UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

SISTEMÁTICA

"FILOGENÓMICA Y BIOGEOGRAFÍA HISTÓRICA DE *TILLANDSIA* SUBGÉNERO *TILLANDSIA* (BROMELIACEAE, POALES)"

TESIS

(**POR ARTÍCULO CIENTÍFICO**)

Plastome phylogenomics reveals an early Pliocene North- and Central America colonization by long-distance dispersal from South America of a highly diverse bromeliad lineage

QUE PARA OPTAR POR EL GRADO DE:

MAESTRA EN CIENCIAS BIOLÓGICAS

PRESENTA:

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CIUDAD UNIVERSITARIA, CD. MX. AGOSTO, 2023

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ASUNTO: Oficio de Jurado

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P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el 8 de mayo del 2023, se aprobó el siguiente jurado para el examen de grado de **MAESTRA EN CIENCIAS BIOLÓGICAS** en el campo de conocimiento de **SISTEMÁTICA** de la alumna **VERA PAZ SANDRA ITZEL** con número de cuenta **311298116** por la modalidad de graduación de **tesis por artículo científico** titulado: **"Plastome phylogenomics reveals an early Pliocene North- and Central America colonization by long-distance dispersal from South America of a highly diverse bromeliad lineage",** que es producto del proyecto realizado en la maestría que lleva por título: **"Filogenómica y biogeografía histórica de *Tillandsia* subgénero *Tillandsia* (Bromeliaceae, Poales)",** ambos realizados bajo la dirección de la **DRA. CAROLINA GRANADOS MENDOZA,** quedando integrado de la siguiente manera:

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Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 01 de junio de 2023

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RESUMEN

Las bromelias son conocidas por haber experimentado una de las radiaciones adaptativas más notables en plantas, sin embargo, el estudio de la evolución en tiempo y espacio de algunos de sus linajes más diversos ha sido obstaculizado por la falta de contextos filogenéticos fechados, resueltos y con alto apoyo. Se estimó el marco espacial y temporal del el origen e historia de dispersión del clado expandido K, un linaje de *Tillandsia* subgen. *Tillandsia* (Bromeliaceae, Poales) altamente diverso que se hipotetiza experimentó una radiación rápida a través del Neotrópico. Para ello, se secuenciaron y ensamblaron 162 plastomas completos nuevos a partir de datos de Hyb-Seq para un muestreo denso del grupo focal, así como para una selección de grupos externos, y se analizaron junto con 38 plastomas adicionales de GenBank para estimar un contexto filogenético fechado. Dicha filogenia fechada fue utilizada para realizar pruebas formales de modelos biogeográficos y reconstrucciones de áreas ancestrales basadas en una compilación significativa de información geográfica. Se encontró que este linaje altamente diverso de *Tillandsia* colonizó Norte- y Centroamérica por la dispersión a larga distancia desde Sudamérica hace ca. 4.86 m.a. y se trazaron varios eventos de dispersión subsecuentes hacia la región Neártica, el Caribe y el dominio Pacífico (desde 2.8 m.a.). El muestreo taxonómico aquí empleado nos permitió calibrar por primera vez varios nodos, no solo dentro del grupo focal, sino también en otros linajes de bromelias. Se espera que este contexto filogenético fechado pueda facilitar futuros estudios macroevolutivos y provea estimaciones de edades de referencia para realizar calibraciones secundarias en otros linajes cercanamente relacionados.

ABSTRACT

Bromeliads are known to have undergone one of the most remarkable plant adaptive radiations, however, the study of the evolution in time and space of some of its more highly diverse lineages has been hindered by the lack of dated, resolved, and strongly supported phylogenetic frameworks. The spatial and temporal frameworks were assessed for the origin and dispersal history of the expanded clade K, a highly diverse *Tillandsia* subgenus *Tillandsia* (Bromeliaceae, Poales) lineage hypothesized to have undergone a rapid radiation across the Neotropics. For this, 162 complete plastomes were newly sequenced and assembled from Hyb-Seq data for a dense taxon sampling of the focal group, plus a careful selection of outgroup species, and analyzed along with 38 additional plastomes from GenBank to estimate a time calibrated phylogenetic framework. This dated phylogenetic hypothesis was then used to perform formal biogeographic model tests and ancestral area reconstructions based on a comprehensive compilation of geographic information. This highly diverse *Tillandsia* lineage colonized the North-and Central America by long-distance dispersal from South America ca. 4.86 Mya and traced several subsequent dispersal events to the southern Nearctic region, the Caribbean, and the Pacific dominion (from 2.8 Mya). The herein employed taxon sampling allowed us to calibrate for the first time several nodes, not only within the focal group, but also in other bromeliad lineages. It is expected that this dated phylogenetic framework will facilitate future macroevolutionary studies and provide reference age estimates to perform secondary calibrations for other closely related lineages.

INTRODUCCIÓN

En linajes de diversificación rápida es común obtener baja resolución filogenética con secuenciación tradicional (i.e. Sanger). El uso de estrategias de Secuenciación de Nueva Generación (NGS) ha permitido incrementar la resolución filogenética de eventos de divergencia en sucesión rápida debido a que su aplicación resulta en la generación de una gran cantidad de datos moleculares. Un método de NGS ampliamente usado en la actualidad es el protocolo Hyb-Seq que combina enriquecimiento híbrido, una estrategia de partición de genoma, con secuenciación somera (*genome skimming*). Dicha estrategia permite recuperar datos de origen nuclear, heredados biparentalmente, y de organelos (mitocondria y cloroplasto), heredados uniparentalmente (Dodsworth et al., 2019; Weitemier et al., 2014). Una estrategia de partición del genoma es el Enriquecimiento Híbrido Anclado (AHE), el cual se basa en la captura por sondas de loci nucleares objetivo altamente conservadas, las cuales están flanqueadas por regiones más variables (Lemmon et al., 2012). El AHE fue originalmente diseñado en animales (Lemmon et al., 2012) y, posteriormente, nuevas sondas de captura fueron diseñadas para angiospermas (Buddenhagen et al., 2016). Este kit "universal" de angiospermas ha permitido generar una gran cantidad de datos moleculares y resolver relaciones filogenéticas profundas a someras en diferentes linajes de plantas con flores (Fragoso-Martínez et al., 2017; Granados Mendoza et al., 2020; Léveillé-Bourret et al., 2018; Mitchell et al., 2017; Wanke et al., 2017). Estudios recientes que han combinado Hyb-Seq con AHE lograron recuperar plastomas completos, de las porciones de datos no objetivo y de secuenciación somera, cuyo análisis resultó en hipótesis filogenéticas con alto soporte estadístico en variedad de profundidades evolutivas (Vera-Paz et al., 2022).

Las hipótesis filogenéticas resueltas en combinación con información sobre la distribución geográfica de las especies permiten modelar la historia biogeográfica de los linajes. Actualmente, se encuentran disponibles una gran cantidad de bases de datos geográficas de libre acceso como el Sistema Global de Información sobre Biodiversidad (GBIF, por sus siglas en inglés; https://www.gbif.org/es/) y Plantas del Mundo en línea (POWO, por sus siglas en inglés; https://powo.science.kew.org/). Para asegurar la incorporación de información de alta calidad en los modelos biogeográficos, los registros disponibles en dichas bases de datos deben ser curados y filtrados para eliminar información mal georreferenciada (e.g. coordenadas en el mar) o con identificaciones taxonómicas erróneas, por mencionar algunos ejemplos. Para ello se han desarrollado paqueterías como CoordinateCleaner (Zizka et al., 2020) que permiten curar las bases de datos de manera automática y aplicaciones como ArcGis (https://www.arcgis.com/index.html) que permiten visualizar los registros geográficos sobre un mapa base. Adicionalmente, es importante incorporar el conocimiento de expertos en los grupos de estudio, que puedan proveer identificaciones taxonómicas certeras.

La familia de la piña (Bromeliaceae) constituye una de las radiaciones adaptativas más importantes del Neotrópico (Givnish et al., 2014) e incluye ca. de 3,742 spp. (Gouda et al., continuamente actualizado; acceso: 15-04-2023) divididas en ocho subfamilias (Brocchinioideae, Bromelioideae, Hechtioideae, Navioideae, Lindmanioideae, Pitcairnioideae, Puyoideae y Tillandsioideae; Givnish et al., 2007). La subfamilia Tillandsioideae es la más diversa, con ca. 1,549 spp. (Gouda & Butcher, continuamente actualizado; acceso: 15-04-2023) actualmente clasificadas en cuatro tribus (i.e. Catopsideae, Glomeropitcairnieae, Tillandsieae y Vrieseseeae) y 21 géneros (Barfuss et al. 2016). *Tillandsia* (tribu Tillandsieae) es el género más rico en especies de Tillandsioideae (780 spp.; Gouda et al., continuamente actualizado; acceso: 15-04-2023) y está clasificado en siete subgéneros: *T.* subgen. *Aerobia* (50 spp.), *T.* subgen. *Anoplophytum* (33 spp.), *T.* subgen. *Diaphoranthema* (30 spp.), *T.* subgen. *Phytarrhiza* (7 spp.), *T.* subgen. *Pseudovriesea* (49 spp.), *T.* subgen. *Viridantha* (23) y *T.* subgen. *Tillandsia* (ca. 270 spp.; Barfuss et al., 2016).

En su estudio de la subfamilia Tillandsioideae Barfuss et al. (2005) analizaron los genes plastídicos codificantes para proteína *rbc*L y *mat*K, los intrones de los genes codificantes para proteína *trn*K, *rps*16 y *trn*L y los espaciadores intergénicos *trn*L-*trn*F y *atp*B-*rbc*L. Dichos autores recuperaron un linaje al cual denominaron clado K compuesto por 11 spp., distribuidas en México, Centroamérica, el Caribe y Sudamérica, pertenecientes a *T.* subgen. *Tillandsia*, así como al entonces reconocido *T.* subgen. *Allardtia*. En una revisión posterior de Tillandsioideae, Barfuss et al. (2016) ampliaron su muestreo taxonómico y analizaron los marcadores de cloroplasto *rpo*B-*trn*C-*pet*N, *trn*K-*mat*K-*trn*K y *ycf*1, así como el gen nuclear *phy*C, recuperado tres linajes al interior de *T.* subgen. *Tillandsia*. El primer linaje está compuesto por *T. hildae*, *T. ferreyrae*, *T. heliconioides*, *T. malzinei* y *T. paniculata* distribuidas en México, Centroamérica y Sudamérica, en adelante referido como "clado de *T. paniculata*". El segundo linaje está integrado por *T. propagulifera*, *T. spiraliflora*, *T. ecarinata*, *T. secunda* y *T. adpressiflora* distribuidas en Sudamérica, en lo subsecuente referido como "clado de *T. secunda*". El tercer linaje corresponde al clado K y es el grupo focal del presente estudio. En 2017 Granados Mendoza et al. utilizaron la región del cloroplasto *mat*K-*trn*K e incrementaron considerablemente el muestreo de especies de *Tillandsia* distribuidas en México y Centroamérica. Estos autores encontraron que el clado K es considerablemente más diverso con al menos 85 spp., lo renombraron a clado expandido K e identificaron dos clados internos con alto apoyo estadístico nombrados K.1 y K.2. Como fue definido por Granados Mendoza et al. (2017) las especies del clado expandido K están distribuidas principalmente en México y Centroamérica, aunque también se encuentran presentes en el Sur de Estados Unidos, el Caribe y Sudamérica.

Las especies del clado expandido K se distribuyen desde el nivel del mar hasta los 3,000 msnm (Espejo-Serna et al., 2004; Gouda et al., continuamente actualizado; acceso: 15-04-2023) y están presentes en diversos tipos de vegetación, incluidos el bosque mesófilo de montaña, los bosques de pino y/o encino, la selva alta perennifolia, la selva baja caducifolia, el chaparral, el matorral xerófilo, el pastizal y la sabana, aunque también suelen encontrarse en vegetación secundaria y zonas urbanas (CONABIO, 2020).

Las especies de este linaje presentan un gradiente morfológico que va desde rosetas del tipo atmosférico, de tamaño pequeño y con hojas densamente cubiertas por tricomas que acumulan detritos, hasta rosetas mesofíticas, que forman grandes tanques capaces de almacenar agua y detritos (Fitotelmatas; Benzing et al., 2000; Reyes-García et al., 2022). Estas especies presentan una serie de adaptaciones morfo-fisiológicas a los hábitos epífitos y epilíticos que les permiten habitar una amplia gama de ambientes xéricos a húmedos (Givnish et al., 2014), como tricomas especializados para la absorción de agua y nutrientes, raíces especializadas en el anclaje (Iii y Martin, 1986) y rutas fotosintéticas C3 y Metabolismo Ácido de las Crasuláceas (CAM, por sus siglas en inglés; Crayn et al., 2015).

La polinización en las especies del clado expandido K se lleva a cabo principalmente por colibríes (Benzing et al., 2000). Estas especies además presentan diversas interacciones ecológicas debido que pueden formar fitotelmatas, donde se alojan diversos animales que se alimentan de la materia orgánica disponible y que pueden pasar parte o su ciclo de vida completo (p. ej. Lounibos y Frank, 2009; Palacios-Vargas y Castaño-Meneses, 2002).

En la actualidad solo existen dos trabajos con muestreos taxonómicos amplios y representativos del clado expandido K, el trabajo de Pinzón et al. (2016), quienes se enfocaron en el complejo de *T. utriculata* (embebido en el clado K.2), y el estudio de Granados Mendoza et al. (2017), quienes incorporaron un amplio muestreo al interior de *T.* subgen. *Tillandsia*. Ambos trabajos reconstruyeron el nodo ancestral del clado expandido K en el norte de los Andes, sugiriendo que, a partir de esta zona,

el clado expandido K de dispersó a su actual centro de diversidad en Centroamérica y México. Pinzón et al. (2016) además sugirió que desde dicha área ancestral las especies por ellos muestreadas del clado expandido K invadieron el oriente de Mesoamérica, las Antillas y Florida. Varios estudios han abordado las relaciones filogenéticas y los límites específicos de diversos complejos y grupos de especies, incluyendo el complejo de *T. ionantha* (Ancona et al., 2022), el grupo de especies pseudobulbosas (Chew et al., 2010), el complejo de *T. erubescens* (Granados Mendoza, 2008; Martínez-García et al., 2022), el complejo de *T. utriculata* (Pinzón et al., 2016) y el complejo de *T. fasciculata* (Sidoti, 2015).

Los trabajos antes mencionados utilizaron caracteres morfológicos y/o loci de cloroplasto, nuclear-ribosomales y nucleares generados con la estrategia de secuenciación Sanger (Ancona et al., 2022; Chew et al., 2010; Granados Mendoza, 2008; Martínez-García et al., 2022; Pinzón et al., 2016; Sidoti, 2015). Debido a muestreos taxonómicos limitados o al escaso número de loci empleados, las relaciones filogenéticas al interior del clado expandido K no fueron resueltas en estudios previos aplicando secuenciación Sanger. Recientemente, un número creciente de estudios filogenéticos en el género *Tillandsia* han utilizado diferentes estrategias de NGS, incrementando la resolución filogenética entre linajes cercanamente relacionados y proporcionado un mayor apoyo estadístico (De La Harpe et al., 2020; Groot Crego et al., 2023; Loiseau et al., 2019; Machado et al., 2020; Möbus et al., 2021; Vera-Paz et al., 2022; Yardeni et al., 2022).

Por otro lado, los estudios de fechamiento filogenético que han incluido representantes de *T.* subgen. *Tillandsia* se han enfocado en niveles taxonómicos más inclusivos, como la familia Bromeliaceae (Bouchenak-Khelladi et al., 2014; Givnish et al., 2004, 2007, 2011, 2014; Janssen & Bremer, 2004; Kessous et al., 2020; Magallón et al., 2015; Möbus et al., 2021; Montes-Azcué, 2020; Ramírez-Barahona et al., 2020; Sulman et al., 2013; Tank et al., 2015; Zhou et al., 2018), la subfamilia Tillandsioideae (Givnish et al., 2007, 2011, 2014; Kessous et al., 2020; Möbus et al., 2021, 2021; Montes-Azcué, 2020; Ramírez-Barahona et al., 2020), la tribu Tillandsieae (Givnish et al., 2007, 2011, 2014; Kessous et al., 2020; Möbus et al., 2021; Montes-Azcué, 2020; Ramírez-Barahona et al., 2020), el clado Core Tillandsioideae (Givnish et al., 2011, 2014; Kessous et al., 2020; Möbus et al., 2021) y el género *Tillandsia* (Möbus et al., 2021; Montes-Azcué, 2020), la mayoría de ellos basados en datos generados con secuenciación Sanger. Usando información del cloroplasto recuperada a partir de secuenciación somera, Möbus et al. (2021) estimaron una edad corona de 6.65 m.a. para *T.* subgen. *Tillandsia*, representando este linaje por las especies *T. malzinei* (clado *T. paniculata*) y *T. utriculata* (clado expandido K).

En conjunto la falta de un contexto filogenético resuelto, fechado y con un muestreo taxonómico amplio y representativo ha impedido conocer la historia biogeográfica del clado expandido K. El presente estudio tiene por objetivo la obtención de una filogenia fechada resuelta y altamente apoyada para un muestreo denso del clado expandido K por medio de la combinación del protocolo Hyb-Seq y el Enriquecimiento Híbrido Anclado. El contexto filogenético fechado resultante es utilizado como base para reconstruir el origen y ruta de dispersión a través del Neotrópico del clado expandido K.

OBJETIVOS

1. Obtener una filogenia resuelta y altamente apoyada entre especies cercanamente relacionadas del clado expandido K de *T*. subgen. *Tillandsia*.
2. Determinar el marco temporal del origen y diversificación del clado expandido K.
3. Inferir la ruta de dispersión del clado expandido K en el Neotrópico.
4. Investigar qué factores climáticos o tectónicos pudieron influir en la ruta de dispersión del clado expandido K.

Plastome phylogenomics reveals an early Pliocene North- and Central America colonization by long-distance dispersal from South America of a highly diverse bromeliad lineage

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Abstract

Understanding the spatial and temporal frameworks of species diversification is fundamental in evolutionary biology. Assessing the geographic origin and dispersal history of highly diverse lineages of rapid diversification can be hindered by the lack of appropriately sampled, resolved, and strongly supported phylogenetic contexts. The use of currently available cost-efficient sequencing strategies allows for the generation of a substantial amount of sequence data for dense taxonomic samplings, which together with well-curated geographic information and biogeographic models allow us to formally test the mode and tempo of dispersal events occurring in quick succession. Here, we assess the spatial and temporal frameworks for the origin and dispersal history of the expanded clade K, a highly diverse *Tillandsia* subgenus *Tillandsia* (Bromeliaceae, Poales) lineage hypothesized to have undergone a rapid radiation across the Neotropics. We assembled full plastomes from Hyb-Seq data for a dense taxon sampling of the expanded clade K plus a careful selection of outgroup species and used them to estimate a time calibrated phylogenetic framework. This dated phylogenetic hypothesis was then used to perform biogeographic model tests and ancestral area reconstructions based on a comprehensive compilation of geographic information. The expanded clade K colonized North- and Central America, specifically the Mexican transition zone and the Mesoamerican dominion, by long-distance dispersal from South America at least 4.86 Mya, when most of the Mexican highlands were already formed. Several dispersal events occurred subsequently northwards to the southern Nearctic region, eastwards to the Caribbean, and southward to the Pacific dominion during the last 2.8 Mya, a period characterized by pronounced climate fluctuations, derived from glacial-interglacial climate oscillations, and substantial volcanic activity, mainly in the Trans-Mexican Volcanic Belt. Our taxon sampling design allowed us to calibrate for the first time several nodes, not only within the expanded clade K focal group, but also in other Tillandsioideae lineages. We expect that this dated phylogenetic framework will facilitate future macroevolutionary studies and provide reference age estimates to perform secondary calibrations for other Tillandsioideae lineages.

Introduction

Modeling when and where diversification of a lineage took place requires sound and appropriately sampled time-calibrated phylogenetic hypotheses, along with information about the geographic distribution of the species of interest and testing of explicit biogeographic models. Species occurrence records can nowadays be readily accessed through several public databases, such as the Global Biodiversity Information Facility (https://www.gbif.org/es/), which, combined with automated curation strategies (e.g. CoordinateCleaner; Zizka et al., 2020), expert knowledge, and newly collected field information, represent a valuable and reliable source of geographic information. Biogeographic models using a priori-defined discrete areas are particularly useful for the reconstruction of biogeographic patterns of lineages above the species level, where continuous biogeographic models can be obscured by the more likely movement of organism within areas (Ronquist and Sanmartín, 2011). Generating molecular phylogenetic hypotheses for species-rich lineages with rapid diversification still poses serious challenges associated with the scarcity of informative data for divergences occurring in quick succession (short internodes, e.g. Leaché and Rannala, 2011; Wickett et al., 2014; Prum et al., 2015; Wanke et al., 2017), as well as with the effort required to generate sequence data for dense taxon samplings. Hyb-Seq is a cost-effective sequencing strategy that allows the simultaneous generation of nuclear, biparentally inherited loci through target enrichment, along with uniparentally inherited organellar (i.e. plastome and mitochondrial) data via genome skimming (Weitemier et al., 2014; Dodsworth et al., 2019). When properly designed, Hyb-Seq strategies allow for the recovery of significant amounts of plastid data (Granados Mendoza et al., 2020) or even complete organellar genomes (Vera-Paz et al., 2022), which represent a significant source of data for phylogenetic inference. Plastome-scale data, and particularly non-coding regions, have shown to substantially improve phylogenetic resolution and statistical support compared to approaches incorporating only or a few coding regions (Givnish et al., 2018). In addition to the amount of sequence data required to achieve phylogenetic resolution for rapidly evolving lineages, generating dated phylogenetic frameworks requires sampling key nodes where fossil-based or secondary calibrations can be established, which often requires sampling much deeper divergences than those of the group of interest (e.g. Givnish et al., 2004, 2011).

Bromeliads are known to have undergone one of the most remarkable plant adaptive radiations in the Neotropics (Givnish et al., 2007, 2011). The phylogenetic position of Bromeliaceae relative to the other families of the order Poales is still uncertain. A recent monocot-wide plastome phylogenetic study placed Bromeliaceae as sister to all other Poales families, albeit with low statistical support (Givnish et al., 2018), which, for instance, differs from the highly supported hypothesis of Bromeliaceae sister of Typhaceae recovered in the multi-locus nuclear analysis of the Tree of Life Explorer (https://treeoflife.kew.org/tree-of-life). A single fossil, *Karatophyllum bromelioides* L.D. Gómez, has unambiguously been assigned to Bromeliaceae (Gómez, 1972; Kessous et al., 2021), however; its use in phylogenetic dating studies has been hampered by the uncertainty around its collection locality and, therefore, its age (Baresch et al., 2011). Regardless of the phylogenetic position of Bromeliaceae within Poales, phylogenetic dating studies using secondary calibrations coincide in that after diverging from its sister lineage, this family underwent an evolutionary stasis of about 81-100 My before diversifying into its modern lineages (Givnish et al., 2011, 2018; Ramírez-Barahona et al., 2020). The geographic patterns of Bromeliaceae diversification have been addressed at subfamily level in a key study by Givnish and collaborators (2011). However, the study of the evolution in time and space of some highly diverse lineages of the bromeliad genus *Tillandsia* (subfamily Tillandsioideae, tribe Tillandsieae; Barfuss et al., 2016) has been hindered by the lack of resolved and strongly supported phylogenetic frameworks. Molecular phylogenetic studies in this genus have mostly used Sanger sequenced plastid,

nuclear-ribosomal, or nuclear low copy markers (Terry et al., 1997; Barfuss et al., 2005; Granados Mendoza, 2008; Chew et al., 2010; Sidoti, 2015; Barfuss et al., 2016; Castello et al., 2016; Pinzón et al., 2016; Granados Mendoza et al., 2017; Montes-Azcué, 2020; Ancona et al., 2022; Donadío et al., 2022). However, studies applying Next Generation Sequencing strategies to *Tillandsia* representatives significantly increased in recent years, (Loiseau et al., 2019, 2021; Möbus et al., 2021; Vera-Paz et al., 2022; Yardeni et al., 2022; Groot Crego et al., 2023), opening exciting possibilities for addressing fundamental macroevolutionary and biogeographic questions in this genus.

Tillandsia, as circumscribed by Barfuss et al. (2016), is the most diverse bromeliad genus, with ca. 780 species distributed from the southern USA to Argentina and Chile (Zizka et al., 2020; Gouda et al., continuously updated). Seven subgenera are currently recognized for this genus, of which *T.* subg. *Tillandsia* is the most diverse (> 270 spp.; Barfuss et al., 2016). Barfuss et al. (2005) proposed that *T.* subg. *Tillandsia*, therein equivalent to the later referred clade K, migrated from the Andes northwards to North- and Central America and identified Mexico and Central America as its centers of diversity. Möbus et al. (2021) recovered stem and crown ages of ca. 7.4 and 6.6 Mya, respectively, for this subgenus, suggesting a recent and rapid diversification from the mid-Neogene and onwards. According to Barfuss et al. (2016), three main lineages in successive sister relationship compose *T.* subg. *Tillandsia*. The first of them including *T. hildae*, *T. ferreyrae*, *T. heliconioides*, *T. malzinei*, and *T. paniculata* from Mexico, Central America, the Caribbean, and South America (hereafter referred to as "*T. paniculata* clade"). The second includes *T. propagulifera*, *T. spiraliflora*, *T. ecarinata*, *T. secunda*, and *T. adpressiflora* from South America (here after referred to as "*T. secunda* clade"). The third lineage, named "clade K" and focus of the present study, was first identified by Barfuss et al. (2005) and at that time it was known to include 11 species from Mexico, Central America, the Caribbean, and South America. Subsequent studies have consistently recovered clade K with strong statistical support. Despite the limited resolution of their phylogenetic context, resulting from the use of a single plastid region (*mat*K-*trn*K), Granados Mendoza et al. (2017) uncovered that this lineage is considerably more diverse (82 spp.), renamed it as "expanded clade K", and named its two main internal clades as K.1 and K.2. The species of the expanded clade K are mainly distributed in North- and Central America, with some species also in northern South America and the Caribbean (Granados Mendoza et al., 2017; Fig. 1). This clade includes species with both Crassulacean Acid Metabolism (CAM) and C3 photosynthesis (Crayn et al., 2015; De La Harpe et al., 2020; Groot Crego et al., 2023), and its species inhabit a wide range of mesic to xeric habitats, from sea level to 3300 m elevation (Espejo-Serna et al., 2004; Gouda et al., continuously updated; Fig. 1). To our knowledge, no study has provided a resolved and strongly supported dated phylogeny representing the various lineages composing the expanded clade K, therefore, hindering further macroevolutionary and biogeographic studies in this highly diverse lineage. A series of molecular and morphological studies have focused on exploring phylogenetic relationships and/or testing species delimitations of specific species complexes and groups within the expanded clade K (Granados Mendoza, 2008; Chew et al., 2010; Sidoti, 2015; Pinzón et al., 2016; Ancona et al., 2022; Martínez-García et al., 2022), many of them identified by Gardner (1986), albeit, with a limited sampling of other lineages of the focal group.

The present study aims at reconstructing the spatial and temporal frameworks of the origin and diversification of the expanded clade K. To achieve this, we generated high-quality plastome assemblies from Hyb-Seq data for a dense taxon sampling of our focal group plus a careful selection of outgroup species. We then used this substantial amount of sequence data to estimate a time-calibrated phylogeny and perform ancestral area reconstructions based on information from a well-curated geographic occurrence database.

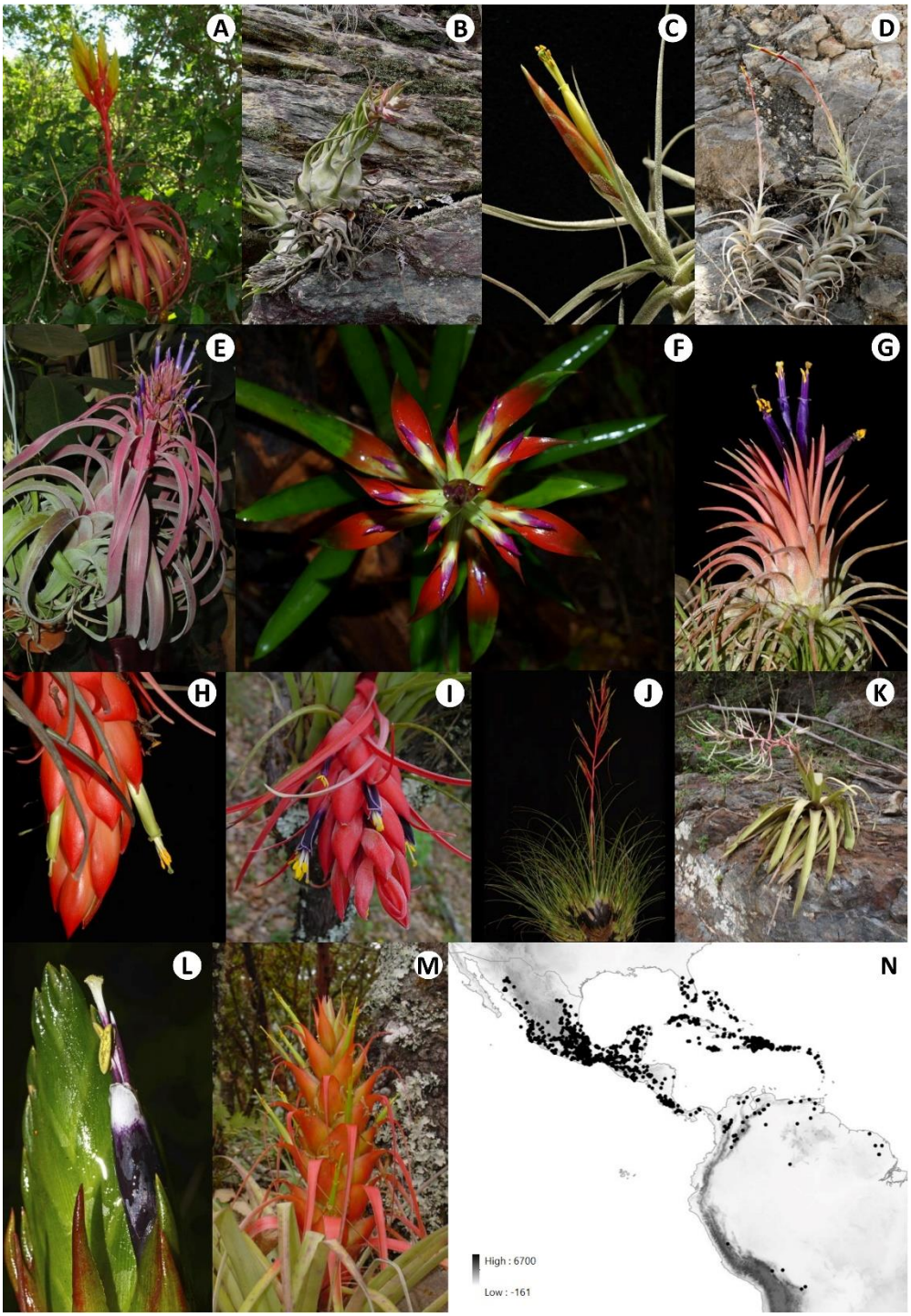

Figure 1. Morphological diversity and geographic distribution of the focal group expanded clade K. Representatives of the expanded clade K: **(A)** *Tillandsia rothii* (Granados Mendoza, 1058), **(B)** *T. seleriana* (Ramírez-Morillo,971), **(C)** *T. schiedeana* (Granados Mendoza, 297), **(D)** *T. albida* (Hornung-Leoni, s/n), **(E)** *T. jaguactalensis* (Ramírez-Morillo, s/n), **(F)** *T. leiboldiana* (Granados Mendoza, 832), **(G)** *T. ionantha* (Salazar, s/n), **(H)** *T. quaquaflorifera* (Granados Mendoza, 836), **(I)** *T. oaxacana* (Ramírez-Morillo, 1396), **(J)** *T. kirchhoffiana* (Granados Mendoza, 445), **(K)** *T. joel-mandimboensis* (Flores-Cruz, 2286), **(L)** *T. punctulata* (Salazar, s/n), and **(M)** *T. carlos-hankii* (Ramírez-Morillo, 1395). **(N)** Geographic distribution of the expanded clade K. Source of Map: Modified from https://www.worldclim.org/data/worldclim21.html (elev 30s, Fick and Hijmans, 2017) and http://tapiquen-sig.jimdo.com (Carlos Efraín Porto Tapiquén. Orogénesis Soluciones Geográficas. Porlamar, Venezuela 2015. Based on layers from the Enviromental Systems Research Institute -

ESRI). Photographers: Granados Mendoza (A, F, H, J, K), Ramírez-Morillo (B, E, I, M), Loyola (C), Hornung-Leoni (D), and Salazar (G, L).

Material and methods

Taxon Sampling

Full plastomes of a total of 200 species were analyzed, of which 162 were newly sequenced and assembled for this study and 38, generated by Guisinger et al. (1 sp.; 2010) and Vera-Paz et al. (37 spp.; 2022), were downloaded from GenBank (Supplementary Material 1). The sampled species were selected to construct a phylogenetic context with key nodes represented for 1) performing secondary calibrations, i.e. stem and crown nodes of Bromeliaceae and the crown node of Tillandsioideae, and 2) reconstructing the geographic origin and dispersal history of the expanded clade K. We aimed at achieving a dense geographic and taxonomic sampling of the expanded clade K. For this, we included 56 species previously recovered by Barfuss et al. (2016) and Granados Mendoza et al. (2017) within the expanded clade K, plus 37 additional *Tillandsia* subg. *Tillandsia* species mainly distributed in Central America and Mexico, which we hypothesized might belong in the expanded clade K given the geographic distribution pattern reported by Granados Mendoza et al. (2017). Sampling within *T.* subg. *Tillandsia* was complemented with three and five species previously recovered by Barfuss et al. (2016) within the *T. secunda* and *T. paniculata* clades, respectively, plus two additional species distributed in the Caribbean and South America and included here for the first time in a phylogenetic context, resulting in a total of 103 sampled species for *T.* subg. *Tillandsia*. Other subgenera and species complexes within *Tillandsia*, recognized by Barfuss et al. (2016), were sampled as follows: *T.* subg. *Aerobia* (8 spp.), *T.* subg. *Anoplophytum* s. str. (5 spp.), *T.* subg. *Diaphoranthema* (5 spp.), *T.* subg. *Phytarrhiza* s. str. (3 spp.), *T.* subg. *Pseudovriesea* (2 spp.), *T.* subg. *Viridantha* (4 spp.), *T. australis* complex (1 sp.), *T. biflora* complex (21 spp.), *T. disticha* complex (1 sp.), *T. gardneri* complex (3 spp.), *T. purpurea* complex (1 sp.), *T. rauhii* complex (2 spp.), *T. sphaerocephala* complex (5 spp.), as well as the unclassified species *T. albertiana* and *T. esseriana*. Tribe Tillandsieae was further represented by species of the genera *Barfussia* (2 spp.), *Guzmania* (2 spp.), *Lemeltonia* (1 sp.), *Pseudalcantarea* (2 spp.), *Racinaea* (3 spp.), and *Wallisia* (2 spp.). Within tribe Vrieseeae, subtribe Cipuropsidinae was represented by the genera *Goudaea* (2 spp.), *Lutheria* (2 spp.), *Mezobromelia* (1 sp.), *Werauhia* (3 spp.), and *Zizkaea* (1 sp.), whereas for subtribe Vrieseinae the genera *Alcantarea* (2 spp.), *Stigmatodon* (2 spp.), and *Vriesea* (4 spp.) were sampled. We included three species of the genus *Catopsis* as representatives of tribe Catopsideae. *Brocchinia micrantha* (subfamily Brocchinioideae) and *Typha latifolia* (Typhaceae) were sampled to represent the crown and stem nodes of Bromeliaceae, respectively. *Typha latifolia* was used to root the phylogenetic tree following the Poales familial phylogenetic relationships from the Kew Tree of Life Explorer (https://treeoflife.kew.org/). Lineages of Tillandsioideae not available to us include tribe Glomeropitcairnieae, and genera *Gregbrownia* (tribe Tillandsieae), *Josemania*, *Cipuropsis*, *Jagrantia*, and *Waltillia* (tribe Vrieseeae; Barfuss et al., 2016; Leme et al., 2017). Classification within Tillandsioideae follows Barfuss et al. (2016) and Gouda et al. (continuously updated), whereas the subfamilial classification of Bromeliaceae follows Givnish et al. (2007).

DNA isolation, library preparation, enrichment, and sequencing

New plastomes were assembled from raw data derived from a Hyb-Seq approach that applies a modified version of the universal probe kit for angiosperms of Buddenhagen et al. (2016), which includes additional Bromeliaceae target sequences. A previous publication (Vera-Paz et al., 2022) demonstrated the feasibility of assembling complete plastomes from data derived from this Hyb-Seq

project and herein-employed molecular methods were according to these authors. DNA isolation was performed at the "Laboratorio de Biología Molecular, Laboratorio Nacional de Biodiversidad (LANABIO)" of the Institute of Biology of the National Autonomous University of Mexico (IB-UNAM). The DNeasy Plant Pro Kit (Qiagen) was used to isolate genomic DNA from silica-gel dried leaf tissue. A minimum DNA concentration of 1.0 µg/µl and a DNA purity 260/280 ratio ≥1 was targeted. DNA concentration and purity were quantified with a Qubit™ fluorometer v. 3.0 (Invitrogen™ Thermo Fisher Scientific) using the Qubit™ dsDNA BR Assay Kit (Invitrogen, TM Thermo Fisher Scientific) and a Nanodrop 2000 spectrophotometer (Thermo Scientific™), respectively. Genomic DNA molecular weight was assessed in 1% agarose test gels, ran at 85 V and 500 mA for 50 min. Library preparation, enrichment, and sequencing were carried out by Daicel Arbor Biosciences (https://arborbiosci.com/). There, DNA concentration was additionally assessed by spectrofluorimetric assays with a PicoGreen assay kit (ThermoFisher Scientific). A Qsonica Q800 ultrasonicator was used to fragment genomic DNA to a target insert length of 300–800 nt. A proprietary modification by Daicel Arbor Biosciences of the KAPA HyperPrep kit (Roche) protocol was applied for library preparation, using custom unique dual-index combinations for the samples. Spectrofluorimetric assays and quantitative PCR assays using a KAPA Library Quantification Kit (Roche) were carried out to quantify the indexed libraries. Capture was carried out in pools of 12 libraries per reaction following the myBaits v. 5 protocol (https://arborbiosci.com/mybaits-v5-chemistry/). After capture, enrichment reactions were amplified for 12 cycles and quantified with spectrofluorimetric and quantitative PCR assays. Based on the number of libraries in each capture, the captures were pooled in approximately equimolar ratios and combined with equimolar pools of non-captured libraries at a 70:30 ratio. Sequencing was performed on the Illumina NovaSeq 6000 platform on S4 PE150 lanes targeting 2 Gbp of sequencing effort per library.

Plastome assembly and annotation

In general, we followed the plastome assembly and annotation methods described in Vera-Paz et al. (2022). In short, raw read quality was visualized in FastQC v. 0.11.9 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Then, the reads' leading and trailing low quality or N bases, as well as adapters were trimmed with Trimmomatic v. 39 (Bolger et al., 2014). Success of the trimming process was verified in FastQC. Trimmed reads were used to perform *de novo* assemblies in GetOrganelle v. 1.5.0 (Jin et al., 2020), applying default settings for 150 bp long paired data. In cases where GetOrganelle assembled partial plastome sequences as separated scaffolds, the SPAdes v. 3.15.5 (Prjibelski et al., 2020) assembler was used in an effort to try and expand the scaffolds further. Using *Tillandsia utriculata* (ON398158; Vera-Paz et al., 2022) as reference, annotations were automatically transferred to the newly assembled plastomes in Geneious Prime® 2021.2.2 (https://www.geneious.com/) and manually curated. Inverted repeat regions were identified with the repeat finder plugin of Geneious Prime®. Herein newly sequenced and assembled full plastomes can be found in GenBank under the accession numbers OQ935587– OQ935748.

Phylogenetic reconstruction and divergence time estimation

Full plastomes were aligned using the MAFFT v. 7.490 (Katoh and Standley, 2013) plugin of Geneious Prime©. The resulting alignment was manually curated and inspected to localize inversions. Gblocks v. 0.91b (Castresana, 2000) was then used to identify ambiguously aligned regions using default settings, except for the allowed gap positions parameter which was set to the option "all". Then, the -E command of RAxML v. 8.2.10 (Stamatakis, 2014) was applied to exclude: 1) small (<200 pb long) inversions, 2) ambiguously aligned regions detected by Gblocks, and 3) one of the inverted repeat regions. Long inversions (>200 pb long) detected in genus *Vriesea*, three species from the *Tillandsia*

biflora complex clade N (i.e. *T. deppeana, T. heterophylla,* and *T. imperialis*), and *Werauhia gladioliflora*, were replaced by "?" in the species where they were present and considered as missing data. This modified alignment was partitioned by protein-coding genes, tRNAs genes, rRNAs genes, introns, and intergenic spacers (IGS) and analyzed in PartitionFinder v. 2.1.1 (Lanfear et al., 2016) to select the best-fit partitioning scheme and corresponding models of molecular evolution using the Bayesian Information Criterion (BIC).

Estimation of phylogenetic relationships and divergence times were carried out simultaneously in BEAST2 v. 2.6.1 (Bouckaert et al., 2014). In BEAUti v. 2.5 we set the analysis priors for two data partitions retrieved as the best-fit partitioning scheme by PartitionFinder, applying the GTR and GTR+G models of molecular evolution, respectively, and empirical base frequencies for both site models. An uncorrelated relaxed clock model was applied, with rates sampled from a log-normal prior (Drummond et al., 2006) and a birth-death model tree prior. Based on the phylogenetic relationships recovered by Vera-Paz et al. (2022) and to reduce the computational cost, the topology was constrained for the major clades Bromeliaceae, Tillandsioideae, Catopsideae, Vrieseeae, and Tillandsieae, leaving unconstrained relationships among and within these clades. Given the lack of fossils that can be unambiguously assigned to Bromeliaceae and dated with confidence (e.g. Baresch et al., 2011), three secondary calibrations were applied using uniform prior distributions obtained from the relaxed (conservative fossil set) calibration performed by Ramírez-Barahona et al. (2020), as follows: 1) the root of the tree, i.e. stem node of Bromeliaceae, (lower: 90.8908 Ma, upper: 123.4522 Ma), 2) the crown node of Bromeliaceae (lower: 15.3615 Ma, upper: 37.7002 Ma), and 3) the crown node of Tillandsioideae (lower: 7.3867, upper: 19.3439). Seven independent Markov Monte Carlo (MCMC) chains were run for 271-300 million generations each, sampling every 5000 steps, until convergence was achieved, which was verified in Tracer V1.7.1 (Suchard et al., 2018). MCMC chains results were summarized with LogCombiner V2.6.1, setting the burn-in to 10% and sampling every 50,000 trees. The Maximum Clade Credibility (MCC) tree was generated in TreeAnnotator v. 2.6.0 and visualized in FigTree V1.4.4. A second phylogenetic analysis without topological constraints was carried out under Maximum Likelihood in IQ-TREE (Nguyen et al., 2015) with an automatic selection of the best-fit substitution model for the individual partitions and assessing node support with 1,000 ultrafast bootstrap replicates.

Estimation of Biogeographic History

Geographic data compilation and delimitation of distribution areas

The geographic distribution of the species included in our phylogenetic context was obtained from various resources including online public repositories (e.g. https://www.gbif.org/ and https://www.jacq.org/), taxonomic literature, own field records, and the study of Zizka et al. (2020; Supplementary Material 2). In the case of public databases, we restricted our search to records that could be associated to an image (e.g. photographs of herbarium vouchers), so that taxonomic identification could be verified if needed. Depending on the data source, geographic distribution data was retrieved as geographic coordinates, distribution polygons (as in Plants of the World Online, https://powo.science.kew.org/), and description of localities.

Geographic coordinates were filtered in R v. 4.2.1 (R Core Team, 2022) and R studio v. 2022.12.0.353 (Posit team, 2022) with the packages *maps* v. 3.4.1 (Becker et al., 2022), *dplyr* v. 1.1.0 (Wickham et al., 2023), *raster* v. 3.6-14 (Hijmans, 2023), *rgdal* v. 1.6-4 (Bivand et al., 2023), and *sp* (Pebesma and Bivand, 2005; Bivand et al., 2013) by plotting them on the map of America, then filtering out coordinates falling in the sea, duplicated values, as well as coordinates that did not match their

associated locality description. After this data filtering, the distributional range of every species was validated with the ArcMap tool of ArcGIS v. 10.5 using the Bromeliaceae species distribution polygons modelled by Zizka et al. (2020) as reference, which were obtained with the R package speciesgeocodeR (https://github.com/azizka/speciesgeocodeR). Validation of species lacking a distribution polygon in Zizka et al. (2020) was performed manually based on the geographic distribution reported in taxonomic literature, as well as the online databases Encyclopaedia of Bromeliads v. 4 (http://bromeliad.nl/encyclopedia/) and Plants of the World Online (https://powo.science.kew.org/).

We estimated ancestral areas based on the biogeographic regionalization of the Neotropical region proposed by Morrone et al. (2019; 2022) and the terrestrial ecoregions of North America (https://www.epa.gov/eco-research/ecoregions-north-america). Because analysis of a high number of areas can hinder the interpretation of biogeographic reconstructions, we performed two separate analyses to infer both the origin and dispersal history of our focal group, the expanded clade K. The first analysis, designed for reconstructing the origin of the focal group, was performed on the dated phylogeny including the complete taxon sampling and the following general discrete areas: Chacoan subregion (A), Boreal and South Brazilian dominions (B), South American transition zone (C), Pacific dominion (D), Antillean subregion + SE USA (E), and Mexican transition zone + Mesoamerican dominion + Nearctic region (F). The second analysis aimed at reconstructing the biogeographic history of the expanded clade K. For this, a subtree corresponding to *Tillandsia* subg. *Tillandsia* was extracted from the original dated phylogeny and analyzed with the following specific discrete areas: Pacific and Boreal and South Brazilian dominions + South American transition zone (Y), Antillean subregion + SE USA (E), Pacific Lowlands and Balsas provinces + El Cabo de Baja California district (G), Mexican transition zone (H), Mosquito, Veracruzan and Yucatán Peninsula provinces (I), and Nearctic region (J). The ArcMap tool of ArcGIS v. 10.5 was used to delimit the above proposed areas and to convert them into shapefiles. These shapefiles were plotted along with the compiled species geographic distribution information (i.e. polygons, geographic coordinates, and georeferenced localities) to extract the species presence in each area with the tool "select layer by location" of ArcMap. The codification of the distribution areas for all the sampled species can be found in the Supplementary Material 2.

Ancestral area reconstructions

Both ancestral area reconstruction analyses were performed in R v. 4.1.1 and R studio v. 1.4.17.17 with the package BioGeoBEARS 0.2.1 (Matzke, 2013). First, widely distributed species, namely *Catopsis sessiliflora, Tillandsia balbisiana, T. fasciculata, T. juncea, T. polystachia, T. pruinosa, T. recurvata, T. usneoides,* and *T. utriculata* were excluded in RASP v4.2 (Yu et al., 2015) with the tool "remove selected groups" to avoid reconstructing an excessive number of ancestral areas. Then, we tested the relative fit of the data to the biogeographic models Dispersal-Extinction-Cladogenesis (DEC), Dispersal-Vicariance-Like (DIVALIKE), BayArea-like (BAYAREALIKE), and the same models plus the founder-event speciation (*j*) parameter. The *j* parameter was considered in model comparison because Tillandsioideae species have seeds with hair-like appendages that form a flight apparatus (wind-dispersed seeds; Barfuss et al., 2016), suggesting that founder-event speciation is a factor potentially influencing the biogeographic history of this group. For all models, we set the maximum number of areas that any species could occupy to five and allowed *j*, as well as the rate of dispersal/range addition (*d*) and rate of extinction/range contraction (*e*) parameters, to vary freely.

Selection of the best-fitting biogeographic model was performed in a likelihood framework, using the Akaike's information criterion weight (AIC-wt) and contrasting the original models versus these models + *j* as alternative hypotheses with the AICstats_2models function of BioGeoBEARS. Ancestral area probabilities reconstructed under the best-fitting biogeographical model for each node of the

analyzed phylogenies were printed to a CSV file, along with a tree with reference node number labels (Supplementary material 2 and 3). The most probable areas were plotted at the nodes of the dated phylogenies in R. Phylogenetic inference, dating, and ancestral area reconstruction were performed on the servers BEAGLE of IB-UNAM and CIPRES Science Gateway (Miller et al., 2010).

Results

Phylogenetic relationships

The alignment of the complete plastomes resulted in a 180,766 bp matrix. A total of 48,040 bp corresponding to one inverted repeat region, small inversions, and ambiguously aligned regions were excluded resulting in a 132,726 bp long matrix that was used for phylogenetic inference. Nearly all phylogenetic relationships of the BEAST2 analysis received strong statistical support (PP≥0.85), excepting as noted below (Figs. 2-5). Genera, subgenera, and species complexes from which we included more than one accession were recovered as monophyletic, except for *Goudaea*, *Lutheria*, *Tillandsia*, and the *T. biflora* complex. Tribe Catopsideae was recovered as the sister lineage of tribes Vrieseeae plus Tillandsieae. In subtribe Vrieseinae, *Alcantarea* is sister of a clade including *Stigmatodon* and *Vriesea*, while subtribe Cipuropsidinae is further divided in two lineages, one including *Lutheria splendens* as sister of *Werauhia* and the other including *Goudaea ospinae* as successive sister of *Goudaea* aff. *chrysostachys*, *Zizkaea*, *Mezobromelia* and *Lutheria bibeatricis* (Fig. 2).

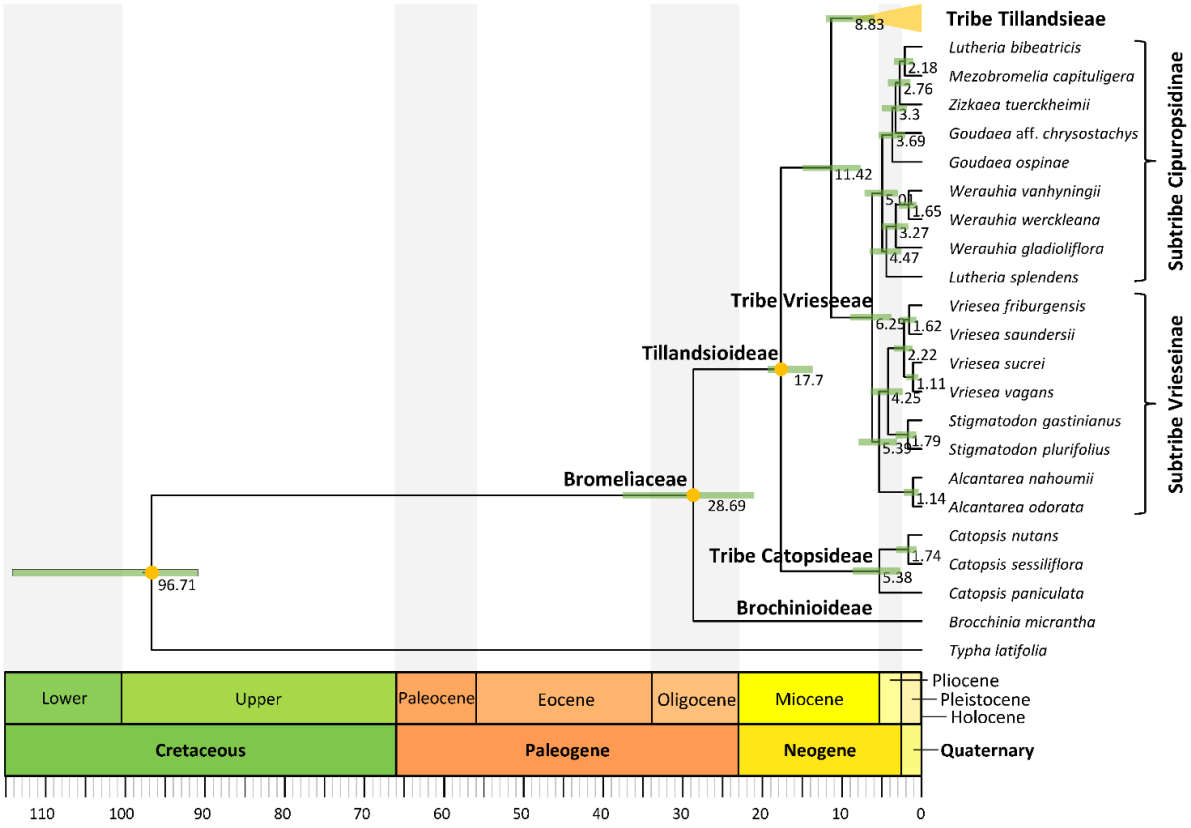

Figure 2. Tillandsioideae tribe and subtribe phylogenetic relationships. Maximum clade credibility tree derived from the analysis of full plastomes. Green bars associated to age values are the 95% highest posterior density (HPD) intervals of

highly supported (PP≥0.85) nodes. Yellow circles denote calibrated nodes. Clade names follow Barfuss et al. (2016) and Givnish et al. (2007).

Within tribe Tillandsieae, *Guzmania* is the sister lineage of a clade containing the remaining sampled species, which is divided into two main lineages. The first of these lineages (PP=0.60) consists of a clade including the *Tillandsia disticha* complex and *Pseudalcantarea* as successive sisters (PP=0.74) of *T*. subg. *Pseudovriesea*, *Barfussia*, *Lemeltonia*, and *Wallisia* plus *Racinaea*. The second lineage (PP=0.34) is further divided into two clades, the first of them including a clade of *T. purpurea* complex and *T*. subg. *Viridantha* as the successive sister of 1) a clade including *T. australis* and *T. sphaerocephala* complexes, 2) the *T. biflora* complex clade N, 3) a clade including the *T. rauhii* complex and the *T. biflora* complex clade P, 4) the *T. gardneri* complex, 5) *T. albertiana*, 6) *T. esseriana*, 7) *T*. subg. *Anoplophytum* s. str., 8) *T*. subg. *Diaphoranthema*, and 9) a clade of *T*. subg. *Aerobia* and *T*. subg. *Phytarrhiza* s. str. (Fig. 3).

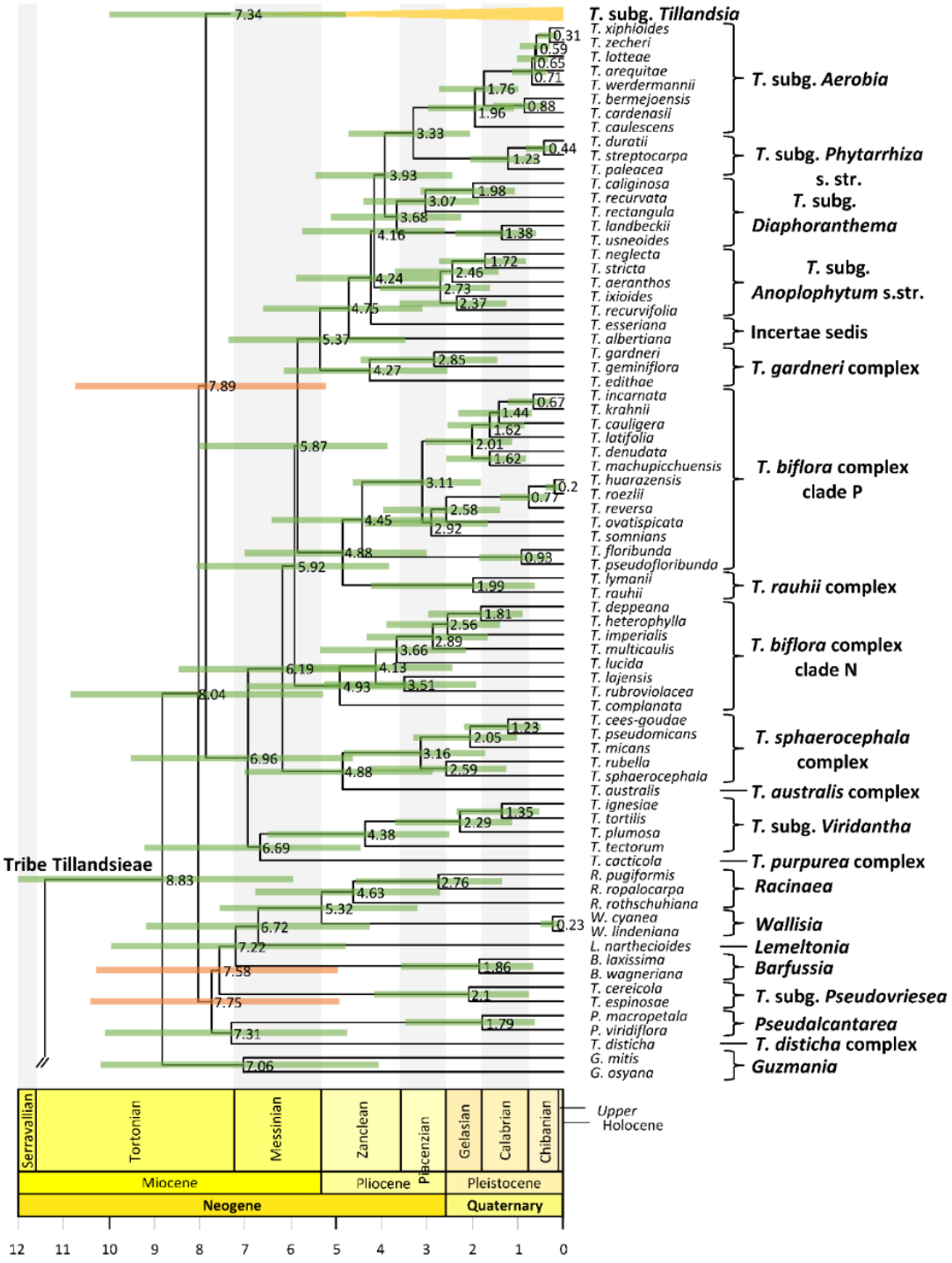

Figure 3. Tribe Tillandsieae phylogenetic relationships. Maximum clade credibility tree derived from the analysis of full plastomes. Bars associated to age values are the 95% highest posterior density (HPD) intervals. Green and orange HPD bars denote highly supported (PP≥0.85) and non-highly supported (PP≤0.85) nodes, respectively. Clade names follow Barfuss et al. (2016).

The second clade corresponds to *T.* subg. *Tillandsia*, where all but a few recent and shortly spaced apart nodes within the later mentioned clade K.2 were strongly supported (Figs. 4-5). Within *T.* subg.

Tillandsia, three main clades were recovered as successive sister lineages (Fig. 4). In the first of them, *T. extensa* is the sister species of a clade composed of *T. marnier-lapostollei*, *T. spiraliflora*, and *T. propagulifera* (corresponding to *T. secunda* clade). The second main clade, *T. paniculata* clade, includes two lineages, the first one including *T. hildae* as sister of *T. funckiana*–*T. flexuosa*, whereas in the second *T. elongata* is sister to *T. malzinei*–*T. heliconioides*. The third main clade is highly diverse, herein including 93 spp., equivalent to the expanded clade K, which consists of two main clades herein identified as K.1 (31 spp.) and K.2 (62 spp.) following the clade nomenclature proposed in that work. Given the substantial number of species integrating these two clades, internal phylogenetic relationships will not be described in detail, but they are shown in full in Figures 4-5.

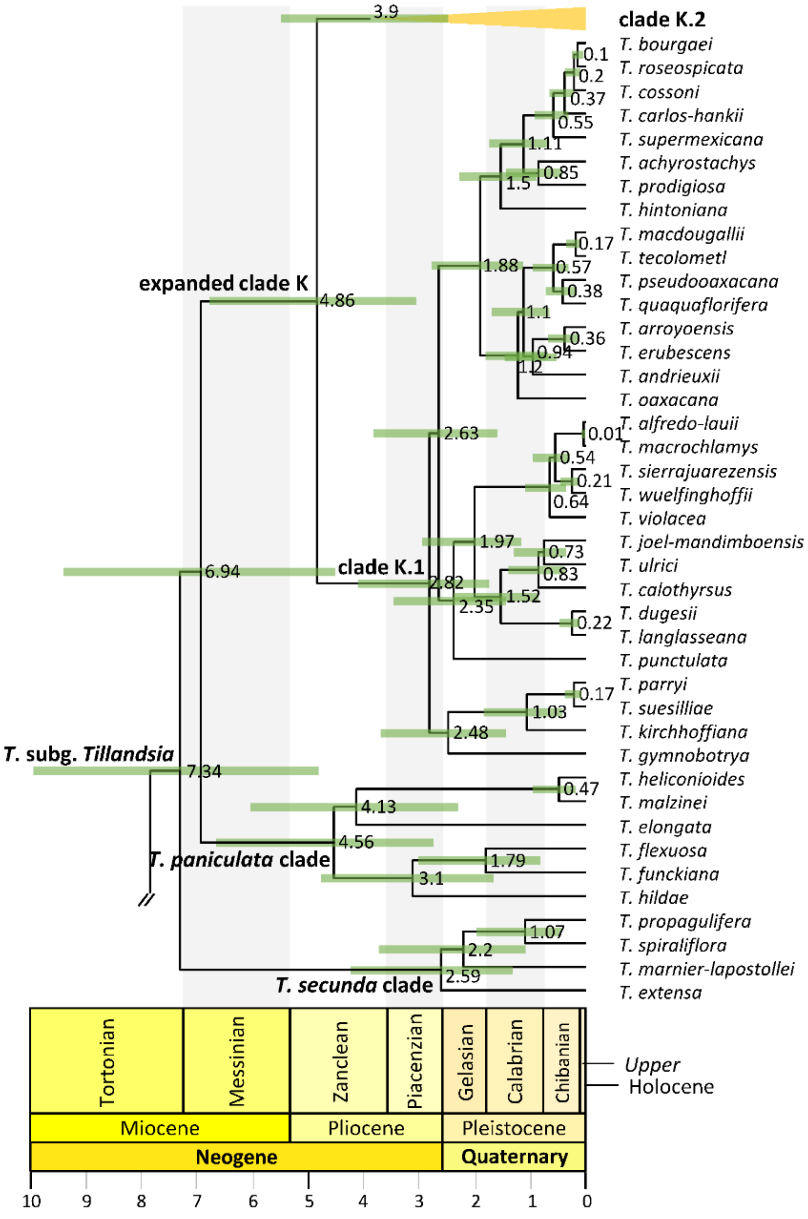

Figure 4. *Tillandsia* subg. *Tillandsia* phylogenetic relationships. Maximum clade credibility tree derived from the analysis of full plastomes. Green bars associated to age values are the 95% highest posterior density (HPD) intervals of highly supported (PP≥0.85) nodes. Clade names follow Barfuss et al. (2016) and Granados Mendoza et al. (2017).

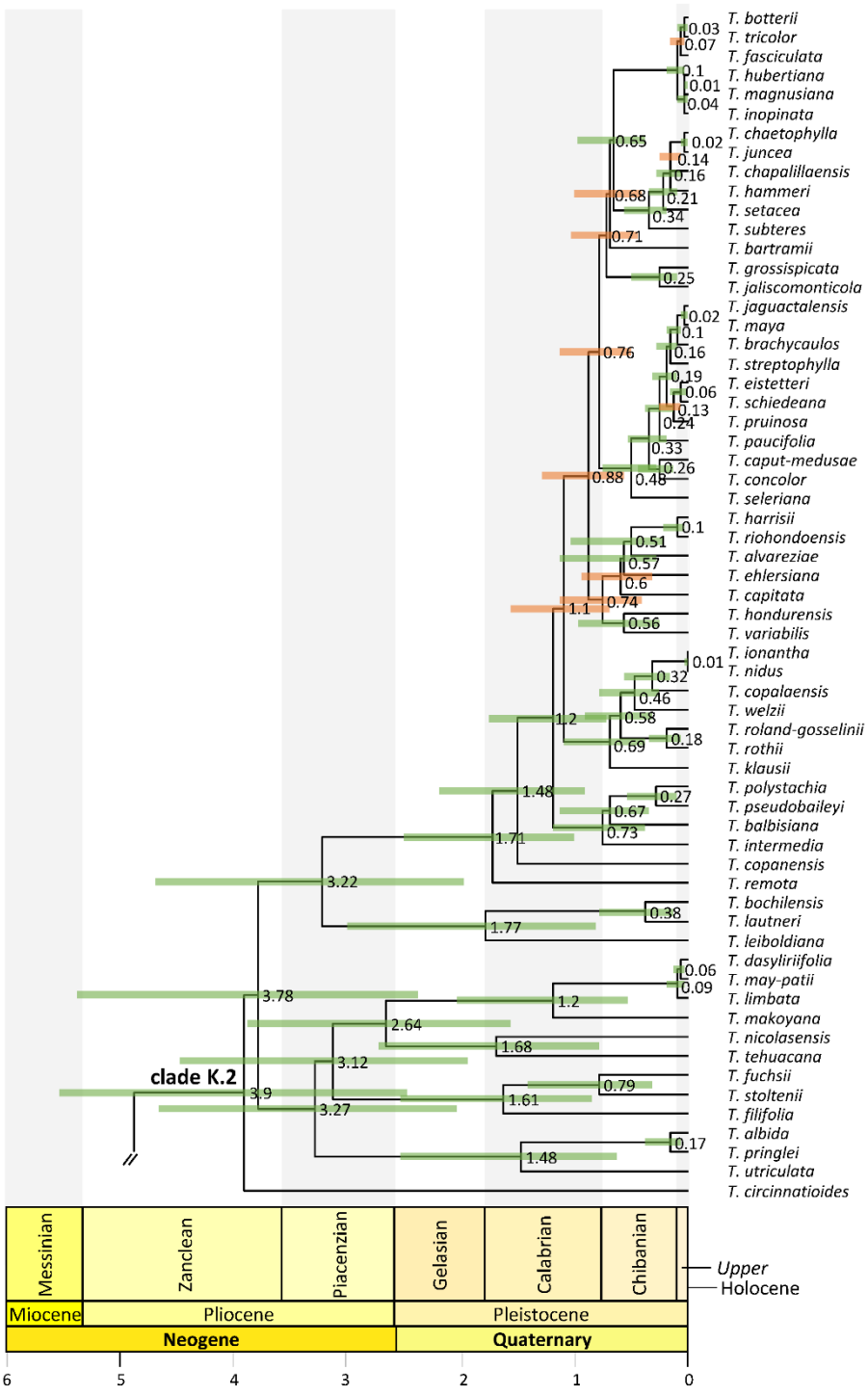

Figure 5. Clade K.2 phylogenetic relationships. Maximum clade credibility tree derived from the analysis of full plastomes. Bars associated to age values are the 95% highest posterior density (HPD) intervals. Green and orange HPD bars denote highly supported (PP≥0.85) and non-highly supported (PP≤0.85) nodes, respectively. Clade names follow Granados Mendoza et al. (2017).

When considering only strongly supported relationships (BS≥85 and PP≥0.85), the ML analysis resulted in a tree topology almost entirely congruent with the BEAST2 analysis, except for the alternative sister position of *T. somnians* and *T. ovatispicata* relative to the clade of *T. huarazensis*, *T. roezlii*, and *T. reversa*, as well as the position of *Goudaea* aff. *chrysostachys* as sister of *Mezobromelia capituligera* instead of sister to the clade of *Mezobromelia capituligera*, *Lutheria bibeatricis*, and *Zizkaea tuerckheimii*.

Lineage Divergence Time

Divergence between Bromeliaceae and Typhaceae was estimated to have occurred 96.71 Mya (95% HPD= 90.89-114.14 Mya), whereas estimated crown ages of Bromeliaceae and Tillandsioideae were 28.69 Mya (95% HPD= 21.06-37.58 Mya) and 17.7 Mya (95% HPD= 13.72-19.34 Mya), respectively. Tribe Catopsideae, with a crown age of 5.38 Mya (95% HPD= 2.73-8.69 Mya), diverged from tribes Vrieseeae and Tillandsieae 17.7 Mya (95% HPD= 13.72-19.34 Mya), while divergence of the latter two tribes was 11.42 Mya (95% HPD= 7.66-15.01 Mya). The crown age of tribe Vrieseeae was 6.25 Mya (95% HPD= 3.81-8.98 Mya), whereas as that of its subtribes Vrieseinae and Cipuropsidinae were 5.39 Mya (95% HPD= 3.16-7.92 Mya) and 5.01 Mya (95% HPD= 2.97-7.25 Mya), respectively (Fig. 2). Crown age of tribe Tillandsieae was 8.83 Mya (95% HPD= 5.94-12 Mya), and within it, *Tillandsia* subg. *Tillandsia* had stem and crown ages of 7.89 (95% HPD= 5.22-10.73 Mya) and 7.34 Mya (95% HPD= 4.8-9.98 Mya), respectively (Fig. 3). Stem and crown ages of the expanded clade K focal group were 6.94 (95% HPD= 4.49-9.42 Mya) and 4.86 Mya (95% HPD= 3.03-6.79 Mya), respectively, whereas the crown ages of its two subclades K.1 and K.2 were 2.82 (95% HPD= 1.72-4.1 Mya) and 3.9 Mya (95% HPD= 2.45-5.5 Mya), respectively (Figs. 4-5). Estimated stem and crown ages of other sampled genera and species complexes are shown in Supplementary Material 4 and point-age estimations for all recovered nodes are shown in Figs. 2-5.

Biogeographic History

Log-likelihoods and estimated *j*, *d*, and *e* parameter values for each tested biogeographic model for both analyses are shown in Table 1. The model that best fitted the data in both cases is BAYAREALIKE+*j*, explaining ca. 99% and 72% of the total predictive power of all assessed models in the analyses with the complete taxon sampling and *Tillandsia* subg. *Tillandsia*, respectively. Among all analyzed areas, the combined area of the Boreal and South Brazilian dominions (B), South American transition zone (C), and Pacific dominion (D) was recovered as the most probable ancestral area for the nodes corresponding to subfamily Tillandsioideae (8%), core Tillandsioideae (45%), tribe Tillandsieae (59%), and a node including all tribe Tillandsieae representatives except *Guzmania* (59.20%). Then, the three nodes preceding the expanded clade K, including that of *T.* subg. *Tillandsia* (53%), were reconstructed as having experienced a range contraction to the Boreal and South Brazilian dominions (B) and South American transition zone (C), followed by a non-contiguous area shift to the Mexican transition zone + Mesoamerican dominion + Nearctic region individual area (F; 98.60%). Within *T.* subg. *Tillandsia*, the node subtending the *T. secunda* clade is similarly reconstructed in the combined area of the Boreal and South Brazilian dominions (B) and South American transition zone (C; 78.60%), whereas the *T. paniculata* clade expanded its ancestral area back to the Pacific dominion (D; 48.50%; Fig. 6, see Supplementary Material 3C for the second most probable ancestral area).

Key nodes relevant for the biogeographic discussion of the expanded clade K are indicated in Figure 7 with the numbers 1–20 (see Supplementary Material 3D for the second most probable ancestral area). The most recent common ancestor of this focal group is estimated to have occupied the combined area of the Pacific Lowlands and Balsas Basin provinces + El Cabo de Baja California district (G), Mexican

transition zone (H), and Mosquito, Veracruzan and Yucatán Peninsula provinces (I; 31%). From this ancestral area, four lineages within clade K.1 were reconstructed to have reduced their range to either the Mexican transition zone (H) and Mosquito, Veracruzan and Yucatán Peninsula provinces (I; node 1 and 5; 36% and 89%) or to the Pacific Lowlands and Balsas Basin provinces + El Cabo de Baja California district (G) and the Mexican transition zone (H; nodes 4 and 6; 50% and 38%), while one lineage experienced a range expansion to the Nearctic region (J; node 3; 53%). Further colonization of the Nearctic region (J) occurred via additional area range expansions from the Mexican transition zone (H) and Mosquito, Veracruzan and Yucatán Peninsula provinces (I; node 2, 47%); and the Pacific Lowlands and Balsas Basin provinces + El Cabo de Baja California district (G) and the Mexican transition zone (H; nodes 7, 12, 28% and 62%). Additional dispersals to the Mosquito, Veracruzan and Yucatán Peninsula provinces (I) involved range expansions from the combined area of the Pacific Lowlands and Balsas Basin provinces + El Cabo de Baja California district (G), the Mexican transition zone (H), and the Nearctic region (J; node 8, 54%) or from the Pacific Lowlands and Balsas Basin provinces + El Cabo de Baja California district (G) and the Mexican transition zone (H), however, with a simultaneous area expansion to the Nearctic region (J, node 11, 54%). Successive area contraction and expansion were modeled at nodes 9 (47.9%) and 10 (80%) in combined areas involving the Pacific Lowlands and Balsas Basin provinces + El Cabo de Baja California district (G), the Mexican transition zone (H), and the Nearctic region (J). Within clade K.1, a single area range expansion back to the Pacific, Boreal and South Brazilian dominions + South American transition zone (Y) is reconstructed in a terminal branch for *Tillandsia punctulata*. Ancestral area reconstruction within clade K.2 is only described for nodes that received strong statistical support (PP≥0.85) in the phylogenetic analysis. Within this clade, several range contractions were reconstructed to either the Pacific Lowlands and Balsas Basin provinces + El Cabo de Baja California district (G) and Mexican transition zone (H; nodes 14, 15, and 19; 74%, 72.50% and 83.88%); the Mexican transition zone (H; node 13, 80.20%); or the Mosquito, Veracruzan and Yucatán Peninsula provinces (I; node 18, 99%). Expansion to the Nearctic region (J) in clade K.2 occurred at nodes 17 (80%) and 20 (79%) from the ancestral area of the expanded clade K and from the Pacific Lowlands and Balsas Basin provinces + El Cabo de Baja California district (G) and Mexican transition zone (H; node 16, 57%), however, with a simultaneous area range expansion to the Mosquito, Veracruzan and Yucatán Peninsula provinces (I) and back to the Pacific, Boreal and South Brazilian dominions + South American transition zone (Y). Eight additional area range expansions back to the Pacific, Boreal and South Brazilian dominions + South American transition zone (Y) were estimated at terminal branches of the clade K.2 corresponding to *Tillandsia filifolia*, *T. leiboldiana*, *T. pseudobaileyi*, *T. ionantha*, *T. variabilis*, *T. schiedeana*, *T. brachycaulos*, and *T. tricolor*. Colonization of the Antillean subregion + SE USA (E) occurred exclusively within clade K.2 in six independent events from either the combined area of the Pacific Lowlands and Balsas Basin provinces + El Cabo de Baja California district (G), Mexican transition zone (H), and Mosquito, Veracruzan and Yucatán Peninsula provinces (I, *T. schiedeana*, *T. variabilis*, and *T. streptophylla*), from the latter combined area plus the Nearctic region (J; *T. setacea*) or from an unknown area due to low statistical support of the phylogenetic relationships (*T. bartramii* and *T. capitata*).

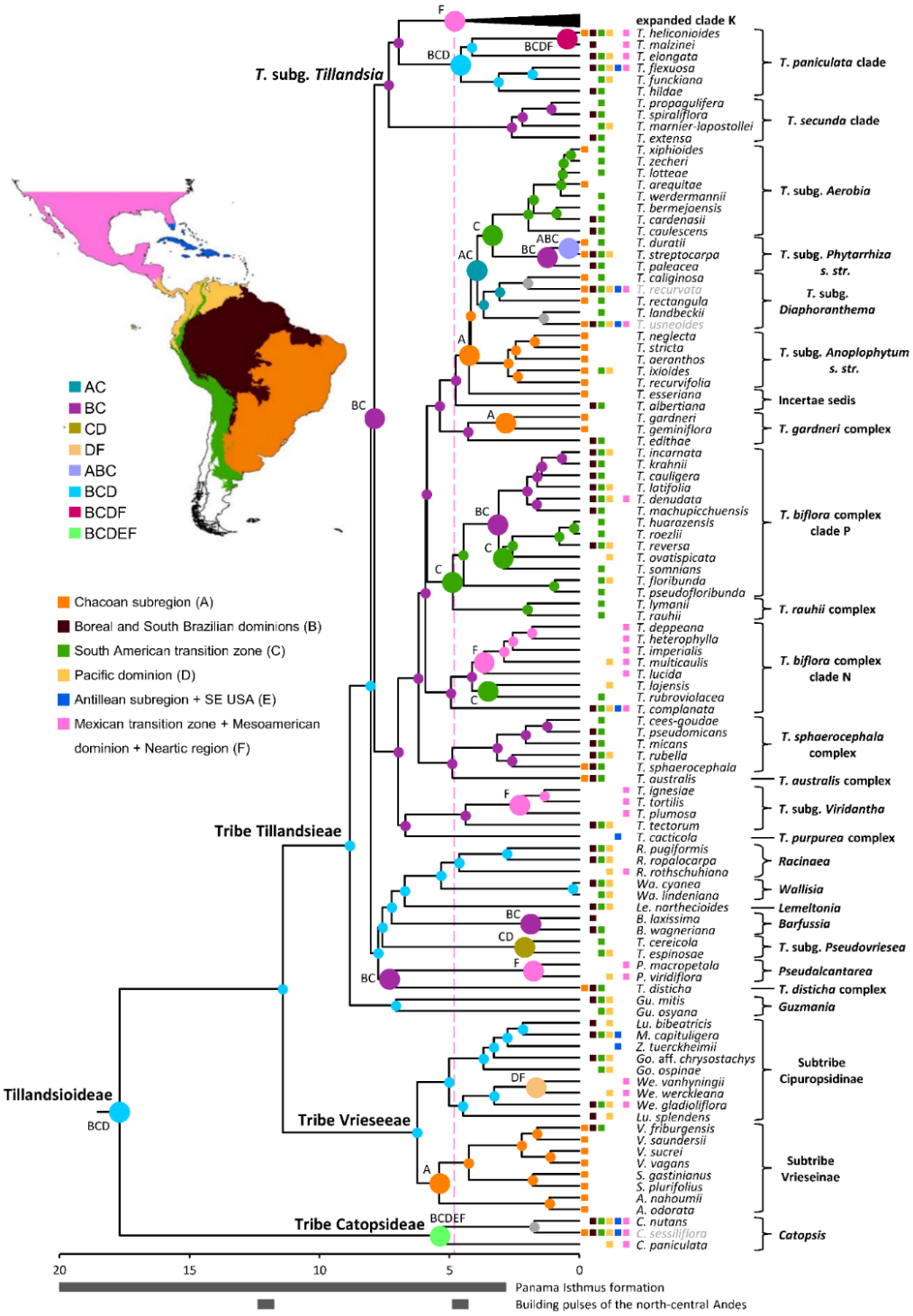

Figure 6. **Geographic origin of the focal group expanded clade K.** Colored circles denote the most probable individual or combined ancestral area under the BAYAREALIKE+J model for highly supported (PP ≥ 0.85) internal nodes. Larger circles denote area shifts. Current geographic distribution of the species is denoted by colored squares. Excluded cosmopolitan species and their associated nodes are shown in gray. Proposed periods of main tectonic events discussed in the text are denoted by gray bars below time scale. Clade names follow Barfuss et al. (2016), Givnish et al. (2007), and Granados Mendoza et al. (2017). Source of Map: http://tapiquen-sig.jimdo.com (Carlos Efraín Porto Tapiquén.

Orogénesis Soluciones Geográficas. Porlamar, Venezuela 2015. Based on layers from the Enviromental Systems Research Institute - ESRI).

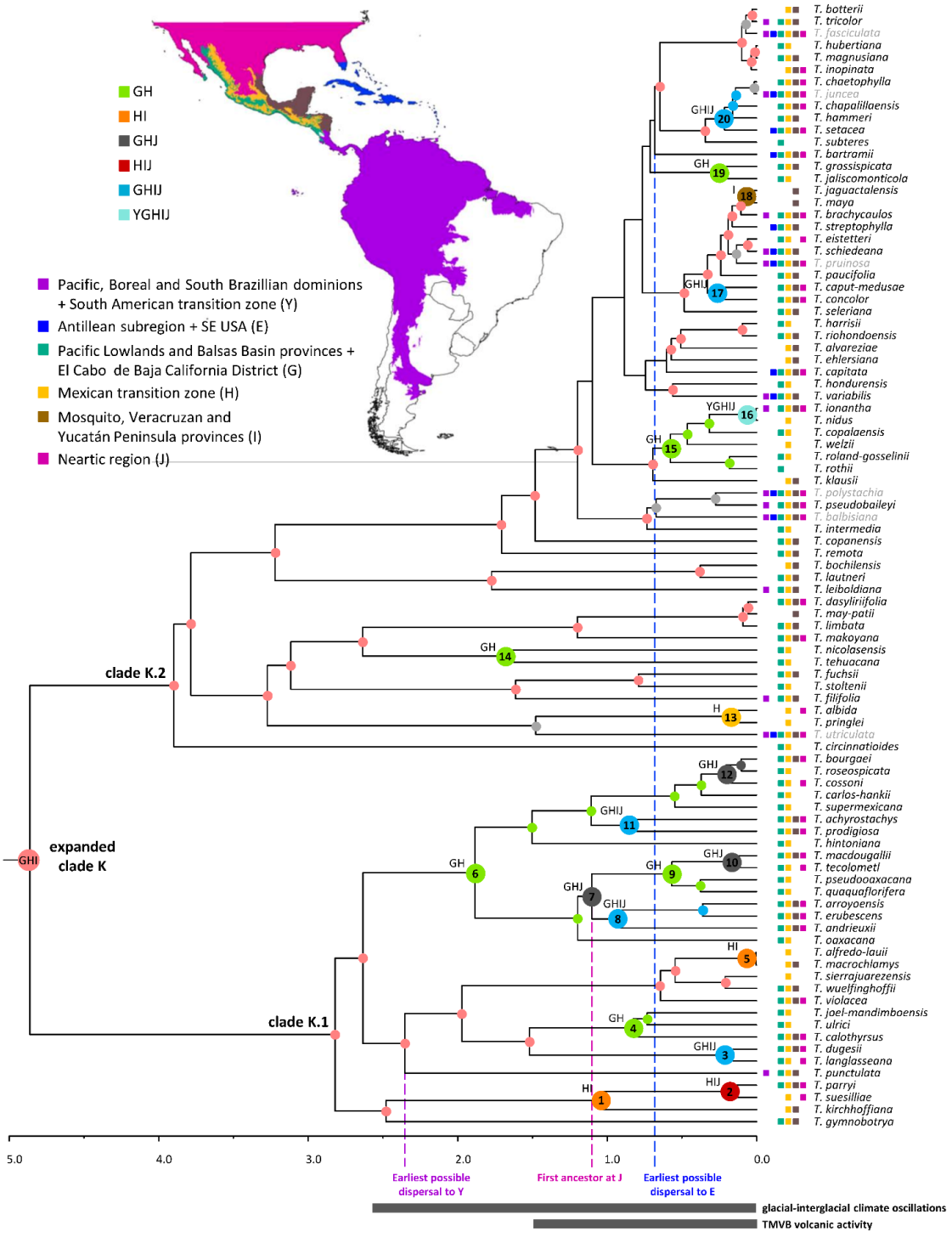

Figure 7. Dispersal history of the focal group expanded clade K. Colored circles denote the most probable individual or combined ancestral area under the BAYAREALIKE+J model for highly supported (PP ≥ 0.85) internal nodes. Larger circles at internal nodes denote area shifts. Node numbers correspond to those discussed in the text. Current geographic distribution of the species is denoted by colored squares. Excluded cosmopolitan species and their associated nodes are

shown in gray. Proposed periods of main tectonic events discussed in the text are denoted by gray bars below time scale. Clade names follow Granados Mendoza et al. (2017). Source of Map: http://tapiquen-sig.jimdo.com (Carlos Efraín Porto Tapiquén. Orogénesis Soluciones Geográficas. Porlamar, Venezuela 2015. Based on layers from the Enviromental Systems Research Institute - ESRI).

Discussion

Evolutionary context of the expanded clade K

Our study contributes 162 newly sequenced and assembled complete plastomes to Bromeliaceae genomic resources, more densely sampled within the expanded clade K of *Tillandsia* subg. *Tillandsia*, but also representing many other *Tillandsia* subgenera and species complexes, as well as other genera of Tillandsioideae. Additionally, the complete plastome of *Brocchinia micrantha* (subfamily Brocchinioideae) was generated here as part of our outgroup sampling. Our analyses of 200 complete plastomes derived in an overall strongly supported phylogenetic hypothesis, with only 13 out of the 198 nodes (excluding the root) with PP<0.85. At tribe and subtribe levels, our results agree with previously proposed plastid phylogenetic contexts (Barfuss et al., 2016; Machado et al., 2020; Loiseau et al., 2021; Vera-Paz et al., 2022). As in the plastid-based tree of Loiseau et al. (2021), we here recovered the genus *Goudaea* (subtribe Cipuropsidinae) as non-monophyletic (see also Supplementary Material 5), albeit herein with strong statistical support. However, the nuclear-based inference of Loiseau et al. (2021) support the monophyly of this genus. Interestingly, those authors propose a potential hybrid origin for *Goudaea chrysostachys*, which could potentially explain the discordance between nuclear and plastid data sources across studies. The genus *Lutheria* is also non-monophyletic in our phylogenetic context with strong statistical support. Remarkably, the alternative phylogenetic positions shown by the herein analyzed *Lutheria* species (*L. splendens* and *L. bibeatricis*) follow the same alternative positions proposed by Loiseau et al. (2021) for this genus upon comparing their plastid and nuclear trees. Our study is the first testing the phylogenetic position of *L. bibeatricis*, therefore its affiliation to the genus *Lutheria* and the reasons (e.g. hybridization) for its unexpected phylogenetic position should be further investigated. Other non-monophyletic groups recovered in our analysis with strong statistical support are the genus *Tillandsia* and the *T. biflora* complex. The non-monophyly of *Tillandsia* results from the sister relationship of *T. disticha* and *Pseudalcantarea*, since the obtained sister relationship of *T.* subg. *Pseudovriesea* to the clade including *Barfussia, Racinaea*, *Wallisia*, and *Lemeltonia* received low statistical support. A comparatively long branch subtends *T. disticha* in a maximum likelihood tree (Supplementary Material 5), a result shared with previous studies (e.g. Barfuss et al., 2016), suggesting that the phylogenetic position of this species could also be influenced by long branch attraction, leaving open the question if *Tillandsia*, as circumscribed by Barfuss et al. (2016), is or is not monophyletic. Additional statistical support is required to resolve recalcitrant nodes along the backbone of Core Tillandsieae (genus *Tillandsia* s.l., Smith and Downs, 1977) to validate or refute the segregation of various genera from *Tillandsia* s.l. (Grant and Zijlstra, 1998; Smith and Till, 1998; Barfuss et al., 2016). Since its recognition in Barfuss et al. (2016), the *T. biflora* complex (136 spp.) was defined as a polymorphic assemblage of species with contrasting geographic distributions, highlighting the presence of Caribbean and Mexican species as part of this complex. Although highly supported in the Bayesian analysis of Barfuss et al. (2016), the sister relationship of the *T. biflora* clades N and P received low statistical support in their maximum likelihood and parsimony analyses, which is in favor of the non-monophyly of this group. Also, *Tillandsia biflora* complex clades N and P show distinct biogeographical patterns, discussed below. The herein obtained phylogenetic relationships could help define which morphological attributes characterize these two independent lineages.

Three strongly supported lineages in successive sister relationship conform *Tillandsia* subg. *Tillandsia*, namely the *T. secunda* clade, the *T. paniculata* clade, and the expanded clade K. These results are consistent with Barfuss et al. (2016), except that these authors recovered the *T. secunda* clade as the sister lineage of the expanded clade K instead of the *T. paniculata* clade as in our phylogeny. While we obtained strong statistical support for these relationships, Barfuss et al. (2016) obtained low statistical support in the ML and parsimony analyses, albeit a PP=0.85 in the Bayesian analysis. The expanded clade K and its internal clades K.1 an K.2 have consistently been recovered as strongly supported monophyletic groups in previous (Chew et al., 2010; Sidoti, 2015; Barfuss et al., 2016; Pinzón et al., 2016; Montes-Azcué, 2020; Vera-Paz et al., 2022; Yardeni et al., 2022) and the present study. Furthermore, species relationships and composition of clades K.1 and K.2 are congruent across previous (Sidoti, 2015; Barfuss et al., 2016; Pinzón et al., 2016; Montes-Azcué, 2020; Vera-Paz et al., 2022; Yardeni et al., 2022) and our study. While all internal relationships of the less diverse clade K.1 are strongly supported, several relationships within clade K.2, which contains the type species of the genus *Tillandsia*, *T. utriculata*, are subtended by short branches and lack strong statistical support. Resolution of these low supported phylogenetic relationships would require the use of more variable sequence data, such as low- or single copy nuclear loci. Although the *mat*K-*trn*K region analysis of Granados Mendoza et al. (2017) included a dense taxon sampling of the expanded clade K, internal resolution of clades K.1 and K.2 was extremely limited. Other studies focused on particular species complexes or groups, such as the *T. ionantha* complex (Ancona et al., 2022), pseudobulbous species (Chew et al., 2010), the *T. erubescens* complex (Granados Mendoza, 2008; Martínez-García et al., 2022), the *T. utriculata* complex (2016), and the *T. fasciculata* complex (Sidoti, 2015), used combinations of Sanger-sequenced DNA markers and/or morphology. When considering strongly supported phylogenetic relationships, our study is in overall agreement with plastid-based phylogenetic relationships recovered in those previous studies. However, there are some disagreements with the nuclear ribosomal phylogenetic context of Chew et al. (2010), who recovered a pair of species from clade K.2 (*T. pseudobaileyi*) and K.1 (*T. achyrostachys*) as sister to one another, with strong support.

Many of the phylogenetic relationships within clades K.1 and K.2 are assessed here for the first time. Phylogenetic position of species of proposed hybrid origin, such as *Tillandsia maya* (*T. balbisiana* × *T. brachycaulos*, Ramírez and Carnevali, 2003), *T. may-patii* (*T. brachycaulos* × *T. dasyliriifolia*, Ramírez and Carnevali, 2003), and *T. rothii* (*T. jaliscomonticola* × *T. rolandgosselinii*, Rauh, 1976) were consistently recovered as sister lineages of one of their proposed parental lineages, in agreement with the proposed hybrid origin of these species. Future studies using nuclear sequence data and phylogenetic network analyses could explicitly test these hybrid origin hypotheses and explore the prevalence of hybridization across the expanded clade K and its influence in the diversification of the group.

Time of origin and diversification of the expanded clade K

We present a time calibrated phylogenetic framework for the expanded clade K based on a comprehensive amount of plastome sequence data. To include key nodes for secondary calibrations, a representative sampling (16 out of 22 genera) of other Tillandsioideae lineages was incorporated, allowing to date the age of the reconstructed ancestors of the expanded clade K all the way back to the stem node of Bromeliaceae. The following discussion will focus on the nodes preceding this focal group.

Regardless of the differences in phylogenetic dating method, taxonomic sampling, and calibration sources (fossils vs. secondary calibrations), most of our crown age estimates fall within the age ranges estimated in previous studies, including the crown ages of Bromeliaceae (28.69 Mya vs. 96-19 Mya;

Givnish et al., 2004, 2007, 2011, 2014; Janssen and Bremer, 2004; Bouchenak-Khelladi et al., 2014; Zhou et al., 2018; Ramírez-Barahona et al., 2020), Core Tillandsioideae (11.42 Mya vs. 12.9-8.7 Mya; Givnish et al., 2011, 2014; Kessous et al., 2020; Loiseau et al., 2021; Möbus et al., 2021), and tribe Tillandsieae (8.8 Mya vs. 8.8-6.5 Mya; Kessous et al., 2020; Loiseau et al., 2021; Möbus et al., 2021). In contrast, crown ages of subfamily Tillandsioideae (17.7 Mya vs. 15.2-13.3 Mya; Givnish et al., 2011, 2014; Ramírez-Barahona et al., 2020; Möbus et al., 2021) and *Tillandsia* subg. *Tillandsia* (7.34 Mya vs. 6.6 Mya; Möbus et al., 2021) are estimated to be older than in previous studies. In both cases, differences in the employed taxon samplings and the use herein of a considerably greater amount of sequence data could explain these age differences. Our sampling, for instance, does not include representatives from tribe Glomeropitcairnieae, sampled by Givnish et al. (2011, 2014) and Ramírez-Barahona et al. (2020), which could have influenced our crown age estimates of subfamily Tillandsioideae. In the case of *T.* subg. *Tillandsia*, Möbus et al. (2021) only sampled two species, *T. utriculata* from the expanded clade K and *T. malzinei* from the *T. paniculata* clade, therefore representing the node succeeding *T.* subg. *Tillandsia* from our phylogeny for which we estimated an age of 6.94 Mya, which is closer to their age estimate for *T.* subg. *Tillandsia*. Our taxon sampling design, in combination with a comprehensive amount of sequence data, allowed us to calibrate for the first time several nodes, not only within our focal group, the expanded clade K, but also in other Tillandsioideae lineages. We expect that this dated phylogenetic framework will facilitate future macroevolutionary studies and provide reference age estimates for performing secondary calibrations for other Tillandsioideae lineages.

Evolution in time and space of the expanded clade K

Origin of the expanded clade K

Our aim was to trace the geographic origin of the focal group expanded clade K and we found the combined area of the Boreal and South Brazilian dominions (B), South American transition zone (C), and Pacific dominion (D) as the most probable ancestral area for the nodes subtending subfamily Tillandsioideae, Core Tillandsioideae, tribe Tillandsieae, and a clade including all tribe Tillandsieae species, excepting the genus *Guzmania*. The three remaining preceding nodes of the expanded clade K, including the one corresponding to *Tillandsia* subg. *Tillandsia*, were reconstructed in a smaller ancestral combined area restricted to the Boreal and South Brazilian dominions (B) and South American transition zone (C). To our knowledge, few studies have performed ancestral area reconstructions for these relevant nodes, focusing on different lineages and taxonomic scales within Bromeliaceae (Givnish et al., 2011; Pinzón et al., 2016; Granados Mendoza et al., 2017; Kessous et al., 2020). In their study of the family Bromeliaceae, Givnish et al. (2011) estimated the area of the Andes and south Chile as the most probable ancestral area for the node subtending subfamily Tillandsioideae. The latter area roughly corresponds to our area delimitation of the South American transition zone (C) and Pacific dominion (D), except for the North East portion of South America that Givnish et al. (2011) circumscribed as part of a different area that also included the Caribbean and southeastern USA, recovered as the second most probable ancestral area for this node by these authors. However, our estimation for this node needs to be taken with caution, since our taxon sampling lacks representatives of tribe Glomeropitcairnieae, which is restricted to the Lesser Antilles and Northeastern Venezuela and, together with tribe Catopsideae, forms the sister lineage (non-Core Tillandsioideae) of the remaining Tillandsioideae species (Core Tillandsioideae). Therefore, this is a key lineage for inferring where subfamily Tillandsioideae arose.

The area of the ancestor of Core Tillandsioideae has been previously reconstructed in either the individual area of the Andes and southern Chile (Givnish et al., 2011) or the combined area of the

Andes and southern Chile, Chacoan dominion, and Atlantic Forest (Kessous et al., 2020). Our results for this node are similar to those obtained by Givnish et al. (2011), with the exception that we recovered a combined ancestral area that also includes the Boreal and South Brazilian dominions (B). The reconstruction of the Atlantic Forest and Chacoan dominion as part of the most probable area for Core Tillandsioideae by Kessous et al. (2020) could be explained by the emphasis that these authors put on sampling Tillandsioideae representatives from these two regions. Both tribe Tillandsieae (Givnish et al., 2011; Granados Mendoza et al., 2017; Kessous et al., 2020) and *Tillandsia* subg. *Tillandsia* (Pinzón et al., 2016; Granados Mendoza et al., 2017) have been reconstructed in previous studies in areas overlapping the herein circumscribed South American transition zone (C) and Pacific dominion (D). The latter is in partial agreement with our results since, for tribe Tillandsieae, we recovered a combined area that also includes the Boreal and South Brazilian dominions (B), whereas for *T.* subg. *Tillandsia* we reconstructed the combined area of the Boreal and South Brazilian dominions (B) and the South American transition zone (C).

Differences in the estimated ancestral areas for the nodes preceding the expanded clade K among our and previous studies are most probably the result of the alternative area circumscriptions and reconstruction strategies used across studies. Additionally, both previous studies and ours have different focal groups, which could result in geographically biased taxon samplings, impacting the obtained ancestral area probabilities at deeper nodes. The recovery of the Boreal and South Brazilian dominions (B) as part of the most probable combined area for all nodes preceding the expanded clade K in our study could be related to our denser representation of species from this area (46 spp.) compared with previous studies (1 sp., Givnish et al., 2011; 28 spp., Pinzón et al., 2016; 93 spp., Granados Mendoza et al., 2017; Kessous et al., 2020). Furthermore, various studies have documented an active interchange between the Amazonia (≈ Boreal and South Brazilian dominions) and the Andes (≈ South American transition zone; Pérez-Escobar et al., 2022), which, although not formally tested herein, could have occurred in early Tillandsioideae lineages, maintaining to a certain extent these two areas as a single unit.

Our taxonomic sample includes 17 species of *Tillandsia* subg. *Tillandsia* distributed in the Pacific dominion (D). Nonetheless, this individual area or combined areas including it were not recovered as the most probable ancestral area for this subgenus, as well as one preceding and one succeeding node, suggesting an area shift from the combined area of the Boreal and South Brazilian dominions (B) and the South American transition zone (C) to the noncontiguous individual area of the Mexican transition zone + Mesoamerican dominion + Nearctic region (F) at the node subtending the expanded clade K. Our temporal framework suggests that this noncontiguous area shift occurred at least 4.86 Mya, well after the first (12 Mya), but before the last intense building pulse of the north-central Andes (4.5 Mya; Rull and Carnaval, 2020), when mean elevation of Central and Venezuelan Andes had already reached ca. 4000 m and the Northern Andes ca. 3500 m (Hoorn et al., 2010). The timing of the formation of the Panama Isthmus has been intensely debated, with studies proposing the presence of land-bridges as early as 23 Mya (Bacon et al., 2015) and land closure dating estimates as recent as 2.8 Mya (O'Dea et al., 2016). Regardless of whether a land or mountain chain connection was already established by the time the ancestor of the expanded clade K dispersed from South- to North- and Central America, our analysis failed to identify the Pacific dominion (D), which includes the region previously occupied by the Panama Isthmus and the Northern and Venezuelan Andes, for the nodes immediately preceding the expanded clade K, suggesting long-distance dispersal as a plausible explanation. Three additional, independent colonization events from the combined area of the Boreal and South Brazilian dominions (B) and the South American transition zone (C) to Central- and North America are herein reconstructed for lineages within the *T. biflora* complex (clade N) and *T.* subg. *Viridantha*, as well as the genus

Pseudalcantarea, at 3.66, 2.29, and 1.79 Mya, respectively (Fig. 3 and Supplementary material 4). These results suggest that long-distance dispersal could have occurred several times in the evolution of Tillandsioideae. However, the different modes of dispersal of the latter lineages should be formally assessed in future studies including denser taxon samplings for these focal groups and their close relatives.

Our biogeographical model selection favored a founder-event speciation, in support of an hypothesis of North- and Central America having been colonized, if not entirely, at least by some long-distance dispersal events from larger South American tillandsioid ancestral populations, which could have been facilitated by the characteristic hair-like appendages (plumose coma) of their anemochorous seeds (Smith and Downs, 1977; Barfuss et al., 2016). *Tillandsia* seeds have small sizes, large comas, and seed coats composed of dead air-filled cells (Chilpa-Galván et al., 2018), attributes that are thought to aid in air flotation (Madison, 1977). Furthermore, compared to other anemochorous species, terminal velocity of falling seeds in still air is slow in *Tillandsia* (Chilpa-Galván et al., 2018), characteristic of seeds with high dispersal ability, being more prone to be dispersed over long distances (Nathan et al., 2011). Long-distance dispersal from South- to North- and Central America has been suggested for other non-Tillandsioideae bromeliad lineages including the genus *Hechtia* (Hechtioideae), from the Andes and southern Chile at least 10.3 Mya, a species of *Aechmea* (Bromelioideae), from the Brazilian Shield ca. 5 Mya, and the genus *Fosterella* (Pitcairnioideae), from the Central Andes (Givnish et al., 2011). Additionally, Bromeliaceae are known to have undergone a transcontinental long-distance dispersal with the colonization of western Africa by *Pitcairnia feliciana* (Pitcairnioideae) from South America ca 9.3 Mya (Givnish et al., 2004). Similar long dispersal events to North- and Central America have been reported in other angiosperm lineages possessing wind-dispersed seeds, such as the epiphytic genus *Cycnoches* (Orchidaceae; Pérez-Escobar et al., 2017) and the lianescent genus *Amphilophium* (Bignoniaceae; 11.2 Mya; Thode et al., 2019) from the Amazonia and the Atlantic Forest, respectively.

Dispersal route of the expanded clade K in the Neotropics

Within North- and Central America, a region spanning the Mesoamerican dominion, herein Pacific Lowlands and Balsas Basin provinces + El Cabo de Baja California district (G) and Mosquito, Veracruzan and Yucatán Pensinsula provinces (I), and the Mexican transition zone (H; sensu Morrone et al., 2022) is reconstructed as the ancestral area of the expanded clade K. To our knowledge, only two other studies have performed ancestral area reconstructions for this node. Granados Mendoza et al. (2017) recovered North- and Central America as the most probable ancestral area for the expanded clade K, whereas Pinzón et al. (2016) reconstructed this node at an ancestral area, therein referred to as Pacific Ocean coast and mountainous region, equivalent to the Mesoamerican dominion (sensu Morrone et al., 2022; herein areas G and I). This is in general agreement with our results; however, comparison to Granados Mendoza et al. (2017) within the expanded clade K is limited by the broad area circumscription and low phylogenetic resolution of their study. Failure to reconstruct the Mosquito, Veracruzan and Yucatán Peninsula provinces (I) as part of the ancestral area of the expanded clade K by Pinzón et al. (2016) could be attributed to differences in taxon sampling designs, which in their case was strongly focused on sampling representatives from their focal group, the *Tillandsia utriculata* species complex, which is one of the various lineages of clade K.2. Regardless of the taxon sampling differences, reconstruction for the shared sampled nodes between Pinzon et al. (2016) and the present study coincide in general.

The ancestor of the expanded clade K is herein dated to have colonized the Mesoamerican dominion and the Mexican transition zone by 4.86 Mya, a time when most of the Mexican highlands were already formed (Sierra Madre Oriental, 60–40 Mya; Sierra Madre Occidental, 54–6 Mya; Sierra Madre del

Sur, 60–18 Mya; Mastretta-Yanes et al., 2015). Models suggest that during the Pliocene (5.33–2.58 Mya) these highlands were mostly dominated by tropical semi-deciduous and deciduous forest and warm-temperate mixed forest in their central to southernmost portions, and temperate conifer forest in the northernmost portion of the Sierra Madre Oriental (Salzmann, et al., 2011). Among these vegetation types, the conifer forest, which together with the tropical-subtropical dry and moist broadleaf forests concentrate most of the expanded clade K species diversity, is modeled to have expanded to the Sierra Madre Occidental towards the present, following the global mean temperature colling trend (Salzmann, et al., 2011; Mastretta-Yanes et al., 2015). The two major lineages of the expanded clade K, clades K.1 and K.2, began diversifying ca. 1 to 2 My later within the same ancestral area. Then, a series of distribution range expansions and contractions occurred within these two clades, the majority of them in a period (2.8 Mya and onwards) characterized by pronounced climate fluctuations, derived from the glacial-interglacial climate oscillations (2.58 Mya to 11.7 kya; Cohen and Gibbard, 2011), and pronounced volcanic activity, mainly in the Trans-Mexican Volcanic Belt (1.5 Mya to date; revised by Mastretta-Yanes et al., 2015). Pleistocene temperature oscillations are thought to have generated changes in precipitation and seasonality, promoting highland vegetation elevational shifts, deriving in cycles of reduced gene flow at high-elevation refugia (interglacial periods) and admixture at lower elevations (glacial periods; Mastretta-Yanes et al., 2015). In addition to these climatic oscillations, the volcanic activity of the Trans-Mexican Volcanic Belt during the Pleistocene, persistent until today, derived in pronounced landscape modifications with the formation of large (> 3500 m) stratovolcanoes, which could not only offer new habitats for colonization, but also built new highland areas where the cycles of reduced gene flow and admixture could have taken place (Mastretta-Yanes et al., 2015). The genetic effects of such elevation shifts are expected to vary across plant lineages depending on their ecological affinities, with less cold tolerant groups being more prone to experience gene flow at lower elevations during glacial periods (Oaks, Cavender-Bares et al., 2011, and pines, Moreno-Letelier et al., 2013). Additionally, taxa particularly sensitive to changes in precipitation could have experienced relevant genetic consequences resulting from expansions and contractions of habitats with suitable humid conditions, such as those distributed in cloud forests, which are known to hold a substantial diversity of epiphytic lineages (Ramírez-Barahona and Eguiarte, 2013; Pérez-Escobar et al., 2022). The expanded clade K is composed of mostly epiphytic and epilithic species, two lifestyles particularly sensitive to water availability (Zotz and Hietz, 2001), therefore, Pleistocene precipitation oscillations could have had a significant effect in their genetic diversity and geographic distribution. Furthermore, the resulting mosaic of dry, semi-dry, and humid forest that characterize the Mexican highlands (Soberón Mainero, 2008) could have been associated with the evolution of photosynthetic metabolisms, as the expanded clade K is known to comprise both C3 and CAM representatives (Crayn et al., 2015; De La Harpe et al., 2020; Groot Crego et al., 2023). Additionally, as discussed in the previous section, the high dispersal capacity of the *Tillandsia* seeds could have played a significant role in their ability to colonize new areas within North-, Central America, and the Caribbean.

Colonization of the Antillean subregion + SE USA (E) is here reconstructed exclusively within clade K.2 in six independent events occurring not earlier than 0.68 Mya (*Tillandsia bartramii*) and as recent as 60 Kya (*T. schiedeana*). Within the Antillean subregion + SE USA (E), *T. schiedeana*, *T. capitata*, and *T. streptophylla* are only found on the Caribbean islands, *T. variabilis* and *T. setacea* are distributed both on the Caribbean islands and in southeastern USA, and *T. bartramii* is restricted to southeastern USA. These six species display the CAM photosynthetic pathway (Crayn et al., 2015), have ample geographic distributions, and some of them are known to form natural hybrids (*T. schiedeana*, *T. bartramii*, *T. variabilis*, and *T. streptophylla*; Rauh, 1976; Luther, 1985; Ramírez et al., 2000; Ehlers, 2004). Nine out of ten dispersal events to the Pacific and Boreal and South Brazilian dominions + South American transition zone (Y) were similarly only reconstructed within clade K.2 from 1.77-0.01

Mya, except for *T. punctulata* of clade K.1, from 2.35 Mya. The latter dispersal events exclusively involved range expansions to the Pacific dominion (D), suggesting that equatorward dispersal of the expanded clade K occurred only at shallower divergences, at the earliest 2.35 Mya. Future phylogeographic studies combining population level samplings representing the breadth of the geographic distribution of the species (as in Pinzón et al., 2016; Ancona et al., 2022), nuclear NGS-derived multilocus data, and explicit biogeographical hypotheses, could permit addressing how and when these widely distributed species colonized the Caribbean region and Pacific dominion, and formally testing which morpho-physiological attributes could have facilitated their dispersal.

Although older than the expanded clade K (crown age 6.65 Mya), the genus *Hechtia* (Hechtioideae) shows a similar biogeographical pattern, with most of its diversification occurring during the last 4 My, albeit specializing into arid and semi-arid biomes, rather than into the ample range of climatic conditions inhabited by the species of the expanded clade K. Several taxa have been proposed to have diversified synchronously with the expanded clade K within the Mexican highlands (e.g. *Pseudotsuga menziesii*, Gugger et al., 2011; *Juniperus deppeana*, Martínez de León et al., 2022; *Quercus series Virentes*, Cavender-Bares et al., 2011; *Podocarpus matudae*, Ornelas et al., 2010; and *Dioon*, Dorsey et al., 2018) and, although their dispersal histories could have been shaped by the same climatic and orogenic processes, their biogeographic histories are as diverse as their phylogenetic and ecological affinities, adaptations, and geographic origins.

Conclusion

We newly sequenced and assembled 162 Tillandsioideae species using the cost-effective sequencing strategy Hyb-Seq, considerably expanding existing bromeliad genomic resources. While some recalcitrant nodes at the backbone of Core Tillandsieae and at some shallower divergences within clade K.2 remain with low statistical support, the analysis of complete plastomes resulted in an overall highly supported phylogenetic framework. The additional phylogenetic resolution herein obtained allowed to uncover new relationships that could aid in define which morphological attributes characterize previously delimited polymorphic species groups, such as the *Tillandsia biflora* complex. Our phylogenetic context points towards the need for further revision of the currently adopted classification in the light of additional sequence data and considering evolutionary processes, such as hybridization, to interpret the consistently recovered phylogenomic discordance between nuclear and plastid evidence. The expanded clade K colonized the Mexican transition zone and Mesoamerican dominion by long-distance dispersal from a combined area of the Boreal and South Brazilian dominions and South American transition zone at least 4.86 Mya. Several subsequent dispersion events occurred northwards to the Nearctic, eastwards to the Caribbean, and southward to the Pacific dominion during the last 2.8 Mya. The pronounced climate fluctuations, derived from the glacial-interglacial climate oscillations, and the volcanic activity of the Trans-Mexican Volcanic Belt could have played an important role in the dispersal history of the expanded clade K. Future phylogeographic studies combining population level sampling representing the breadth of the geographic distribution of the species, nuclear NGS-derived multilocus data, and explicit biogeographical hypotheses, could address how and when widely distributed species colonized the Caribbean region and Pacific dominion, and formally test which morpho-physiological attributes could have facilitated their dispersal. Finally, future studies using nuclear sequence data and phylogenetic network analyses could formally test previous hybrid origin hypotheses and explore the prevalence of hybridization across the expanded clade K, as well as other Tillandsioideae lineages, and its influence in the diversification of these groups.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

SIV-P and CGM conceived and designed the study. SIV-P, CGM, DD, AR, CM-A, GS, EG, IR-M, MF-C, XG-A, MB, AM-G, CH-L, MB, SW designed the taxon sampling and collected or provided the samples. SIV-P, CGM, DD, LC, and GS performed the laboratory work. SIV-P, DD, MJ, AR, CM-A, LC performed the bioinformatic process. SIV-P, CGM, CM-A, RH-G, SM, LS-G designed and performed the analyses. SIV-P drafted the manuscript. SIV-P, CGM, DD, MJ, AR, CM-A, RH-G, SM, LS-G, GS, EG, IR-M, LC, MF-C, XG-A, AM-G, CH-L, MB, SW proofread and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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Tables

Table 1. Tested biogeographic models are Dispersal-Extinction-Cladogenesis (DEC), Dispersal-Vicariance-Like (DIVALIKE), and BayArea-like (BAYAREALIKE) with and without the founder-event speciation (*j*) parameter. The rate of dispersal/range addition (d) and rate of extinction/range contraction (e) parameters were allowed to vary freely. Resulting log-likelihood (LnL) are indicated for each model and that of the best-fitting model is highlighted in bold for each analysis as selected by the Akaike's information criterion weight (AICwt).

Models	LnL	*d*	*e*	*j*	AIC	AIC-wt
Full sampling analysis						
DEC	-461.300	0.521	0.212	0	926.600	0.730
DEC+*j*	-461.300	0.790	0.958	1.0E-05	928.600	0.270
	p = 1					
DIVALIKE	-486.800	1.036	1.526	0	977.600	0.730
DIVALIKE+*j*	-486.800	1.591	2.015	1.0E-05	979.600	0.270
	p = 1					
BAYAREALIKE	-465.400	0.081	0.220	0	934.800	0.007
BAYAREALIKE+*j*	**-459.400**	0.085	0.239	0.005	924.800	0.993
	p = 5.0E-04					
***Tillandsia* subg. *Tillandsia* analysis**						
DEC	-464.300	0.048	0.015	0	932.600	6.8E-08
DEC+*j*	-446.800	0.048	0.015	1.00E-05	899.600	0.999
	p = 3.3E-9					
DIVALIKE	-467.400	0.054	0.021	0	938.700	0.058
DIVALIKE+*j*	-463.600	0.054	0.021	1.00E-05	933.100	0.942
	p = 0.006					
BAYAREALIKE	-357.000	0.035	0.087	0	718.00	0.280
BAYAREALIKE+*j*	**-355.100**	0.027	0.057	1.00E-05	716.200	0.720
	p = 0.049					

DISCUSIÓN GENERAL

El presente estudio contribuye con 162 plastomas nuevos a los recursos genómicos de la familia Bromeliaceae. La mayoría de ellos corresponden a especies del grupo focal clado expandido K, del *T.* subgen. *Tillandsia*, pero también a especies representantes de otros subgéneros y complejos de especies de *Tillandsia*, así como otros géneros de Tillandsioideae. El análisis de 200 plastomas completos derivó en un contexto filogenético altamente apoyado, con únicamente 13 de 198 nodos (excluyendo la raíz) con PP <0.85.

Tillandsia subgen. *Tillandsia* está conformado por tres linajes altamente apoyados en hermandad sucesiva, incluyendo el clado de *T. secunda*, el clado de *T. paniculata* y el clado expandido K. Este resultado es consistente con Barfuss et al. (2016), quien recuperó estos mismos tres linajes aunque en su estudio el clado de *T. secunda* es hermano del clado expandido K, en lugar del clado de *T. paniculata* como fue recuperado en el presente trabajo. El clado expandido K y sus principales linajes K.1 y K.2 han sido recuperados consistentemente como grupos monofiléticos con alto apoyo aquí y estudios previos (Barfuss et al., 2016; Chew et al., 2010; Granados Mendoza et al., 2017; Montes-Azcué, 2020; Pinzón et al., 2016; Sidoti, 2015; Vera-Paz et al., 2022; Yardeni et al., 2022). Muchas de las relaciones filogenéticas al interior de los clados K.1 y K.2 fueron evaluadas por primera vez en el presente estudio, sin embargo, varias de las relaciones obtenidas al interior de dichos linajes, así como la composición de sus especies son congruentes con estudios previos (Barfuss et al., 2016; Granados Mendoza et al., 2017; Montes-Azcué, 2020; Pinzón et al., 2016; Sidoti, 2015; Vera-Paz et al., 2022; Yardeni et al., 2022). Todas las relaciones internas del clado K.1 están fuertemente apoyadas, mientras que algunas relaciones dentro del clado K.2, el cual contiene a la especie tipo del género *Tillandsia*, *T. utriculata*, presentan bajo apoyo estadístico. La resolución de dichas relaciones filogenéticas recalcitrantes podría abordarse en estudios futuros que combinen el uso de loci nucleares de pocas copias o copia simple, los cuales son generalmente más variables que las regiones del cloroplasto (Sang, 2002; Zimmer & Wen, 2012). Varias especies propuestas como de origen híbrido (e.g. Tillandsia maya (*T. balbisiana* × *T. brachycaulos*; Ramírez & Carnevali, 2003) fueron recuperadas como linajes hermanos de uno de sus linajes parentales putativos, en concordancia con el origen híbrido propuesto para estas especies.

El marco filogenético fechado aquí obtenido para el clado expandido K se basó en el análisis de una cantidad considerable de datos de secuencias plastídicas e incorporó un muestreo representativo (16 de 22 géneros) de otros linajes de Tillandsioideae, permitiendo estimar la edad de los ancestros del clado expandido K hasta el nodo troncal de Bromeliaceae. Lo anterior permitió fechar por primera vez varios nodos, no solo dentro de nuestro grupo focal, el clado expandido K, sino también en otros linajes de Tillandsioideae. El contexto filogenético fechado aquí propuesto podría facilitar futuros estudios macroevolutivos, siendo fuente de edades de referencia para realizar calibraciones secundarias para otros linajes de Tillandsioideae. Resultado del análisis de fechamiento aquí implementado, se estimó una edad corona para *T.* subgen. *Tillandsia* más antigua (7.34 m.a.) que el estudio de Möbus et al. (6.6 m.a.; 2021). Diferencias entre los muestreos taxonómicos empleados en el presente estudio y el estudio de Möbus y colaboradores (2021), así como el uso aquí de una cantidad considerablemente más amplia de datos de secuencias, podrían explicar las diferencias en los estimados de edad para este subgénero. Por ejemplo, Möbus et al. (2021) solo muestrearon dos especies de *T.* subgen. *Tillandsia*, i.e. *T. utriculata* del clado expandido K y *T. malzinei* del clado de *T. paniculata*, lo que representa un nodo posterior al correspondiente a *T.* subg. *Tillandsia* de la filogenia aquí presentada, para el cuál se estimó una edad de 6.94 m.a., que es más cercano a la edad estimada por dichos autores para *T.* subgen. *Tillandsia.*

Nuestro objetivo fue trazar el origen geográfico del grupo focal clado expandido K y reconstruimos el área combinada de los dominios Boreal y Sur Brasileños (B) y la zona de transición de Sudamérica (C) como el área ancestral para los tres nodos que preceden al clado expandido K, incluyendo el que corresponde a *T.* subgen. *Tillandsia*. Este subgénero ha sido reconstruido en estudios previos en áreas que se sobreponen con la zona de transición Sudamericana y dominio del Pacífico aquí circunscritas (Granados Mendoza et al., 2017; Pinzón et al., 2016), lo cual concuerda parciamente con nuestros resultados. Las diferencias en las áreas ancestrales estimadas para los nodos que preceden el clado expandido K entre el presente y estudios previos son probablemente el resultado de los diferentes grupos focales, áreas propuestas y estrategias de reconstrucción empleadas.

Nuestro muestreo taxonómico incluye 17 especies de *T.* subgen. *Tillandsia* distribuidas en el dominio del Pacífico (D). No obstante, esta área individual o áreas combinadas incluyéndola no fueron recuperadas como el área ancestral más probable para este subgénero, así como un nodo anterior y uno posterior, sugiriendo un cambio de área desde el área combinada de los dominios Boreal y Sur Brasileños (B) y la zona de transición Sudamericana (C) hacia el área individual no continua de la zona de transición Mexicana + el dominio Mesoamericano + la región del Neártico (F) en el nodo que sustenta el clado expandido K. Nuestro marco temporal sugiere que este cambio entre áreas no contiguas ocurrió al menos hace 4.86 m.a., mucho después del primero (12 m.a.), pero cerca del último pulso intenso de formación de los Andes Norte-Centrales (4.5 m.a.; Rull and Carnaval, 2020), cuando la elevación media de los Andes Centrales y los Andes Venezolanos habían ya alcanzado ca. 4,000 m y los Andes Centrales ca. 3,500 m (Hoorn et al., 2010). El tiempo de formación del Istmo de Panamá ha sido debatido intensamente, con estudios que proponen la presencia de puentes terrestres tan antiguos como 23 m.a. (Bacon et al., 2015) y estimaciones de tiempo del cierre tan recientes como 2.8 m.a. (O'Dea et al., 2016).

Independientemente de si estaba o no establecida una conexión de tierra o una cadena montañosa al tiempo en que el ancestro del clado expandido K se dispersó del Sudamérica a Norte- y Centroamérica, nuestro análisis no logró identificar el dominio del Pacífico (D), que incluye la región previamente ocupada por el Istmo de Panamá y los Andes del Norte y Venezolanos, para los nodos que preceden inmediatamente el clado expandido K, sugiriendo la dispersión a larga distancia como la explicación plausible. El modelo biogeográfico elegido favorece la especiación por evento fundador, en apoyo de una hipótesis de que Norte- y Centroamérica fueron colonizadas, si no por completo, al menos por algunos eventos de dispersión a larga distancia desde poblaciones ancestrales más grandes de Tillandsioideae Sudamericanas, lo que pudo haber sido facilitado por los apéndices plumosos (coma plumosa) característicos de sus semillas anemócoras (Barfuss et al., 2016; Smith & Downs, 1977).

El presente estudio reconstruye que el ancestro del clado expandido K colonizó el dominio Mesoamericano y zona de transición Mexicana hace 4.86 m.a., cuando la mayoría de las cadenas montañosas mexicanas estaban ya formadas (Sierra Madre Oriental, 60-40 m.a.; Sierra Madre Occidental, 54-6 m.a.; Sierra Madre del Sur, 60-18 m.a.; Mastretta-Yanes et al., 2015). Los dos linajes principales del clado expandido K, los clados K.1 y K.2, comenzaron a diversificar ca. 1 a 2 m.a. después dentro de la misma área ancestral. Posteriormente, una serie de expansiones y contracciones en los rangos de distribución ocurrieron dentro de esos dos clados, la mayoría de ellos en un periodo (2.8 m.a. al presente) caracterizado por fluctuaciones climáticas pronunciadas, derivadas de las oscilaciones climáticas del periodo glacial-interglacial (2.58 m.a. a 11.7 miles de años; Cohen and Gibbard, 2011), así como la pronunciada actividad volcánica, principalmente en la Faja Volcánica Transmexicana (fechada en 1.5 m.a.; revisado por Mastretta-Yanes et al., 2015). La colonización de la región de las Antillas + SE EUA (E) se reconstruyó exclusivamente dentro del clado K.2, en seis

eventos independientes que ocurrieron hace no más de 0.68 m.a. (*Tillandsia bartramii*) y el más reciente hace 60 mil años (*T. schiedeana*). Nueve de los diez eventos de dispersión hacia los dominios Pacífico y Boreal y Sur Brasileños + zona de transición Sudamericana (Y) fueron reconstruidos de forma similar exclusivamente dentro del clado K.2 hace 1.77-0.01 m.a., excepto por *T. punctulata* del clado K.1 hace 2.35 m.a. Los eventos de dispersión posteriores solo involucraron expansiones del rango de distribución hacia el dominio del Pacífico (D), sugiriendo que la dispersión hacia el ecuador del clado expandido K ocurrió únicamente en divergencias someras, hace no más de 2.35 m.a. Estudios filogeográficos futuros que combinen muestreos poblacionales que representen la amplitud de la distribución geográfica de las especies (como en Pinzón et al., 2016; Ancona et al., 2022), datos nucleares derivados de NGS, e hipótesis biogeográficas explícitas, podrían ayudar a contestar cómo y cuándo esas especies ampliamente distribuidas colonizaron la región del Caribe y el dominio del Pacífico, así como poner formalmente a prueba qué atributos morfo-fisiológicos pudieron haber facilitado dicha dispersión.

CONCLUSIONES Y PERSPECTIVAS A FUTURO

Se secuenciaron y ensamblaron plastomas nuevos correspondientes a 162 especies de Tillandsioideae utilizando la estrategia de secuenciación de alto rendimiento Hyb-Seq, lo que representa una contribución significativa a los recursos genómicos existentes para Bromeliaceae. Aunque algunos nodos profundos de Core Tillandsieae y otros al interior del clado K.2 permanecen con poco apoyo estadístico, el análisis de plastomas completos resultó en general en un marco filogenético altamente apoyado. El clado expandido K colonizó la zona de transición Mexicana y el dominio Mesoamericano por dispersión a larga distancia desde un área combinada de los dominios Boreal y del Sur de Brasil y la zona de transición Sudamericana al menos hace 4.86 millones de años. Posteriormente, varios eventos de dispersión ocurrieron hacia el norte al Neártico, hacia el este al Caribe y hacia el sur al dominio del Pacífico durante los últimos 2,8 millones de años. Las fluctuaciones climáticas pronunciadas, derivadas de las oscilaciones climáticas glaciales-interglaciales, y la actividad volcánica de la Faja Volcánica Transmexicana podrían haber jugado un papel importante en la historia de dispersión del clado expandido K. Futuros estudios filogeográficos que combinen muestreos a nivel poblacional que representen la amplitud de la distribución geográfica de las especies, datos nucleares multilocus derivados de NGS e hipótesis biogeográficas explícitas podrían abordar cómo y cuándo las especies de amplia distribución colonizaron la región del Caribe y el dominio del Pacífico, y probar formalmente qué atributos morfofisiológicos podrían haber facilitado su dispersión. Finalmente, estudios futuros que utilicen datos de secuencias nucleares y análisis de redes filogenéticas podrían probar formalmente hipótesis previas sobre el origen híbrido de algunas especies y explorar la prevalencia de la hibridación en el clado expandido K, así como en otros linajes de Tillandsioideae, y su influencia en la diversificación de estos grupos. La resolución filogenética adicional aquí obtenida permitió descubrir nuevas relaciones que podrían ayudar a definir qué atributos morfológicos caracterizan grupos de especies polimórficas previamente delimitados, como el complejo *Tillandsia biflora*. Los resultados aquí obtenidos apuntan hacia la necesidad de una revisión de la clasificación actualmente adoptada, usando datos de secuenciación adicionales y considerando los procesos evolutivos (e.g. hibridación) para interpretar la discordancia filogenómica recuperada consistentemente entre la evidencia nuclear y plastídica.

El contexto filogenético y la información geográfica aquí generados podría además ser utilizados para estimar las tasas de especiación, extinción y diversificación neta del clado expandido K, así como para poner a prueba si la colonización de los linajes a diferentes tipos de ambientes tuvo un efecto en dichas tasas evolutivas. El análisis de variables bioclimáticas para la modelación de nichos,

a partir de los datos geográficos aquí generados, podría ayudar a conocer la capacidad de las especies del clado K para adaptarse a condiciones distintas a las de su nicho ancestral o si hay conservadurismo de nicho ecológico. Los recursos genéticos producidos mediante Hyb-Seq no analizados aquí, como los loci nucleares o mitocondriales, podrían ser analizados para explorar si existe discordancia filogenética entre los diferentes compartimentos genómicos. Adicionalmente, los datos nucleares podrían ser utilizados para estimar por métodos bioinformáticos los niveles de ploidía de las especies del clado expandido K, así como de otros linajes de Tillandsioideae, lo que permitiría estudiar la evolución en los cambios en los niveles de ploidía en este grupo focal.

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