bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Taxon-specific or universal? Using target capture to study the evolutionary history of a rapid radiation

Gil Yardeni[1,*], Juan Viruel[2], Margot Paris[3], Jaqueline Hess[1,4], Clara Groot Crego[1], Marylaure de La Harpe[1], Norma Rivera[1], Michael H. J. Barfuss[1], Walter Till[1], Valeria Guzmán-Jacob[5], Thorsten Krömer[6], Christian Lexer[1,**], Ovidiu Paun[1,**], and Thibault Leroy[1,**]

[1] *Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, A-1030 Vienna, Austria*
[2] *Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS United Kingdom*
[3] *Department of Biology, Unit of Ecology & Evolution, University of Fribourg, Fribourg, Switzerland*
[4] *Department of Soil Ecology, Helmholtz Centre for Environmental Research, UFZ, Halle (Saale), Germany*
[5] *Biodiversity, Macroecology and Biogeography, University of Goettingen, Göttingen, Germany*
[6] *Centro de Investigaciones Tropicales, Universidad Veracruzana, José María Morelos 44–46, Xalapa, Veracruz, Mexico*
[**] *shared last authorship*
[*] *corresponding author: gil.c.yardeni@gmail.com*

Abstract - Target capture emerged as an important tool for phylogenetics and population genetics in non-model taxa. Whereas developing taxon-specific capture probes requires sustained efforts, available universal kits may have a lower power to reconstruct relationships at shallow phylogenetic scales and within rapidly radiating clades. We present here a newly-developed target capture set for Bromeliaceae, a large and ecologically-diverse plant family with highly variable diversification rates. The set targets 1,776 coding regions, including genes putatively involved in key innovations, with the aim to empower testing of a wide range of evolutionary hypotheses. We compare the relative power of this taxon-specific set, Bromeliad1776, to the universal Angiosperms353 kit. The taxon-specific set results in higher enrichment success across the entire family, however, the overall performance of both kits to reconstruct phylogenetic trees is relatively comparable, highlighting the vast potential of universal kits for resolving evolutionary relationships. For more detailed phylogenetic or population genetic analyses, e.g. the exploration of gene tree concordance, nucleotide diversity or population structure, the taxon-specific capture set presents clear benefits. We discuss the potential lessons that this comparative study provides for future phylogenetic and population genetic investigations, in particular for the study of evolutionary radiations. ***Keywords***— target capture, plant radiation, Bromeliaceae, *Tillandsia*, population structure, phylogenomics

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

1 # 1 Introduction

2 Targeted sequencing approaches have emerged as a promise to studying evolutionary
3 relationships in non-model taxa, enabling researchers to retrieve large data sets whereas re-
4 quiring few genomic resources (Bossert & Danforth, 2018; Escudero, Nieto-Feliner, Pokorny,
5 Spalink, & Viruel, 2020; Soto-Gomez et al., 2019). Using custom DNA baits, the method
6 largely retrieves the same loci across a wide taxonomic scale, obtains comparable and merge-
7 able data sets, and may be combined with genome-skimming (E. M. Lemmon & Lemmon,
8 2013; Weitemier et al., 2014). Pre-existing knowledge of the targeted loci further provides
9 opportunities to address specific questions on both deep and shallow timescales (Hale, Gard-
10 ner, Viruel, Pokorny, & Johnson, 2020; A. R. Lemmon, Emme, & Lemmon, 2012). Finally,
11 the method does not necessarily require a reference genome, is highly cost-effective, and with
12 the ability to sequence herbarium samples, reduces the need for extensive sampling cam-
13 paigns (Blaimer, Lloyd, Guillory, & Brady 2016; Hale et al. 2020; Weitemier et al., 2014).
14 Target capture has been successfully applied to resolve phylogenies in diverse groups, from
15 arthropods such as bees (*Xylocopa*, Blaimer et al., 2016; Apidae, Bossert et al., 2019) and
16 Araneae (Hexathelidae, Hedin, Derkarabetian, Ramírez, Vink, & Bond, 2018) to mammals
17 (Cetacea, McGowen et al., 2020), and in numerous plant groups (*Heuchera*, Folk, Mandel,
18 & Freudenstein, 2015; Gesneriaceae, Ogutcen et al., 2021; Zingiberales, Sass, Iles, Barrett,
19 Smith, & Specht, 2016 to name a few). The method's utility for studies at micro-evolutionary
20 scales has been to date marginally explored, but several studies pointed at a good ability
21 to analyse genomic diversity and estimate population genomic parameters (Choquet et al.,
22 2019; Christmas, Biffin, Breed, & Lowe, 2017; de La Harpe et al., 2019; Derrien & Ramos-
23 Onsins, 2020; Sanderson, DiFazio, Cronk, Ma, & Olson, 2020). Nonetheless, the development
24 of probes for target enrichment may pose several challenges: first, the need to identify regions
25 conserved enough to ensure recovery, yet polymorphic enough to provide ample information

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

26 (Soto-Gomez et al., 2019; Villaverde et al., 2018). Second, probe design requires detecting
27 regions without pervasive copy number polymorphism (Kadlec, Bellstedt, Maitre, & Pirie,
28 2017; A. R. Lemmon et al., 2012), a particular challenge for angiosperms and other groups,
29 where duplication events are ubiquitous (Van de Peer, Mizrachi, & Marchal, 2017).

30 In contrast, universal kits offer an attractive alternative that require reduced efforts
31 to establish, and provide comparable data sets across wider ranges of taxa (Johnson et al.,
32 2019; Kadlec et al., 2017). Such kits were designed to retrieve single-copy markers, for
33 example, in the broad scope of amphibians (Hime et al., 2021), anthozoans (Quattrini et al.,
34 2018), vertebrates (A. R. Lemmon et al., 2012) or angiosperms (Johnson et al., 2019). In the
35 latter example, the Angiosperms353 kit is designed to target 353 single-copy sequences across
36 angiosperms. So far the kit has been employed successfully in resolving phylogenies, including
37 but not limited to *Nepenthes* (Murphy et al., 2020), *Schefflera* (Shee, Frodin, Cámara-Leret,
38 & Pokorny, 2020) and the rapid radiation of *Burmeistera* (Bagley, Uribe-Convers, Carlsen,
39 & Muchhala, 2020), establishing the kit as an eminent tool in macroevolutionary research.
40 Its utility at microevolutionary levels is yet to be fully realized, although several works
41 established its suitability to deliver informative signals at a lower taxonomic level (Beck et al.,
42 2021) and in acquiring population genomics parameters (Slimp, Williams, Hale, & Johnson,
43 2020). The use of highly-conserved markers in a universal kit may, however, limit resolution
44 power. Generally, taxon-specific baits are expected to deliver a higher information content
45 and hence more accurate results (Kadlec et al., 2017), as enrichment success is known to drop
46 with the level of divergence between sequences used for probe design and the targeted taxa
47 (Liu et al., 2019). However, a study comparing the power of the universal Angiosperms353
48 kit and a taxon-specific kit to resolve phylogenomic relationship in Cyperaceae reported
49 surprisingly similar performance (Larridon et al., 2020). It remains to be established whether
50 these findings apply to other taxa and other evolutionary scales, including at population
51 level, where ample genomic variability is required to resolve intra-specific relationships and

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

52 investigate patterns of genetic differentiation.

53 Until recently, the technology available to investigate evolutionary questions in rapidly
54 evolving groups featuring high net diversification rates has presented major obstacles, in par-
55 ticular for non-model groups. Decreasing costs of sequencing coupled with an ever-growing
56 plethora of bioinformatic tools for data processing and downstream analysis has led to an in-
57 crease in the use of methods like whole-genome sequencing, RNA sequencing and restriction
58 site associated DNA sequencing (RAD-Seq) in lieu of traditional methods employing few con-
59 served markers (de La Harpe et al., 2017; McKain, Johnson, Uribe-Convers, Eaton, & Yang,
60 2018; Weitemier et al., 2014; Zimmer & Wen, 2013). Whole-genome sequencing however
61 remains costly, posing barriers for research targeting large numbers of samples, organisms
62 with large genomes and non-model organisms, for which the availability of high-quality ge-
63 nomic resources is often limited (Hollingsworth, Li, van der Bank, & Twyford, 2016; Supple
64 & Shapiro, 2018). While RAD-seq is an affordable alternative and widely used in popula-
65 tion genetics, the resulting data sets may fall short when screened for homologous sequences
66 across distantly related lineages (but see e.g., Heckenhauer, Samuel, Ashton, Abu Salim, &
67 Paun, 2018). Additionally, the use of short and inconsistently-represented loci across phylo-
68 genetic sampling may result in low information content and difficulties in assessing paralogy
69 (E. M. Lemmon & Lemmon, 2013; McKain et al., 2018; Jones & Good, 2016).

70 Rapid evolutionary radiations are key stages in the evolutionary history across the
71 Tree of Life and highly recurrent, hence an essential part of biodiversity research (Gavrilets
72 & Losos, 2009; Givnish et al., 2014; Hughes, Nyffeler, & Linder, 2015; Soltis, Folk, & Soltis,
73 2019; Soltis & Soltis, 2004). Fast evolving groups provide potent opportunities to investi-
74 gate important questions in evolutionary biology, such as the interplay between ecological
75 and evolutionary processes in shaping biodiversity. Research on rapidly evolving lineages
76 has provided insight on the fascinating circumstances that drive radiations mostly in model
77 groups. A few notable study systems are the cichlid fish (McGee et al., 2020; Salzburger,

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

78 2018), *Heliconius* butterflies (Dasmahapatra et al., 2012; Moest et al., 2020), *Anolis* lizards
79 (McGlothlin et al., 2018; Stroud & Losos, 2020), Darwin's finches (Lamichhaney et al., 2015;
80 Zink & Vázquez-Miranda, 2019), white-eyes birds (Moyle, Filardi, Smith, & Diamond, 2009)
81 and New World lupins (Nevado, Atchison, Hughes, & Filatov, 2016). Nevertheless, much
82 remains unknown about the genomic basis underlying rapid species diversification outside
83 these intensively studied systems.

84 Research of rapidly diversifying lineages presents several challenges. First, a brief di-
85 versification period typically leads to imperfect reproductive barriers and incomplete lineage
86 sorting, reflected in significant gene tree discordance and ambiguous relationships (Degnan
87 & Rosenberg, 2009; Lamichhaney et al., 2015; Pease, Haak, Hahn, & Moyle, 2016; Straub
88 et al., 2014). In addition, understanding 'speciation through time' poses a methodological
89 challenge, and requires connecting two conceptual worlds: macroevolutionary investigations,
90 concerned with spatial and ecological patterns over deeper timescales, and microevolution-
91 ary approaches, providing insight into the processes acting during population divergence
92 and speciation (Bragg, Potter, Bi, & Moritz, 2016; de La Harpe et al., 2017). Resolving
93 phylogenomic relationships and disentangling the contribution of different genomic processes
94 through time typically requires large-scale genomic datasets and thorough taxon sampling
95 efforts (E. M. Lemmon & Lemmon, 2013; Linder, 2008; Straub et al., 2012).

96 Here, we present Bromeliad1776, a new bait set for targeted sequencing, designed to
97 address a wide range of evolutionary hypotheses in Bromeliaceae: from producing robust
98 phylogenies to studying the interplay of genomic processes during speciation and the ge-
99 netic basis of trait shifts, such as photosynthetic and pollination syndrome. This highly
100 diverse Neotropical radiation provides an excellent research system for studying the drivers
101 and constraints of rapid adaptive radiation (Benzing, 2000; Givnish et al., 2011; Loiseau et
102 al., 2021; Mota et al., 2020; Palma-Silva & Fay, 2020; Wöhrmann, Michalak, Zizka, & Weis-
103 ing, 2020). Bromeliaceae is a species-rich and charismatic monocot family, consisting of over

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

104 3,000 species, including crops in the genus *Ananas* and other economically important species
105 (Luther, 2008). Members of the family are characterized by a distinctive leaf rosette that
106 often impounds rainwater in central tanks (phytotelmata). A diversity of arthropods and
107 other animal species and microbes reside in bromeliad tanks, in some cases even leading to
108 protocarnivory and other forms of nutrient acquisition (Givnish, Burkhardt, Happel, & Wein-
109 traub, 1984; C. Leroy, Carrias, Céréghino, & Corbara, 2016). Bromeliads present a diversity
110 of repeatedly evolving adaptive traits, which allowed them to occupy versatile habitats and
111 ecological niches (Benzing, 2000). CAM photosynthesis, water-absorbing trichomes, forma-
112 tion of tank habit, extensive rates of epiphytism and a diversity of pollination syndromes
113 are some of the adaptations correlated with high rates of diversification within the family
114 (Benzing, 2000; Crayn, Winter, & Smith, 2004; Givnish et al., 2014; Kessler, Abrahamczyk,
115 & Krömer, 2020; Quezada & Gianoli, 2011).

116 To assess the utility of the Bromeliad1776 kit, we performed a comparison between
117 our taxon-specific kit and the universal Angiosperms353 kit using several methods across
118 different evolutionary time-scales. We present Bromeliad1776 in the light of methodological
119 considerations on bait design, data handling, analyses and other practical considerations.

120 2 Materials and Methods

121 2.1 In-house bait design

122 Whole-genome sequences and gene models from *Ananas comosus* v.3 (Ming et al., 2015)
123 were used to design a bait set aiming to target i) random protein coding genomic regions,
124 ii) genes previously described as associated with key innovation traits in Bromeliaceae (see
125 below), iii) markers previously used for phylogenomic inference in Bromeliaceae and iv) genes
126 orthologous to those in the Angiosperms353 bait set. The 1776 selected genes are detailed
127 in Supporting information Table S1.

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

128 The random protein coding genes (subset *i* above) were selected based on genetic
129 diversity parameters calculated using whole-genome sequence and RNAseq data previously
130 published de La Harpe et al. (2020) with the PopGenome R package v.2.1.6 (Pfeifer, (Pfeifer,
131 Wittelsbürger, Ramos-Onsins, & Lercher, 2014). Genomic regions were retained in this cat-
132 egory if they shared at least 70% identity between *A. comosus* and *T. sphaerocephala*, and
133 if they had nucleotide diversity (π) values not exceeding the 90% quantile of the (π) distri-
134 bution across genes for four *Tillandsia* species (*Tillandsia australis*, *Tillandsia fasciculata*,
135 *Tillandsia floribunda* and *T. sphaerocephala*). We further excluded genes with a total exonic
136 size smaller than 1,100 bp, or individual exons smaller than 120 bp. Next, copy-number
137 variation was calculated based on clustering of *A. comosus* and *Tillandsia* transcriptome
138 assemblies to generate three copy number categories - "single copy", "low copy" (i.e., less
139 than five copies) and "high copy" (i.e., five or more copies). For the random genes (i.e., bait
140 subset *i*) we included in the design only "single copy" genes. Finally, we excluded genes that
141 were located in genomic regions outside those assigned to linkage groups in the *A. comosus*
142 reference (Ming et al., 2015). A total of 1,243 genes were identified for this part.

143 The bait subset of genes associated with key innovative traits in Bromeliaceae (subset
144 *ii* above) included (1) genes putatively under positive selection along branches relevant to
145 C3/CAM shifts (de La Harpe et al., 2020), (2) genes that exhibit differential gene expression
146 between CAM and C3 *Tillandsia* species (de La Harpe et al., 2020) and (3) genes putatively
147 associated with photosynthetic and developmental functions, or with flavonoid and antho-
148 cyanin biosynthesis, according to the literature (e.g. Ming et al., 2015; Palma-Silva, Ferro,
149 Bacci, & Turchetto-Zolet, 2016; Wai et al., 2017; Goolsby, Moore, Hancock, Vos, & Edwards,
150 2018). *Ananas comosus* genes with the highest match scores (calculated as lowest E-score
151 in BLASTP, Madden (2013) against the sequences of genes from the literature were added
152 to the bait set (see Supporting information Table S2 for details). A total of 1,612 genes
153 underpinning innovative traits were included in the bait design, regardless of criteria used

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

154 for subset *i* for size, similarity and duplication rate.

155 Markers previously used for phylogenomic inference in Bromeliaceae (subset *iii*) were
156 obtained from the literature, spanning 13 genes (e.g. Barfuss et al., 2016; Machado et al.,
157 2020; Schulte, Barfuss, & Zizka, 2009, see TS2 for full list). Genes orthologous to those in
158 the Angiosperms353 bait set (Johnson et al., 2019) were identified using the orthologous gene
159 models from *A. comosus* based on gene annotations (Ming et al., 2015) or using BLASTP
160 (Madden, 2013), totalling 281 genes.

161 Finally, we used a draft genome of *T. fasciculata* (Jaqueline Hess, personal communi-
162 cation) to exclude from all candidates genes that exhibited multiple BLASTN hits, if they
163 have not been previously described as duplicated within the genus (de La Harpe et al., 2020).
164 Specifically, we excluded genes that matched another genomic sequence of at least 100bp with
165 high similarity score ($> 80\%$) and low E-value ($< 10^{-5}$). In an additional round of filtering
166 was performed by the manufacturer of the final bait set, Arbor Biosciences (Ann Arbor, MI,
167 USA), multi-copy genes with sequences that are more than 95% identical were collapsed into
168 a single sequence, and baits with more than 70% GC content or containing at least 25% re-
169 peated sequences were excluded. In addition, targets including exons smaller than 80 bp were
170 completed with regions flanking the exons according to the *A. comosus* reference genome.
171 The final kit included 1776 genes: 801 random protein coding genes, 681 genes associated
172 with key innovative traits, 13 genes representing phylogenetic markers and 281 genes ortholo-
173 gous to the Angiosperms353 set. Probes were designed with 57,445 80-mer baits tiling across
174 targets in 2x coverage, targeting approximately 2.3Mbp. The kit is subsequently referred to
175 as the Bromeliad1776 bait set. Further specifications can be found in Supporting information
176 Tables S1 and S2 and in the github repository: https://github.com/giyany/Bromeliad1776.

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

177 ## 2.2 Plant material collection

178 We sampled a total of 70/72 Bromeliaceae samples (for Angiosperms353 and for
179 Bromeliad1776, accordingly; Supporting information Table S3), including 56 accessions from
180 the Tillandsioideae subfamily and 16 representing the other subfamilies, except Navioideae
181 (according to Givnish et al. (2014). Within Tillandsioideae, we sampled 38/40 individuals
182 from five species of the *Tillandsia* subgenus *Tillandsia* ('clade K' in Barfuss et al. (2016);
183 Sampling in Mexican populations illustrated in Supporting information Figure S1).

184 ## 2.3 Library preparation & enrichment

185 DNA extractions were performed using a modified CTAB protocol (Doyle & Doyle,
186 1987), purified using Nucleospin® gDNA cleanup kit from Macherey-Nagel (Hudlow et al.,
187 2011) following the supplier's instructions with a two-fold elution step and finally quantified
188 with Qubit® 3.0 Fluorometer (Life Technologies, Ledeberg, Belgium).

189 For each sample, 200ng DNA was sheared using Bioruptor® Pico sonication device
190 (Diagenode, Seraing, Belgium) aiming for an average insert size of 350bp, dried in a speed
191 vacuum Eppendorf concentrator 5301 (Eppendorf, Germany) and eluted in 30µL ddH_2O.
192 Genomic libraries were prepared using the NEBNext® Ultra TM II DNA Library Prep
193 Kit for Illumina® (New England Biolabs, Ipswich, MA, United States) using reagents at
194 half volumes following Hale et al. (2020) and using 11 PCR cycles, increased up to 13 cy-
195 cled for libraries with low genomic output. Samples were double-indexed with NEBNext®
196 Multiplex Oligos for Illumina® (New England Biolabs, Ipswich, MA, USA). Fragment sizes
197 were inspected with Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and
198 concentrations were measured with Qubit® 3.0 Fluorometer. Subpools of 11-14 equimolar
199 genomic libraries were prepared using phylogenetic proximity and DNA concentrations of
200 the genomic libraries, which ranged from 2.62 to 118.0 ng/µL, following Soto-Gomez et al.
201 (2019).

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

202 We used the Angiosperms353 and the Bromeliad1776 bait sets from Arbor Biosciences
203 (Ann Arbor, MI, USA) to enrich each subpool of genomic libraries independently with a single
204 hybridization reaction of myBaits® target capture kits from Arbor Biosciences (Ann Arbor,
205 MI, USA), following Hale et al. (2020). Average fragment size and DNA yield were estimated
206 for each subpool using Agilent Bioanalyzer and Qubit® 3.0 Fluorometer. Subpools were then
207 pooled in equimolar conditions and sequenced at Vienna BioCenter Core Facilities (Vienna,
208 Austria) on Illumina® NextSeq™ 550 (2x150bp, Illumina, San Diego, CA). Sequencing was
209 conducted independently for either bait kit. The sequencing data are publicly available in
210 the NCBI Short Reads Archive (BioProject ID PRJNAxxx, SRA Study SRPxxx).

211 2.4 Data processing

212 The raw sequence data in BAM format was demultiplexed using deML v.1.1.3 (Renaud,
213 Stenzel, Maricic, Wiebe, & Kelso, 2015) and samtools view v.1.7 (Li et al., 2009), converted
214 to fastq using bamtools v.2.4.0 (Barnett, Garrison, Quinlan, Strömberg, & Marth, 2011)
215 and quality checked using FastQC v.0.11.7 (Andrews, 2010). Reads were then trimmed for
216 adapter content and quality using TrimGalore v.0.6.5 (Krueger, 2019), a wrapper tool around
217 FastQC and Cutadapt, using settings –fastqc –retain unpaired. Sequence quality and adapter
218 removal was confirmed with FastQC reports.

219 Quality and adapter-trimmed reads were aligned to *A. comosus* reference genome v.3
220 (Ming et al., 2015) using bowtie2 (Langmead & Salzberg, 2012) with the –very-sensitive-local
221 option to increase sensitivity and accuracy. Samtools (Li et al., 2009) was then used to re-
222 move low quality mapping and sort alignments by position, and PCR duplicates were marked
223 using MarkDuplicates from PicardTools v.2.25 (*Picard Toolkit*, 2019). Summary statistics of
224 the mapping step were generated using samtools stats. Variants were called using freebayes
225 v1.3.2-dirty (Garrison & Marth, 2012) and sites marked as MNP
226 complex were decomposed and normalized using the script 'vcfallelicprimitives' from vcflib

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

227 (Garrison, 2012). Next, AN/AC field was calculated using bcftools v.1.7 (Li, 2011) and
228 variant calls were filtered using vcflib (Garrison & Marth, 2012) and bcftools. Given that
229 freebayes does not perform automatic variant filtering steps, we identified sets of parameters
230 that generate reliable final SNP sets, based on two independent criteria: the highest tran-
231 sition/transversion ratios as reported by SnpSift (SnpEff suite, Cingolani et al., 2012) and
232 the lowest π_N/π_S (see section 2.7 below). After a detailed evaluation, we used the following
233 criteria to generate two high quality SNP sets: we considered genotype calls with per-sample
234 coverage below 10× as missing (NA) and excluded variants (i) marked as indels or neighboring
235 indels within a distance of 3 bp, (ii) with depth of coverage at the SNP level lower than 500×,
236 (iii) with less than ten reads supporting the alternate allele at the SNP level, or (iv) with more
237 than 40% missing data. Summary statistics of the final SNP sets were generated using the
238 script vcf2genocountsmatrix.py (available from https://github.com/giyany/Bromeliad1776),
239 namely the total number of SNPs, the proportion of on-target SNPs and the proportion of
240 SNPs in some specific genomic contexts, with *A. comosus* genome v.3 as a reference. We
241 present an example of data processing with the HybPiper pipeline (Johnson et al., 2016) in
242 the github repository (https://github.com/giyany/Bromeliad1776).

243 2.5 Bait specificity and efficiency

244 To explore bait specificity, we calculated the percentage of high quality trimmed reads
245 on-target using samtools stats and bedtools intersect v2.25.0 (Quinlan & Hall, 2010) us-
246 ing the script calculat_bait_target_specifity.sh (available from https://github.com/giyany/
247 Bromeliad1776). Targets for Bromeliad1776 were defined as the bait sequences plus their
248 500 bp flanking regions. Targets for Angiosperms353 were defined using orthogroups to
249 *A. comosus*: gene annotations from the bait set were used to assign genes to orthogroups
250 using OrthoFinder (Emms & Kelly, 2019), resulting in 559 *A. comosus* genes assigned to
251 orthogroups. Within the orthgroups, targets were again defined as exonic regions plus their

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

252 500 bp flanking regions.

253 To provide insights into determinants of bait capture success, we calculated bait effi-
254 ciency for all baits of Bromeliad1776. For each bait, efficiency was calculated as the number
255 of high-quality reads uniquely mapping to each bait target region, averaged over samples.
256 We then tested for the correlation of capture efficiency to several bait characteristics (copy
257 number, GC content, number and size of exons in targeted gene, size of baits and phyloge-
258 netic distance to *A. comosus*) with a generalized linear model or Kruskal-Wallis test in R
259 v.4.0.3 (R Core Team, 2020) using a negative binomial family.

260 2.6 Phylogenomic analyses

261 We inferred phylogenomic relationships for all samples using two methods: a con-
262 catenation method, and a coalescent-based species tree estimation. The latter method was
263 included as concatenation methods do not account for gene tree incongruence, which may
264 result in high support for an incorrect topology (Kubatko & Degnan, 2007), especially in
265 the presence of notable incomplete lineage sorting. In addition, gene tree incongruence anal-
266 ysis provides insight into molecular genome evolution, including the extent of incomplete
267 lineage sorting and other genomic processes such as hybridization and introgression (Galtier
268 & Daubin, 2008; Wendel & Doyle, 1998).

269 We used the SNP genotypes to create a phylip matrix with vcf2phylip v.2.0 (Ortiz,
270 2019) and constructed a maximum-likelihood species tree for each bait set with RAxML-NG
271 v.0.9.0 (Kozlov, Darriba, Flouri, Morel, & Stamatakis, 2019), using 250 bootstrap replicates
272 and a GTR model with an automatic MRE-based bootstrap convergence test. Next, we
273 constructed a species tree using ASTRAL-III v.5.7.7 (hereafter: ASTRAL, Zhang, Rabiee,
274 Sayyari, & Mirarab, 2018). For both the Angiosperms353 and the Bromeliad1776 sets, we
275 considered genes within each bait set as independent genomic windows. For Angiosperms353,
276 we extracted the 559 genes (assigned to orthogroups as explained above) as genomic windows

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

277 using bedtools intersect. For Bromeliad1776, genomic windows were extracted using the *A.*
278 *comosus* gene sequences included in bait design. All loci and all accessions were included in
279 species tree inference regardless of the percentage of missing data, since taxon completeness
280 of individual gene trees is important for statistical consistency of this approach, and we
281 expected only low levels of fragmentary sequences (Mirarab, 2019; Nute, Chou, Molloy, &
282 Warnow, 2018). After excluding genes with zero coverage, 269 genes and 1,600 genes were
283 included in species tree inference for Angiosperms353 and Bromeliad1776, respectively.

284 For each gene, a maximum-likelihood gene tree was inferred using ParGenes (Morel,
285 Kozlov, & Stamatakis, 2019) with RAxML-NG (Kozlov et al., 2019), using a GTR model
286 with an automatic MRE-based bootstrap convergence test. Loci with insufficient signal may
287 reduce the accuracy of species tree estimation (Mirarab, 2019), hence, in all gene trees, nodes
288 with a bootstrap support smaller than ten were collapsed using Newick utilities (Junier
289 & Zdobnov, 2010). A species tree was then generated in ASTRAL with quartet support
290 and posterior probability for each tree topology. The number of conflicting gene trees was
291 calculated using phyparts and visualized using the script phypartspiecharts.py (available from
292 https://github.com/mossmatters/MJPythonNotebooks).

293 2.7 Population structure and nucleotide diversity estimates

294 To explore the genetic structure within the *Tillandsia* species complex, we focused on
295 five species from 15 localities (Supporting information Table S3 and Supporting information
296 Figure S1). We first used plink v.1.9 (Chang et al., 2015) to filter out SNPs in linkage dise-
297 quilibrium. Population structure was further explored through individual ancestry analysis,
298 with identity-by-descent matrix calculated by plink and inference of population structure
299 using ADMIXTURE v.1.3. with K values ranging from one to ten, and 30 replicates for
300 each K, using a block optimization method (Alexander & Lange, 2011). A summary of the
301 ADMIXTURE results was obtained and presented using pong (Behr, Liu, Liu-Fang, Nakka,

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

302 & Ramachandran, 2016). The set of LD-pruned biallelic SNPs was further filtered to allow a
303 maximum of 10% missing data and used to perform a principal components analysis (PCA)
304 with SNPRelate v.1.20.1 (Zheng et al., 2012). Finally, for each *Tillandsia* species, we used
305 the strategy of T. Leroy et al. (2021) to compute synonymous (π_S) and non-synonymous (π_N)
306 nucleotide diversities and Tajima's D, from fasta sequences using seq_stat_coding (T. Leroy
307 et al., 2021).

308 # 3 Results

309 ## 3.1 Higher mapping rates and capture efficiency for taxon-specific set

310 On average, 4,401,958 (803,464-12,693,516) paired-end reads per accession were gen-
311 erated per Angiosperms353 library and 2,962,023 (1,282,762-6,298,880) per Bromeliad1776
312 library. Overall, the mapping rates to the *A. comosus* reference genome were higher for li-
313 braries enriched with Bromeliad1776, with an average mapping rate of 82.3% (61.8%-95.9%)
314 and 42.8% (22.1%-77.9%), for Bromeliad1776 and Angiosperms353, respectively (Support-
315 ing information Figure S2, Supporting information Table S4). Higher mapping rates were
316 recorded for subfamilies Bromelioideae and Puyoideae, as compared to Tillandsioideae, for
317 both the Angiosperms353 and Bromeliad1776 sets (see Supporting information Figures S3
318 and S4, respectively). This may reflect the effect of reference bias, and in the case of
319 Bromeliad1776, it may be further amplified by our kit design based on *A. comosus* (subfam-
320 ily Bromelioideae). Bait specificity was high for Bromeliad1776 with on average 90.4% reads
321 on-target (76.5%-94.2%), while for Angiosperms353 bait specificity was 14.0% (4.6%-30.1%;
322 see Supporting information Figure S2). Mapping rates and bait specificity were positively
323 correlated for both bait sets (GLM, P<0.01).

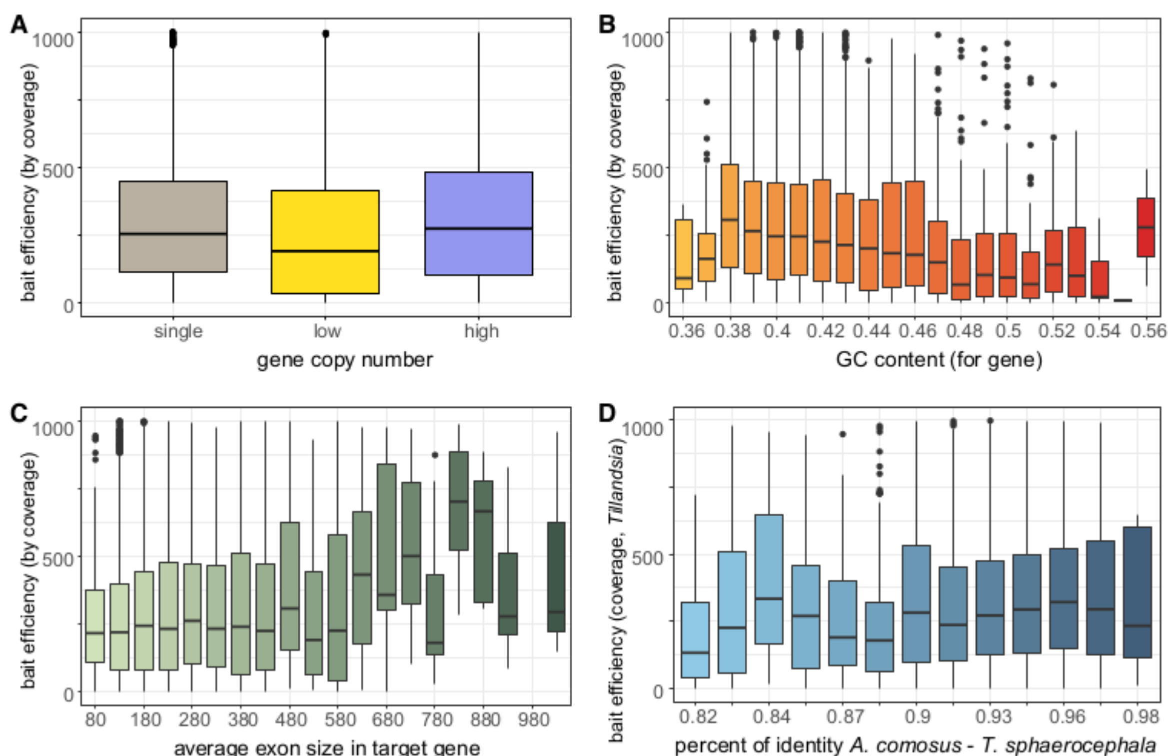
bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Bait capture taxon-specific vs. universal

324 ## 3.2 Bait efficiency depends on the genomic context

325 We investigated factors that may influence bait efficiency, starting with the contribu-
326 tion of gene copy number variation. We assumed three categories regarding the number of
327 paralogs per orthogroup: single copy, low-copy (i.e., less than five copies) and high-copy (i.e.,
328 five or more copies). The number of gene copies had a significant effect on bait efficiency
329 and post-hoc Dunn's test supported significant differences in efficiency for comparisons be-
330 tween low-copy and high-copy, and between single-copy and low-copy (P=2.8^{-44}). Low-copy
331 genes exhibit the lowest enrichment success, suggesting that the bait efficiency is not simply
332 correlated to the number of gene copies (Figure 1). We also recovered a significant effect of
333 the intragenic GC content and GC content of the baits on bait efficiency (GLM, P=1.5^{-68}).
334 Finally, we investigated the possible link between efficiency and gene structure. Average
335 exon sizes ($P< 2.0^{-16}$) and total number of exons per gene (P=1.1^{-89}) were also positively
336 correlated with enrichment success. The size of the smallest exon for all targeted genes was
337 however not correlated with bait efficiency. Sequence similarity, measured as percent of iden-
338 tity between Tillandsia sequences and those of *A. comosus*, was positively correlated with
339 capture success (P=4.8^{-13}; Figure 1).

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.



340

341 **Figure 1** *Effects of (A) putative gene copy number, (B) gene GC content, (C) average*
342 *exon size, and (D) percent of identity on bait efficiency in Bromeliad1776 bait set, measured*
343 *as the number of high-quality reads uniquely mapping to bait target region across samples.*
344 *Continuous variable was binned and y-values higher than 1,000 excluded for visualization in*
345 *B-D.*

346 ## 3.3 Both kits provided a large number of SNPs

347 After variant calling and filtering, we identified 47,390 and 209,186 high-quality SNPs
348 for the Angiosperms353 and the Bromeliad1776 bait sets, respectively. On average, miss-
349 ing data represented 23.7% of genotype calls per individual in Angiosperms353, but only
350 6.3% for the Bromeliad1776 kit. The differences in missingness are likely associated with
351 the higher mean depth per site across the Bromeliad1776 kit (6,602), as compared to An-

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

352 giosperms353 (3,437). Focusing on the subgenus *Tillandsia*, we identified 15,622 SNPs for
353 Angiosperms353 (including a total of 18.9% missing data) compared to 65,473 polymorphic
354 sites (2.9% missing data) for Bromeliad1776. In both full data sets and the subset in-
355 cluding only *Tillandsia* samples, Bromeliad1776 recovered more variants in intronic regions
356 compared with Angiosperms353. Angiosperms353 recovered a large proportion of off-target
357 SNPs, whereas in Bromeliad1776 approximately 15% of the SNPs were recovered from flank-
358 ing regions (Table 1). We discuss ascertainment bias that may rise due to the non-random
359 selection of markers in Supplementary Information.

360 3.4 Similar phylogenomic resolution in concatenation method, Bromeliad1776 361 outperforms Angiosperms353 for species tree reconstruction

362 The Angiosperms353 and Bromeliad1776-based maximum-likelihood phylogenetic trees
363 recovered the same backbone phylogeny of Bromeliaceae, clustering subfamily Tillandsiaoedeae
364 and the subgenus *Tillandsia* with high bootstrap values (Supporting information Figure S5).
365 Neither set obtained high support for inter-population structure for *Tillandsia gymnobotrya*,
366 but highly-supported nodes separated *T. fasciculata* accessions from Mexico and from other
367 locations, and the populations of *T. punctulata* for the Bromeliad1776 data set were similarly
368 separated. The tree topologies were identical, with the notable exception of the placements
369 of*Tillandsia biflora* and *Racinaea ropalocarpa* and the genus *Deuterocohnia* (Supporting in-
370 formation Figure S5, purple arrow). Overall, internal nodes are strongly supported for both
371 sets, except for *Hechtia carlsoniae* as sister to Tillandsioideae, which is poorly supported for
372 both sets. While several internal nodes are slightly less supported for the Angiosperms353
373 set, overall these results demonstrate the efficacy of both kits in phylogenomic reconstruc-
374 tion using concatenation approaches, indicating that as few as 47k SNPs within variable
375 regions provide reliable information to resolve phylogenetic relationships within the recent
376 evolutionary radiation of *Tillandsia*.

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Bait capture taxon-specific vs. universal

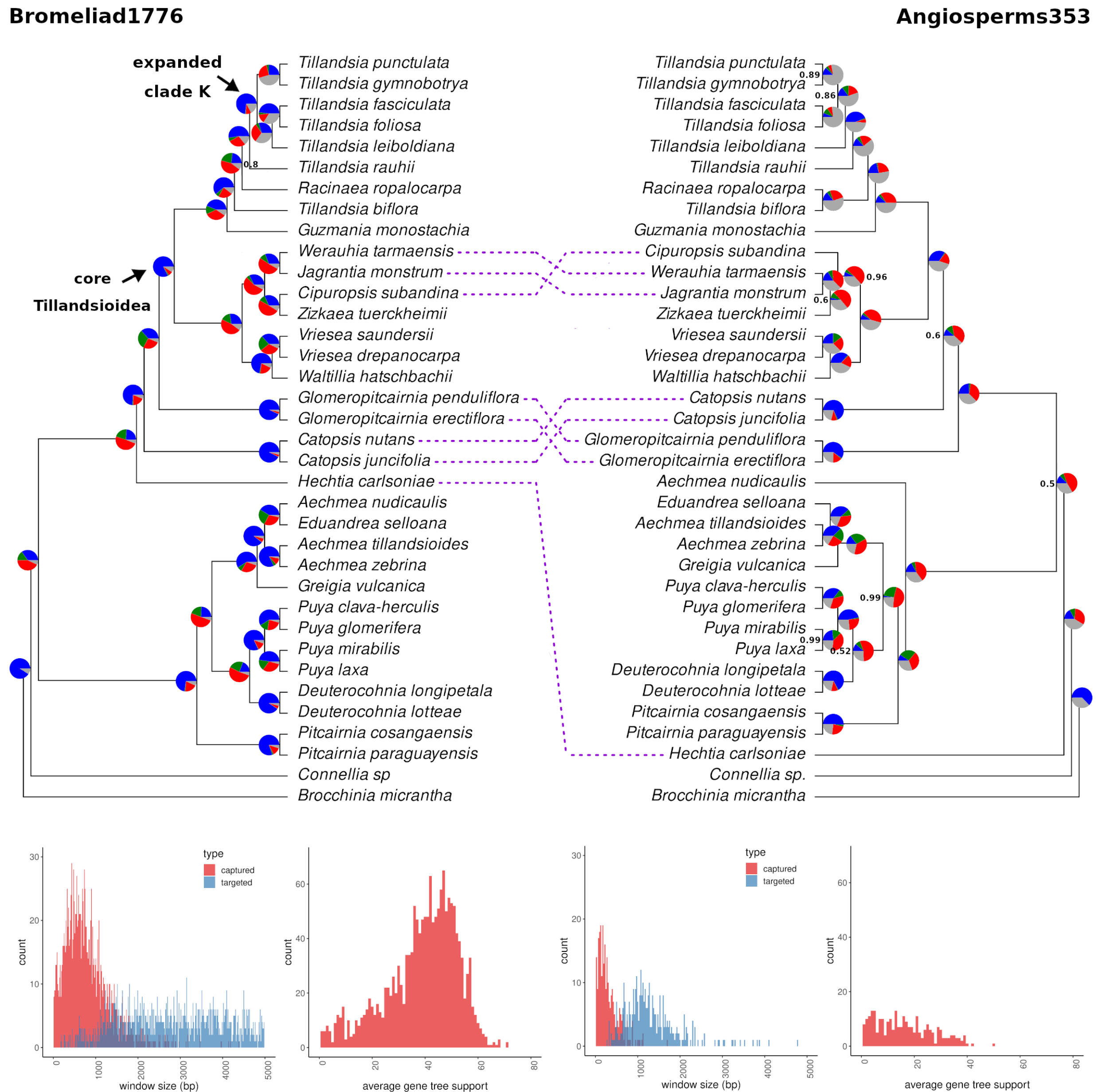
377 Species trees as inferred with ASTRAL for both data sets likewise provided an overall
378 strong local posterior support (Figure 2, see also Supporting information). Several nodes
379 however exhibit lower local posterior support values for the Angiosperms353 tree than for
380 the Bromeliad1776 tree. The topology for the Bromeliad1776 ASTRAL tree was similar to
381 the ML tree, but differed again by placing *Deuterocohnia* as sister taxa to *Puyoideae* only.
382 In the Angiosperms353 tree, the topology differed from both ML trees and the ASTRAL
383 Bromeliad1776 tree in several nodes. *H. carlsoniae* was placed as a sister taxa to all other
384 subfamilies in the Angiosperm353 phylogeny. Notably, the placement of *Catopsis* and *Glom-*
385 *eropitcrania* differed, as well as the placement of *Cipurosis subandinai*, *T. biflora* and *R.*
386 *ropalocarpa*. Several internal nodes were poorly supported, such as the node separating the
387 tribe Catopsideae and core Tillandsioideae, and the nodes separating Tillandsioideae from all
388 other subfamilies. The differences in topology between the Angiosperms353 ASTRAL tree to
389 all other trees (ML trees and Bromeliad1776 ASTRAL tree) together with the low posterior
390 support suggest lower resolution power and a poor fit of this data set for resolving a species
391 tree.

392 The length and average size of the input gene trees different among sets, with average
393 window length of 304.6 bp and 819.9 bp and average gene tree support of 16.9 and 38.9 for
394 Angiosperms353 and Bromeliad1776 bait-sets, respectively (Figure 2). An examination of
395 gene tree concordance constructed with Bromeliad1776 data set allowed to identify variable
396 levels of gene tree conflict among nodes (Figure 2). Gene tree discordance was especially high
397 for the split between Tillandsioideae and other subfamilies, as well as for the split between
398 Puyoideae and taxa assigned to Bromelioideae. Furthermore, gene tree discordance and the
399 proportion of un-informative gene trees was especially high for splits among clades within the
400 K.1 and K.2 clades of subgenus *Tillandsia*. A similar analysis with Angiosperms353 yielded
401 evidence for gene tree discordance, but a considerable number of gene trees were reported
402 to be non-informative (grey part of the pie charts), especially within subgenus *Tillandsia*

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Bait capture taxon-specific vs. universal

403 (Figure 2).



404

405 **Figure 2** *Coalescent-based species trees generated ASTRAL-III for samples enriched with*
406 *Bromeliad1776 (left) and Angiosperms353 (right, flipped for mirroring), on 269 and 1600*
407 *genes for each set, respectively. Node values represent local posterior probabilities (pp) for the*
408 *main topology and are equal to 1 unless noted otherwise. Pie charts at the nodes show levels*

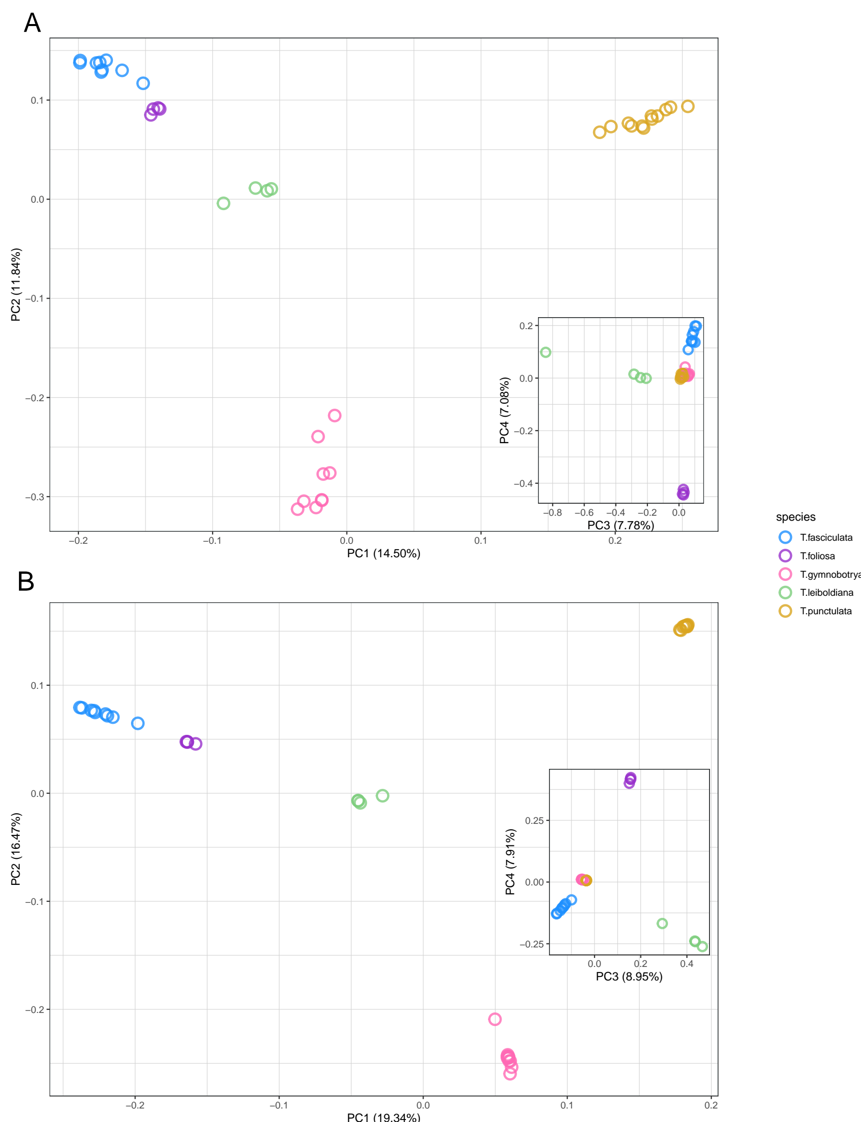
bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

409 *of gene tree discordance: the percentages of concordant gene trees (blue), the top alternative*
410 *bipartition (green), other conflicting topologies (red) and uninformative gene trees (gray). At*
411 *bottom, length and average bootstrap support for gene trees from either data set, according to*
412 *the design of the bait set used for enrichment: Angiosperms353 (right) and Bromeliad1776*
413 *(left). Each gene was considered a single genomic window.*

414 ## 3.5 Strong interspecific structure, but little evidence for within-species popula-
415 tion structure

416 After LD-pruning and retaining maximum 10% missing data, 1,025 and 32,941 biallelic
417 SNPs were included for the *Tillandsia* PCA analysis of the Angiosperms353 and Bromeliad1776
418 data sets, respectively. Overall, both data sets provided evidence for interspecific structure,
419 but not for population structure, with Bromeliad1776 resulting in border-line higher res-
420 olution (slightly better separating *T. foliosa* from *T. fasciculata*). The percentage of ex-
421 plained variance was higher in the Bromeliad1776 set (19.3% and 16.5% for PC1 and PC2)
422 as compared to the Angiosperms353 data set (14.5% and 11.8%, see Figure 3, Supporting
423 information Figure S6). Based on these two PCAs, we found no evidence for spatial genetic
424 structure within each species, since accessions did not cluster by geographic origin on the two
425 PCs presented, or any other PCs we investigated (See Supporting information Figure S6).

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.



426

427 **Figure 3** *Principal Component Analysis (PCA) plot for samples of Tillandsia subgenus*
428 *Tillandsia enriched with two bait sets: A. Angiosperms353 (1,025 variants); B. Bromeliad1776*
429 *(32,941 variants). Colors indicate different species according to legend.*

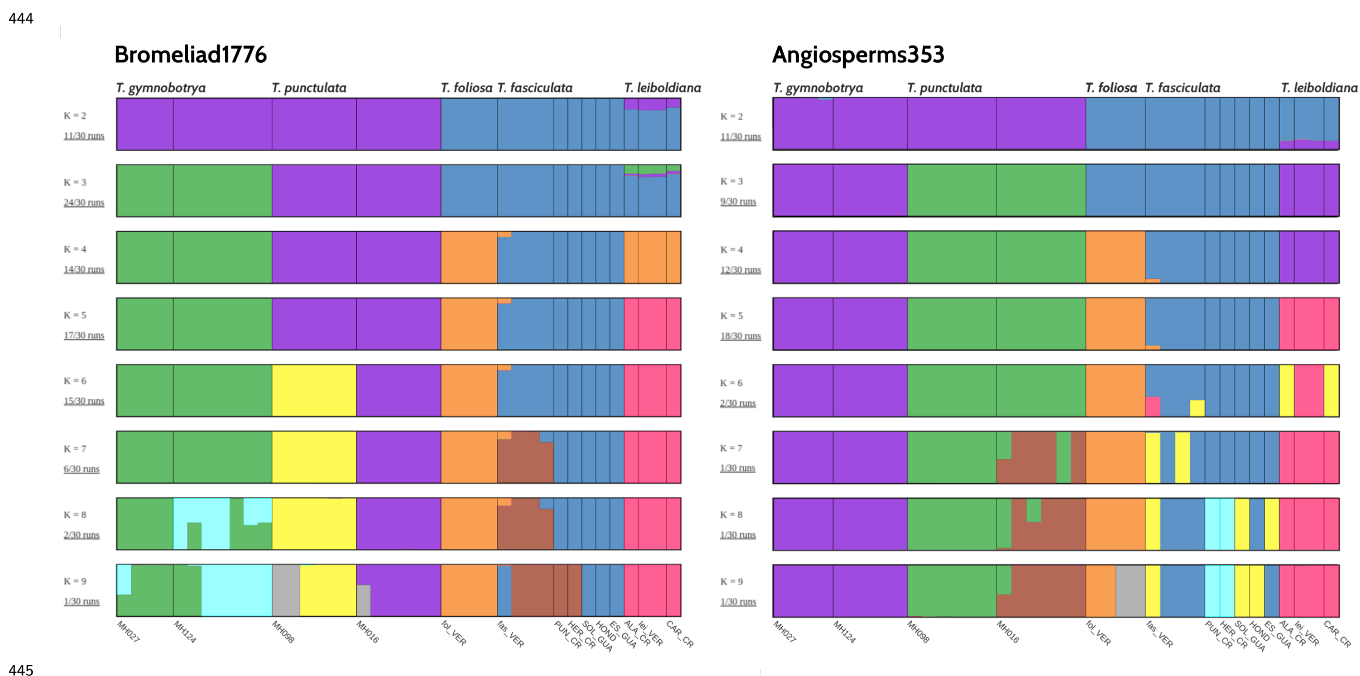
430

431 In addition to PCA, we performed ADMIXTURE analyses based on 9,804 and 42,613
432 variants for the Angiosperms353 and Bromeliad1776 sets, respectively (Figure 4). We used
433 a cross-validation strategy to identify the best K and found clear support for K=5 for the

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

434 Bromeliad1776 set (Supporting information Figure S7). In contrast, the CV pattern for the
435 Angiosperms353 set varied widely, providing limited information about the best K. Low-
436 est CV values were however observed for K=9 with locally low values for K=5 and K=3
437 (Supporting information Figure S7). We further investigated the ADMIXTURE bar plots at
438 different values of K. For K=5, very similar patterns can be observed for both sets, with the
439 recovered clusters reflecting the expected species boundaries. The main difference between
440 the two data sets was the ability of the Bromeliad1776 set to reach a more consistent solution
441 ("consensus") among 30 runs, especially at large K, as compared to the runs based on the
442 Angiosperms353 bait set. The Bromeliad1776 was also able to distinguish between different
443 sampling localities of *T. punctulata* and of *T. fasciculata* at K=7-8 (Figure 4).

444



445

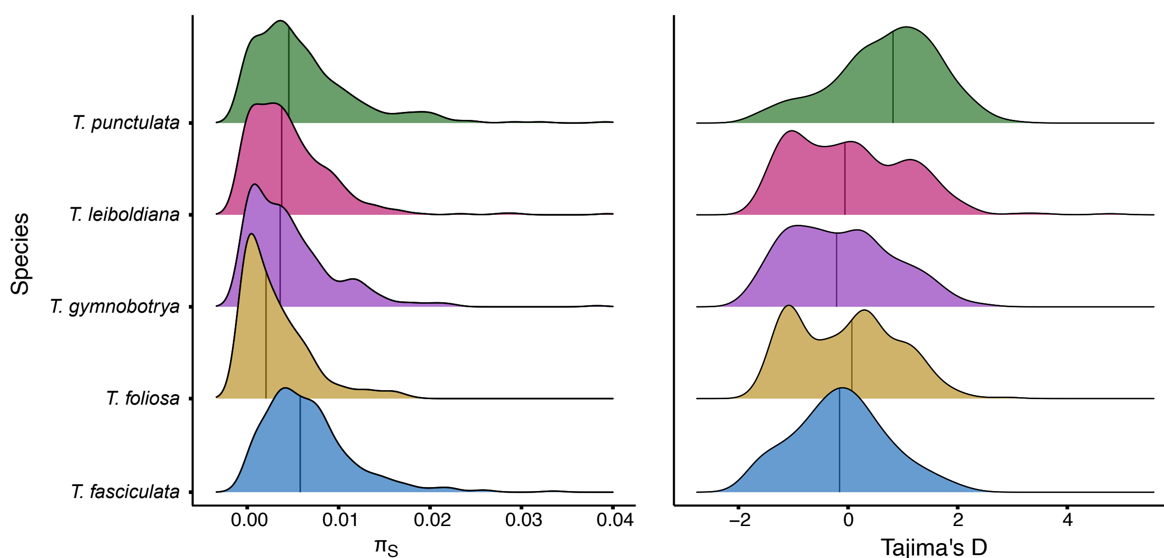
446 **Figure 4** *Population structure of 5 Tillandsia subgenus Tillandsia species from 14 sampling*
447 *locations inferred with the ADMIXTURE software. Samples were enriched with either of two*
448 *bait sets: Angiosperms353 (9,804 variants after LD-pruning) and Bromeliad1776 (42,613*
449 *variants after LD-pruning), showing values of K=2 to K=9. Colors represent genetically*

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

450 *differentiated groups while each accession is represented by a vertical bar.*

451 3.6 Distinct diversities hint at different demographic processes

452 Averaged levels of nucleotide diversity at synonymous sitesπ_S greatly varied among 453 species, from $4.1x10^{-3}$ to $8.1x10^{-3}$ for *T. foliosa* and *T. fasciculata*, respectively (Support-454 ing information Table S5; Figure 5). Given the recent divergence of these different species 455 and their roughly similar life history traits, they are expected to share relatively similar mu-456 tation rates, hence the observed differences in π_S are expected to translate into differences of 457 long-term N_e. Looking at the distribution of π_S across genes, we foundbroader or narrower 458 distributions depending on the species, which explains the observed differences in averaged 459 π_S, as typically represented by the median of the distribution (vertical bars, Figure 5). Most 460 species exhibit distributions of Tajima's D (Fig 5) that are centered around zero, with the 461 notable exception of *T. punctulata*. The distribution of this species is shifted toward positive 462 Tajima's D values, therefore indicating a recent population contraction, suggesting that this 463 species experienced a unique demographic trajectory as compared to the other species.



464

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

465 **Figure 5** *Distribution of Tajima's D and synonymous (π_S) nucleotide diversity within each*
466 *species for the Bromeliad1776 kit.*

467 4 Discussion

468 4.1 A taxon-specific bait set performs marginally better for phylogenomics

469 In this study, we compared the information content and performance of a taxon-specific
470 bait set and a universal bait set for addressing questions on evolutionary processes at different
471 scales in a highly diverse Neotropical plant group, including recently radiated clades. We
472 found that the taxon-specific kit provided a greater number of segregating sites, yet contrary
473 to our expectations, the abundance of information content did directly translate to a greater
474 resolution power.

475 The universal and taxon-specific sets performed comparably when investigating macroevo-
476 lutionary patterns: the inferred species trees are remarkably consistent between the two bait
477 sets (Supporting information Figure S5, Figure 2). Notably, both sets were sufficiently in-
478 formative to reconstruct the relationships among the fastest radiating clades. These results
479 resonate with previous comparative works (e.g. in *Burmeistera*, Bagley et al., 2020; in *Bud-*
480 *dleja,* Chau, Rahfeldt, & Olmstead, 2018; and in *Cyperus*, Larridon et al., 2020), where
481 taxon-specific markers provided higher gene assembly success, but a comparable number of
482 segregating sites for phylogenetic inference, indicating that universal bait sets are nearly as
483 effective as taxon-specific bait sets, even in fast evolving taxa. The main advantage of the
484 bromeliad taxon-specific set is its ability to provide additional resolution for deeper exami-
485 nation of gene tree incongruence (Figure 2), currently a fundamental tool in phylogenomic
486 research (Edwards, 2009; Morales-Briones et al., 2020; Pease et al., 2016).

487 The taxon-specific bait set performed marginally better to address hypotheses at more
488 recent evolutionary scales and provided arguably clearer evidence for inference of species

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

489 genomic structure using clustering methods. In fact, genetic markers obtained from both
490 data sets provided sufficient information to infer species but no geographic structure, sug-
491 gesting that *Tillandsia* could be characterized by high gene dispersal among populations.
492 Considering that the Angiosperms353 kit has shown potential to provide within-species sig-
493 nal, as recently demonstrated by Beck et al. (2021) on *Solidago ulmifolia*, and to estimate
494 demographic parameters from herbarium specimen (Slimp et al., 2020), we would expect the
495 taxon-specific set to accurately reveal a geographical genetic structure. However, the present
496 study is generally based on small sample sizes per species (n=4-8), mostly sampled within
497 a limited geographic range, limiting our ability to draw robust conclusions on the levels of
498 intra-specific population structure.

499 The Bromeliad1776 kit provided a substantially larger number of segregating sites
500 (more than 200k vs. 47k in Angiosperms353; Table 1, Supporting information Figure S2)
501 due to higher enrichment success, following the expectation for higher sequence variation in
502 custom-made loci (Figure 1, see also Bragg et al., 2016; de La Harpe et al., 2019; Kadlec et
503 al., 2017). We accordingly found that rates of molecular divergence are distinctly correlated
504 with enrichment success in our sampling (Figure 1), following the expectation that a universal
505 kit will provide fewer segregating sites.

506 However, the difference in resolution power between the kits cannot be ascribed solely
507 to the different numbers of SNPs, but rather to the length and variability of the obtained
508 regions. The topology obtained with the Angiosperm353 data set under the multi-species
509 coalescent model was substantially different from all other inferred trees and the input gene
510 trees provided a low power to detect patterns of gene tree discordance (Figure 2). We addi-
511 tionally observed that the highly conserved regions targeted by Angiosperms353 are shorter
512 in comparison to Bromeliad1776 targets and thus result in shorter input windows for species
513 tree inference (Figure 2). Hence, the patterns of gene tree discordance in the Angiosperms353
514 data set likely indicate incorrect gene tree estimation or other model misspecifications, rather

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

515 than a biological signal. Specifically, coalescence-based methods are sensitive to gene tree
516 estimation error (Zhang et al., 2018) and perform better with gene trees estimated from
517 unlinked loci long enough and variable enough to render sufficient signal per gene tree - this
518 is especially true for data sets with many taxa. The high rates of uninformative genes trees,
519 found in almost half of the intergenic nodes in the Angiosperms353 data set, is expected with
520 increased levels of gene tree error which in turn reduce the accuracy of ASTRAL (Mirarab,
521 2019; Sayyari & Mirarab, 2016). In contrast, the Bromeliad1776 ASTRAL tree (Figure 2,
522 left) resolved phylogenetic relationships among taxa with high posterior probability and a
523 topology similar to the ML tree. Gene tree discordance analysis revealed high incongruence
524 around certain nodes, possibly reflecting rapid speciation events.

525 Since inference of phylogenetic relationships under the multi-species coalescent and
526 exploration of gene tree discordance are both pivotal to phylogenomic research (Degnan &
527 Rosenberg, 2009; Edwards et al., 2016; Pease et al., 2016), a taxon-specific kit provides a
528 clear advantage especially in recent rapid radiations, where gene tree conflict and incomplete
529 lineage sorting are expected to be prevalent (Dornburg, Su, & Townsend, 2019; Kubatko &
530 Degnan, 2007; Roch & Warnow, 2015). In that regard, inference of the species tree with the
531 Bromeliad1776 is a tool to drive further hypotheses concerning evolutionary and demographic
532 processes in the evolution of *Tillandsia*. Moreover, the features of the loci targeted provide
533 an important opportunity to study selection (see section 4.3).

534 4.2 Insights on Bromeliaceae phylogeny and demographic processes in *Tillandsia*
535

536 Both bait sets resolved the phylogeny of Bromeliaceae, including the fastest evolving
537 lineages of the subfamily Tillandsioideae. The results generally agreed to previous findings
538 of the relationships among taxa (Givnish et al., 2011, 2014). Several findings which contrast
539 with the expected known phylogeny may point at a complexity of genomic processes in the

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

540 evolutionary history of Bromeliaceae subfamilies. Both the ML tree and species tree did not
541 support a monophyly of the subfamily Pitcairnioideae, which was represented by four samples
542 and two genera in our phylogeny: *Deuterochonia* and *Pitcarnia*. Rather, the genus *Deute-*
543 *rochonia* was sister to subfamily Puyoideae or sister to both Puyoideae and Bromelioideae
544 subfamilies. Theses findings contrasts results of Barfuss et al. (2016) and Granados Mendoza
545 et al. (2017). Interestingly, in a visualization of gene tree discordance we found high levels
546 of incongruence and a high percentage of trees supporting an alternative topology in the
547 node splitting the genera, indicating that several genomic processes such as hybridization
548 and incomplete lineage sorting may have accompanied divergence in this group, contribut-
549 ing to the phylogenetic conflict and extending the challenges in resolving these evolutionary
550 relationships. Within the core Tillandsioideae, the tribes Tillandsieae and Vrieseeae were
551 found to be monophyletic, in accordance with previous work on the subfamily (Barfuss et
552 al., 2016). Finally, within our focal group *Tillandsia* subgenus *Tillandsia*, clade K as sug-
553 gested by Barfuss et al. (2016) and clades K.1 and K.2 as proposed by Granados Mendoza et
554 al. (2017) were all well supported, further in agreement with their interpretation of Mexico
555 and Central America as a center of diversity for subgenus *Tillandsia*. Within *Tillandsia*,
556 incongruence was prominent at the recent splits within clade K.1. and clade K.2 as expected
557 in a recent rapid radiation, a result of high levels of incomplete lineage sorting, hybridization
558 and introgression (Berner & Salzburger, 2015).

559 When applied to methods in population genetics, we obtained some evidence for a
560 difference in demographic processes and in the level of genetic variation among species. This
561 was especially true for the taxon-specific bait set: for example, the bait set differentiated
562 between populations of *T. punctulata* and *T. fasciculata*, but not *T. gymnobotrya* in a max-
563 imum likelihood tree and ancestry analysis (Supporting information Figure S5, Figure 4),
564 indicating differences in inter-population genetic structure among species. The evidence for
565 different demographic processes in these species extended to estimates of Tajima's D, where

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

566 lower values may indicate a recent bottleneck. In addition, we found a unique distribution of
567 nucleotide diversity for *T. foliosa*, possibly reflecting a low effective population size for this
568 endemic species in contrast with the closely related, but widespread *T. fasciculata*. In all
569 cases, our limited sampling given the large size of the family constrains our ability to draw
570 conclusions of a 'true' phylogeny and to account for population structure. Our finding how-
571 ever suggests that nuclear markers obtained with a target capture technique can highlight
572 genomic processes and be further applied to address questions in population genomics with
573 a wider sampling scheme.

574 4.3 Future prospects and implications for research in Bromeliaceae and other
575 rapid radiations

576 Beyond the scope of this study, the availability of a bait set kit for Bromeliaceae
577 provides a prime genetic resource for investigating several topical research questions on the
578 origin and maintenance of Bromeliaceae diversity. Manyfold studies of bromeliad phyloge-
579 nomics set force the challenges of resolving species-level phylogenies with a small number of
580 markers, particularly in young and speciose groups (Goetze, Zanella, Palma-Silva, Büttow,
581 & Bered, 2017; Granados Mendoza et al., 2017; Loiseau et al., 2021; Versieux et al., 2012).
582 This particularly curated bait set allows highly efficient sequencing across taxa: within our
583 study, we found high mapping success with 82.3% average read mapping. As expected, we
584 documented a difference in enrichment success among taxa, explained by divergence time to
585 the reference used for bait design (see Supporting information Figure S4), suggesting possible
586 deviations from the assumptions of non-randomly distributed missing data that may mislead
587 phylogenetic inference (A. R. Lemmon, Brown, Stanger-Hall, & Lemmon, 2009; Streicher,
588 Schulte, & Wiens, 2016; Xi, Liu, & Davis, 2016). However, given the large enrichment suc-
589 cess, downstream analysis with deliberate methodology can account for possible biases and
590 provide robust inference with strict data filtering (Molloy & Warnow, 2018; Streicher et al.,

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

591 2016). Hence, target enrichment with Bromeliad1776 can produce large data sets with con-
592 sistent representation between taxa, allowing repeatability between studies and retaining the
593 possibility for global synthesis by including sequence baits orthologous to the universal An-
594 giosperms353 bait set. Moreover, with specific knowledge of the loci targeted in this set, the
595 ability to obtain the same sequences across taxa and experiments and to differentiate genic
596 regions with the use of *A. comosus* models, this bait set offers a broad utility for research in
597 population genomics.

598 Another important feature in the Bromeliad1776 set is the inclusion of genes puta-
599 tively associated with key innovative traits in Bromeliaceae with a focus on C3/CAM shifts.
600 Little is known about the molecular basis of the CAM pathway, an adaptation to arid en-
601 vironments which evolved independently and repeatedly in over 36 plant families (Heyduk,
602 Moreno-Villena, Gilman, Christin, & Edwards, 2019; Chen, Xin, Wai, Liu, & Ming, 2020;
603 Silvera et al., 2010). CAM phenotypes are considered key adaptations in Bromeliaceae, as-
604 sociated with expansion into novel ecological niches. In *Tillandsia*, C3/CAM shifts were
605 found to be particularly associated with increased rates of diversification (Crayn et al., 2004;
606 de La Harpe et al., 2020; Givnish et al., 2014). The Bromeliad1776 bait set offers oppor-
607 tunities to address specific questions on the relationship between rapid diversification and
608 photosynthetic syndromes in this clade, including testing for gene sequence evolution. Ad-
609 ditionally, the inclusion of multi-copy genes, combined with newly developed pipelines for
610 studying gene duplication and ploidy (Morales-Briones et al., 2020; Viruel et al., 2019), are
611 beneficial for studying the role of gene duplication and loss in driving diversification. With
612 the increasing ubiquity of target baits as a genomic tool we expect to see additional pipelines
613 and applications emerging, further expanding the utility of target capture for both macro-and
614 microevolutionary research.

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

615 # 5 Conclusions

616 Even as whole genome sequencing becomes increasingly economically feasible, tar-
617 get capture is expected to remain popular due to its extensive applications in research. We
618 found that evaluating the differences in resolution power between universal and taxon-specific
619 bait sets is far from a trivial task, and we attempted to lay out a methodological roadmap
620 for researchers wishing to reconstruct the complex evolutionary history of rapidly diversify-
621 ing lineages. While a taxon-specific set offers exciting opportunities beyond phylogenomic
622 and into research of molecular evolution, its development is highly time-consuming, requires
623 community-based knowledge and may cost months of work when compared with out-of-the-
624 box universal kits. Our results suggest that universal kits can continue to be employed when
625 aiming to reconstruct phylogenies, in particular as this may offer the possibility to use pre-
626 viously published data to generate larger data sets. However, for those wishing to deeply
627 investigate evolutionary questions in certain lineages, a taxon-specific kit offers certain ben-
628 efits during data processing stages, where knowledge of the design scheme and gene models
629 is extremely useful, and the possible return of costs is especially high for taxa emerging as
630 model groups. We furthermore encourage groups designing taxon-specific kits to include also
631 universal probes, furthering the mission to complete the tree of life.

632 # 6 Acknowledgments

633 This paper is dedicated to Christian Lexer, a wonderful mentor, friend and colleague.
634 The research was supported with funding from the Christian Lexer professorship. The anal-
635 yses benefited from the Vienna Scientific Cluster (VSC) and the Montpellier Bioinformatics
636 Biodiversity (MBB) platform services. We thank Huiying Shang and Aram Drevekenin for
637 help with code, Kelly Swarts and Claus Vogl for insightful discussions and advice. We thank
638 the members of Swiss SNSF Sinergia project CRSII3_147630 for accession sampling.

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

639 # 7 Author Contribution

640 CL, MP and GY conceived the study. CL provided funding. TK coordinated sample
641 collection, MdLH, VGJ and GY collected data. Species identification was performed by
642 MHJB and WT. Targets were selected by GY, with guidance from JH and MP. Molecular
643 work was performed by CGC, JV, NR, MHJB. The data was analyzed by GY and TL with
644 feedback by JV and OP. The manuscript was written by GY with significant input from all
645 co-authors. TL and OP should be considered joint senior author.

646 # References

647 Alexander, D. H., & Lange, K. (2011). Enhancements to the ADMIXTURE algorithm for
648 individual ancestry estimation. *BMC Bioinformatics*, *12*(1), 246. doi: 10.1186/1471-2105
649 -12-246

650 Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data.*
651 Babraham Bioinformatics, Babraham Institute, Cambridge, United Kingdom.

652 Bagley, J. C., Uribe-Convers, S., Carlsen, M. M., & Muchhala, N. (2020). Utility of targeted
653 sequence capture for phylogenomics in rapid, recent angiosperm radiations: Neotropical
654 *Burmeistera* bellflowers as a case study. *Molecular Phylogenetics and Evolution*, *152*,
655 106769. doi: 10.1016/j.ympev.2020.106769

656 Barfuss, M. H. J., Till, W., Leme, E. M. C., Pinzón, J. P., Manzanares, J. M., Halbritter,
657 H., . . . Brown, G. K. (2016). Taxonomic revision of Bromeliaceae subfam. Tillandsioideae
658 based on a multi-locus DNA sequence phylogeny and morphology. *Phytotaxa*, *279*(1),
659 1–97. doi: 10.11646/phytotaxa.279.1.1

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

660 Barnett, D. W., Garrison, E. K., Quinlan, A. R., Strömberg, M. P., & Marth, G. T. (2011).
661 BamTools: A C++ API and toolkit for analyzing and managing BAM files. *Bioinformatics*,
662 *27*(12), 1691–1692. doi: 10.1093/bioinformatics/btr174

663 Beck, J. B., Markley, M. L., Zielke, M. G., Thomas, J. R., Hale, H. J., Williams, L. D., &
664 Johnson, M. G. (2021). Is Palmer's elm leaf goldenrod real? The Angiosperms353 kit
665 provides within-species signal in Solidago ulmifolia s.l. *bioRxiv*. doi: 10.1101/2021.01.07
666 .425781

667 Behr, A. A., Liu, K. Z., Liu-Fang, G., Nakka, P., & Ramachandran, S. (2016). Pong: Fast
668 analysis and visualization of latent clusters in population genetic data. *Bioinformatics*,
669 *32*(18), 2817–2823. doi: 10.1093/bioinformatics/btw327

670 Benzing, D. H. (2000). *Bromeliaceae: Profile of an Adaptive Radiation*. Cambridge University
671 Press.

672 Berner, D., & Salzburger, W. (2015). The genomics of organismal diversification illuminated
673 by adaptive radiations. *Trends in Genetics*, *31*(9), 491–499. doi: 10.1016/j.tig.2015.07.002

674 Blaimer, B. B., Lloyd, M. W., Guillory, W. X., & Brady, S. G. (2016). Sequence Capture and
675 Phylogenetic Utility of Genomic Ultraconserved Elements Obtained from Pinned Insect
676 Specimens. *PLOS ONE*, *11*(8), e0161531. doi: 10.1371/journal.pone.0161531

677 Bossert, S., & Danforth, B. N. (2018). On the universality of target-enrichment baits for
678 phylogenomic research. *Methods in Ecology and Evolution*, *9*(6), 1453–1460. doi: 10.1111/
679 2041-210X.12988

680 Bossert, S., Murray, E. A., Almeida, E. A. B., Brady, S. G., Blaimer, B. B., & Danforth,
681 B. N. (2019). Combining transcriptomes and ultraconserved elements to illuminate the
682 phylogeny of Apidae. *Molecular Phylogenetics and Evolution*, *130*, 121–131. doi: 10.1016/
683 j.ympev.2018.10.012

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

684 Bragg, J. G., Potter, S., Bi, K., & Moritz, C. (2016). Exon capture phylogenomics: Efficacy
685 across scales of divergence. *Molecular Ecology Resources*, *16*(5), 1059–1068. doi: 10.1111/
686 1755-0998.12449

687 Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015).
688 Second-generation PLINK: Rising to the challenge of larger and richer datasets. *Giga-*
689 *Science*, *4*(1). doi: 10.1186/s13742-015-0047-8

690 Chau, J. H., Rahfeldt, W. A., & Olmstead, R. G. (2018). Comparison of taxon-specific versus
691 general locus sets for targeted sequence capture in plant phylogenomics. *Applications in*
692 *Plant Sciences*, *6*(3), e1032. doi: 10.1002/aps3.1032

693 Chen, L.-Y., Xin, Y., Wai, C. M., Liu, J., & Ming, R. (2020). The role of cis -elements
694 in the evolution of crassulacean acid metabolism photosynthesis. *Horticulture Research*,
695 *7*(1), 1–8. doi: 10.1038/s41438-019-0229-0

696 Choquet, M., Smolina, I., Dhanasiri, A. K. S., Blanco-Bercial, L., Kopp, M., Jueterbock,
697 A., ... Hoarau, G. (2019). Towards population genomics in non-model species with large
698 genomes: A case study of the marine zooplankton *Calanus finmarchicus*. *Royal Society*
699 *Open Science*, *6*(2), 180608. doi: 10.1098/rsos.180608

700 Christmas, M. J., Biffin, E., Breed, M. F., & Lowe, A. J. (2017). Targeted capture to assess
701 neutral genomic variation in the narrow-leaf hopbush across a continental biodiversity
702 refugium. *Scientific Reports*, *7*(1), 41367. doi: 10.1038/srep41367

703 Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., ... Ruden, D. M.
704 (2012). A program for annotating and predicting the effects of single nucleotide polymor-
705 phisms, SnpEff. *Fly*, *6*(2), 80–92. doi: 10.4161/fly.19695

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

706 Crayn, D. M., Winter, K., & Smith, J. A. C. (2004). Multiple origins of crassulacean acid
707 metabolism and the epiphytic habit in the Neotropical family Bromeliaceae. *Proceedings*
708 *of the National Academy of Sciences*, *101*(10), 3703–3708. doi: 10.1073/pnas.0400366101

709 Dasmahapatra, K. K., Walters, J. R., Briscoe, A. D., Davey, J. W., Whibley, A., Nadeau,
710 N. J., ... The Heliconius Genome Consortium (2012). Butterfly genome reveals promis-
711 cuous exchange of mimicry adaptations among species. *Nature*, *487*(7405), 94–98. doi:
712 10.1038/nature11041

713 de La Harpe, M., Hess, J., Loiseau, O., Salamin, N., Lexer, C., & Paris, M. (2019). A ded-
714 icated target capture approach reveals variable genetic markers across micro- and macro-
715 evolutionary time scales in palms. *Molecular Ecology Resources*, *19*(1), 221–234. doi:
716 10.1111/1755-0998.12945

717 de La Harpe, M., Paris, M., Hess, J., Barfuss, M. H. J., Serrano-Serrano, M. L., Ghatak, A.,
718 ... Lexer, C. (2020). Genomic footprints of repeated evolution of CAM photosynthesis
719 in a Neotropical species radiation. *Plant, Cell & Environment*, *43*(12), 2987–3001. doi:
720 10.1111/pce.13847

721 de La Harpe, M., Paris, M., Karger, D. N., Rolland, J., Kessler, M., Salamin, N., & Lexer,
722 C. (2017). Molecular ecology studies of species radiations: Current research gaps, oppor-
723 tunities and challenges. *Molecular Ecology*, *26*(10), 2608–2622. doi: 10.1111/mec.14110

724 Degnan, J. H., & Rosenberg, N. A. (2009). Gene tree discordance, phylogenetic inference
725 and the multispecies coalescent. *Trends in Ecology & Evolution*, *24*(6), 332–340. doi:
726 10.1016/j.tree.2009.01.009

727 Derrien, T., & Ramos-Onsins, S. (2020). Assessing a novel sequencing-based approach for
728 population genomics in non-model species. *Peer Community in Genomics*, *1*, 100002. doi:
729 10.24072/pci.genomics.100002

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

730 Dornburg, A., Su, Z., & Townsend, J. P. (2019). Optimal Rates for Phylogenetic Inference
731 and Experimental Design in the Era of Genome-Scale Data Sets. *Systematic Biology*,
732 *68*(1), 145–156. doi: 10.1093/sysbio/syy047

733 Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of
734 fresh leaf tissue. *PHYTOCHEMICAL BULLETIN*, *19*(1), 11–15.

735 Edwards, S. V. (2009). Is a New and General Theory of Molecular Systematics Emerging?
736 *Evolution*, *63*(1), 1–19. doi: 10.1111/j.1558-5646.2008.00549.x

737 Edwards, S. V., Xi, Z., Janke, A., Faircloth, B. C., McCormack, J. E., Glenn, T. C., ... Davis,
738 C. C. (2016). Implementing and testing the multispecies coalescent model: A valuable
739 paradigm for phylogenomics. *Molecular Phylogenetics and Evolution*, *94*, 447–462. doi:
740 10.1016/j.ympev.2015.10.027

741 Emms, D. M., & Kelly, S. (2019). OrthoFinder: Phylogenetic orthology inference for com-
742 parative genomics. *Genome Biology*, *20*(1), 238. doi: 10.1186/s13059-019-1832-y

743 Escudero, M., Nieto-Feliner, G., Pokorny, L., Spalink, D., & Viruel, J. (2020). Editorial:
744 Phylogenomic Approaches to Deal With Particularly Challenging Plant Lineages. *Frontiers*
745 *in Plant Science*, *11*. doi: 10.3389/fpls.2020.591762

746 Folk, R. A., Mandel, J. R., & Freudenstein, J. V. (2015). A protocol for targeted enrichment
747 of intron-containing sequence markers for recent radiations: A phylogenomic example from
748 *Heuchera* (Saxifragaceae). *Applications in Plant Sciences*, *3*(8), 1500039. doi: 10.3732/
749 apps.1500039

750 Galtier, N., & Daubin, V. (2008). Dealing with incongruence in phylogenomic analyses.
751 *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1512), 4023–
752 4029. doi: 10.1098/rstb.2008.0144

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

753 Garrison, E. (2012). *Vcflib: A C++ library for parsing and manipulating VCF files.* Github.
754 https://github. com/ekg/vcflib.

755 Garrison, E., & Marth, G. (2012). Haplotype-based variant detection from short-read se-
756 quencing. *arXiv:1207.3907 [q-bio]*.

757 Gavrilets, S., & Losos, J. B. (2009). Adaptive Radiation: Contrasting Theory with Data.
758 *Science*, *323*(5915), 732–737. doi: 10.1126/science.1157966

759 Givnish, T. J., Barfuss, M. H. J., Ee, B. V., Riina, R., Schulte, K., Horres, R., ... Sytsma,
760 K. J. (2011). Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae:
761 Insights from an eight-locus plastid phylogeny. *American Journal of Botany*, *98*(5), 872–
762 895. doi: 10.3732/ajb.1000059

763 Givnish, T. J., Barfuss, M. H. J., Ee, B. V., Riina, R., Schulte, K., Horres, R., ... Sytsma,
764 K. J. (2014). Adaptive radiation, correlated and contingent evolution, and net species
765 diversification in Bromeliaceae. *Molecular Phylogenetics and Evolution*, *71*, 55–78. doi:
766 10.1016/j.ympev.2013.10.010

767 Givnish, T. J., Burkhardt, E. L., Happel, R. E., & Weintraub, J. D. (1984). Carnivory in
768 the Bromeliad *Brocchinia reducta*, with a Cost/Benefit Model for the General Restriction
769 of Carnivorous Plants to Sunny, Moist, Nutrient-Poor Habitats. *The American Naturalist*,
770 *124*(4), 479–497. doi: 10.1086/284289

771 Goetze, M., Zanella, C. M., Palma-Silva, C., Büttow, M. V., & Bered, F. (2017). Incomplete
772 lineage sorting and hybridization in the evolutionary history of closely related, endemic
773 yellow-flowered *Aechmea* species of subgenus *Ortgiesia* (Bromeliaceae). *American Journal*
774 *of Botany*, *104*(7), 1073–1087. doi: 10.3732/ajb.1700103

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

775 Goolsby, E. W., Moore, A. J., Hancock, L. P., Vos, J. M. D., & Edwards, E. J. (2018). Molec-
776 ular evolution of key metabolic genes during transitions to C4 and CAM photosynthesis.
777 *American Journal of Botany*, *105*(3), 602–613. doi: 10.1002/ajb2.1051

778 Granados Mendoza, C., Granados-Aguilar, X., Donadío, S., Salazar, G. A., Flores-Cruz, M.,
779 Hágsater, E., ... Magallón, S. (2017). Geographic structure in two highly diverse lineages
780 of *Tillandsia* (Bromeliaceae). *Botany*, *95*(7), 641–651. doi: 10.1139/cjb-2016-0250

781 Hale, H., Gardner, E. M., Viruel, J., Pokorny, L., & Johnson, M. G. (2020). Strategies for
782 reducing per-sample costs in target capture sequencing for phylogenomics and population
783 genomics in plants. *Applications in Plant Sciences*, *8*(4), e11337. doi: 10.1002/aps3.11337

784 Heckenhauer, J., Samuel, R., Ashton, P. S., Abu Salim, K., & Paun, O. (2018). Phyloge-
785 nomics resolves evolutionary relationships and provides insights into floral evolution in the
786 tribe Shoreeae (Dipterocarpaceae). *Molecular Phylogenetics and Evolution*, *127*, 1–13. doi:
787 10.1016/j.ympev.2018.05.010

788 Hedin, M., Derkarabetian, S., Ramírez, M. J., Vink, C., & Bond, J. E. (2018). Phylogenomic
789 reclassification of the world's most venomous spiders (Mygalomorphae, Atracinae), with
790 implications for venom evolution. *Scientific Reports*, *8*(1), 1636. doi: 10.1038/s41598-018
791 -19946-2

792 Heyduk, K., Moreno-Villena, J. J., Gilman, I. S., Christin, P.-A., & Edwards, E. J. (2019).
793 The genetics of convergent evolution: Insights from plant photosynthesis. *Nature Reviews*
794 *Genetics*, *20*(8), 485–493. doi: 10.1038/s41576-019-0107-5

795 Hime, P. M., Lemmon, A. R., Lemmon, E. C. M., Prendini, E., Brown, J. M., Thomson,
796 R. C., ... Weisrock, D. W. (2021). Phylogenomics Reveals Ancient Gene Tree Discordance
797 in the Amphibian Tree of Life. *Systematic Biology*, *70*(1), 49–66. doi: 10.1093/sysbio/
798 syaa034

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

799 Hollingsworth, P. M., Li, D.-Z., van der Bank, M., & Twyford, A. D. (2016). Telling plant
800 species apart with DNA: From barcodes to genomes. *Philosophical Transactions of the*
801 *Royal Society B: Biological Sciences*, *371*(1702), 20150338. doi: 10.1098/rstb.2015.0338

802 Hudlow, W. R., Krieger, R., Meusel, M., Sehhat, J. C., Timken, M. D., & Buoncristiani, M. R.
803 (2011). The NucleoSpin® DNA Clean-up XS kit for the concentration and purification
804 of genomic DNA extracts: An alternative to microdialysis filtration. *Forensic Science*
805 *International. Genetics*, *5*(3), 226–230. doi: 10.1016/j.fsigen.2010.03.005

806 Hughes, C. E., Nyffeler, R., & Linder, H. P. (2015). Evolutionary plant radiations: Where,
807 when, why and how? *New Phytologist*, *207*(2), 249–253. doi: 10.1111/nph.13523

808 Johnson, M. G., Gardner, E. M., Liu, Y., Medina, R., Goffinet, B., Shaw, A. J., ... Wickett,
809 N. J. (2016). HybPiper: Extracting coding sequence and introns for phylogenetics from
810 high-throughput sequencing reads using target enrichment. *Applications in Plant Sciences*,
811 *4*(7), 1600016. doi: 10.3732/apps.1600016

812 Johnson, M. G., Pokorny, L., Dodsworth, S., Botigué, L. R., Cowan, R. S., Devault, A., ...
813 Wickett, N. J. (2019). A Universal Probe Set for Targeted Sequencing of 353 Nuclear Genes
814 from Any Flowering Plant Designed Using k-Medoids Clustering. *Systematic Biology*,
815 *68*(4), 594–606. doi: 10.1093/sysbio/syy086

816 Jones, M. R., & Good, J. M. (2016). Targeted capture in evolutionary and ecological
817 genomics. *Molecular Ecology*, *25*(1), 185–202. doi: 10.1111/mec.13304

818 Junier, T., & Zdobnov, E. M. (2010). The Newick utilities: High-throughput phylogenetic
819 tree processing in the UNIX shell. *Bioinformatics (Oxford, England)*, *26*(13), 1669–1670.
820 doi: 10.1093/bioinformatics/btq243

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

821 Kadlec, M., Bellstedt, D. U., Maitre, N. C. L., & Pirie, M. D. (2017). Targeted NGS for
822 species level phylogenomics: "made to measure" or "one size fits all"? *PeerJ*, *5*, e3569.
823 doi: 10.7717/peerj.3569

824 Kessler, M., Abrahamczyk, S., & Krömer, T. (2020). The role of hummingbirds in the
825 evolution and diversification of Bromeliaceae: Unsupported claims and untested hypothe-
826 ses. *Botanical Journal of the Linnean Society*, *192*(4), 592–608. doi: 10.1093/botlinnean/
827 boz100

828 Kozlov, A. M., Darriba, D., Flouri, T., Morel, B., & Stamatakis, A. (2019). RAxML-
829 NG: A fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference.
830 *Bioinformatics*, *35*(21), 4453–4455. doi: 10.1093/bioinformatics/btz305

831 Krueger, F. (2019). *Trim Galore: A wrapper tool around Cutadapt and FastQC to con-*
832 *sistently apply quality and adapter trimming to FastQ files, with some extra functionality*
833 *for MspI-digested RRBS-type (Reduced Representation Bisufite-Seq) libraries.* Babraham
834 Bioinformatics, Babraham Institute, Cambridge, United Kingdom.

835 Kubatko, L. S., & Degnan, J. H. (2007). Inconsistency of Phylogenetic Estimates from
836 Concatenated Data under Coalescence. *Systematic Biology*, *56*(1), 17–24. doi: 10.1080/
837 10635150601146041

838 Lamichhaney, S., Berglund, J., Almén, M. S., Maqbool, K., Grabherr, M., Martinez-Barrio,
839 A., ... Andersson, L. (2015). Evolution of Darwin's finches and their beaks revealed by
840 genome sequencing. *Nature*, *518*(7539), 371–375. doi: 10.1038/nature14181

841 Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature*
842 *Methods*, *9*(4), 357–359. doi: 10.1038/nmeth.1923

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

843 Larridon, I., Villaverde, T., Zuntini, A. R., Pokorny, L., Brewer, G. E., Epitawalage, N., ...
844 Baker, W. J. (2020). Tackling Rapid Radiations With Targeted Sequencing. *Frontiers in*
845 *Plant Science*, *10*. doi: 10.3389/fpls.2019.01655

846 Lemmon, A. R., Brown, J. M., Stanger-Hall, K., & Lemmon, E. M. (2009). The Effect
847 of Ambiguous Data on Phylogenetic Estimates Obtained by Maximum Likelihood and
848 Bayesian Inference. *Systematic Biology*, *58*(1), 130–145. doi: 10.1093/sysbio/syp017

849 Lemmon, A. R., Emme, S. A., & Lemmon, E. M. (2012). Anchored Hybrid Enrichment
850 for Massively High-Throughput Phylogenomics. *Systematic Biology*, *61*(5), 727–744. doi:
851 10.1093/sysbio/sys049

852 Lemmon, E. M., & Lemmon, A. R. (2013). High-Throughput Genomic Data in Systematics
853 and Phylogenetics. *Annual Review of Ecology, Evolution, and Systematics*, *44*(1), 99–121.
854 doi: 10.1146/annurev-ecolsys-110512-135822

855 Leroy, C., Carrias, J.-F., Céréghino, R., & Corbara, B. (2016). The contribution of microor-
856 ganisms and metazoans to mineral nutrition in bromeliads. *Journal of Plant Ecology*, *9*(3),
857 241–255. doi: 10.1093/jpe/rtv052

858 Leroy, T., Rousselle, M., Tilak, M.-K., Caizergues, A. E., Scornavacca, C., Recuerda, M.,
859 ... Nabholz, B. (2021). Island songbirds as windows into evolution in small populations.
860 *Current Biology*. doi: 10.1016/j.cub.2020.12.040

861 Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association map-
862 ping and population genetical parameter estimation from sequencing data. *Bioinformatics*,
863 *27*(21), 2987–2993. doi: 10.1093/bioinformatics/btr509

864 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., ... Durbin, R. (2009).
865 The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079.
866 doi: 10.1093/bioinformatics/btp352

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

867 Linder, H. P. (2008). Plant species radiations: Where, when, why? *Philosophical*
868 *Transactions of the Royal Society B: Biological Sciences*, *363*(1506), 3097–3105. doi:
869 10.1098/rstb.2008.0075

870 Liu, Y., Johnson, M. G., Cox, C. J., Medina, R., Devos, N., Vanderpoorten, A., ... Goffinet,
871 B. (2019). Resolution of the ordinal phylogeny of mosses using targeted exons from
872 organellar and nuclear genomes. *Nature Communications*, *10*(1), 1485. doi: 10.1038/
873 s41467-019-09454-w

874 Loiseau, O., Machado, T. M., Paris, M., Koubínová, D., Dexter, K., Versieux, L., ... Salamin,
875 N. (2021). Genome skimming reveals widespread hybridization in a Neotropical flowering
876 plant radiation. *Frontiers in Ecology and Evolution*, *9*. doi: 10.3389/fevo.2021.668281

877 Luther, H. E. (2008). *An alphabetical list of bromeliad binomials* (Eleventh ed.). The Marie
878 Selby Botanical Gardens Sarasota, Florida, USA: the Bromeliad Society International.

879 Machado, T. M., Loiseau, O., Paris, M., Weigand, A., Versieux, L. M., Stehmann, J. R., ...
880 Salamin, N. (2020). Systematics of *Vriesea* (Bromeliaceae): Phylogenetic relationships
881 based on nuclear gene and partial plastome sequences. *Botanical Journal of the Linnean*
882 *Society*, *192*(4), 656–674. doi: 10.1093/botlinnean/boz102

883 Madden, T. L. (2013). The BLAST sequence analysis tool. In *The NCBI Handbook [Internet]*
884 (Second ed.). US: National Center for Biotechnology Information.

885 McGee, M. D., Borstein, S. R., Meier, J. I., Marques, D. A., Mwaiko, S., Taabu, A., ...
886 Seehausen, O. (2020). The ecological and genomic basis of explosive adaptive radiation.
887 *Nature*, 1–5. doi: 10.1038/s41586-020-2652-7

888 McGlothlin, J. W., Kobiela, M. E., Wright, H. V., Mahler, D. L., Kolbe, J. J., Losos, J. B.,
889 & Brodie, E. D. (2018). Adaptive radiation along a deeply conserved genetic line of least
890 resistance in Anolis lizards. *Evolution Letters*, *2*(4), 310–322. doi: 10.1002/evl3.72

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

891 McGowen, M. R., Tsagkogeorga, G., Álvarez-Carretero, S., dos Reis, M., Struebig, M.,
892 Deaville, R., … Rossiter, S. J. (2020). Phylogenomic Resolution of the Cetacean
893 Tree of Life Using Target Sequence Capture. *Systematic Biology*, *69*(3), 479–501. doi:
894 10.1093/sysbio/syz068

895 McKain, M. R., Johnson, M. G., Uribe-Convers, S., Eaton, D., & Yang, Y. (2018). Practical
896 considerations for plant phylogenomics. *Applications in Plant Sciences*, *6*(3), e1038. doi:
897 10.1002/aps3.1038

898 Ming, R., VanBuren, R., Wai, C. M., Tang, H., Schatz, M. C., Bowers, J. E., … Yu, Q.
899 (2015). The pineapple genome and the evolution of CAM photosynthesis. *Nature Genetics*,
900 *47*(12), 1435–1442. doi: 10.1038/ng.3435

901 Mirarab, S. (2019). Species Tree Estimation Using ASTRAL: Practical Considerations.
902 *arXiv:1904.03826 [q-bio]*.

903 Moest, M., Belleghem, S. M. V., James, J. E., Salazar, C., Martin, S. H., Barker, S. L.,
904 … Jiggins, C. D. (2020). Selective sweeps on novel and introgressed variation shape
905 mimicry loci in a butterfly adaptive radiation. *PLOS Biology*, *18*(2), e3000597. doi:
906 10.1371/journal.pbio.3000597

907 Molloy, E. K., & Warnow, T. (2018). To Include or Not to Include: The Impact of Gene
908 Filtering on Species Tree Estimation Methods. *Systematic Biology*, *67*(2), 285–303. doi:
909 10.1093/sysbio/syx077

910 Morales-Briones, D. F., Gehrke, B., Huang, C.-H., Liston, A., Ma, H., Marx, H. E., …
911 Yang, Y. (2020). Analysis of paralogs in target enrichment data pinpoints multiple ancient
912 polyploidy events in *Alchemilla* s.l. (Rosaceae). *bioRxiv*, 2020.08.21.261925. doi: 10.1101/
913 2020.08.21.261925

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

914 Morel, B., Kozlov, A. M., & Stamatakis, A. (2019). ParGenes: A tool for massively parallel
915 model selection and phylogenetic tree inference on thousands of genes. *Bioinformatics*,
916 *35*(10), 1771–1773. doi: 10.1093/bioinformatics/bty839

917 Mota, M. R., Pinheiro, F., Leal, B. S. d. S., Sardelli, C. H., Wendt, T., & Palma-Silva, C.
918 (2020). From micro- to macroevolution: Insights from a Neotropical bromeliad with high
919 population genetic structure adapted to rock outcrops. *Heredity*, 1–18. doi: 10.1038/
920 s41437-020-0342-8

921 Moyle, R. G., Filardi, C. E., Smith, C. E., & Diamond, J. (2009). Explosive Pleistocene di-
922 versification and hemispheric expansion of a "great speciator". *Proceedings of the National*
923 *Academy of Sciences*, *106*(6), 1863–1868. doi: 10.1073/pnas.0809861105

924 Murphy, B., Forest, F., Barraclough, T., Rosindell, J., Bellot, S., Cowan, R., … Cheek, M.
925 (2020). A phylogenomic analysis of *Nepenthes* (Nepenthaceae). *Molecular Phylogenetics*
926 *and Evolution*, *144*, 106668. doi: 10.1016/j.ympev.2019.106668

927 Nevado, B., Atchison, G. W., Hughes, C. E., & Filatov, D. A. (2016). Widespread adaptive
928 evolution during repeated evolutionary radiations in New World lupins. *Nature Commu-*
929 *nications*, *7*(1), 12384. doi: 10.1038/ncomms12384

930 Nute, M., Chou, J., Molloy, E. K., & Warnow, T. (2018). The performance of coalescent-
931 based species tree estimation methods under models of missing data. *BMC Genomics*,
932 *19*(5), 286. doi: 10.1186/s12864-018-4619-8

933 Ogutcen, E., Christe, C., Nishii, K., Salamin, N., Möller, M., & Perret, M. (2021). Phyloge-
934 nomics of Gesneriaceae using targeted capture of nuclear genes. *Molecular Phylogenetics*
935 *and Evolution*, 107068. doi: 10.1016/j.ympev.2021.107068

936 Ortiz, E. M. (2019). *Vcf2phylip v2.0: Convert a VCF matrix into several matrix formats for*
937 *phylogenetic analysis.* Zenodo. doi: 10.5281/zenodo.2540861

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

938 Palma-Silva, C., & Fay, M. F. (2020). Bromeliaceae as a model group in understanding the
939 evolution of Neotropical biota. *Botanical Journal of the Linnean Society*, *192*(4), 569–586.
940 doi: 10.1093/botlinnean/boaa003

941 Palma-Silva, C., Ferro, M., Bacci, M., & Turchetto-Zolet, A. C. (2016). De novo assembly
942 and characterization of leaf and floral transcriptomes of the hybridizing bromeliad species
943 (Pitcairnia spp.) adapted to Neotropical Inselbergs. *Molecular Ecology Resources*, *16*(4),
944 1012–1022. doi: 10.1111/1755-0998.12504

945 Pease, J. B., Haak, D. C., Hahn, M. W., & Moyle, L. C. (2016). Phylogenomics Reveals
946 Three Sources of Adaptive Variation during a Rapid Radiation. *PLOS Biology*, *14*(2),
947 e1002379. doi: 10.1371/journal.pbio.1002379

948 Pfeifer, B., Wittelsbürger, U., Ramos-Onsins, S. E., & Lercher, M. J. (2014). PopGenome:
949 An Efficient Swiss Army Knife for Population Genomic Analyses in R. *Molecular Biology*
950 *and Evolution*, *31*(7), 1929–1936. doi: 10.1093/molbev/msu136

951 *Picard toolkit.* (2019). Broad Institute, GitHub repository.
952 http://broadinstitute.github.io/picard/.

953 Quattrini, A. M., Faircloth, B. C., Dueñas, L. F., Bridge, T. C. L., Brugler, M. R., Calixto-
954 Botía, I. F., ... McFadden, C. S. (2018). Universal target-enrichment baits for anthozoan
955 (Cnidaria) phylogenomics: New approaches to long-standing problems. *Molecular Ecology*
956 *Resources*, *18*(2), 281–295. doi: 10.1111/1755-0998.12736

957 Quezada, I. M., & Gianoli, E. (2011). Crassulacean acid metabolism photosynthesis in
958 Bromeliaceae: An evolutionary key innovation. *Biological Journal of the Linnean Society*,
959 *104*(2), 480–486. doi: 10.1111/j.1095-8312.2011.01713.x

960 Quinlan, A. R., & Hall, I. M. (2010). BEDTools: A flexible suite of utilities for comparing
961 genomic features. *Bioinformatics*, *26*(6), 841–842. doi: 10.1093/bioinformatics/btq033

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

962 R Core Team. (2020). *R: A language and environment for statistical computing.* R Foundation
963 for Statistical Computing. Vienna, Austria.

964 Renaud, G., Stenzel, U., Maricic, T., Wiebe, V., & Kelso, J. (2015). deML: Robust demulti-
965 plexing of Illumina sequences using a likelihood-based approach. *Bioinformatics (Oxford,*
966 *England)*, *31*(5), 770–772. doi: 10.1093/bioinformatics/btu719

967 Roch, S., & Warnow, T. (2015). On the Robustness to Gene Tree Estimation Error (or lack
968 thereof) of Coalescent-Based Species Tree Methods. *Systematic Biology*, *64*(4), 663–676.
969 doi: 10.1093/sysbio/syv016

970 Salzburger, W. (2018). Understanding explosive diversification through cichlid fish genomics.
971 *Nature Reviews Genetics*, *19*(11), 705–717. doi: 10.1038/s41576-018-0043-9

972 Sanderson, B. J., DiFazio, S. P., Cronk, Q. C. B., Ma, T., & Olson, M. S. (2020). A tar-
973 geted sequence capture array for phylogenetics and population genomics in the Salicaceae.
974 *Applications in Plant Sciences*, *8*(10), e11394. doi: 10.1002/aps3.11394

975 Sass, C., Iles, W. J. D., Barrett, C. F., Smith, S. Y., & Specht, C. D. (2016). Revisiting the
976 Zingiberales: Using multiplexed exon capture to resolve ancient and recent phylogenetic
977 splits in a charismatic plant lineage. *PeerJ*, *4*, e1584. doi: 10.7717/peerj.1584

978 Sayyari, E., & Mirarab, S. (2016). Fast Coalescent-Based Computation of Local Branch
979 Support from Quartet Frequencies. *Molecular Biology and Evolution*, *33*(7), 1654–1668.
980 doi: 10.1093/molbev/msw079

981 Schulte, K., Barfuss, M. H. J., & Zizka, G. (2009). Phylogeny of Bromelioideae (Bromeliaceae)
982 inferred from nuclear and plastid DNA loci reveals the evolution of the tank habit within
983 the subfamily. *Molecular Phylogenetics and Evolution*, *51*(2), 327–339. doi: 10.1016/
984 j.ympev.2009.02.003

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

985 Shee, Z. Q., Frodin, D. G., Cámara-Leret, R., & Pokorny, L. (2020). Reconstructing the Com-
986 plex Evolutionary History of the Papuasian *Schefflera* Radiation Through Herbariomics.
987 *Frontiers in Plant Science*, *11*. doi: 10.3389/fpls.2020.00258

988 Silvera, K., Neubig, K. M., Whitten, W. M., Williams, N. H., Winter, K., & Cushman, J. C.
989 (2010). Evolution along the crassulacean acid metabolism continuum. *Functional Plant*
990 *Biology*, *37*(11), 995–1010. doi: 10.1071/FP10084

991 Slimp, M., Williams, L. D., Hale, H., & Johnson, M. G. (2020). On the potential of
992 Angiosperms353 for population genomics. *bioRxiv*, 2020.10.11.335174. doi: 10.1101/
993 2020.10.11.335174

994 Soltis, P. S., Folk, R. A., & Soltis, D. E. (2019). Darwin review: Angiosperm phylogeny and
995 evolutionary radiations. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1899),
996 20190099. doi: 10.1098/rspb.2019.0099

997 Soltis, P. S., & Soltis, D. E. (2004). The origin and diversification of angiosperms. *American*
998 *Journal of Botany*, *91*(10), 1614–1626. doi: 10.3732/ajb.91.10.1614

999 Soto-Gomez, M. S., Pokorny, L., Kantar, M. B., Forest, F., Leitch, I. J., Gravendeel, B.,
1000 ... Viruel, J. (2019). A customized nuclear target enrichment approach for developing a
1001 phylogenomic baseline for *Dioscorea* yams (Dioscoreaceae). *Applications in Plant Sciences*,
1002 *7*(6), e11254. doi: 10.1002/aps3.11254

1003 Straub, S. C. K., Moore, M. J., Soltis, P. S., Soltis, D. E., Liston, A., & Livshultz, T. (2014).
1004 Phylogenetic signal detection from an ancient rapid radiation: Effects of noise reduction,
1005 long-branch attraction, and model selection in crown clade Apocynaceae. *Molecular Phy-*
1006 *logenetics and Evolution*, *80*, 169–185. doi: 10.1016/j.ympev.2014.07.020

1007 Straub, S. C. K., Parks, M., Weitemier, K., Fishbein, M., Cronn, R. C., & Liston, A. (2012).

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

1008 Navigating the tip of the genomic iceberg: Next-generation sequencing for plant system-
1009 atics. *American Journal of Botany*, *99*(2), 349–364. doi: 10.3732/ajb.1100335

1010 Streicher, J. W., Schulte, J. A., & Wiens, J. J. (2016). How Should Genes and Taxa be
1011 Sampled for Phylogenomic Analyses with Missing Data? An Empirical Study in Iguanian
1012 Lizards. *Systematic Biology*, *65*(1), 128–145. doi: 10.1093/sysbio/syv058

1013 Stroud, J. T., & Losos, J. B. (2020). Bridging the Process-Pattern Divide to Understand the
1014 Origins and Early Stages of Adaptive Radiation: A Review of Approaches With Insights
1015 From Studies of Anolis Lizards. *Journal of Heredity*, *111*(1), 33–42. doi: 10.1093/jhered/
1016 esz055

1017 Supple, M. A., & Shapiro, B. (2018). Conservation of biodiversity in the genomics era.
1018 *Genome Biology*, *19*(1), 131. doi: 10.1186/s13059-018-1520-3

1019 Van de Peer, Y., Mizrachi, E., & Marchal, K. (2017). The evolutionary significance of
1020 polyploidy. *Nature Reviews Genetics*, *18*(7), 411–424. doi: 10.1038/nrg.2017.26

1021 Versieux, L. M., Barbará, T., Wanderley, M. d. G. L., Calvente, A., Fay, M. F., & Lexer, C.
1022 (2012). Molecular phylogenetics of the Brazilian giant bromeliads (*Alcantarea*, Bromeli-
1023 aceae): Implications for morphological evolution and biogeography. *Molecular Phylogenet-*
1024 *ics and Evolution*, *64*(1), 177–189. doi: 10.1016/j.ympev.2012.03.015

1025 Villaverde, T., Pokorny, L., Olsson, S., Rincón-Barrado, M., Johnson, M. G., Gardner, E. M.,
1026 … Sanmartín, I. (2018). Bridging the micro- and macroevolutionary levels in phyloge-
1027 nomics: Hyb-Seq solves relationships from populations to species and above. *New Phytol-*
1028 *ogist*, *220*(2), 636–650. doi: 10.1111/nph.15312

1029 Viruel, J., Conejero, M., Hidalgo, O., Pokorny, L., Powell, R. F., Forest, F., … Leitch, I. J.
1030 (2019). A Target Capture-Based Method to Estimate Ploidy From Herbarium Specimens.
1031 *Frontiers in Plant Science*, *10*. doi: 10.3389/fpls.2019.00937

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

1032 Wai, C. M., VanBuren, R., Zhang, J., Huang, L., Miao, W., Edger, P. P., . . . Ming, R. (2017).
1033 Temporal and spatial transcriptomic and microRNA dynamics of CAM photosynthesis in
1034 pineapple. *The Plant Journal*, *92*(1), 19–30. doi: 10.1111/tpj.13630

1035 Weitemier, K., Straub, S. C. K., Cronn, R. C., Fishbein, M., Schmickl, R., McDonnell, A., &
1036 Liston, A. (2014). Hyb-Seq: Combining target enrichment and genome skimming for plant
1037 phylogenomics. *Applications in Plant Sciences*, *2*(9), 1400042. doi: 10.3732/apps.1400042

1038 Wendel, J. F., & Doyle, J. J. (1998). Phylogenetic Incongruence: Window into Genome
1039 History and Molecular Evolution. In D. E. Soltis, P. S. Soltis, & J. J. Doyle (Eds.),
1040 *Molecular Systematics of Plants II: DNA Sequencing* (pp. 265–296). Boston, MA: Springer
1041 US. doi: 10.1007/978-1-4615-5419-6_10

1042 Wöhrmann, T., Michalak, I., Zizka, G., & Weising, K. (2020). Strong genetic differentiation
1043 among populations of *Fosterella rusbyi* (Bromeliaceae) in Bolivia. *Botanical Journal of*
1044 *the Linnean Society*, *192*(4), 744–759. doi: 10.1093/botlinnean/boz096

1045 Xi, Z., Liu, L., & Davis, C. C. (2016). The Impact of Missing Data on Species Tree Estimation.
1046 *Molecular Biology and Evolution*, *33*(3), 838–860. doi: 10.1093/molbev/msv266

1047 Zhang, C., Rabiee, M., Sayyari, E., & Mirarab, S. (2018). ASTRAL-III: Polynomial time
1048 species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics*, *19*(6),
1049 153. doi: 10.1186/s12859-018-2129-y

1050 Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A
1051 high-performance computing toolset for relatedness and principal component analysis of
1052 SNP data. *Bioinformatics*, *28*(24), 3326–3328. doi: 10.1093/bioinformatics/bts606

1053 Zimmer, E. A., & Wen, J. (2013). Reprint of: Using nuclear gene data for plant phylogenetics:
1054 Progress and prospects. *Molecular Phylogenetics and Evolution*, *66*(2), 539–550. doi:
1055 10.1016/j.ympev.2013.01.005

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

1056 Zink, R. M., & Vázquez-Miranda, H. (2019). Species Limits and Phylogenomic Relationships
1057 of Darwin's Finches Remain Unresolved: Potential Consequences of a Volatile Ecological
1058 Setting. *Systematic Biology*, *68*(2), 347–357. doi: 10.1093/sysbio/syy073

1059
8 Data Accessibility

1060 NCBI

1061 **Table 1** Number and characteristics of the variants obtained for Angiosperms353 and
1062 Bromeliad1776.
1063

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

	indv Nr.	SNP Nr.	site mean depth	SNPs in exonic regions	SNPs in intronic regions	SNPs in intergenic regions	on-target SNPs	flanking SNPs	off-target SNPs
intragenic vcf									
Angiosperm353	70	47,390	3447	40,628 (85.7%)	4,376 (9.2%)	2,386 (5.1%)	8,424 (17.8%)	3,488 (7.4%)	35,478 (74.8%)
Bromeliad1776	72	209,186	6601.7	170,893 (81.7%)	35,790 (17.1%)	2,503 (1.2%)	162,924 (77.9)%	37,661 (18.0%)	8,601 (4.11%)
pop-level vcf									
Angiosperm353	38	15,622	1,837.8	13,345 (85.5%)	1,442 (9.2%)	835 (5.3%)	3,032 (19.4%)	1,129 (7.22%)	11,461 (73.4%)
Bromeliad1776	40	65,473	3914.9	54,636 (83.5%)	9,967 (15.2%)	870 (1.3%)	51,405 (78.5%)	10,588 (16.2%)	3,480 (5.3%)

bioRxiv preprint doi: https://doi.org/10.1101/2021.05.20.444989; this version posted May 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

1064 # 9 Supporting information

1065 ## 9.1 Tables

1066 **Table S1** Genes included in the Bromeliad1776 bait design, with identifiers as annotated
1067 in *Ananas comosus* genome v.3 (Ming et al., 2015). The table includes details about exon
1068 composition, copy number and putatively associated pathways.

1069 **Table S2** Categories of pathways and traits used to choose genes of interest for the Bromeliad1776
1070 bait set, including literature source and number of genes in each category.

1071 **Table S3** List of accessions used in this study. For samples of Tillandsia subgenus Tilland-
1072 sia locality codes are also indicated.

1073 **Table S4** Number of reads, numbers and percentage of read mapping to target in all
1074 samples for both bait sets.

1075 **Table S5** Averaged levels of nucleotide diversity at synonymous (π_S) and non-synonymous
1076 (π_N) for 5 *Tillandsia* subgenus *Tillandsia* species.