstruct the phylogeny of the subfamily Bromelioideae with a specific focus on early diversification of major lineages within the core Bromelioideae, (2) to identify new and/or confirm previously recognized monophyletic groups, and (3) to provide a basis for taxonomic and nomenclatural changes necessary to make currently recognized taxa monophyletic.

■ MATERIALS AND METHODS

Plant material. — Altogether 87 samples were included in the presented study, comprising 31 genera and 86 species of Bromelioideae. Thus, our sampling represents 73.7% and 8.7% of the Bromelioideae genera and species, respectively. Several studies consistently showed good statistical support for "early diverging Bromelioideae" including genera *Bromelia*, *Ochagavia*, *Greigia*, *Fascicularia* and *Deinacanthon*, *Ananas* as well as the Cryptanthoid complex (e.g., Schulte & al., 2009; Silvestro & al., 2014; Leme & al., 2021, 2022). Here, we focused on the diversification of the core Bromelioideae and included *Deinacanthon urbanianum* (Mez) Mez as an outgroup along with two *Fernseea* Baker species and few representatives of the Cryptanthoid complex and *Ananas* group (8 and 2 species respectively), omitting samples of *Ananas*, *Disteganthus* Lem., *Forzzaea* Leme & al., and *Lapanthus* Louzada & Versieux. Focusing on the core Bromelioideae sensu Silvestro & al. (2014) and Paule & al. (2020b), the small genera *Acanthostachys* Klotzsch and *Neoglaziovia* Mez are well-represented, each with two out of three recognized species. Other well-circumscribed and previously studied groups like the *Ronnbergia* E.Morren & André alliance and the new genus *Karawata* J.R.Maciel & G.Sousa (Aguirre-Santoro & al., 2016; Aguirre-Santoro, 2017; Maciel & al., 2019) were also included with few representatives (5 and 1, respectively). For the remaining core Bromelioideae, we included representatives of all genera except the monospecific *Pseudaechmea* L.B.Sm. & Read and *Eduandrea* (Baker) Leme & al., due to lack of available material. Of the polyphyletic genus *Aechmea*,

we included representatives of the recognized subgenera except *Ae*. subg. *Ortgiesia* (Regel) Mez, the only one for which monophyly was already shown by Silvestro & al. (2014) and comprehensively confirmed by Goetze & al. (2016). Moreover, we included at least one representative of each clade identified by Heller (2018) and Leme & al. (2021) and of most of the clades identified by Sass & Specht (2010). A list of all studied samples with geographical origin and herbarium voucher information is included in Appendix 1.

DNA extraction. — Total genomic DNA was isolated either from ca. 20 mg of silica-gel dried leaves or from fresh material according to the modified CTAB method (Doyle & Doyle, 1990) as described in Horres & al. (2000) including an additional purifying step to precipitate remaining polysaccharides (Michaels & al., 1994). The DNA concentration was quantified using a Qubit dsDNA BR Assay Kit and a Qubit v.2.0 fluorometer (Invitrogen, Carlsbad, California, U.S.A.), and the quality was visually assessed using 1.5% agarose gel electrophoresis.

Target enrichment, library preparation, and DNA sequencing. — Library preparation was done using KAPA Hyperplus Library Prep Kit (Roche, Basel, Switzerland), according to the manufacturer's recommendations. Fragmentation of the DNA was done using an ultrasonicator E220 (Covaris, Woburn, Massachusetts, U.S.A.). The fragment-length of the libraries was chosen to be on average at 500 bp. The resulting libraries were then checked for quality on an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, California, U.S.A.) and quantified with a Qubit v.3.0 fluorometer. Equimolar pools (1 µg) were enriched using the Angiosperms353 v.4 kit (Johnson & al., 2019) following the manufacturer's (Daicel Arbor Biosciences, Ann Arbor, Michigan, U.S.A.) recommendations. Up to 20 samples were pooled in a single hybridization reaction for hybridization with the Angiosperms353 v.4 kit RNA-baits. Hybridizations were carried out at 65°C for 48 hours. Enriched products were amplified using KAPA HiFi 2x HotStart ReadyMix (Roche, Basel, Switzerland). PCR products were cleaned via AMPure XP magnetic-beads (Beckmann Coulter, Indianapolis, Indiana, U.S.A.). Products were quantified using a Qubit v.3.0 fluorometer and quality checked by Agilent Bioanalyzer. Multiple enriched library pools were then multiplexed and sequenced at the Georgia Genomics and Bioinformatics Core (GGBC) Facility (University of Georgia, Athens, Georgia, U.S.A.) on an Illumina NextSeq 500 platform (Illumina, San Diego, California, U.S.A.). The sequencing runs generated about 800 million paired-end 150 bp reads, with 82% at or above quality-score Q30. The read number distribution across libraries was between 1,218,422 and 24,743,086 reads per sample. Raw reads are deposited in the NCBI Sequence Read Archive (SRA) database under the BioProject ID PRJNA704933.

Data treatment. — Illumina raw reads were trimmed using Trimmomatic v.0.33 (Bolger & al., 2014). As suggested for our particular downstream procedure (Johnson & al., 2019), reads with a quality score below 20 and those with 4 bp window below 20 were removed (LEADING: 20 TRAILING: 20 SLIDING WINDOW: 4:20 MINLEN: 50). The recovered sequences were then assembled using HybPiper v.1.3 (Johnson & al., 2016), using the target file available at https://github.com/mossmatters/Angiosperms353. In HybPiper, the reads are mapped using BWA v.0.7.17 (Li & Durbin, 2009), de novo gene assembly is done using SPAdes v.3.14.1 (Bankevich & al., 2012). Coding sequences were then extracted using Exonerate v.2.4.0 (Slater & Birney, 2005). Additionally, sequences flanking the exons (i.e., introns and untranslated regions) were recovered using the *intronerate.py* script included in HybPiper. Statistical analysis of sequence recovery was done using the *get_seq_lengths.py* and the *hybpiper_stats.py* function respectively, as described in the HybPiper manual, and by using R v.3.6.0 (R Core Team, 2020). The nuclear exon sequences, as well as the supercontigs (exon + flanking non-coding sequences), were additionally trimmed using trimAL v.1.4.1 applying *automated1* setting (Capella-Gutiérrez & al., 2009). For phylogenetic reconstruction, we chose all trimmed alignments for which at least 50% of the mean exon length (according to Johnson & al., 2019) was recovered for at least 75% of the species included.

In order to explore the off-target fraction of the sequence data, raw reads were first filtered using BBDuk v.1.0 from BBTools (Bushnell, 2017) as implemented in Geneious v.11.1.5 (Kearse & al., 2012) to remove adapters, known Illumina artifacts, phiX, and to quality-trim both ends to <Q20 or discard if read length was below 10 bp after trimming. The reads of each sample were subsequently mapped to the plastome of *Ananas comosus* (L.) Merr. (GenBank NC_026220.1; Nashima & al., 2015) edited to include only one inverted repeat (IR) copy (132,862 bp). Reads were also mapped to five mitochondrion genes previously sequenced in the family Bromeliaceae (apocytochrome b gene: DQ916699, F1-ATPase alpha subunit gene: AY299710, maturase gene: DQ401378, NADH dehydrogenase subunit 5 gene: DQ406945, ribosomal protein S3 gene: GU351853; total length 6650 bp) using the Geneious mapper, custom sensitivity (gaps per read 5%, maximum gap size 5, word length 20, index word length 15, maximum mismatches per read 10%, maximum ambiguity 4, ignoring words repeated more than 20 times) and 25 iterations. The majority consensus sequences were called with a minimum coverage of 3×. In the case of the plastome dataset consensus sequences were trimmed to the reference sequence, in the case of the 5 separate mitochondrion genes also nucleotides mapped beyond the reference were kept, gene alignment sites containing more than 80% gaps were masked and the separate alignments were concatenated.

Phylogenetic reconstruction. — All datasets were separately aligned with MAFFT v.7.407 (Katoh & Standley, 2013) using default parameters and automatic selection of the appropriate alignment strategy (*auto* mode). For the nuclear dataset, introns and untranslated regions were concatenated.

Maximum likelihood (ML) phylogenetic analyses of individual nuclear, plastome, and mitogenome supermatrices were performed with RAxML-HPC BlackBox v.8.2.12 (Stamatakis, 2014) on CIPRES Science Gateway v.3.3 (Miller

& al., 2010) and XSEDE (Towns & al., 2014) using default settings and automatic bootstrapping halt. Support values for resulting tree branches are given as bootstrap percentage support values (BS). Bayesian phylogenetic inferences (BI) were also conducted on CIPRES Science Gateway using MrBayes v.3.2.7a (Ronquist & al., 2012) and GTR + Γ model. Two independent runs with four Markov chains each (one cold chain and three heated) were carried out for 10^6 generations sampling trees every 1000th generation. We assessed convergence of the parameters by evaluating the estimated sample size (ESS > 200) in Tracer v.1.7 (Rambaut & al., 2018) and the potential scale reduction factor (PSRF→1) (Gelman & Rubin, 1992). The first 25% of the sampled trees were discarded as burn-in, and the two runs were combined. A majority rule consensus of the remaining trees was computed to calculate Bayesian posterior probabilities (PP).

ML and BI analyses were carried out for all three concatenated datasets (nuclear, plastome, mitogenome). Additionally, a coalescence approach using unrooted gene trees was performed for the nuclear exon sequences. Separate trees were inferred for each exon using RAxML v.8.2.11 as implemented in Geneious, GTRGAMMA model, 100 randomized tree searches, rapid bootstrap analysis and search for best-scoring ML tree. The resulting unrooted gene trees were then used to calculate a species tree using ASTRAL III v.5.7.7 (Zhang & al., 2018). Support values of the gene trees were calculated using quartet scores (Q1, Q2, Q3) as well as local posterior probabilities (LPP) (Sayyari & Mirarab, 2016). The quartet scores for each node were assessed using the logic of D statistics: Q2 ~ Q1, Q1 < 50 – ILS; Q2 >> Q3 – HGT; Q2 << Q3 – HGT to evaluate whether gene tree discordance could be due to incomplete lineage sorting (ILS) alone or in addition to horizontal gene transfer (HGT; i.e., reticulation) (Mirarab & al., 2014; Pease & Hahn, 2015; Solís-Lemus & al., 2016).

Output trees were visualized and further processed using FigTree v.1.4.4 (Rambaut, 2016). Cophyloplots were produced with the function *cophylo* using R package phytools v.0.7-70 (Revell, 2012; R Core Team, 2020). Alignments as well as the input and output files of the presented analyses are deposited in Zenodo (https://doi.org/10.5281/zenodo.7228016). Concerning phylogenetic trees, we considered moderate branch support with BS: 75%–84%/(L)PP: 0.90–0.94/Q1: 70–89 and high support BS: 85%–100%/(L)PP: 0.95–1/Q1: 90–100, respectively.

■ RESULTS

Datasets. — In order to assess the hybridization effectiveness of the Angiosperms353 probe set, for each sample, we measured the percentage of reads that successfully mapped on target sequences (target enrichment) and the gene recovery rate (number of target genes recovered). Coding sequences were recovered for all studied species, and the numbers of recovered target loci range between 330 and 353. Target-enrichment rates were low, ranging between 0.74% and 6.72% (suppl. Tables S1, S2). After applying the criteria described above, 125 loci in total were considered further (suppl. Table S2) and the resulting concatenated supermatrix for the nuclear dataset comprised 193,914 bp. Concerning the plastome dataset, 47,585 to 2,336,547 reads per sample were mapped to the *Ananas comosus* reference with 46.17× to 2246.56× mean coverage matching 97.6% to 100% of the reference sequence. The total of 450 to 53,007 reads per sample were mapped on particular mitochondrial genes with 10.99× to 887.57× mean coverage almost always matching 100% (except five cases of >97.5%) of the reference (suppl. Table S1). The final plastome alignment, which contained one copy of the inverted repeat (IR) and included both coding and non-coding regions, comprised 139,238 bp. The final length of the mitogenome alignment was 34,208 bp.

Phylogenetic reconstruction: Nuclear sequence data. — The ML and the Bayesian analyses of the concatenated nuclear dataset of both exons and introns revealed trees of identical topology and with most branches receiving maximum statistical support (Fig. 1). Clades formed by early diverging events within Bromelioideae are recovered with high support with the exception of the core Bromelioideae sensu Silvestro & al. (2014) (clade A, BS: 41, but PP: 0.99). When rooted with the monospecific genus *Deinacanthon*, *Fernseea* is sister to the remainder of the subfamily. The remaining species of the early diverging lineages grouped in two sister clades, one composed of the *Ananas* group and the Cryptanthoid complex (Leme & al., 2017).

Whereas the monophyly of the core Bromelioideae (clade A) is not well supported in the ML supermatrix nor the ASTRAL analyses (Fig. 2), *Acanthostachys* is clearly placed outside of clade B with the remaining core Bromelioideae. Within clade B, *Neoglaziovia* is sister to clade C, and *Karawata* is sister to the diverse clade D including the *Ronnbergia* alliance (Aguirre-Santoro, 2017) as a sister to clade E. All of these nested clades have maximum support in both ML and Bayesian analyses. The Nidularioid group also forms a robust clade including the genera *Nidularium* Lem., *Canistropsis* (Mez) Leme, *Edmundoa* Leme, *Neoregelia* L.B.Sm., and *Wittrockia* Lindm. In the sister clade, *Aechmea nudicaulis* (L.) Griseb., *Ae. distichantha* Lem. and *Quesnelia quesneliana* (Brongn.) L.B.Sm. group together (*Aechmea nudicaulis* group) and are sister to clade G (BS: 73/PP: 1; Fig. 1) composed of the *Billbergia* Thunberg species sister to the Billbergiopsis group comprising species of *Quesnelia* subg. *Billbergiopsis* Mez plus *Ae. fasciata* (Lindl.) Baker and *Ae. flavorosea* E.Pereira. Clade G is missing from the ASTRAL tree as the *Aechmea nudicaulis* and Billbergiopsis groups form a poorly supported clade (LPP: 0.49; Fig. 2). Clade H (BS: 92/PP: 1; Fig. 1), a morphologically heterogenous group of species, includes the *Aechmea sphaerocephala* Baker group (Maciel & al., 2018), which receives low to moderate statistical support in the supermatrix (BS: 27/PP: 0.91; Fig. 1) analysis, in a sister position to the remainder. However, the *Aechmea sphaerocephala* group except *Ae. serragrandensis* Leme & J.A.Siqueira is sister to clade F in the ASTRAL analysis, and clade H is therefore missing in the ASTRAL tree

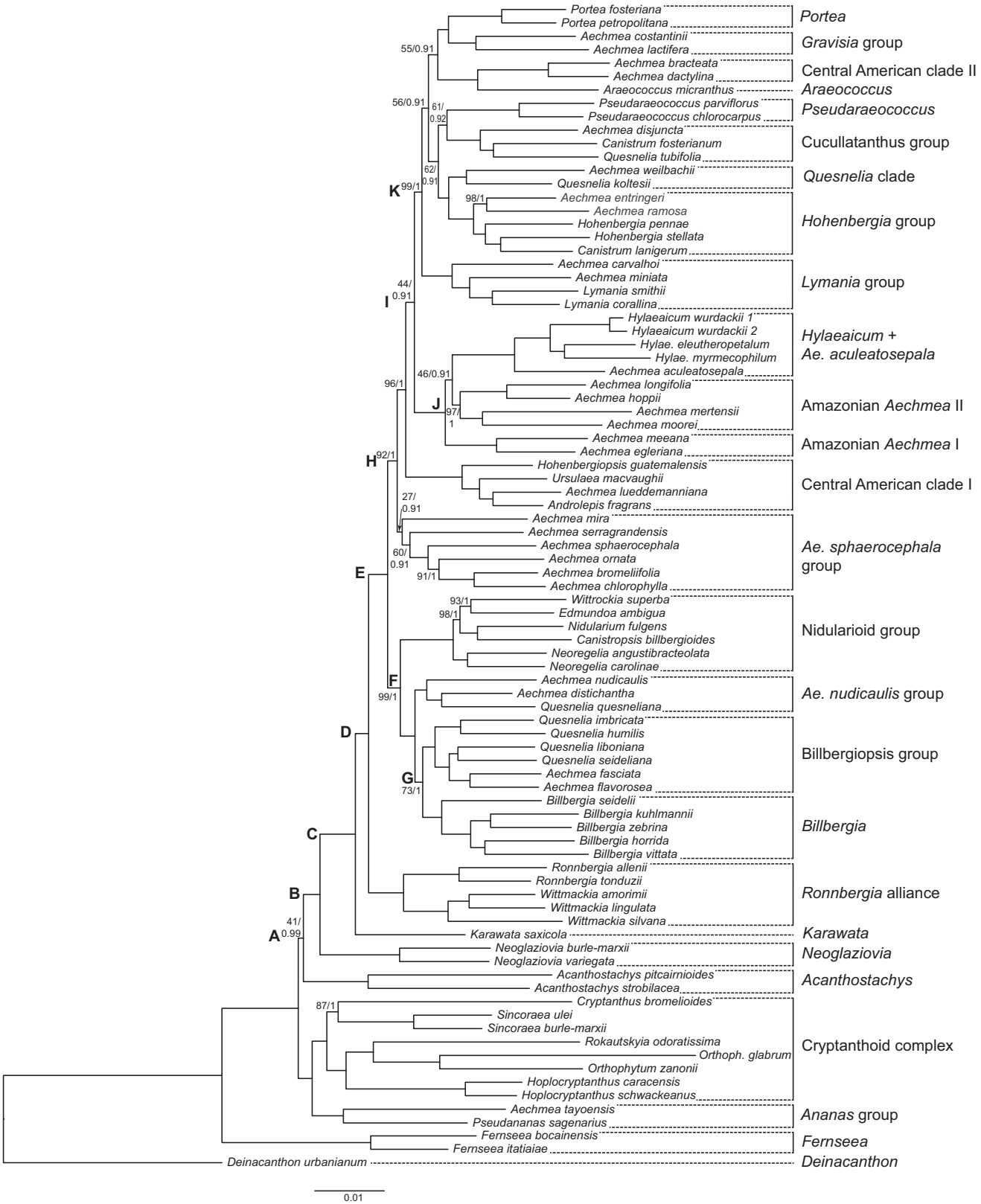

Fig. 1. Best-scoring ML tree based on the nuclear supermatrix. Numbers above or below branches show bootstrap support percentages and posterior probabilities from corresponding Bayesian analysis (BS/PP). BS/PP of 100%/1 are not shown. The scale bar indicates the branch length for 0.01 substitutions per nucleotide position. Nested focal clades are labelled at their root nodes with capital letters. Groups recovered by the phylogenetic analyses are shown on the right.

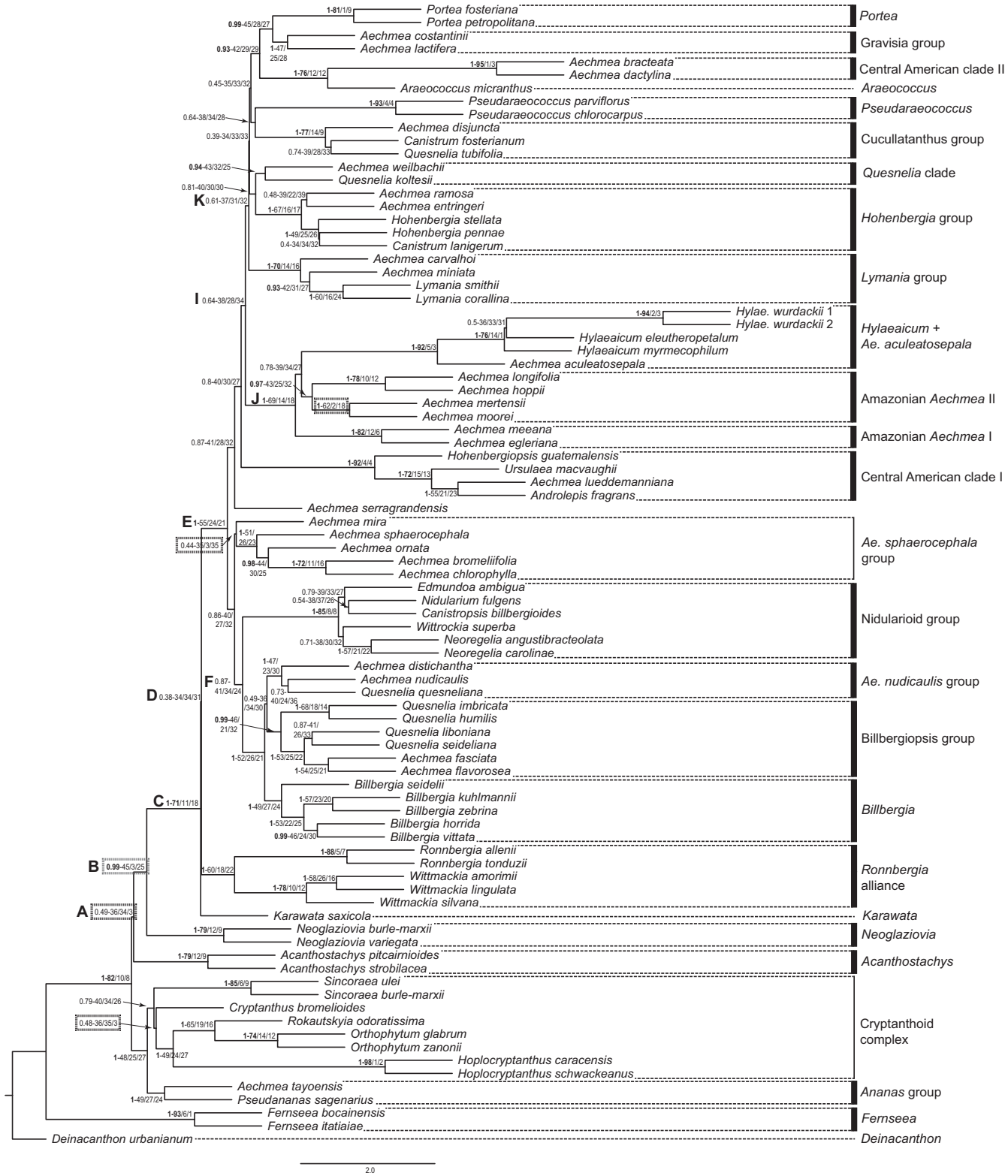

Fig. 2. Species tree generated using ASTRAL-III based on 125 gene trees. Numbers above or below branches are local posterior probabilities (LPP) followed by the respective quartet support for the main topology (Q1), and the first (Q2) and the second alternative (Q3) topology (LPP-Q1/Q2/Q3). LPP-Q1/Q2/Q3 in squares indicates cases with large differences between Q2 and Q3. Focal groups recovered in phylogenetic analyses are named on the right. Thick vertical bar indicates moderate to high support either by LPP, Q1 or both. Nested focal clades are labelled at their root nodes with capital letters. ASTRAL-III measures branch lengths in coalescent units (the scale bar shown corresponds to two coalescent units) for internal branches and not terminal branches (branch lengths of terminal branches are therefore arbitrary and meaningless).

(Fig. 2). The next branching highly supported clade combines species of the genera *Aechmea*, *Androlepis* Brongn. ex Houllet, *Hohenbergiopsis* L.B.Sm. & R.W.Read, and *Ursulaea* Read & H.U.Baensch, all of them distributed in Central America (Central American clade I, Ramirez-Diaz & al., 2019). Clade I (supermatrix BS: 44/PP: 0.91, Fig. 1; ASTRAL LPP: 0.64, Fig. 2) consists of a group of species principally distributed in Amazonia (clade J) as sister to the remainder (clade K). In clade J, three major clades can be discerned: Amazonian *Aechmea* I with *Ae. egleriana* L.B.Sm. and *Ae. meeana* E.Pereira & Reitz, Amazonian *Aechmea* II with *Ae. hoppii* (Harms) L.B.Sm., *Ae. longifolia* (Rudge) L.B.Sm. & M.A.Spencer, *Ae. mertensii* (Meyer) Schult. & Schult.f. and *Ae. moorei* H.Luther, and genus *Hylaeaicum* (Ule ex Mez) Leme & al. with associated *Ae. aculeatosepala* (Rauh & Barthlott) Leme (Leme & al., 2021). The remaining clade K comprises the *Lymania* Read group, which includes two *Lymania* species together with *Ae. carvalhoi* E.Pereira & Leme and *Ae. miniata* (Beer) hort. ex Baker, in highly supported sister position to the *Hohenbergia* Schult. & Schult.f. group including representatives of the genera *Hohenbergia*, *Canistrum* E.Morren, as well as *Ae. entringeri* Leme and *Ae. ramosa* Mart. ex Schult. & Schult.f., the *Quesnelia* clade with *Ae. weilbachii* F.Didr. and *Quesnelia koltesii* Amorim & Leme, Cucullatanthus group, the genera *Pseudaraeococcus* (Mez) R.A.Pontes & Versieux and *Araeococcus* Brongn. (Pontes & al., 2020), together with the Central American clade II and *Portea* Brongn. ex C.Koch and the *Gravisia* group (Heller & al., 2015). Although all of the defined lineages in clade K are highly supported in both supermatrix and ASTRAL analyses, the backbone in this part of the phylogenetic tree is poorly supported in the ASTRAL and supermatrix ML analyses and only moderately supported in the Bayesian supermatrix analysis.

The comparison of the phylogenetic trees obtained from supermatrix (Fig. 1) and ASTRAL analyses (Fig. 2, suppl. Fig. S1) revealed a single moderately supported difference in the placement of *Aechmea serragrandensis*. Poorly supported incongruences in resolution of deeper nodes were found in three parts of the tree. Additional poorly supported differences were found in several terminal clades: the Cryptanthoid complex, *Ae. nudicaulis*, and the Nidularioid clade. Significant differences between the quartet scores for alternative resolutions (highlighted nodes in Fig. 2) raise the possibility of reticulation events influencing the inferred placement of *Acanthostachys*, *Cryptanthus bromelioides* Otto & A.Dietr., and *Neoglaziovia* within the Cryptanthoid complex, the *Ae. sphaerocephala* group, and the Amazonian *Aechmea* II clade (Fig. 2). In general, the supermatrix and ASTRAL analyses of single-copy nuclear genes provide congruent implications for the pace and pattern of cladogenesis in the Bromelioideae.

Phylogenetic reconstruction: Plastome sequence data. — The ML tree based on 139,238 bp plastome data (Fig. 3, suppl. Fig. S2) is largely consistent with the nuclear tree topologies (Figs. 1, 2) and almost all of the recognized clades. However, it revealed some differences concerning the placement of a few species, which are described in the following. Interestingly, the *Ananas* clade comprises *Neoglaziovia burlemarxii* Leme, which is in the nuclear tree part of the *Neoglaziovia* clade. The plastome data revealed high support for the Cryptanthoid complex. However, both *Acanthostachys* species were also placed in the Cryptanthoid complex clade and not in the core Bromelioideae clade as recovered with poor support in the nuclear trees. The clade of the *Ronnbergia* alliance, which is highly supported in the nuclear trees, receives low support in the plastome ML analysis (BS: 56), but moderate support in the Bayesian analysis (PP: 0.93; Fig. 3). It is sister to the remaining core Bromelioideae and has the only *Karawata* species nested within. Clades F and H comprise the same species and also principally the same subclades as described in the nuclear trees, but with few species in diverging placements and differences in the deeper branching (suppl. Fig. S2). However, in clade H, several of the deeper nodes receive poor bootstrap support (BS: 45–62) and a range of low posterior probabilities. In clade F, the *Aechmea nudicaulis* group is sister to the Nidularioid group. However, the former comprises also *Ae. ornata* Baker, which is part of the *Ae. sphaerocephala* group in the nuclear tree. The *Ae. nudicaulis* and Nidularioid groups are sisters to two *Quesnelia* species that in the nuclear tree group with the Billbergiopsis group. In clade G, the *Billbergia* group is well supported and sister to a clade composed of a smaller Billbergiopsis group (excluding *Q. humilis* Mez and *Q. imbricata* L.B.Sm.) sister to *Billbergia seidelii* L.B.Sm. & Reitz, which is included in the *Billbergia* clade in the nuclear tree. However, the branch comprising the Billbergiopsis group and *Billbergia seidelii* received no statistical support. In clade H, all clades found in the nuclear tree are present too, but most of the basal nodes receive no statistical support. Two species (*Aechmea mira* Leme & H.Luther, *Ae. serragrandensis*) that are part of the *Ae. sphaerocephala* group in the nuclear tree, are placed differently. *Aechmea mira* is sister to *Ae. weilbachii* and *Quesnelia koltesii*, and *Ae. serragrandensis* is sister to a large clade of species of principally Amazonian or east Brazilian distribution. It should be noted that the *Ae. sphaerocephala* group received no support in the nuclear tree.

Phylogenetic reconstruction: Mitogenome sequence data. — The phylogenetic tree resulting from the ML analysis based on 34,208 bp mitogenome data is generally characterized by poor statistical support for most of the branches (suppl. Fig. S3). It is therefore not described here in detail. Similar to the other analyses, *Deinacanthon* and *Fernseea* are consecutive sisters to the remainder and highly supported groups. Additional clades that were also found in the analyses above and which received moderate to high statistical support are the following: *Acanthostachys*, Amazonian *Aechmea* I, Central American clade I, *Hylaeaicum* + *Ae. aculeatosepala* group, *Portea*, *Gravisia* group, and *Pseudaraeococcus*.

■ DISCUSSION

On-target data. — The efficiency in terms of the number of raw captured loci was in our study in the range of moderate

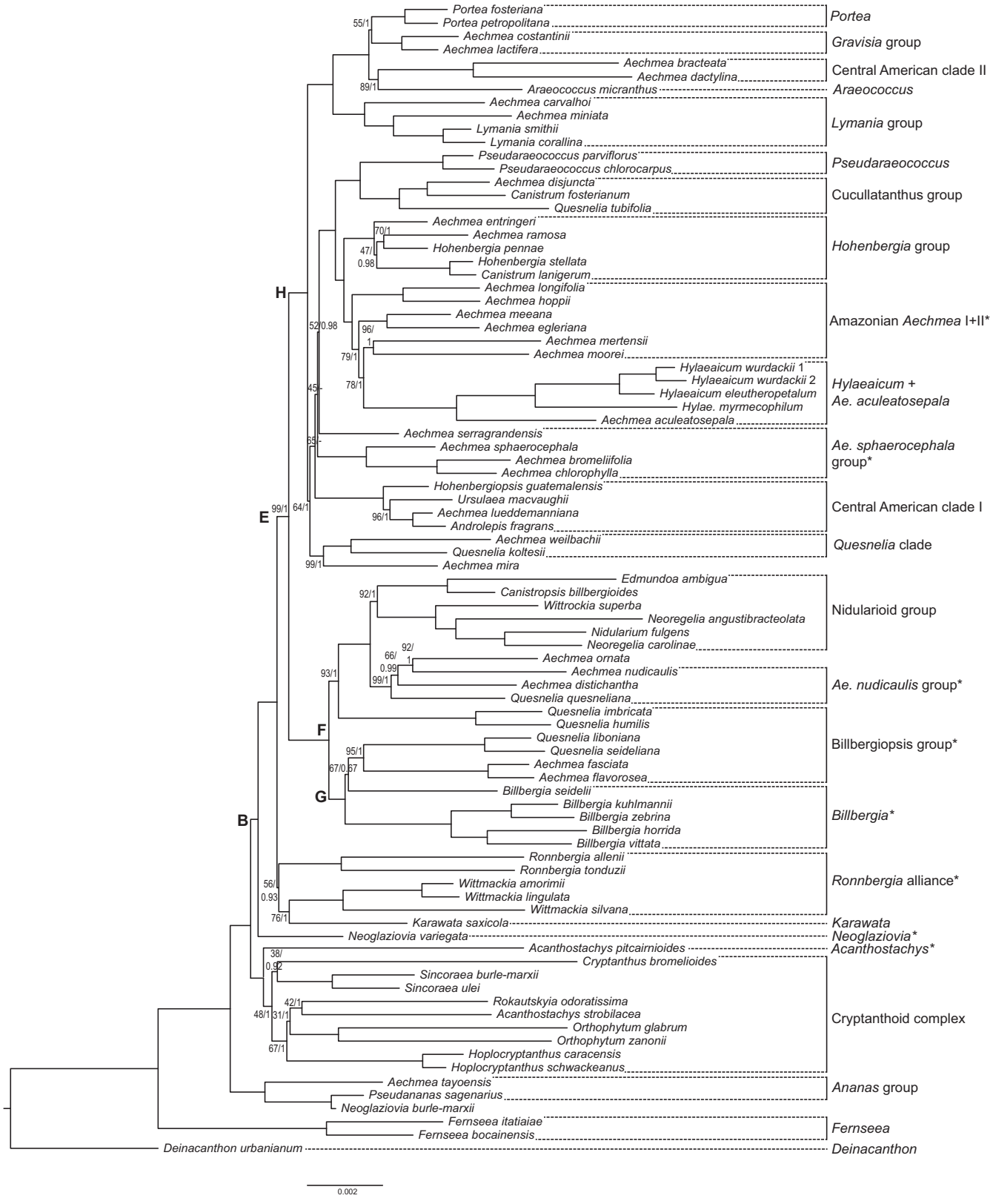

Fig. 3. Best-scoring ML tree based on the plastome alignments. Numbers above or below branches show bootstrap support percentages and posterior probabilities from corresponding Bayesian analysis (BS/PP). BS/PP of 100%/1 are not shown. The scale bar indicates the branch length for 0.002 substitutions per nucleotide position. Nested focal clades are labelled at their root nodes with capital letters. Groups recovered by the phylogenetic analyses are shown on the right. Asterisk indicates that these groups are not monophyletic when compared with the nuclear phylogenetic analyses.

to high across targeted species (177–322, mean = 281, "Genes with Seqs", suppl. Table S1). However, 50% of the mean exon length was recovered only for 173 loci and after subtraction of loci for which at least 75% of the species were covered, only ca. 35% (125 loci) of the original targeted nuclear loci have been utilized for phylogenetic inference.

Studies of phylogenetic relationships within monocots employing the Angiosperms353 bait set have been relatively rare so far. However, in these, a higher fraction of the targeted loci could be utilized, as in, e.g., Orchidaceae (294 loci; Pérez-Escobar & al., 2021) or Cyperaceae (215 loci; Larridon & al., 2020) compared to our results. On one hand, the criteria applied herein might be considered rather stringent. By using this conservative filtering strategy, we aimed to reconstruct more robust relationships by decreasing noise due to missing information as previously established by Shah & al. (2021) for Ochnaceae.

On the other hand, the design strategy of the Angiosperms353 kit incorporated only single-copy protein-coding genes with no more than 30% sequence divergence from any target instance for each gene (Johnson & al., 2019). During the design and specificity tests of the probes, only a rather small proportion of commelinids (45) were employed, including one bromeliad from the distantly related subfamily Brocchinioideae, and no other representatives of Poales were included in the taxon set used for testing (Johnson & al., 2019). Given that the sequence divergence between Bromeliaceae subfamilies can reach up to 20% (Givnish & al., 2011) we suppose that the lower sequence homoplasy between our dataset and the Angiosperms353 target sequences can be at least partially attributed to the high divergence and resulting in lower capture success. Alternatively, given the effect of filtering genes by recovery of a minimum of 50% of the mean reference exon length, we might also consider that the exons in bromeliads have undergone changes in structure (due to intron separation) and/or length in our target. Additionally, technical artifacts such as PCR- and/or sequencing bias might have further contributed to this issue. Taxon-specific probes or a combination of universal and taxon-specific ones would most probably have improved the recovery of the target loci as already shown by, e.g., Larridon & al. (2020), Ogutcen & al. (2021), and Yardeni & al. (2022) for Bromeliaceae. Nevertheless, this study demonstrates that even 125 universal target-loci can be very effective in solving relationships when compared to Sanger sequencing of a few loci. Moreover, the employment of universal probes saves labor and enables merging datasets of multiple studies. However, standardized, evidence-based bioinformatics workflows need to be developed in order to objectively assess these effects of filtering criteria and parameters on phylogenetic reconstruction, especially in light of evidence that employing no alignment filtering does not always inhibit recovery of robust and well-supported phylogenetic trees based on Angiosperms353 loci (e.g., Baker & al., 2021; Shah & al., 2021).

Off-target data. — In addition to the analyses of the target-enriched nuclear loci we explored also the potential of recovering plastome and mitogenome data from the off-target sequence data. The off-target fraction comprised in all samples more than 93% of the reads, which represented at least 1.68×10^6 reads per sample (suppl. Table S1). The proportion of reads that mapped to the plastome reference varied between 0.43% and 16.27% comprising at least 47,584 bp. For the majority of the samples, sequences matching the full reference plastome were achieved. The proportion of mitogenome data was much lower, ranging from 0.11% to 1.83% (suppl. Table S1). However, the reads in all except five samples matched 100% of the five reference genes at the given coverage (3×), although, due to limited reference length we most probably did not recover a substantial portion of the mitogenome. Accordingly, using high-quality DNA extractions from fresh or well-preserved silica-gel dried material together with relatively deep sequencing, sufficient amount of plastome data can be generated using target-enrichment approaches similarly as previously shown (Granados Mendoza & al., 2020; Schneider & al., 2021b). In addition, we show, to our knowledge for the first time, that a potentially informative portion of the mitogenome could be recovered as flow through of target-enrichment studies.

Phylogenetic resolution. — In our approach, ML and Bayesian analyses of the concatenated datasets received the best statistical support. The amount and the variability of the sequence data were sufficient to obtain good resolution and high statistical support in most of the backbone nodes. This has not been achieved before in Bromelioideae, which are characterized by rapid radiation and low genetic variability (e.g., Sass & Specht, 2010; Silvestro & al., 2014; Evans & al., 2015; Leme & al., 2021). The plastome data provided a well-supported tree highly congruent with the nuclear trees. Concerning the mitogenome data, several highly supported clades congruent with nuclear and plastome analyses were recovered too. However, mitochondrial genes evolve rather slowly when compared to plastome and nuclear genomes (Wolfe & al., 1987), and although substitution rates can vary among particular genes and between species (e.g., Mower & al., 2007) the majority of the recovered clades were unsupported. This could be attributed to the relatively young age of the subfamily (crown age of the extant lineages: 10.9 Ma, Silvestro & al., 2014) as well as to insufficient data, as we explored only five genes and 34,208 bp in total, whereas full mitogenomes in Poales may be as long as 700 kbp (e.g., *Zea mays* L. GenBank DQ645536.1).

Implications for Bromelioideae systematics. — In combination with taxonomic, distributional, morphological, and ecological information we recognize here 26 well-supported clades. Some of these clades are morphologically well characterized and have long been recognized as genera, viz. (1) *Deinacanthon*, (2) *Fernseea*, (3) *Acanthostachys*, and (4) *Neoglaziovia* (e.g., Smith & Downs, 1979; Rauh & Barthlott, 1982; Forzza & al., 2020; Monteiro, 2021) and supported by several phylogenetic studies (Schulte & Zizka, 2008; Schulte & al., 2009; Silvestro & al., 2014; Evans & al., 2015; Aguirre-Santoro & al., 2016; Matuszak-Renger & al.,

2018). In the latter two genera, our nuclear data support this view and identify them as successive sister lineages to a clade including the remainder of the core Bromelioideae. Previously, *Neoglaziovia* was either placed as sister to the remaining core Bromelioideae (Evans & al., 2015; Aguirre-Santoro & al., 2016; Matuszak-Renger & al., 2018; Leme & al., 2021) or *Neoglaziovia* and *Acanthostachys* formed a clade sister to the *Ronnbergia* alliance (Silvestro & al., 2014). However, these relationships were supported rather weakly. In our nuclear supermatrix ML and coalescence analyses, the monophyly of clade A was poorly supported, although it received good support in Bayesian analysis (Figs. 1, 2). Interestingly, the analyses based on uniparentally inherited organelle data revealed contrasting scenarios. Plastome analyses placed both *Acanthostachys* species within the Crypthanthoid complex and *Neoglaziovia burle-marxii* within the *Ananas* group (Fig. 3). Mitogenome analyses also placed *Neoglaziovia burle-marxii* within the *Ananas* group and moderately supported the sister position of *Acanthostachys* to the Cryptanthoid complex and the remainder of the later-diverging taxa (suppl. Fig. S3). This points either towards ILS or reticulation events in the evolutionary history of *Acanthostachys* and *Neoglaziovia*. Given the difference between Q2 and Q3 values for resolution of relationships around nodes subtending clades B and C in the coalescent-based ASTRAL analysis (Fig. 2), together with the consistent conflict between phylogenetic inferences drawn from the nuclear data and cytoplasmic plastome and mitogenome data, we consider reticulation events (e.g., Fehrer & al., 2007; Kim & Donoghue, 2008) in addition to ILS as plausible sources of gene tree incongruence and poor support for the monophyly of the core Bromelioideae (clade A).

Other groups identified here have been recognized only recently as monophyletic groups and received corresponding taxonomic treatments. This applies to the somewhat enigmatic (5) *Ananas* group, including also species like *Aechmea tayoensis* Gilmartin, *Ae. fernandae* (E.Morren) Baker (both from *Ae.* subg. *Chevaliera* (Gaudich. ex Beer) Baker) and *Disteganthus basilateralis* Lem. (Matuszak-Renger & al., 2018). Taxonomic consequences still have to be made and are hampered by the controversy concerning the taxonomic rank of lineages within the *Ananas/Pseudananas* clade (Butcher & Gouda, 2014; Coppens d'Eeckenbrugge & Govaerts, 2015). The *Ananas* group has been well-supported in previous phylogenetic studies (Silvestro & al., 2014; Aguirre-Santoro & al., 2016) and is also highly supported in our analyses. However, the phylogenetic position of the *Ananas* group relative to the remainder of the clade differs among the nuclear and plastome/mitogenome analyses (suppl. Figs. S2, S3) as *Ananas* group is either the earliest diverging lineage from the *Fernseea* sister clade (plastome dataset, Fig. 3) or sister to the Cryptanthoid complex (nuclear, Fig. 1). Due to ASTRAL Q1, Q2 and Q3 values we interpret this as a consequence of rapid diversification and ILS (Fig. 2).

The taxonomy of the (6) Cryptanthoid complex has received considerable attention (e.g., Leme & Kollmann, 2007, 2010; Louzada & Versieux, 2010; Louzada & Wanderley, 2010; Louzada & al., 2014; Leme, 2015; Cruz & al., 2017) and has recently been monographed by Leme & al. (2017, 2022). Our analyses revealed congruent topologies of plastome and nuclear trees and the included genera were monophyletic (suppl. Fig. S2), although in the plastome tree several branches were not well-supported. Hence, we assume that remaining polytomies and unsupported clades (Leme & al., 2017, 2022) can be resolved after more representative sampling is studied using nuclear phylogenomics.

(7) *Karawata* is represented here only by *K. saxicola* (L.B.Sm.) J.R.Maciel & G.Sousa, but has been studied and characterized in more detail by Maciel & al. (2018, 2019). The species of this genus have been part of *Aechmea* subg. *Chevaliera*, which is clearly polyphyletic—as all recognized subgenera of *Aechmea* except subg. *Ortgiesia* (Goetze & al., 2016). Interestingly, the phylogenetic placement of *K. saxicola* was also incongruent among the studied datasets. In the nuclear tree, *K. saxicola* was sister to clade D, in the plastome tree part of the *Ronnbergia* alliance with moderate support (suppl. Fig. S2), similarly as recovered also previously (Maciel & al., 2018, 2019).

The (8) *Ronnbergia* alliance has also recently been revised and has undergone considerable nomenclatural adjustments (Aguirre-Santoro, 2017; Aguirre-Santoro & al., 2016). The *Ronnbergia* alliance has three centers of distribution: (i) from Central America (Costa Rica) southward to the Andes of Peru (genus *Ronnbergia*), (ii) the Atlantic Forest in Brazil (*Wittmackia* Mez p.p.) and (iii) the Caribbean (*Wittmackia* p.p. – the Wittmackiopsis group – Aguirre-Santoro, 2017). Five species from this clade were included in our study; however, it does not include a species of the Caribbean Wittmackiopsis group. The topology recovered here is congruent between plastome and nuclear datasets and in line with the current delimitation of *Ronnbergia* and *Wittmackia* (Aguirre-Santoro, 2017).

The remaining core Bromelioideae fall into two large clades (F and H, Fig. 1). Clade F comprises four highly supported clades (Nidularioid group, *Aechmea nudicaulis* group, *Billbergia*, and Billbergiopsis group) that all have their center of species diversity in eastern to southeastern Brazil. Few species have a distribution extending to Paraguay and Argentina, and *Ae. nudicaulis*, the most widespread bromelioid species, is extending to Central America (Zizka & al., 2020). The highly supported (9) Nidularioid group comprises six species from members of the five genera *Canistropsis*, *Edmundoa*, *Neoregelia*, *Nidularium*, and *Wittrockia*. All of them are distributed in the Atlantic Forest in southeastern Brazil, and characterized by compact, head-like inflorescences nested in the center of the rosette or involucrate with large primary bracts impounding water, and usually nearly symmetrical sepals (Leme & al., in prep.).

Studied species of (10) *Billbergia* are all occurring in the Atlantic Forest of southeastern Brazil (sometimes extending to the Cerrado) and in the nuclear analysis form a highly supported clade. However, the topology of the clade with fully supported branches does not reflect the present subdivision into *B.* subg. *Billbergia* and subg. *Helicodea* (Lem.) Baker,

as recovered in the morphological phylogenetic study (Almeida & al., 2009). Interestingly, Ramirez-Diaz & al. (2019) found *Billbergia viridiflora* H.Wendl. as part of the enigmatic Central American clade I (see below). This highlights the need for a revision of this genus including also species from the Amazonian center of distribution as well as species occurring in Central America.

Sister to the *Billbergia* clade is the (11) Billbergiopsis group. It includes the representatives of *Quesnelia* subg. *Billbergiopsis*, *Aechmea fasciata* and *Ae. flavorosea* (both belonging to *Ae*. subg. *Aechmea*) nested among the *Quesnelia* representatives. The *Quesnelia* species grouped here share the same leaf anatomical type (Mantovani & al., 2012). According to our data, *Quesnelia* is polyphyletic, with its species grouping in four different, highly supported clades (members of *Q*. subg. *Quesnelia* distributed over three clades, see also below). Recent morphological, anatomical and molecular studies in the genus already regarded *Quesnelia* as polyphyletic and found evidence for discerning three groups (Faria & al., 2004; Almeida & al., 2009; Mantovani & al., 2012).

It is noteworthy that one included member of *Quesnelia* subg. *Quesnelia*, *Q. quesneliana*, is part of the (12) *Aechmea nudicaulis* group, which is sister to the *Billbergia* and Billbergiopsis groups and comprises also *Ae. nudicaulis* (*Ae*. subg. *Pothuava* (Baker) Baker) and *Ae. distichantha* (*Ae*. subg. *Platyaechmea* (Baker) Baker). Mantovani & al. (2012) found anatomical similarities between members of *Quesnelia* subg. *Quesnelia* and *Aechmea* subg. *Pothuava*. Interestingly, the highly supported clade (Fig. 1) combines the most widespread and ecologically variable *Q. quesneliana* with two exceptionally widely distributed and ecologically variable *Aechmea* (*Ae. distichantha*, *Ae. nudicaulis*). These taxa overlap in their southeastern distribution area; however, they differ considerably in their morphology. In the tree obtained from the plastome data, another species from *Ae*. subg. *Pothuava*, *Ae. ornata*, is placed in this clade (Fig. 3).

In the remaining core Bromelioideae (clade H), we identified the (13) *Aechmea sphaerocephala* group as sister to the remainder, but statistical support is lacking. It combines species from *Ae*. subg. *Chevaliera* (*Ae. mira*, *Ae. serragrandensis*, *Ae. sphaerocephala*) with representatives of *Ae*. subg. *Macrochordion* (de Vriese) Baker (*Ae. bromeliifolia* (Rudge) Baker ex Benth. & Hook.f., *Ae. chlorophylla* L.B.Sm.) and *Ae*. subg. *Pothuava* (*Ae. ornata*). Four species from core Bromelioideae are grouped differently in the plastome tree (Fig. 3, suppl. Fig. S2), and three of them are from the *Ae. sphaerocephala* group. However, only the positions of *Ae. ornata* in the *Ae. nudicaulis* group and *Ae. mira* (in a clade with *Ae. weilbachii* and *Quesnelia koltesii*) in the plastome tree received high statistical support, the position of *Ae. serragrandensis* remains doubtful. This is most probably due to reticulation events involved in the evolutionary history of the group as suggested by the significantly different ASTRAL Q2 and Q3 values (Fig. 2).

The (14) Central American clade I (Sass & Specht, 2010; Silvestro & al., 2014; Ramirez-Diaz & al., 2019) is consistently supported (nuclear, plastome, mitogenome) as sister to clade I. The Central American clade I includes species distributed in Central America from five different genera (*Aechmea*, *Androlepis*, *Billbergia* [not included here], *Hohenbergiopsis*, *Ursulaea*). This genetically very well-defined clade has also been found in previous studies with relevant sampling and resolution (Schulte & al., 2005; Horres & al., 2007; Schulte & Zizka, 2008; Schulte & al., 2009; Sass & Specht, 2010; Silvestro & al., 2014; Díaz, 2019). From the morphological point of view, the members of this clade are strikingly different (Ramirez-Diaz & al., 2020). However, the consistent results of molecular phylogenetic studies very much contradict the current taxonomy. Further studies including also Central American *Billbergia* species would be most interesting.

The remaining sister clades J and K received full support, although their sister position is only moderately supported by nuclear Bayesian analyses. In clade J, three highly supported clades can be recognized. All of them are characterized by a principally Amazonian distribution. The distribution of the (15) Amazonian *Aechmea* I with *Ae. egleriana* and *Ae. meeana* (both *Ae*. subg. *Aechmea*) seems to be centered more eastwards in Amazonia than of (16) Amazonian *Aechmea* II (*Ae. hoppii* (Harms) L.B.Sm., *Ae. longifolia* [both were members of the genus *Streptocalyx* Beer], *Ae. moorei* [subg. *Platyaechmea*], *Ae. mertensii* [subg. *Aechmea*]). The species of the remaining clade have recently been united in the genus (17) *Hylaeaicum* (Leme & al., 2021), also supported by the mitogenome data in our analysis. According to Leme & al. (2021) the new genus comprises altogether 12 species. Groups 15, 16, and 17 are also closely related in the plastome tree. However, the tree topology is slightly altered, and some relationships are only moderately supported. In the quartet sampling, no strong signal for reticulations was recovered. The remaining clade K (Figs. 1, 2) received maximum support and—in contrast to clade J—comprises species principally distributed in (south)eastern Brazil, except for one clade with Central American species. The first branching is the (18) *Lymania* group. Besides the two *Lymania* species it includes *Ae. carvalhoi* and *Ae. miniata*, both from *Ae*. subg. *Lamprococcus* (Beer) Baker. Sass & Specht (2010) and Silvestro & al. (2014) found a similar highly supported clade composed of the studied *Lymania* species and representatives of *Ae*. subg. *Lamprococcus* (*Ae. farinosa* (Regel) L.B.Sm., *Ae. fulgens* Brongn., *Ae. miniata*). All species in the *Lymania* clade share a very similar distribution in the Atlantic Forest of (south)eastern Brazil. Concerning the next branching clades, differences in the nuclear and plastome analyses were recovered. Whereas in the nuclear analyses the *Lymania* group was sister to the remainder (Figs. 1, 2), in the plastome tree (Fig. 3), it was in a sister position to a group of well-supported terminal clades: *Portea*, *Gravisia* group, Central American clade II and *Araeococcus*. (19) Central American clade II (*Ae. bracteata* (Sw.) Griseb., *Ae. dactylina* Baker, both from *Ae*. subg. *Aechmea*) was recovered in sister position to (20) *Araeococcus. Araeococcus* has been recognized also in previous studies (Sass & Specht, 2010; Silvestro & al., 2014) and is a morphologically well-defined genus with Amazonian distribution (Pontes

& al., 2020). Two additional well-supported clades are the (21) *Gravisia* group and the genus (22) *Portea*, the latter one also concerning the mitogenome data. Both groups have been studied in detail and considered monophyletic previously (Heller & al., 2015). In the sister clade (nuclear), three terminal clades with full statistical support were found. The (23) *Hohenbergia* group includes *H. stellata* Schult. & Schult.f., *H. pennae* Pereira, *Canistrum lanigerum* H.Luther & Leme and two species from *Ae*. subg. *Aechmea*. All species are distributed in eastern Brazil, only *H. stellata* extends to northern South America and the Caribbean. In sister position is the (24) *Quesnelia* clade, combining *Q. koltesii* and *Ae. weilbachii* (*Ae*. subg. *Lamprococcus*) from southeastern Brazil, both species with surprising morphological similarities (Leme & al., in prep.).

In the sister clade, the last two groups can be discerned: the species of the genus (25) *Pseudaraeococcus*, supported also by mitogenome analyses, and—in sister position—species that are characterized by stoloniferous habit, cucullate petal apex, tubular corolla, and unappendaged seeds. This (26) Cucullatanthus group comprises *Quesnelia tubifolia* Leme & L.Kollmann, *Canistrum fosterianum* L.B.Sm., and *Ae. disjuncta* (L.B.Sm.) Leme & J.A.Siqueira (*Ae*. subg. *Aechmea*). A revision of this group is in progress (Leme & al., in prep.). Cucullate petals are a prominent morphological feature of the group, whose members nevertheless are currently spread over at least three bromelioid genera according to present taxonomy. Based on morphological, biogeographical, and anatomical differences, Pontes & al. (2020) recently elevated *Araeococcus* subg. *Pseudaraeococcus* Mez to genus rank. The group is noteworthy in few species having no or only an inconspicuous water-impounding tank, while core Bromelioideae, in general, are characterized by pronounced tank habit (Silvestro & al., 2014).

■ CONCLUSION

In the first Bromelioideae phylogeny with a well-resolved backbone, 26 monophyletic groups were discriminated, which form a basis for further revisional work. A comparison of nuclear and plastome data revealed only a few differences in the topology, which can be explained by fast diversification followed by ILS or reticulation (i.e., hybridization, introgression) events. We confirmed the feasibility of plastome and mitogenome mining from off-target reads when using the Angiosperms353 kit, generating complementary sequence data of uniparental inheritance at no extra sequencing cost. Our analyses are in general congruent across methods and data sources, although the mitogenome analyses did not prove to have sufficient statistical support.

Concerning the Bromelioideae systematics, generic delimitation has been confirmed in several cases, but not in the highly problematic *Aechmea*. Obviously, the genus and subgenus concept in the core Bromelioideae has to be revised considerably. Besides *Aechmea*, *Canistrum*, *Hohenbergia*, and *Neoregelia*, the genus *Quesnelia* is especially problematic with its species distributed over four clades. Several of the monophyletic groups have already been detected by studies based on Sanger sequencing and revised recently, leading to the recognition of discriminatory morphological characters. Other groups have been defined based on the revision of morphological and anatomical features as well as distributional patterns and are supported here by the extensive molecular data.

It is evident that several detected monophyletic groups are more consistent with respect to the distribution than to current generic circumscription. Remarkable in this respect are the two fully supported Central American clades as well as clades comprising *Aechmea* species with Amazonian distribution. Hence, the presented robust phylogenetic framework will enable future studies aiming to better understand the evolutionary drivers and processes in this exciting subfamily, including biogeographical phenomena and evolution of particular traits and key innovations.

■ AUTHOR CONTRIBUTIONS

GZ, EMCL, and JP designed the study. FB and JLM carried out the targeted gene selection and the lab work; SH contributed data; JP, FB, JLM, and GZ analyzed the data. GZ, JP, and FB wrote the first draft of the manuscript. EMCL, SH, RCF, and MAK contributed tissue samples for sequencing. All co-authors contributed to the interpretation of the results and writing of the final version of the manuscript. — JP, https://orcid.org/0000-0001-5375-7689; JLM, https://orcid.org/0000-0003-4811-2231; EMCL, https://orcid.org/0000-0003-4712-9832; RCF, https://orcid.org/0000-0002-7035-9313; MAK, https://orcid.org/0000-0002-1693-6829; SH, https://orcid.org/0000-0003-1027-2926; GZ, https://orcid.org/0000-0002-5030-7049

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Appendix 1. List of studied accessions and corresponding data.

Taxon name, project DNA ID, origin, sourced living collection and accession number, original collector and collection number, herbarium code (in brackets), NCBI SRA accession number. Type species for genera and subgenera are marked with *. All samples have been newly sequenced for this study.

Acanthostachys pitcairnioides (Mez) Rauh & Barthlott, BE214b, Brazil, Espírito Santo, Domingos Martins, Mata Roberto Kautsky, Collection E. Leme 483, *E. Leme 483* (RB), SRS8361495; ***Acanthostachys strobilacea*** (Schult. & Schult.f.) Klotzsch*, FB047, Brazil, Guaíra, BG Heidelberg 103630, *W. Rauh 36780* (FR-0105523), SRS8361559; ***Aechmea aculeatosepala*** (Rauh & Barthlott) Leme, BE389.3, Ecuador, Collection E. Leme 3234, *E. Leme 3234* (HB), SRS8361500; ***Aechmea bracteata*** (Sw.) Griseb., FB059, Ecuador, Los Ríos Province, Babahojo, BG Heidelberg 130274, *W. Rauh 37682* (WU 0009731, WU 0009732), SRS8361569; ***Aechmea bromeliifolia*** (Rudge) Baker ex Benth. & Hook.f., FB058, Brazil, Minas Gerais, Grão Mogol, BG Heidelberg 130276, *W. Rauh 67356* (N/A), SRS8361567; ***Aechmea carvalhoi*** E.Pereira & Leme, BE566, Brazil, Bahia, Itamarajú, Collection E. Leme 579, *A.M. de Carvalho s.n.* (RFA, HB), SRS8361514; ***Aechmea chlorophylla*** L.B.Sm., BE468, Brazil, Bahia, near Itabuna, BG Heidelberg 130239, *W. Rauh 67645a* (FR-0035868), SRS8361504; ***Aechmea costantinii*** (Mez) L.B.Sm., BE058.2, Brazil, Alagoas, Murici, Res. Ecol. Murici, Mata da Bananeira, Pedra do Bonito, Collection E. Leme 6596, *E. Leme 6596* (HB), SRS8361509; ***Aechmea dactylina*** Baker, FB045, N/A, BG Heidelberg 103874, ex BG Hamburg (FR-0035867), SRS8361555; ***Aechmea disjuncta*** (L.B.Sm.) Leme & J.A.Siqueira, BE507, Brazil, Bahia, Itaju da Colonia to Jussari, Collection E. Leme 4013, *P. Nahoum s.n.* (HB), SRS8361510; ***Aechmea distichantha*** Lem.*, BE600, Brazil, Rio Grande do Sul, Viamão, Parque Estadual de itapuã, Collection E. Leme 1444, *E. Leme 1444* (HB), SRS8361524; ***Aechmea egleriana*** L.B.Sm., BE403, Brazil, Amazonas, Collection E. Leme 4159, *J.B.F. da Silva 2156* (HB), SRS8361503; ***Aechmea entringeri*** Leme, BE604, Brazil, Espírito Santo, Domingos Martins, Boqueirão, prop. João Thomas, Collection E. Leme 1054, *R.A. Kautsky 936* (HB), SRS8361523; ***Aechmea fasciata*** (Lindl.) Baker, FB002, Brazil, BG Berlin 118-25-74-63, *Berndt s.n.* (B GH 12203), SRS8361537; ***Aechmea flavorosea*** E.Pereira, FB003, N/A, BG Berlin 119-73-74-83 (B GH 10638a), SRS8361536; ***Aechmea hoppii*** (Harms) L.B.Sm., FB020, Colombia, Putumayo, Orito, BG Heidelberg 103873, *W. Rauh 37422* (FR-0035363, HEID 602548), SRS8361547; ***Aechmea lactifera*** Leme & J.A.Siqueira, BE589, Brazil, Pernambuco, Ipojuca, Fazenda Merepe, Praia do Cupe, Collection E. Leme 4812, *J.A. Siqueira-Filho 796* (UFP, HB), SRS8361521; ***Aechmea longifolia*** (Rudge) L.B.Sm. & M.A.Spencer, BE546, Brazil, Pará, without exact place, Collection E. Leme 6275, *J.B.F. da Silva s.n.* (HB), SRS8361513; ***Aechmea lueddemanniana*** (K.Koch) Mez*, FB043, Costa Rica, Cartago Province, Ruta 10 from Cervantes to Birrisito, 8 km after Cervantes, BG Heidelberg 108134, *W. Till 7048* (N/A), SRS8361557; ***Aechmea meeana*** (L.B.Sm.) L.B.Sm., BE497, Brazil, Amazonas, baixo Rio Negro, Igapó, Collection E. Leme 3357, *R. & K. Ryde s.n.* (HB), SRS8361508; ***Aechmea mertensii*** (Meyer) Schult. & Schult.f., BE048.3, Brazil, Pará, Santa Isabel do Pará Caraparu, Collection E. Leme 6288, *E. Leme 6288* (RB01387146), SRS8361573; ***Aechmea miniata*** (Beer) hort. ex Baker, FB018, Brazil, State of Pernambuco, near Gravatá, BG Heidelberg 104304, *G. Pfister s.n.* (N/A), SRS8361545; ***Aechmea mira*** Leme & H.Luther, BE154.3, Brazil, Bahia, Guaibim Terrestre, formando grandes touceiras na restinga arbórea, Collection E. Leme 3722, *E. Leme 3722* (RB00557707, HB), SRS8361572; ***Aechmea moorei*** H.Luther, FB015, Colombia, Garzon-Altamira-La Plata, BG Heidelberg 130154, *W. Rauh 37203* (HEID 602781), SRS8361544; ***Aechmea nudicaulis*** (L.) Griseb.*, FB039, Panama, BG Heidelberg 130250, *W. Rauh 65212* (N/A), SRS8361556; ***Aechmea ornata*** Baker, BE045.1, Brazil, Santa Catarina, Porto Belo, Praia de Zimbros, Collection E. Leme 1529, *E. Leme 1529* (RB), SRS8361571; ***Aechmea ramosa*** Mart. ex Schult. & Schult.f., BE385, Brazil, Campos, Pedra Lisa, road to Santa Rita, Collection E. Leme 8094, *E. Leme 8094* (HB), SRS8361499; ***Aechmea serragrandensis*** Leme & J.A.Siqueira, BE515, Brazil, Alagoas, Ibateguara, Usina Serra Grande, Engenho Coimbra, Collection E. Leme 6679, *J.A. Siqueira Filho 1500* (HB), SRS8361511; ***Aechmea sphaerocephala*** Baker*, FB048, Brazil, Rio de Janeiro, Armação dos Búzios, BG Heidelberg 130262, ex BG Rio de Janeiro (HEID 607585), SRS8361562; ***Aechmea tayoensis*** Gilmartin, BE264b, Ecuador, Collection E. Leme 3240, *E. Leme 3240* (RB01380678), SRS8361496; ***Aechmea weilbachii*** F.Didr., BE228b, Brazil, Rio de Janeiro, Rio de Janeiro, Serra da Pedra Branca, Collection E. Leme 168, *E. Leme 168* (RB), SRS8361494; ***Androlepis fragrans*** (L.B.Sm.) L.B.Sm. & Read, BE606, Mexico, Veracruz, ca. 3 miles North of Fortin de Las Flores, Collection E. Leme 5451, *A. Lau Jr. s.n.* (HB), SRS8361525; ***Araeococcus micranthus*** Brongn.*, FB008, French Guiana, Saint-Laurent-du-Maroni (Arrondissement), Saint-Laurent-du-Maroni, Chutes Voltaire, along trail between Camp Voltaire and waterfall, BG Berlin 146-30-15-20, *N. Kösters s.n.* (B GH 51312), SRS8361539; ***Billbergia horrida*** Regel, FB049, Brazil, BG Heidelberg 103028, *R. Kautsky s.n.* (N/A), SRS8361560; ***Billbergia kuhlmannii*** L.B.Sm., BE432, N/A, BG Heidelberg 102974, ex Bundesgärten Wien (N/A), SRS8361506; ***Billbergia seidelii*** L.B.Sm. & Reitz, BE580, Brazil, Espírito Santo, Domingos Martins, Galo, Collection E. Leme 1702, *R.A. Kautsky 997* (HB), SRS8361517; ***Billbergia vittata***

Appendix 1. Continued.

Brongn. ex Morel, FB051, Brazil, Bahia, Aocon, BG Heidelberg 103607, *A.F.H. Buining s.n.* (HEID 602908), SRS8361564; ***Billbergia zebrina*** (Herb.) Lindl., BE577, Brazil, Rio de Janeiro, Rio de Janeiro, Parque Nacional da Tijuca, Collection E. Leme 128, *E. Leme 128* (RB01405203), SRS8361515; ***Canistropsis billbergioides*** (Schult.f.) Leme, FB034, Brazil, Rio de Janeiro, Campos, Morro de Côco, BG Heidelberg 109834, *W. Till 11142* (N/A), SRS8361553; ***Canistrum fosterianum*** L.B.Sm., FB012, N/A, BG Berlin 213-15-87-83, ex Palmengarten Frankfurt (N/A), SRS8361542; ***Canistrum lanigerum*** H.H.Luther & Leme, BE611, Brazil, Bahia, north of Valença, road to Salvador, near Nazaré, Collection E. Leme 4664, *W. Berg & J. Anderson BAB 195* (HB), SRS8361526; ***Cryptanthus bromelioides*** Otto & A.Dietr.*, BE657, Brazil, Rio de Janeiro, Rio de Janeiro, Barra da Tijuca, Collection E. Leme 2229, *E. Leme 2229* (RB, SEL 1996-0500, WU 0008978), SRS8361535; ***Deinacanthon urbanianum*** (Mez) Mez*, FB054, N/A, BG Heidelberg 105010, *D. Muhr s.n.* (HEID 602945), SRS8361565; ***Edmundoa ambigua*** (Wanderley & Leme) Leme*, BE140.4, Brazil, Rio de Janeiro, Parati Mirim, Estr. Parati a Cunha, P. N. Bocaina, Collection E. Leme 1073, *E. Leme 1073* (RB01371070), SRS8361563; ***Fernseea bocainensis*** Pereira & Moutinho, BE204b, Brazil, São Paulo, Bananal, Sertão das Cobras, Fazenda Albion (de Francisco Pontual), Collection E. Leme 1422, *E. Leme 1422* (RB01207183), SRS8361493; ***Fernseea itatiaiae*** (Wawra) Baker*, FB055, Brazil, State of Minas Gerais, Serra da Mantiqueira, BG Heidelberg 102174, *W. Barthlott 90-6* (HEID 206327), SRS8361566; ***Hohenbergia pennae*** Pereira, FB050, Brazil, Bahia State, Mucugé, BG Heidelberg 104043, *W. Rauh 70094* (HEID 603412), SRS8361561; ***Hohenbergia stellata*** Schult. & Schult.f.*, BE585, Brazil, Bahia, Igrapiúna, Fazenda Michelin, Mata da Vila 5, Margem direita do Rio Serinhaém, Collection E. Leme 8504, *E. Leme 8504* (RB01395308), SRS8361518; ***Hohenbergiopsis guatemalensis*** (L.B.Sm.) L.B.Sm. & Read*, FB019, Guatemala, BG Heidelberg 103058, *W. Rauh 38822* (FR-0035736, HEID 201352), SRS8361595; ***Hoplocryptanthus caracensis*** (Leme & E.Gross) Leme, S.Heller & Zizka, BE641, Brazil, Minas Gerais, Itabirito, Serra de Capanema. Mina de Capanema, Collection E. Leme 7230, *E. Leme 7230* (RB), SRS8361530; ***Hoplocryptanthus schwackeanus*** (Mez) Leme, S.Heller & Zizka, BE651, Brazil, Minas Gerais, Morro do Pilar, Morro do Caxinguelê, Collection E. Leme 4912, *E. Leme 4912* (RB), SRS8361533; ***Hylaeaicum* aff. *eleutheropetalum*** (Ule) Leme & Forzza*, BE592, Ecuador, Morona-Santiago, Mendez-Morona, Collection E. Leme 1976, *H. Luther s.n.* (RB), SRS8361522; ***Hylaeaicum myrmecophilum*** (Ule) Leme & Forzza, BE587, Brazil, Collection E. Leme 2555, *J. Kent s.n.* (SEL), SRS8361519; ***Hylaeaicum wurdackii*** (L.B.Sm.) Leme, Zizka & Aguirre-Santoro, FB023, Colombia, BG Heidelberg 103272, *W. Rauh 37406A* (N/A), SRS8361546; ***Hylaeaicum wurdackii*** (L.B.Sm.) Leme, Zizka & Aguirre-Santoro, BE451, Colombia, BG Heidelberg 103272, *W. Rauh 37406A* (N/A), SRS8361505; ***Karawata saxicola*** (L.B.Sm.) J.R.Maciel & G.Sousa, BE166.2, Brazil, Rio de Janeiro, Campos, Parque Estadual do Desengano, Bacia do Rio Mocotó, Fazenda São Julião, Collection E. Leme 1318, *E. Leme 1318* (RB), SRS8361575; ***Lymania corallina*** (Brongn. ex Beer) Read, FB046, N/A, BG Heidelberg 103674, ex BG Wien (N/A), SRS8361558; ***Lymania smithii*** Read, FB009, Brazil, Bahia, Ilheus, 6 km W Olivenca, BG Berlin 166-23-83-23, *B. Leuenberger 3109* (B GH 17529), SRS8361540; ***Neoglaziovia burle-marxii*** Leme, BE276, Brazil, Minas Gerais, Jequitinhonha, Collection E. Leme 7900, *Z. Miranda s.n.* (HB), SRS8361502; ***Neoglaziovia variegata*** (Arruda) Mez*, BE618, Brazil, Pernambuco, road Ferreiro-Bodocó, Collection E. Leme 6677, *E. Leme 6677* (HB), SRS8361528; ***Neoregelia angustibracteolata*** Pereira & Leme, FB024, Brazil, State of Espírito Santo, Domingo Martins, BG Heidelberg 104378, *R. Kautsky s.n.* (HEID 201366), SRS8361548; ***Neoregelia carolinae*** (Beer) L.B.Sm., FB031, Brazil, State of Rio de Janeiro, Petropolis, Correas, Mata Porcus, 15 km N Petropolis, BG Heidelberg 109836, *W. Till 14004* (N/A), SRS8361551; ***Nidularium fulgens*** Lem.*, FB035, Brazil, State of São Paulo, Guarujá Island, BG Heidelberg 130426, *W. Rauh 35716* (N/A), SRS8361554; ***Orthophytum glabrum*** (Mez) Mez*, BE639, Brazil, Minas Gerais, Collection E. Leme 3281, *E. Leme 3281* (RB), SRS8361527; ***Orthophytum zanonii*** Leme, BE644, Brazil, Espírito Santo, Pancas, Laginha, Pedra do Vidal, propr. Vidal Krause, Collection E. Leme 5941, *E. Leme 5941* (HB), SRS8361532; ***Portea fosteriana*** L.B.Sm., BE560, Brazil, Espírito Santo Vargem Alta Mono de Sal, Collection E. Leme 7809, *E. Leme 7809* (RB01395434), SRS8361512; ***Portea petropolitana*** (Wawra) Mez, FB004, N/A, BG Berlin 120-26-74-83 (B GH 34552), SRS8361538; ***Pseudananas sagenarius*** (Arruda) Schult. & Schult.f.*, BE179, Brazil, Bahia, Lagedão, Collection E. Leme 5579, *E. Leme 5579* (RB), SRS8361576; ***Pseudaraeococcus chlorocarpus*** (Wawra) R.A.Pontes & Versieux, BE257, Brazil, Alagoas, Murici, Res. Ecol. Murici, Mata da Bananeira, Collection E. Leme 6600, *E. Leme 6600* (HB), SRS8361497; ***Pseudaraeococcus parviflorus*** (Mart. ex. Schult.f.) R.A.Pontes & Versieux*, FB063, Brazil, State of Bahia, BG Heidelberg 130605, ex BG Selby (FR-0035876), SRS8361570; ***Quesnelia humilis*** Mez, BE065.2, Brazil, São Paulo, Collection E. Leme 3473, *E. Leme 3473* (RB00648352), SRS8361520; ***Quesnelia imbricata*** L.B.Sm., BE051, Brazil, Santa Catarina, Campo Alegre, Próximo à divisa com PR, Morro do Quiriri (Iquererim), Collection E. Leme 1661, *E. Leme 1661* (RB00648797), SRS8361498; ***Quesnelia koltesii*** Amorim & Leme, BE112.1, Brazil, Bahia, Camaçã, Faz. Serra Bonita, RPPN Serra Bonits, 9,7 km W from Camaçã and then 6 km SW to the road to the reserve and Embratel tower, Collection E. Leme 6743, *A. Amorim 5443* (HB), SRS8361552; ***Quesnelia liboniana*** (De Jonghe) Mez*, BE071.1, Brazil, Rio de Janeiro, Teresópolis Soberbo, Morro do Elefante, Collection E. Leme 2361, *E. Leme 2361* (R010007239, RB01414259), SRS8361531; ***Quesnelia quesneliana*** (Brongn.) L.B.Sm., BE095.2, Brazil, Minas Gerais, Rio Preto, Riacho Santana, Vale Sul S/A, Collection E. Leme 3402, *M. Brugger s.n.* (HB), SRS8361541; ***Quesnelia seideliana*** L.B.Sm. & Reitz, FB027, Brazil, State of Rio de Janeiro, Novo Friburgo, Lumiar, BG Heidelberg 109404, *L.F.N. Carvalho s.n.* (N/A), SRS8361549; ***Quesnelia tubifolia*** Leme & L.Kollmann, BE582, Brazil, Minas Gerais, Santa Maria do Salto Near the border with Bahia, Santa Maria do Salto, Talismã, RPPN Fazenda Duas Barras, border of the Alto Cariri State Park, Collection E. Leme 8210, *E. Leme 8210* (RB00850726, RB01397248), SRS8361516; ***Rokautskyia odoratissima*** (Leme) Leme, S.Heller & Zizka, BE654, Brazil, Espírito Santo, Santa Leopoldina, Reserva Florestal Roberto Kautsky, Collection E. Leme 5216, *R. Menescal & al. s.n.* (HB 73797), SRS8361534; ***Ronnbergia allenii*** (L.B. Sm.) Aguirre-Santoro, FB013, Panama, Panamá Province, Cerro Jefe, BG Heidelberg 103813, *W. Rauh 65213* (FR-0035510, HEID 603947), SRS8361543; ***Ronnbergia tonduzii*** (Mez & Pittier ex Mez) Aguirre-Santoro, BE246, Ecuador, Collection E. Leme 3226, *W. Berg 92* (HB), SRS8361594; ***Sincoraea burle-marxii*** (L.B.Sm. & Read) Louzada & Wand., BE640, Brazil, Bahia, Riachinho, between Palmeiras and Caeté-Açu, Collection E. Leme 3821, *R. Oliveira s.n.* (RB), SRS8361529; ***Sincoraea ulei*** (Louzada & Wand.) Louzada & Wand., BE486, Brazil, Bahia, Rio de Contas, Collection E. Leme 6054, *E. Leme 6054* (RB), SRS8361507; ***Ursulaea macvaughii*** (L.B.Sm.) Read & H.U.Baensch*, FB057, N/A, BG Heidelberg 105622, ex BG Wien (FR-0035514), SRS8361568; ***Wittmackia amorimii*** (Leme) Aguirre-Santoro, BE392, Brazil, Bahia, Wenceslau Guimarães, Nova Esperança, Est. Ecológica de Nova Esperança, Collection E. Leme 4376, *E. Leme 4376* (RB01397278, RB01207270), SRS8361501; ***Wittmackia lingulata*** (L.) Mez*, BE156.2, Dominican Republic, Riviera La Croix, Collection E. Leme 2860, *H. Rabinowitz s.n.* (SEL), SRS8361574; ***Wittmackia silvana*** (Leme) Aguirre-Santoro, BE231b, Brazil, Bahia, Nova Cana Serra da Boa Vista (Oricana), Collection E. Leme 5305, *E. Leme 5305* (CEPEC00107893), SRS8361492; ***Wittrockia superba*** Lindm.*, FB028, Brazil, BG Heidelberg 102741, *Dumke s.n.* (FR-0032052), SRS8361550.