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ARTICLE

Geographic structure in two highly diverse lineages of *Tillandsia* (Bromeliaceae)

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Abstract: The Neotropical genus *Tillandsia* (Bromeliaceae) is an excellent model system for macroevolutionary and biogeographic studies owing to its remarkable species diversity (ca. 650 spp.) and varied morphological and ecological adaptations to epiphytic and saxicolous habitats. Recent phylogenetic studies have greatly improved our knowledge about generic limits and infrageneric classification of *Tillandsia*. These studies have identified two clades of *Tillandsia* characterized by a distinct geographic distribution: (*i*) a North and Central American clade that includes species from subgenus *Tillandsia*; and (*ii*) a central South American clade containing species from subgenera *Aerobia*, *Anoplophytum*, *Diaphoranthema*, and *Phytarrhiza*. Our study aimed to determine the size, composition, and potential geographic structure of these two clades within the context of a global phylogeny of Tillandsioideae. With the addition of 100 newly sequenced species to previous studies to cover the now ca. 30% of the known species diversity of *Tillandsia*, our analyses found both clades to be strongly supported, and revealed that their species richness is much greater than previously known. Ancestral area estimation suggests that most of the diversification of the first of these clades took place in North and Central America, whereas within the second, most of the migratory events occurred from the Andes to the Brazilian shield.

Key words: ancestral area reconstruction, *matK–trnK* region, Neotropics, phylogenetics, Tillandsioideae.

Résumé : Le genre néotropical *Tillandsia* (Bromeliaceae) constitue un excellent système modèle à des fins d'études macroévolutives et biogéographiques à cause de sa remarquable diversité (environ 650 espèces) et de ses différentes adaptations morphologiques et écologiques aux habitats épiphytes et saxicoles. Des études phylogénétiques récentes ont grandement amélioré nos connaissances des limites génériques et de la classification infragénérique de *Tillandsia*. Ces études ont permis d'identifier deux clades de *Tillandsia* caractérisés par des distributions géographiques distinctes : (*i*) un clade d'Amérique du Nord et d'Amérique Centrale qui comprend les espèces du sous-genre *Tillandsia* et (*ii*) un clade d'Amérique du Sud qui comprend les espèces des sous-genres *Aerobia, Anoplophytum, Diaphoranthema* et *Phytarrhiza*. L'étude des auteurs visait à déterminer la taille, la composition et la structure géographique potentielle de ces deux clades dans le contexte d'une phylogénie globale des *Tillandsioideae*. Avec l'ajout de 100 nouvelles espèces séquencées à celles d'études précédentes afin de couvrir environ 30 % de la diversité connue d'espèces de *Tillandsia*, les analyses des auteurs ont révélé que les deux clades sont fortement supportés et que leur richesse en espèces est beaucoup plus grande que ce qui était connu jusqu'à présent. L'estimation de la zone ancestrale suggère que la plus grande partie de la diversification du premier de ces clades a pris place en Amérique du Nord et en Amérique Centrale, alors qu'à l'intérieur du second, la plupart des événements migratoires sont survenus des Andes au bouclier brésilien. [Traduit par la Rédaction]

Mots-clés : reconstruction de la zone ancestrale, région *matK–trnK*, Néotropiques, phylogénétique, Tillandsioideae.

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Introduction

Tillandsia L. is one of the largest genera of monocotyledons, including ca. 650 spp. distributed from the southeastern United States and northern Mexico, south through Mesoamerica and the Caribbean region to central Argentina (Gouda et al. continuously updated; Barfuss et al. 2016). Its species exhibit a remarkable diversity of morphological and physiological adaptations to epiphytic and saxicolous habitats such as specialized roots for anchoring to various substrates, reduced stems with leaves in compact rosettes, modified trichomes for water and nutrient uptake, and crassulacean acid metabolism (Crayn et al. 2004; Givnish et al. 2011). Species of *Tillandsia* frequently are important to the ecology of many organisms. Their leaf rosettes often function as humus- or water-gathering organs for the plant, but they are also the source of nutrients, water, and shelter for multiple animal species living in the forest canopy (Benzing 2000). In addition, the nectar produced by their flowers is an important food source for pollinators such as hummingbirds, moths, and bats (Benzing 2000; Aguilar-Rodríguez et al. 2014).

Tillandsia belongs to tribe Tillandsieae (subfamily Tillandsioideae), which now, following the recognition of monophyletic units, includes the genera *Barfussia* Manzan. & W. Till, *Gregbrownia* W. Till & Barfuss, *Guzmania* Ruiz & Pav., *Lemeltonia* Barfuss & W. Till, *Pseudalcantarea* (Mez) Pinzón & Barfuss, *Racinaea* M.A. Spencer & L.B. Sm., *Tillandsia*, and *Wallisia* (Regel) E. Morren (Barfuss et al. 2016). The infrageneric classification of *Tillandsia* recently proposed by Barfuss et al. (2016) recognizes seven subgenera, namely *Aerobia* Mez in C. DC., *Anoplophytum* (Beer) Baker in G. Nicholson, *Diaphoranthema* (Beer) Baker, *Phytarrhiza* (Vis.) Baker, *Pseudovriesea* Barfuss & W. Till, *Tillandsia*, and *Viridantha* (Espejo) W. Till & Barfuss, plus several unclassified species complexes.

Barfuss et al. (2016) analyzed 93 species of *Tillandsia* and DNA sequences of one nuclear and three plastid markers. That study confirmed several strongly supported major clades within tribe Tillandsieae (referred as clades G to R) previously identified by Barfuss et al. (2005). Some of these major clades showed distinct geographic distributions, such as clade K, which is entirely composed of species of subgenera *Tillandsia* distributed in North and Central America. Andean clade Q, which consist of species from subg. *Aerobia*, and southeastern Brazilian clade R, that includes species from subg. *Anoplophytum* s. str., are nested within a more inclusive clade (hereinafter referred to as clade Q-R) which also contains *Tillandsia albertiana* Verv. and *Tillandsia esseriana* Rauh & L.B.Sm., as well as species from subgenera *Diaphoranthema* and *Phytarrhiza* s. str. (Barfuss et al. 2016).

Molecular phylogenetic studies including *Tillandsia* representatives have used plastid markers from coding (i.e., genes *matK*, *ndhF*, *rbcL*, and *ycf1*) and noncoding regions (i.e., *trnK*, *rps16*, and *trnL* introns; *atpB–rbcL*, *psbA–trnH*, *rbcL–accD*, *rpl32–trnL*, *trnD–trnT*, *trnH–psbA*, and *trnL–trnF* intergenic spacers, IGS), as well as nuclear sequences from the ribosomal internal and external transcribed spacers (ITS and ETS, respectively), and the 5.8S gene (Horres et al. 2000; Barfuss et al. 2005; Givnish et al. 2007, 2011; Granados Mendoza 2008; Chew et al. 2010; Donadío 2013; Castello et al. 2016; Pinzón et al. 2016). Among these, the *matK–trnK* region, including the *matK* gene and at least part of the noncoding portions of the *trnK* intron in which it is embedded, has been the most widely used marker in phylogenetic studies of Bromeliaceae (revised by Palma-Silva et al. 2016), for which a number of sequences are currently available in GenBank.

Although the study of Barfuss et al. (2016) included the most comprehensive sampling of *Tillandsia* species so far, the ca. 93 species sampled by them represent only about 14.3% of the species diversity of the genus, and the phylogenetic position of many other species is still unknown. In this study, we assess the phylogenetic position of numerous North, Central, and South American species of *Tillandsia* that have not previously been analyzed in a global phylogenetic context of subfamily Tillandsioideae. We also test whether the geographic structure within *Tillandsia* suggested by Barfuss et al. (2005) still holds true as species sampling increases, and, if so, advance our knowledge about the size and species composition of two particular lineages previously identified by those authors: (*i*) the North and Central American clade K; and (*ii*) the South American clade Q-R. These two lineages represent the extremes of the distribution of *Tillandsia* in the northernmost and southernmost portions of the Neotropical region, and appear to represent two important, independent diversifications corresponding roughly, on the one hand, to subgenus *Tillandsia*, and on the other to subgenera *Aerobia*, *Anoplophytum* s. str., *Phytarrhiza* s. str., and *Diaphoranthema*.

With these goals in mind, we sequenced the *matK–trnK* region of 100 species of *Tillandsia*, which when added to the species sampled by Barfuss et al. (2005, 2016), now represent ca. 30% of the total number of species currently recognized for the genus. As noted in previous studies (Barfuss et al. 2005; Granados Mendoza 2008), this region presents a level of sequence variation suitable for resolving phylogenetic relationships in Bromeliaceae at various taxonomic levels, thus promising to be useful to establish the position of the target *Tillandsia* species within subfamily Tillandsioideae. Both Bayesian inference and maximum likelihood analyses were performed, and the resulting phylogenetic hypotheses served as the basis to discuss relationships at multiple taxonomic levels in subfamily Tillandsioideae, but with emphasis on the K and Q-R clades. To assess formally the aforementioned geographic structure of the target clades, we conducted an ancestral distribution area analysis with the Bayesian Binary MCMC (BBM) method.

Materials and methods

Taxon sampling

One hundred species of *Tillandsia* were newly sequenced for this work, and these were pooled with other sequences of Tillandsioideae available from GenBank. In total, our generic and specific sample was as follows: *Alcantarea* (E. Morren ex Mez) Harms (6 spp.); *Barfussia* (3 spp.); *Catopsis* Griseb. (7 spp.); *Glomeropitcairnia* (Mez) Mez (2 spp.); *Goudaea* W. Till & Barfuss (2 spp.); *Gregbrownia* (2 spp.); *Guzmania* (20 spp.); *Jagrantia* Barfuss & W. Till (1 sp.); *Josemania* W. Till & Barfuss (2 spp.); *Lemeltonia* (3 spp.); *Lutheria* Barfuss & W. Till (2 spp.); *Mezobromelia* L.B. Sm. (3 spp.); *Pseudalcantarea* (3 spp.); *Racinaea* (12 spp.); *Stigmatodon* Leme, G.K. Br. & Barfuss (3 spp.); *Tillandsia* (204 spp.); *Vriesea* Lindl. (24 spp.); *Wallisia* (3 spp.); *Werauhia* J.R. Grant (8 spp.); and *Zizkaea* W. Till & Barfuss (1 sp.). Additionally, the analysis included a representative species for each of the subfamilies Brocchinioideae, Bromelioideae, Hechtioideae, Lindmanioideae, Navioideae, Pitcairnioideae, and Puyoideae. *Brocchinia steyermarkii* L.B. Sm. was used to root the phylogenetic trees based on previous phylogenetic analyses of Bromeliaceae (Givnish et al. 2007, 2011, 2014). Plant material was obtained through several field expeditions in Latin America, as well as from the collection of live plants held at the Centro Universitario de Conservación e Investigación de Bromelias Mexicanas (CUCIBROM). A list of the species analyzed, including voucher information or reference (as applicable) and GenBank accessions, is provided in Supplementary data, Tables S1[1] and S2. Species names follow Gouda et al. (continuously updated) and Barfuss et al. (2016).

Molecular methods

Genomic DNA was extracted from fresh or silica gel-dried tissue with a modification of the CTAB method of Doyle and Doyle (1987) that included an additional treatment with RNAse A (QIAGEN) and proteinase K (Promega). The *matK–trnK* region was amplified using the external primers 19F (Molvray et al. 2000) and trnK 2R (Steele and Vilgalys 1994), and sequenced using the aforementioned external primers plus the internal primers 731F (Molvray et al. 2000) and 1326R (Cuénoud et al. 2002). In some cases a new internal primer designed by us (matK–tillF: 5′-AAATCTTGGTTCAAATCCTTCAAT-3′) was used for sequencing. PCR reactions (15 μL) were performed with the commercial mix '*Taq* PCR Core Kit' (QIAGEN) and included 1.5 μL (1×) of 10× PCR buffer, 0.6 μL of 0.4% aqueous solution of bovine serum albumin (BSA), 0.3 μL (200 μmol/L) of dNTP mix (10 mmol/L each), 0.15 μL of each primer (10 pmol/μL), 0.3 μL (500 μmol/L) of 25 mmol/L $MgCl^2$, 0.3 μL of 4% *v*/*v* dimethyl sulfoxide (DMSO), 0.075 μL (0.375 units) of *Taq* DNA polymerase, and 0.1–1 μL of template DNA. The PCR program consisted of an initial denaturation at 94 °C for 2.5 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 45 s, and extension at 72 °C for 2.5 min. A final extension at 72 °C for 7 min was applied. PCR products were run in 1% agarose gels and sequencing was performed at the Laboratorio de Biología Molecular de la Biodiversidad y de la Salud, Instituto de Biología, Universidad Nacional Autónoma de México, and the Canadian Museum of Nature.

Sequence edition and alignment

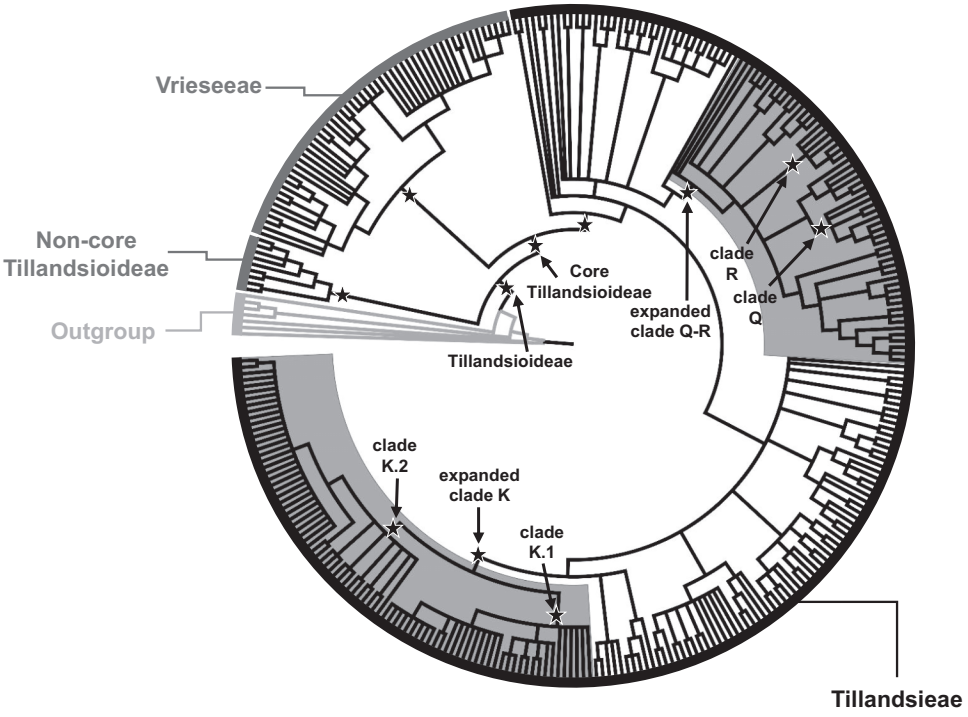
Sequences were edited and assembled with the program Sequencher version 4.10 (Gene Codes Corp., Ann Arbor, Michigan, USA). Alignment was first performed with default settings of the online version of the program MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/; Katoh and Toh 2008) and subsequently manually adjusted and checked for a correct reading frame of gene *matK* in PhyDE (Müller et al. 2005).

Phylogeny estimation

We conducted Bayesian inference (BI) and maximum likelihood (ML) analyses of the *matK–trnK* region with the programs MrBayes version 3.2.6 (Ronquist et al. 2012) and RAxML-HPC version 8.2.9 (Stamatakis 2014), respectively, on XSEDE in the CIPRES Science Gateway (Miller et al. 2010). PartitionFinder version 1.1.1 (Lanfear et al. 2012) was used to analyze three given partitions: two corresponding each to the 5′ and 3′ amplified non-coding portions of the *trnK* intron, and the other to the *matK* gene, to estimate the best fitting model to identified partitions subsets. The General Time Reversible plus Gamma (GTR+G) model was found to best fit the partition subset including both amplified non-coding portions of the *trnK* intron, whereas the same model, but with proportion of invariable sites (GTR+G+I), was found to best fit the partition subset corresponding to the *matK* gene. This partition scheme and best-fitting models were implemented in the BI analysis, which consisted of two independent and simultaneous runs of 10 million generations. These runs included four chains, each with a different random starting tree and sampled every 200 generations. Burn-in was set at 10% of the samples after runs were examined using Tracer version v1.6.0 (Rambaut and Drummond 2007) and a 50% majority rule consensus tree, as well as posterior probabilities (PP) of nodes were calculated from the remaining trees. As recommended in the RAxML manual, for ML tree inference the GTR+G model was applied to both data partition subsets, using the "rapid bootstrapping and search for the best-scoring ML tree" algorithm (Stamatakis et al. 2008), with 1000 bootstrap replicates under GTRCAT. The resulting tree was compiled and edited in FigTree version 1.4.0 (Rambaut 2009).

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjb-2016-0250.

Fig. 1. Bayesian 50% majority rule consensus tree resulting from the analysis of the plastid *matK–trnK* region, summarizing phylogenetic relationships of 314 representatives of Tillandsioideae and seven outgroup species. Black stars denote strongly supported (PP ≥ 0.85) main clades discussed in the text.



Ancestral area estimation

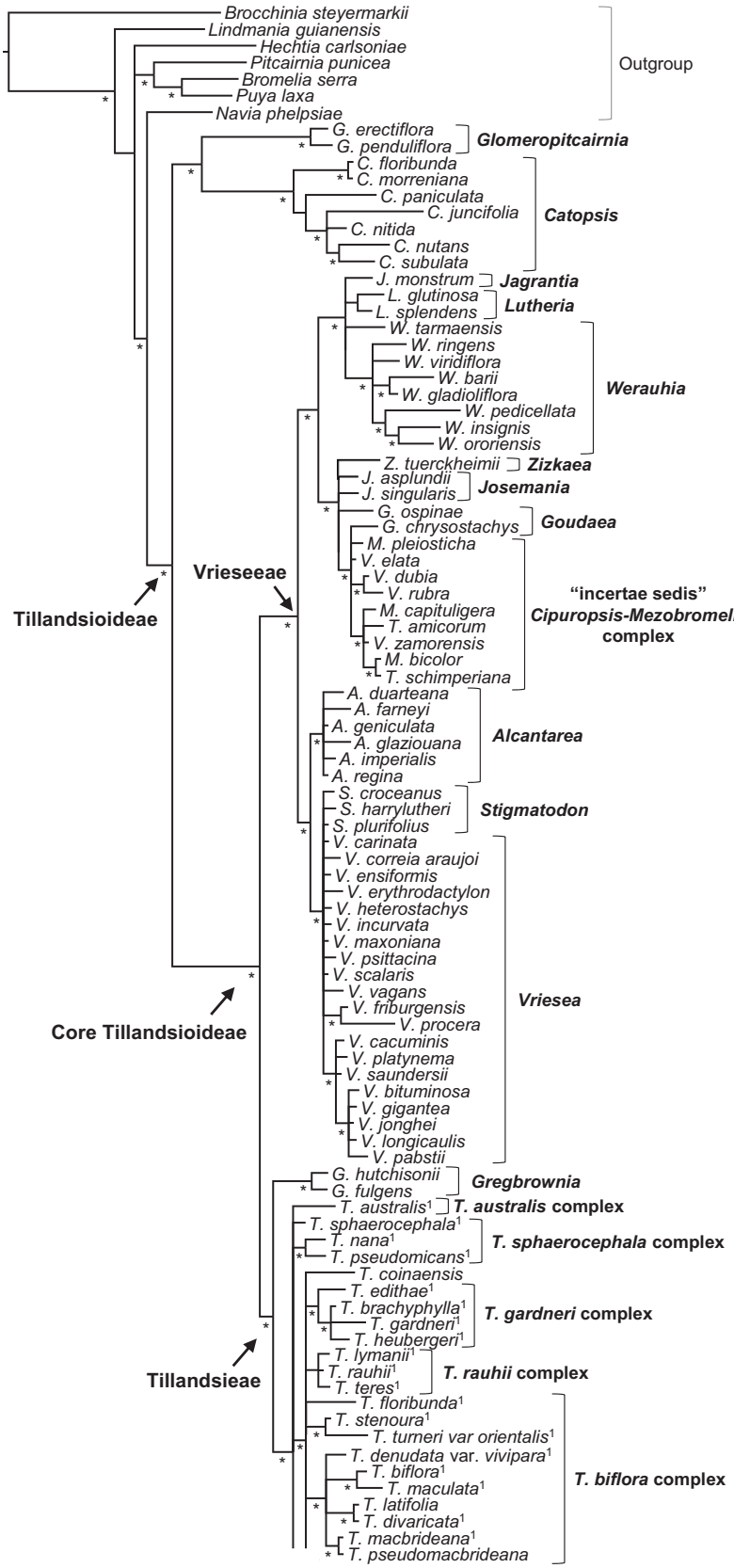
Estimation of ancestral areas of geographic distribution was performed with the program RASP version 3.2 (Yu et al. 2015), using the BI 50% majority rule consensus tree and including only members of Tillandsioideae. The BBM method for ancestral states reconstruction was applied because it accepts trees with local polytomies, which was the case for our reference tree. The BBM analysis consisted of 10 chains of 10 000 000 generations each, sampling one step every 2000 generations. Burn-in was set to 1000 of the sampled steps. A model equivalent to Jukes–Cantor was applied for reconstructing ancestral area states of given nodes and among-site rate variation was set to "equal". The distribution areas considered in the analysis correspond to the areas of endemism for Bromeliaceae used by Givnish et al. (2007, 2011) and included: (A) Guiana Shield; (B) Brazilian Shield; (C) Amazonia; (D) Andes and southern Chile (hereafter Andes-S Chile); (E) northern South America and the Caribbean; and (F) North and Central America. Information on the distribution of species was obtained from Smith and Downs (1974, 1977, 1979); McVaugh and Anderson (1989); Lloyd (1991); Utley and Burt-Utley (1994); Espejo-Serna et al. (2004, 2005, 2007, 2010); Ramirez-Morillo et al. (2004); Figueroa-Brito and Guzmán-Rivera (2005); Granados Mendoza (2005); Terreros Olivares (2012); Diego-Escobar et al. (2013); González Rocha (2014); Donadío et al. (2015); and Granados Mendoza et al. (2016). Species distributed in five or more individual areas (i.e., *Racinaea spiculosa* (Griseb.) M.A. Spencer & L.B. Sm., *Tillandsia bulbosa* Hook., *Tillandsia complanata* Benth., *Tillandsia polystachia* (L.) L., and *Tillandsia recurvata* (L.) L.) were excluded from the BBM analysis to keep a maximum number of four coded areas per species, to avoid reconstructing an excessive number of possible ancestral areas.

Results

Phylogenetic analysis

The data matrix consisted of 321 terminals and 1796 aligned characters, of which 224 correspond to the 5′ and 3′ noncoding portions of the *trnK* intron and 1572 to the *matK* gene. Overall, the trees resulting from our BI and ML analyses are highly similar, well-resolved, and include many strongly supported phylogenetic clades at deep to medium phylogenetic levels of the monophyletic subfamily Tillandsioideae (PP 1; bootstrap support, BS 87%; Figs. 1 and 2; Supplementary data, Fig. S1). Two main clades were retrieved and highly supported within Tillandsioideae corresponding to the non-core (PP 1; BS 98%) and core Tillandsioideae (PP 1; BS 100%) clades of Barfuss et al. (2016), respectively. Non-core Tillandsioideae genera *Catopsis* and *Glomeropitcainia*, and therefore, tribes Catopsideae and Glomeropitcairnieae, were herein highly supported as monophyletic (PP 1, BS 100%; PP 1, BS 100%, respectively). Within core Tillandsioideae, tribes Tillandsieae and Vrieseeae were also found as monophyletic (PP 0.94, BS 77%; PP 1, BS 100%, respectively). Despite the low resolution of the backbone and some internal relationships within these two tribes, several genera, subgenera, and species complexes

Fig. 2. Detail of the phylogenetic relationships obtained from the analysis in Fig. 1. Brackets indicate main clades, tribes, genera and species complexes recognized for Tillandsioideae by Barfuss et al. (2016); [1], denotes species analyzed in their revision; *, denotes strongly supported clades (PP ≥ 0.85).



previously identified by Barfuss et al. (2016), of which more than one representative were included in our present study, are confirmed as monophyletic. Monophyly of genera *Alcantarea* (PP 0.97; BS 68%; tribe Vrieseeae), *Gregbrownia* (PP 1; BS 98%), *Barfussia* (PP 1; BS 83%), *Pseudalcantarea* (PP 1; BS 94%), *Wallisia* (PP 1; BS 88%), and *Guzmania* (PP 1; BS 85%) received high to moderate support, as well as that of the *Tillandsia* subgenera *Viridantha* (PP 1; BS 72%), *Pseudovriesea* (PP 0.99; BS 68%), and *Tillandsia* (PP 1; BS 85%), and the species complexes of *Tillandsia gardneri* Lindl. (PP 0.97; BS 85%) and *Tillandsia purpurea* Ruiz & Pav. (PP 1; BS 99%; tribe Tillandsieae).

In our analysis, all *Tillandsia* representatives found by Barfuss et al. (2016) in their clades K and Q-R are nested within two more inclusive clades with strong to moderate support. These clades are herein referred to as "expanded clade K" (PP 1; BS 100%; Figs. 1, 3, and 5) and "expanded clade Q-R" (PP 1; BS 56%; Figs. 1, 3, and 4). Expanded clade K is entirely composed of species belonging to *Tillandsia* subg. *Tillandsia* (82 spp.). Two clades herein referred to as K.1 (PP 0.98; BS 51%) and K.2 (PP 0.85; BS 46%) were found within expanded clade K (Fig. 5).

Within the expanded clade Q-R (55 spp.), *T. albertiana* and *T. peiranoi* A. Cast. form a polytomy with a clade containing three highly supported subclades: (1) *T.* subg. *Anoplophytum* s. str. (clade R; 7 spp.; PP 1; BS 91%); (2) *T.* subg. *Phytarrhiza* s. str. (5 spp.; PP 1; BS 85%); and (3) *T.* subg. *Aerobia* (clade Q; 9 spp., including *T. argentina* C.H.Wright; PP 1; BS 95%). Additionally, over 20 spp. assigned in previous studies to *T.* subg. *Diaphoranthema*, another 11 spp. not classified yet at subgeneric level, and two spp. (*T. bandensis* Baker and *T. crocata* [E.Morren] Baker) previously classified under subg. *Phytarrhiza* (Smith and Downs 1977) were also placed in the expanded clade Q-R. However, relationships among the latter species were poorly resolved. (Fig. 4).

Biogeographic analyses

Our analysis estimated North and Central America (0.94) as the most probable ancestral area for the node subtending the expanded clade K. Clades K.1 and K.2 also showed high probabilities for North and Central America as their ancestral area (0.99 for both). Additionally, two independent migrations to the northern Neotropics (North and Central America) were detected (Fig. 6). The first of them is represented by the genus *Pseudalcantarea* with a probability of 0.95. The second corresponds to *T.* subg. *Viridantha* (0.92). For the node subtending the expanded clade Q-R, Andes-S Chile was identified as the most probable ancestral area (0.98). The Brazilian Shield plus Andes-S Chile were inferred as the most probable ancestral area for clade Q (0.83). For the individual clade R, the Brazilian Shield plus Andes-S Chile was also estimated as the joint ancestral area with highest probability (0.54), and with less probability, the Brazilian Shield (0.34; Fig. 6). Other areas or sets of areas ancestral for the

Fig. 3. Continuation of Fig. 2.

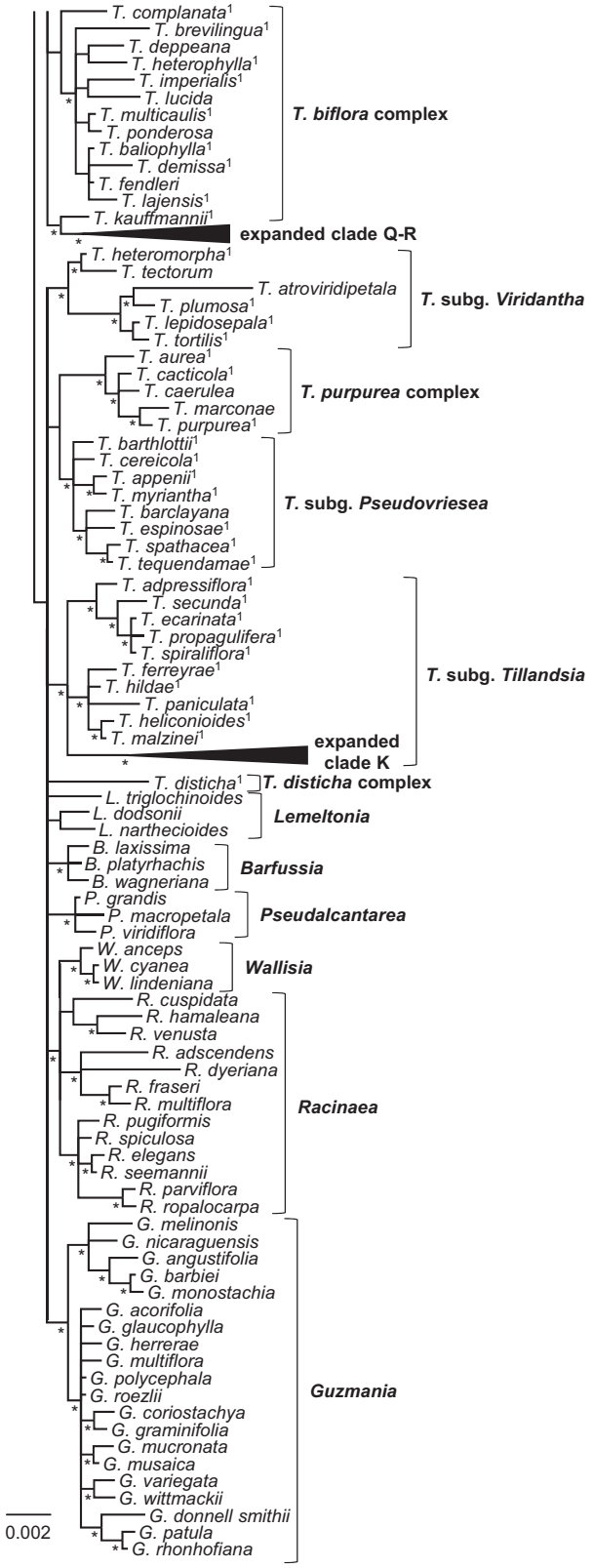
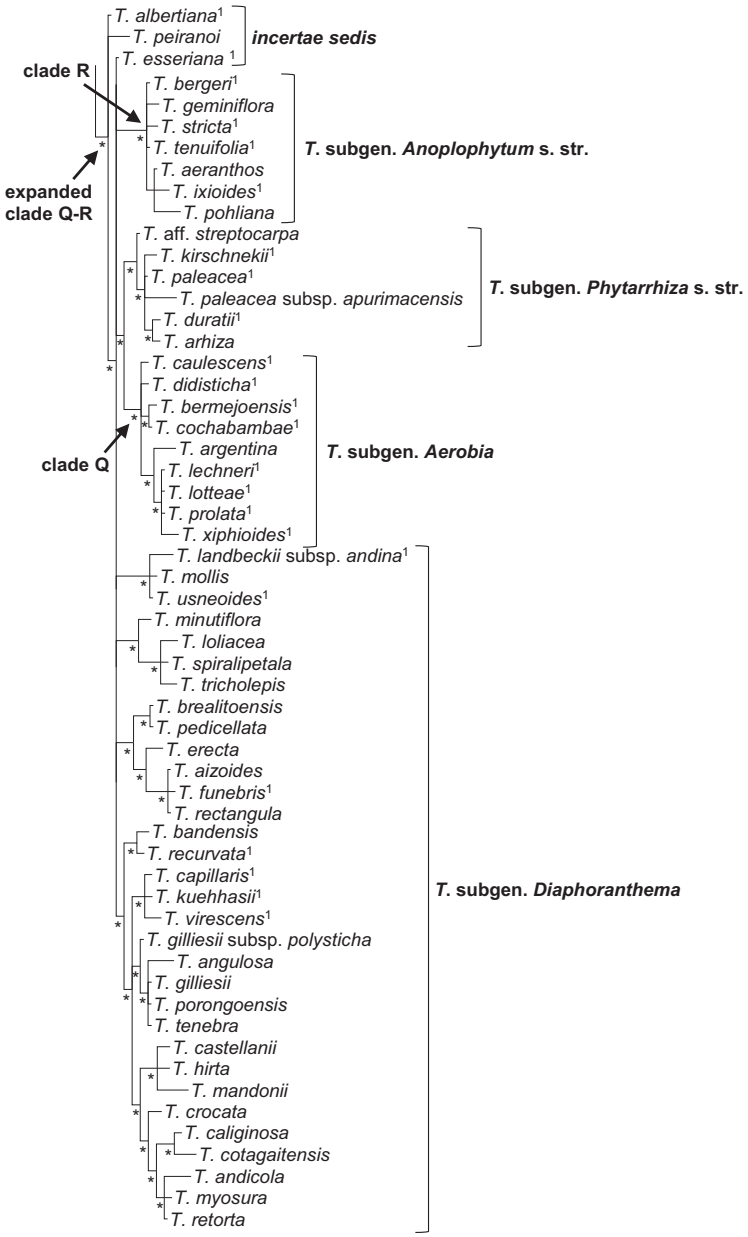


Fig. 4. Continuation of Fig. 3.



aforementioned nodes were estimated with lower probabilities.

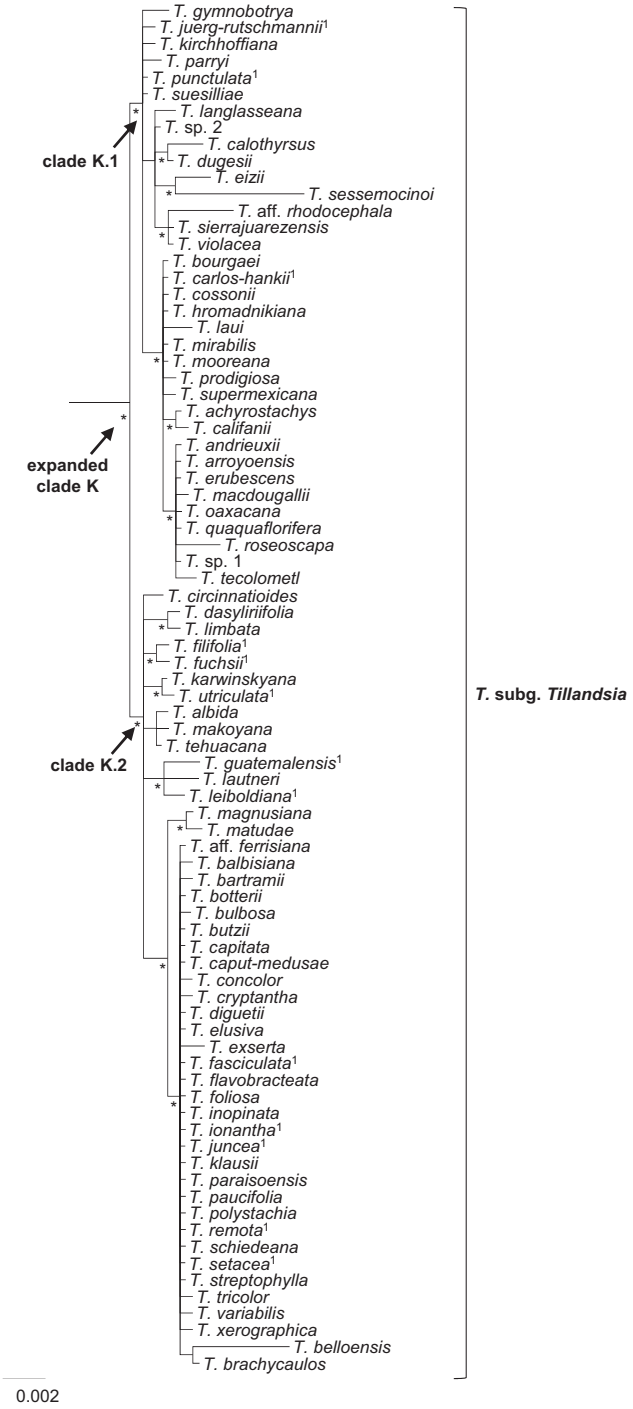
Discussion

Phylogenetic relationships and performance of the *matK–trnK* region

In our analyses, the phylogenetic utility of the *matK–trnK* region is higher at medium to deep taxonomic levels of Tillandsioideae. In general, our results are in agreement with those of Barfuss et al. (2016). All main clades, tribes, genera, and species complexes found by those authors within Tillandsioideae were herein confirmed as monophyletic, with the exception of some genera (Vrieseeae: *Josemania*, *Werauhia*, *Goudaea*, *Lutheria*, *Stigmatodon*, and *Vriesea*; Tillandsieae: *Racinaea*, *Tillandsia*, and *Lemeltonia*) and species complexes (*T. sphaerocephala*, *T. rauhii*, and *T. biflora*) for which the resolution provided solely by the *matK–trnK* region was limited compared with that of the four-locus phylogeny of Barfuss et al. (2016).

Shallower phylogenetic relationships generally lack strong support, and branches are extremely short, particularly within the expanded clade K. In the expanded clade Q-R several subclades were retrieved with strong bootstrap support. However, the "backbone" of this clade remains largely unresolved. Barfuss et al. (2005) suggested that *Tillandsia* is a phylogenetically young lineage, based on the low genetic divergence present among its members. Recently dated phylogenies of Bromeliaceae place the crown age of Tillandsioideae (15.2 ± 0.42 Mya) and "core tillandsioids" (9.6 ± 0.67 Mya) in the middle or late Miocene (Givnish et al. 2014), suggesting a rather

Fig. 5. Continuation of Fig. 4.



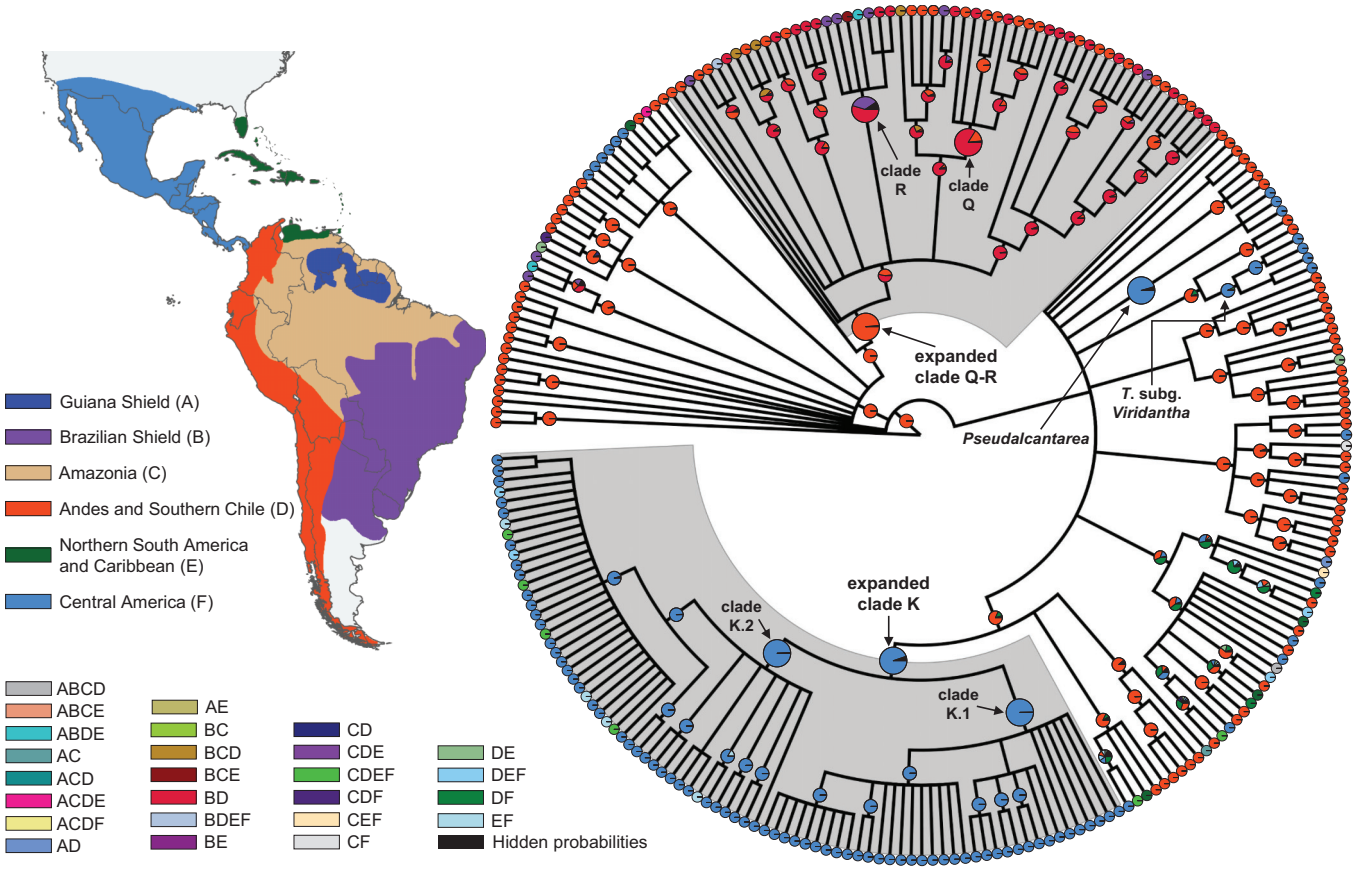
recent diversification of these lineages during which few molecular changes might have accumulated.

Size, composition, and geographic structure of expanded clades K and Q-R

Relative to the sample analyzed by Barfuss et al. (2016), we increased the known diversity of clade K from 13 to 82 species, and that of clade Q-R from 25 to 50 species. All species within the expanded clade K belong to subg. *Tillandsia,* corresponding roughly to 30% of the currently known diversity of this subgenus (Barfuss et al. 2016). Of the species composing the expanded clade Q-R, Barfuss et al. (2016) maintained *T. albertiana* and *T. esseriana* as *incetae sedis*. We found *T. albertiana* and *T. peiranoi* in polytomy with a clade where *T. esseriana* also forms a polytomy with several small clades with species previously assigned, or assignable (see discussion below), to *T.* subg. *Diaphoranthema*, *T.* subg. *Anoplophytum* s. str., *T.* subg. *Phytarrhiza* s. str., and *T.* subg. *Aerobia*. Therefore, *T. peiranoi* is herein also considered as *incertae sedis*. Several species described after Smith and Downs (1977) and not explicitly treated by Barfuss et al. (2016) were here found nested within several small and highly supported clades also containing species assigned in these two previous studies to *T.* subg. *Diaphoranthema*; we therefore considered these species to belong to that subgenus. It is worth noticing that *T. bandensis* and *T. crocata*, two species considered by Smith and Downs (1977) to belong to subg. *Phytarrhiza*, were found here nested in two clades composed by species currently classified under subg. *Diaphorenthema* and not within *T.* subg. *Phytarrhiza* s. str.

Givnish et al. (2011) proposed that Bromeliaceae originated ca. 100 Mya in the Guiana Shield, spreading from there during the middle of the Miocene (16–13 Mya). Furthermore, Barfuss et al. (2005) suggested that migration occurred towards the Caribbean region (*Catopsis* and *Glomeropitcairnia*), eastern Brazil (*Vriesea*, *Alcantarea* and *Werauhia*), and the Andes (*Guzmania*, *Mezobromelia*, *Racinaea*, and *Tillandsia*). Subgenus *Tillandsia* migrated northwards to North and Central America, whereas subgenera *Allardtia* and *Diaphoranthema* migrated southwards through the Andes up to Chile and Argentina, respectively (Barfuss et al. 2005). Our biogeographic analysis corroborates the interpretation of Barfuss et al. (2005) of Mexico and Central America as a center of diversity for subg. *Tillandsia*. North and Central America were identified in our analysis as the most probable ancestral area for the node subtending the expanded clade K. The fact that most of its extant diversity occurs in that area (including Mexico) suggests that its diversification occurred in this region. The remarkable environmental heterogeneity of the mountain tropical forests of North and Central America, coupled with the complex climatic history of this region during the late Miocene and Pliocene, which included increased seasonality and aridity induced by a global cooling trend (Antonelli and Sanmartín 2011; Graham 2011*a*, 2011*b*), could have promoted high diversification rates of subg. *Tillandsia*, resulting in the current high species richness contained within the expanded clade K. Our analysis suggests that diversification of clade K.1 took place entirely in North and Central America. In clade K.2 only one lineage showed a low probability (0.16) to have expanded its distribution to northern South America and the Caribbean. Owing to the current lack of resolution within clade K.2 (i.e., short branches and low BS support), the direction of other potential area shifts are currently unclear and will

Fig. 6. Phylogenetic tree summarizing relationships within tribe Tillandsieae. Pie charts indicate ancestral area reconstruction probabilities for highly supported (PP ≥ 0.85) internal nodes. Clade names correspond to those discussed in the text. Source of Map: John Harvey, Wikimedia Commons (https://upload.wikimedia.org/wikipedia/commons/e/e8/BlankMap-World6-Equirectangular.svg) original text: {PD-USGov-CIA-WF}. Creative Commons License (CC0 1.0).



need to be assessed under a better resolved and supported phylogenetic framework. Aside these local radiations, diversity of core tillandsioids in the northern Neotropics was apparently enhanced further by two other independent and asynchronous migrations (i.e., *Pseudalcantarea* and *T.* subg. *Viridantha*), probably both from the Andes-S Chile area, but further resolution is required to confirm this.

Contrary to an exclusively Andean area of diversification for clade Q proposed by Barfuss et al. (2005), our analysis found the Brazilian Shield plus Andes-S Chile as the most probable joint ancestral area. Like for clade Q, the Brazilian Shield plus Andes-S Chile was estimated as the most probable joint ancestral area for clade R; however, this probability is rather low (0.54) and close to that of the Brazilian Shield alone (0.34). For the expanded Q-R clade, the Andes-S Chile is estimated with high probability (0.98%) as its ancestral area, suggesting that migration within that clade could have occurred mainly from the Andes-S Chile area towards the Brazilian shield.

Our study includes a substantially enlarged taxon sampling of tribe Tillandsieae with respect to previous molecular studies, in particular for the genus *Tillandsia*. However, to achieve enhanced phylogenetic resolution, future studies should continue Barfuss et al.'s (2016) efforts for increasing character sampling, especially of potentially more informative markers such as low- or single-copy nuclear genes. A better resolved and supported phylogenetic framework will serve as the basis for robust reconstructions of the biogeographic history and for studying the morphological and ecological evolution underlying the remarkable diversification of the Tillandsioideae.

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