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Sistemática

La poliploidía y su efecto en los atributos foliares en Asteraceae: una aproximación de la biología comparada

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Me permito informar a usted que en la reunión del Subcomité por Campo de Conocimiento de Ecología, Manejo Integral de Ecosistemas, Biología Evolutiva y Sistemática del Posgrado en Ciencias Biológicas, celebrada el día 13 de mayo de 2019, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la alumna **RIVERA PÉREZ PATRICIA** con número de cuenta **406076144** con la tesis titulada: **"La poliploidía y su efecto en los atributos foliares en Asteraceae: una aproximación de la biología comparada"**, realizada bajo la dirección de la **DRA. TERESA MARGARITA TERRAZAS SALGADO:**

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“And here's to the fools who dream

Crazy as they may seem

Here's to the hearts that break

Here's to the mess we make”

Hurwitz, Pasek, Paul

Audition (The fools who dream), 2016

Índice

La poliploidía y su efecto en los atributos foliares en Asteraceae: una aproximación de la biología comparada

Resumen

La variación en los caracteres vegetativos de las plantas suele asociarse con la adaptación a ambientes específicos. La disponibilidad de agua y nutrientes, y la temperatura media anual son características ambientales que generalmente se encuentran relacionadas con la variación en las plantas. Las hojas, como órganos principalmente especializados para la fotosíntesis y respiración, tienen un gran número de caracteres morfológicos a los cuales se les han dado interpretaciones adaptativas. Sin embargo, la variación foliar también puede estar asociada a la forma de crecimiento y el nivel de ploidía de la planta. En este trabajo utilizamos métodos comparativos filogenéticos para evaluar la hipótesis de que la filogenia de la familia Asteraceae influye en la relación entre caracteres foliares y caracteres citogenéticos a distintos niveles jerárquicos, particularmente a nivel de tribu. Nuestros resultados muestran una gran diversidad en la arquitectura y anatomía foliar de la familia, incluso dentro de un ambiente con disponibilidad restringida de agua y suelo. Los análisis comparativos filogenéticos mostraron que la relación entre los caracteres foliares y los números cromosómicos presenta una señal filogenética. También encontramos que los principales ejes de variación de las hojas

se relacionan significativamente con la forma de crecimiento. Concluimos que la diversidad en los caracteres foliares de la familia está relacionada con la forma de crecimiento y el número cromosómico de la planta, y que probablemente esta diversidad ha sido clave para que Asteraceae se distribuya en todo el mundo y sea exitosa en ambientes perturbados.

Abstract

Vegetative variation in plants is usually associated with adaptation to specific environments. Water and nutrient availability together with mean annual temperature are usually related to plant variation. The leaves, as organs specialized on photosynthesis and respiration, have a large number of characters interpreted as adaptive. However, leaf variation can be also associated with plant growth form and ploidy level. In this work, we use phylogenetic comparative methods to evaluate the hypothesis that Asteraceae phylogeny influences the relationships between leaf characters and cytogenetic characters at different hierarchical levels, particularly at the tribe level. Our results show that there is a great diversity in the leaf architecture and anatomy of the family, even within an environment with restricted water and soil availability. Phylogenetic comparative methods showed that the relationship between leaf characters and chromosome numbers present a phylogenetic signal. We also found that the main axes of leaf variation are significantly related to growth form. We conclude that the diversity in the foliar characters of the family is related to both growth form and chromosome number of the plant and we propose that this leaf diversity could be important for the success of Asteraceae worldwide, particularly in disturbed environments.

Introducción general

Las plantas han jugado un papel importante en la historia de la Tierra. Por ejemplo, al transformar una atmósfera reductora a una oxidante o secuestrando carbono atmosférico (Woodward et al., 1995; Elbert et al., 2012; Niklas 2016). Se estima que 90% de la productividad primaria del planeta es generada por las plantas vasculares (Belnap 2012; Harrison y Morris 2017). Las hojas de cultivos y bosques con predominancia de angiospermas contribuyen a dicha productividad (Brodribb y Feild 2010). La variación en hojas está relacionada con la productividad primaria porque la función principal de las hojas es capturar luz y CO_2 (Brodribb y Feild 2010). Los procesos biofísicos básicos detrás de esta función están fuertemente conservados en los diferentes grupos de plantas; sin embargo, la diversidad en los caracteres micro y macroscópicos foliares aumenta a mayores niveles de organización. Esta variación foliar es un componente importante para entender la biodiversidad porque relaciona explícitamente las estrategias evolutivas de las especies de plantas con los ciclos de materia y energía de los ecosistemas (Harrison y Morris 2017).

A escala global, el espectro de variación foliar suele relacionarse con la adaptación de las plantas al ambiente (Westoby et al., 2002; Wright et al., 2004). Por ejemplo, en ambientes húmedos las hojas suelen ser suaves y tener mayor área foliar que en ambientes secos en los que predominan hojas esclerófilas y más

pequeñas. Sin embargo, también se ha comprobado que existe un amplio espectro de variación foliar dentro de cada comunidad (Diaz et al., 1998; Rivera et al., 2017). La variación microclimática, las interacciones con herbívoros o polinizadores y los diversos orígenes filogenéticos y biogeográficos de las especies contribuyen en gran medida a la variación dentro de las comunidades (Herrera 1992). Particularmente los eventos de duplicación completa del genoma (poliploidia) tienen efectos importantes en la estructura foliar. La poliploidía puede generar interacciones entre distintos genomas parentales y permitir la generación de “novedades evolutivas” a nivel genético, que tienen repercusiones en la planta a nivel morfológico, ecológico y fisiológico (Osborn et al., 2003). Por ejemplo, Balao et al., (2011) encontraron que los niveles de ploidía y los tamaños genómicos tienen un efecto neto significativo sobre la evolución del fenotipo de poblaciones de *Dianthus broteri* Boiss. & Reut. Los autores estudiaron caracteres florales y foliares y concluyeron que para que cualquier relación entre poliploidía/contenido de ADN y fenotipo sea correctamente investigada, las relación de parentesco entre los organismos debe ser considerada.

Una aproximación actual es que la variación foliar refleja compromisos entre los requerimientos de las hojas para realizar sus funciones primarias de adquisición de energía y un conjunto de “restricciones” a nivel celular y organísmico, que incluyen la necesidad de soporte mecánico, la prevención de pérdida de agua, la forma de crecimiento, los patrones de desarrollo y los caracteres citogenéticos (Press 1999; Santiago y Wright 2007; Balao et al., 2011; John et al., 2013). Dado que estos compromisos suelen ser específicos para cada especie, muy probablemente son dependientes de la historia filogenética del organismo. Es decir,

que en especies más cercanamente relacionadas la variación en los caracteres foliares va a responder de forma parecida a un conjunto similar de restricciones.

El estudio de la variación foliar es complejo y requiere un muestreo amplio de plantas con un espectro de variación en los caracteres foliares; por ejemplo, que crezcan en distintos ambientes, con diversas formas de crecimiento y cuyas relaciones filogenéticas sean conocidas. La familia Asteraceae es un buen sistema para estudiar la variación foliar ya que es una de las familias de angiospermas más diversas. Los miembros de la familia presentan gran variación foliar, de formas de crecimiento y de números cromosómicos. Es cosmopolita y se encuentra bien representada en México. Se considera que más de la mitad de las especies presentes en nuestro país son endémicas (Villaseñor 2003, 2004). Las relaciones al interior de la familia están casi completamente resueltas (Panero y Funk 2008; Panero et al., 2014; Panero y Crozier 2016; Mandel et al., 2017) aunque las especies mexicanas se encuentran pobremente representadas en las hipótesis filogenéticas recientes. Con el objetivo de explorar en un contexto filogenético que conjunto de características de las plantas de la familia Asteraceae explican mejor su gran diversidad foliar, se plantearon los siguientes objetivos:

- Describir y documentar la variación de las hojas de representantes de Asteraceae en México.
- Investigar la posición filogenética de especies de Asteraceae nativas de México dentro de la filogenia más reciente de la familia.
- Utilizar la filogenia que incluya a representantes de los géneros más diversos de Asteraceae nativos de México para analizar los patrones de relación entre caracteres anatómicos foliares, citogenéticos y formas de crecimiento.

Para abordar los objetivos, en este trabajo se describe la variación foliar observada en especies de Asteraceae distribuidas en un matorral xerófilo de México (Capítulo 1). El capítulo 2 explora la variación asociada a la adaptación a un ambiente específico: El matorral xerófilo de la Reserva Ecológica del Pedregal de San Angel. El capítulo 3 aborda las relaciones filogenéticas dentro de la familia, poniendo énfasis en la posición filogenética de las especies mexicanas. Finalmente, se integra la información obtenida en este trabajo en un estudio de la variación foliar asociada a caracteres citogenéticos en un contexto filogenético (Capítulo 4).

Capítulo 1.

Descripción de la variación foliar en especies de Asteraceae distribuidas en un matorral xerófilo de México

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Research article

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Leaf architecture and anatomy of Asteraceae species in a xerophytic scrub in Mexico City, Mexico

Arquitectura y anatomía foliar de especies de Asteraceae en un matorral xerófilo de la Ciudad de México, México

Patricia Rivera[1, 2, 3], Teresa Terrazas[1], Alicia Rojas-Leal[1], José Luis Villaseñor[1]

Abstract:

Background and Aims: Leaf architecture and anatomy in the Asteraceae family are extremely diverse and have been studied from ecological, physiological and evolutionary perspectives. The aims of this study are to describe in detail leaf architecture and anatomy for 61 species belonging to 13 tribes of Asteraceae inhabiting a xerophytic scrub in Mexico City, Mexico and to discuss characters common to these tribes.

Methods: Mature and undamaged leaves of 61 species of Asteraceae were collected in southwestern Mexico City in the "Reserva Ecológica del Pedregal de San Ángel" (REPSA). Standard anatomical techniques were used to obtain permanent slides of cleared leaves and transverse and paradermal sections. The permanent slides were analyzed to describe leaf architecture and anatomy by tribe following the standard terminologies.

Key results: The results show a significant variation in leaf architecture although pinnate venation, brochidodromous secondary venation, areoles moderately developed and looped ultimate marginal venation predominate in the material studied. For anatomy, the most common traits are the striate cuticle, occurrence of trichomes and glands, as well as collateral vascular bundles with a parenchymatous sheath with girders in the Asteraceae present in this xerophytic scrub. There are no unique combinations of leaf characters for the family or any tribe.

Conclusions: Leaf diversity in the family and within each tribe is consistent with some previous reports. Anatomical descriptions are a fundamental piece of the evolutionary, ecological and physiological studies in Asteraceae. The results of this descriptive study will allow testing hypotheses about the factors causing leaf diversity in this plant lineage. More leaf anatomical studies of the family are necessary to confirm the patterns proposed for the tribes and the family.

Key words: Asteroideae, Compositae, Heliantheae, leaf anatomy, midvein, vascular bundle sheath, venation pattern.

Resumen:

Antecedentes y Objetivos: La arquitectura y anatomía foliar en la familia Asteraceae son extremadamente diversas y han sido estudiadas desde distintas perspectivas, como la ecológica, fisiológica y evolutiva. Los objetivos de este estudio son describir detalladamente la arquitectura y anatomía foliar de 61 especies incluidas en 13 tribus de la familia Asteraceae que habitan un matorral xerófilo en la Ciudad de México, México y discutir los caracteres comunes de las tribus.

Métodos: Se colectaron hojas maduras y sanas de 61 especies de Asteraceae al sureste de la Ciudad de México en la "Reserva Ecológica del Pedregal de San Ángel" (REPSA). Se usaron técnicas anatómicas estandarizadas para obtener hojas aclaradas y secciones transversales y paradermales. Estas preparaciones permanentes se analizaron para describir la arquitectura y anatomía foliar por tribu con base en la terminología convencional.

Resultados clave: Los resultados muestran una variación significativa en la arquitectura foliar, aunque predominan la venación pinnada, venación secundaria broquidódroma, areolas moderadamente desarrolladas y márgenes en bucles. En la anatomía los atributos más comunes son la cutícula estriada, la presencia de tricomas y glándulas, así como haces vasculares colaterales con una vaina parenquimatosa con extensiones en las Asteráceas presentes en este matorral xerófilo. No hay una combinación única de caracteres foliares para la familia o las tribus.

Conclusiones: La diversidad foliar dentro de la familia y al interior de cada tribu corresponde con reportes previos. Las descripciones anatómicas son una pieza fundamental de los estudios evolutivos, ecológicos y fisiológicos en Asteraceae. Los resultados de este estudio descriptivo permitirán probar hipótesis acerca de los factores que causan la diversidad foliar en este linaje de plantas. Se necesitan más estudios anatómicos foliares en Asteraceae para confirmar los patrones propuestos para las tribus y la familia.

Palabras clave: anatomía foliar, Asteroideae, Compositae, Heliantheae, patrón de venación, vaina del haz vascular, vena media.

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Introduction

As pointed out by Endress et al. (2000) the backbone of plant systematics is the comparative study of plant structure, morphology and anatomy. The information obtained through comparative morpho-anatomical studies is necessary for ecological, phylogenetic and evolutionary studies. For example, anatomical characters, especially of leaves, have been extensively used in the systematics of several plant families, including Bromeliaceae (De Faria et al., 2012), Myrtaceae (Al-Edany and Al-Saadi, 2012), Amaryllidaceae (Lin and Tan, 2015), Malpighiaceae (Araújo et al., 2010) and Asteraceae (Castro et al., 1997; Milan et al., 2006; Adedeji and Jewoola, 2008; Bombo et al., 2012; Akinnubi et al., 2014; Rojas-Leal et al., 2017; Lusa et al., 2018). Particularly in Asteraceae, leaf architecture and anatomical characters are extremely diverse (Bombo et al., 2012; Rojas-Leal et al., 2014, 2018) and have been studied from an ecological (Bercu et al., 2012; Moroney et al., 2013; Rivera et al., 2017; Ferraro and Scremin-Dias, 2018), physiological (Bondarev et al., 2003; McKown and Dengler, 2007; Santiago and Kim, 2009), and medical perspective (Cambi et al., 2006; Hulley et al., 2010; García-Sánchez et al., 2012).

Asteraceae is one of the largest families of angiosperms. While cosmopolitan, it is usually dominant in arid and temperate vegetation. Evidence suggests that North America, particularly Mexico, has been important for diversification in some of the most diverse lineages of the family (Noyes and Rieseberg, 1999; Suárez-Mota and Villaseñor, 2011; Villaseñor, 2018). Leaf diversity in the family has been associated to variable environmental conditions such as drought (Ferraro and Scremin-Dias, 2018), saline soils (Grigore and Toma, 2006; Bercu et al., 2012) and light conditions (Rossatto and Kolb, 2010). However, the environment alone does not explain the variation in leaf anatomical characters, because some of these as well as most leaf architectural characters can be constrained by the phylogenetic history, the growth form or the ploidy level of the species (Rivera et al., 2017).

In this paper, we describe the leaf architecture and anatomy of 61 species belonging to 13 tribes of the Asteraceae growing in a xerophytic scrub natural reserve within the central campus of the Universidad Nacional Autónoma de México. This area represents the remnants of the native flora of Mexico City before the extensive urbanization and it is one of the best-studied protected areas in Mexico in terms of its biodiversity (Lot and Cano-Santana, 2009, Céspedes et al., 2018). The aims of this work are to present a detailed description of the diversity in the leaf architecture and anatomy of Asteraceae occurring in a xerophytic scrub and to discuss characters common to the tribes present.

Materials and Methods

Site description

Plant samples were collected in the "Reserva Ecológica del Pedregal de San Ángel" (REPSA). The REPSA is located in southwestern Mexico City, between coordinates 19°18'21" - 19°20'11"N, 99°10'15" - 99°12'4"W and from 2200 to 2310 m a.s.l. The REPSA has a total area of 237.3 hm (UNAM, 2006). The average annual temperature is 15.6 °C and the total annual rainfall 833 mm. The climate type in the area is temperate subhumid (Cb(w1)w), with a distinctive rain season from June to October and a dry season from November to May. The substratum is volcanic rock from the Xitle volcano eruption, and the soil is scarce and shallow.

Sampling

Samples of mature and healthy leaves of 61 species were collected from August 2008 to December 2009, during two rainy seasons. Leaves were fixed with a formaldehyde-glacial acetic acid-ethyl alcohol solution (Ruzin, 1999) for 24 hours; then rinsed with tap water and stored in a glycerin-ethyl-alcohol-water solution (GAA 1:1:1) until sectioning. All the leaves were scanned with an HP Photosmart Plus scanner (Hewlett-Packard Development Company, Palo Alto, USA), with the highest resolution (2400 dpi) before sectioning. Leaf area was determined using an image analyzer according to the procedure described by Garnier et al. (2001). At least three leaves per species where cleared following Martínez-Cabrera et al. (2007). Three to six leaves per species were dehydrated in increasing concentrations of ter-butanol (10-100%) with an automatic tissue processor (TP1020 Leica, Westlar, Germany) remaining for 24 hours in each concentration. The tissues were embedded in paraffin and transverse and paradermal sections of 10 to 12 µm in thickness were cut with a rotatory microtome (RM2125 Leica, Westlar, Germany).

The sections were stained with safranin-fast green (Ruzin, 1999) and mounted on synthetic resin. Photographs and measurements were obtained through a microscope (BX-51 Olympus, Tokio, Japan) attached to an image analyzer (Image Pro, 2019).

Descriptions were made from cleared leaves and transverse and paradermal sections of three leaves per species. Voucher specimens are at the Herbario Nacional de México (MEXU) of the Instituto de Biología, Universidad Nacional Autónoma de México. Details of the voucher specimens as collector and collection number are given in Appendix. Leaf architecture follows Ellis et al. (2009). Leaf lamina and midvein descriptions follow Metcalfe and Chalk (1979), Dickison (2000) and Koch et al. (2009). Quantitative characters for each species are available in Rivera et al. (2017) and tribe classification follows Anderberg et al. (2007).

Results

The leaf architecture and foliar anatomy are summarized and illustrated (Figs. 1-15) by tribe. When te taxon name is given for a character state meas that the character state occurs only it.

Asteraceae

Tribe Anthemideae

Two species: *Artemisia ludoviciana* Nutt. and *Cotula australis* (Sieber ex Spreng.) Hook. f. (Figs. 1, 2).

Leaves sessile, alternate, simple (*Artemisia* L.), pinnatisect or bipinnatisect (*Cotula* L., Fig. 1A); lamina size microphyll, lamina shape linear to lanceolate, margin entire, revolute (*Artemisia*), apex acuminate, base truncate (*Artemisia*) or concave (*Cotula*); primary vein framework pinnate, primary vein straight or slightly undulate, secondary venation brochidodromous, areole development moderate (*Artemisia*) or lacking (*Cotula*), veinlets simple, straight, unbranched, marginal ultimate venation looped (*Artemisia*) or incomplete (*Cotula*); teeth absent; leaves hypostomatic; in surface view, cells tetragonal-elongated with S-undulate anticlines (Figs. 2A, B), adaxial epidermis glabrate, abaxial epidermis subglabrous (*Cotula*) to tomentose (*Artemisia*), with multicellular trichomes and anomocytic stomata (Fig. 2A), in transverse view, cuticle striate and thin (<0.44 µm), epidermises uniseriate with conical or rectangular cells and thicker outer periclinal walls, adaxial epidermis thicker than the abaxial (*Artemisia*) or both epidermises equally thick (*Cotula*), stomata at the same level as the epidermal cells (*Cotula*) or above (*Artemisia*); mesophyll homogenous or heterogeneous (Figs. 2C, D), palisade parenchyma generally occupying 50% of the mesophyll (*Artemisia*, Fig. 2C); vascular bundles collateral with a parenchymatous bundle sheath, canals associated with vascular bundles (Fig. 2E); midvein contour gently protruded in both surfaces (Fig. 2F) or flat adaxially and projected abaxially (Fig. 2G), cuticle conspicuously striate and thicker than in the lamina, epidermises uniseriate with narrow convex cells and thicker outer periclinal and anticlinal walls, beneath the epidermis, annular collenchyma and towards the vascular bundle parenchyma, a single central collateral vascular bundle, within the bundle, xylem with radial rows of three to five vessels, phloem formed by three to five rows of cells, a cap of sclereids (three to four layers) external to xylem.

Tribe Astereae

Six species: *Baccharis pteronioides* DC., *B. salicifolia* (Ruiz & Pav.) Pers., *Conyza bonariensis* (L.) Cronquist, *C. canadensis* (L.) Cronquist, *C. coronopifolia* Kunth and *Laennecia sophiifolia* (Kunth) G.L. Nesom (Figs. 1, 3).

Leaves sessile or petiolate, alternate, simple (Fig. 1B) or pinnately lobed (*Laennecia* Cass., *C. coronopifolia*); lamina size microphyll to notophyll, lamina shape linear-lanceolate to linear-oblong, margin entire (*C. bonariensis*, *Laennecia*), dentate to serrate (*Baccharis* L., *C. canadensis*, *C. coronopifolia*), margin revolute (*C. bonariensis*), apex acuminate to convex, base cuneate (*Baccharis*), truncate or lobate (*C. bonariensis*, *C. canadensis*, *Laennecia*) to cordate (*C. coronopifolia*); primary vein framework pinnate (*B. salicifolia*, *Laennecia*) or palmate (*B. pteronioides*, *Conyza* Less.), primary vein straight, secondary venation brochidodromous or actinodromous (*B. pteronioides*, *Conyza*), areole development moderate, veinlets simple, curved, unbranched or one-branched (*C. coronopifolia*), marginal ultimate venation looped; teeth lacking principal vein, with accessory vein straight (*Baccharis*, *Conyza*) or absent

Figure 1: Leaf morphology. A. *Cotula australis* (Sieber ex Spreng.) Hook. f.; B. *Baccharis pteronioides* DC.; C. *Schkuhria pinnata* (Lam.) Kuntze ex Thell.; D. *Helminthotheca echioides* (L.) Holub.; E. *Sonchus oleraceus* L.; F. *Taraxacum officinale* F.H. Wigg.; G. *Bidens pilosa* L.; H. *Dahlia coccinea* Cav.; I. *Piqueria trinervia* Cav.; J. *Pseudognaphalium viscosum* (Kunth) Anderb.; K. *Ambrosia cumanensis* Kunth; L. *Montanoa tomentosa* Cerv.; M. *Tithonia tubiformis* (Jacq.) Cass.; N. *Galinsoga parviflora* Cav.; O. *Barkleyanthus salicifolius* (Kunth) H. Rob. & Brettell; P. *Pittocaulon praecox* (Cav.) H. Rob. & Brettell; Q. *Dyssodia papposa* (Vent.) Hitchc.; R. *Tagetes micrantha* Cav. Scale is 0.5 cm in A, C; 1 cm in B, D-R.

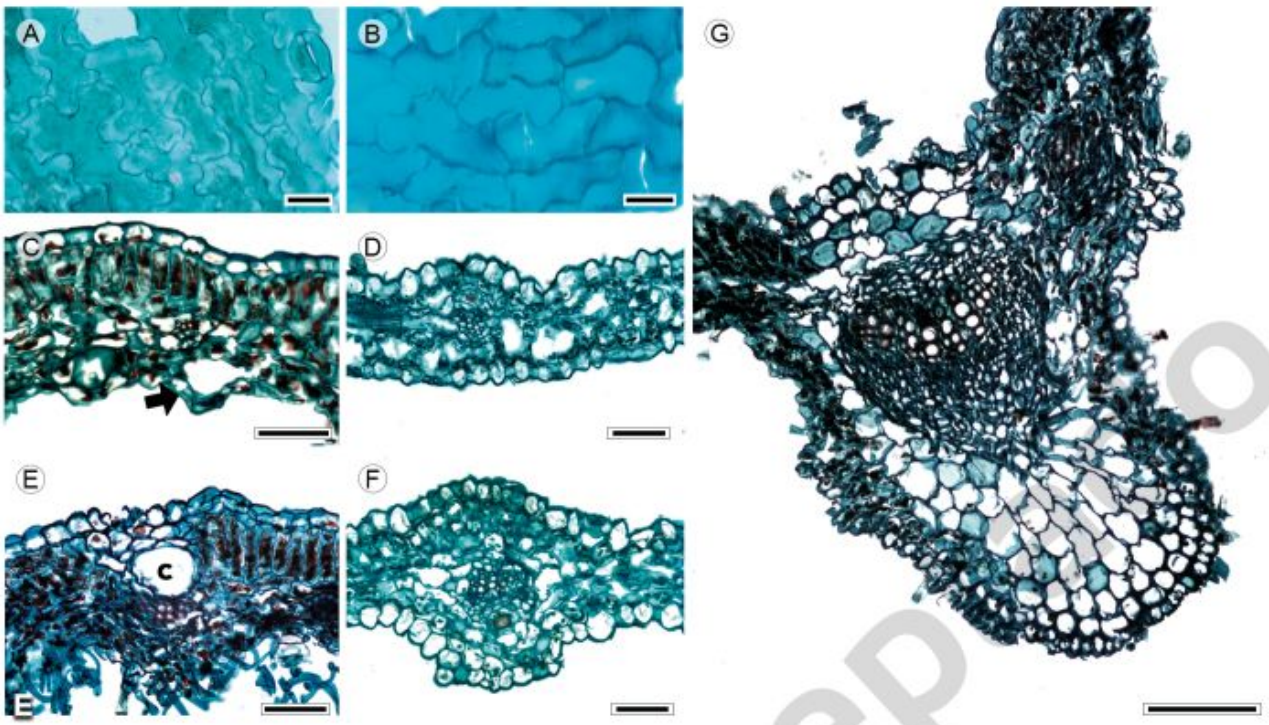

Figure 2: Lamina and midvein in Anthemideae. A, D, F. *Cotula australis* (Sieber ex Spreng.) Hook. f.; B, C, E, G. *Artemisia ludoviciana* Nutt. Scale is 20 µm in A, B; 50 µm in C-F, 100 µm in G. c=canal.

(*Laennecia*); leaves amphistomatic; in surface view, cells tetragonal or polygonal-elongated with straight anticlines (*Baccharis*, Fig. 3A, *C. canadensis*) or tetragonal to tetragonal-elongated with S-undulated to V-undulated anticlines (*C. bonariensis*, *C. coronopifolia*, Fig. 3B, *Laennecia*), adaxial and abaxial surfaces pubescent, with multicellular trichomes and glands and anomocytic or cyclocytic stomata (*Baccharis*, Fig. 3A), in transverse view, cuticle conspicuously striate and thickness between 0.28 and 0.57 µm, epidermises uniseriate with square or cupola cells (Figs. 3C-E) and thicker outer periclinal walls, both epidermis with the same width, stomata at the same level as epidermal cells; mesophyll heterogeneous (Figs. 3C, D) or homogeneous (*Laennecia*, Fig. 3E), palisade parenchyma occupying 33 to 41% of the mesophyll, except for *Laennecia*, paraveinal mesophyll between the vascular bundles; collateral vascular bundles with a parenchymatous bundle sheath, extensions of the sheath (girders) (Fig. 3E), except in *C. bonariensis* and *C. canadensis*, girders across the leaf (*C. coronopifolia*, *Laennecia*) or linked to either adaxial or abaxial surface (*Baccharis*), canals generally associated with vascular bundles (Fig. 3D); midvein contour with a slight central depression adaxially and flat abaxially (Fig. 3F) or flat adaxially and round and protruding abaxially (Fig. 3G), cuticle thicker than in the lamina, epidermises uniseriate with narrow convex cells and thicker outer periclinal walls, annular collenchyma below the epidermises mostly in the central region of the midrib (*Baccharis*) and parenchyma towards the vascular tissue, with palisade parenchyma towards the center of the midvein, a single central collateral vascular bundle (Figs. 3F, G) or three bundles, within the bundle, xylem with radial rows of two to six vessels separated by one or two rows of parenchyma, phloem formed by three to five rows of cells, a cap of sclereids associated with phloem and xylem, more conspicuous in the larger bundle.

Tribe Bahieae

Two species: *Florestina pedata* (Cav.) Cass. and *Schkuhria pinnata* (Lam.) Kuntze ex Thell. (Figs. 1, 4, 5).

Leaves petiolate, opposite at the base and alternate near the apex, pinnatisect or bipinnatisect (*Schkuhria* Roth,

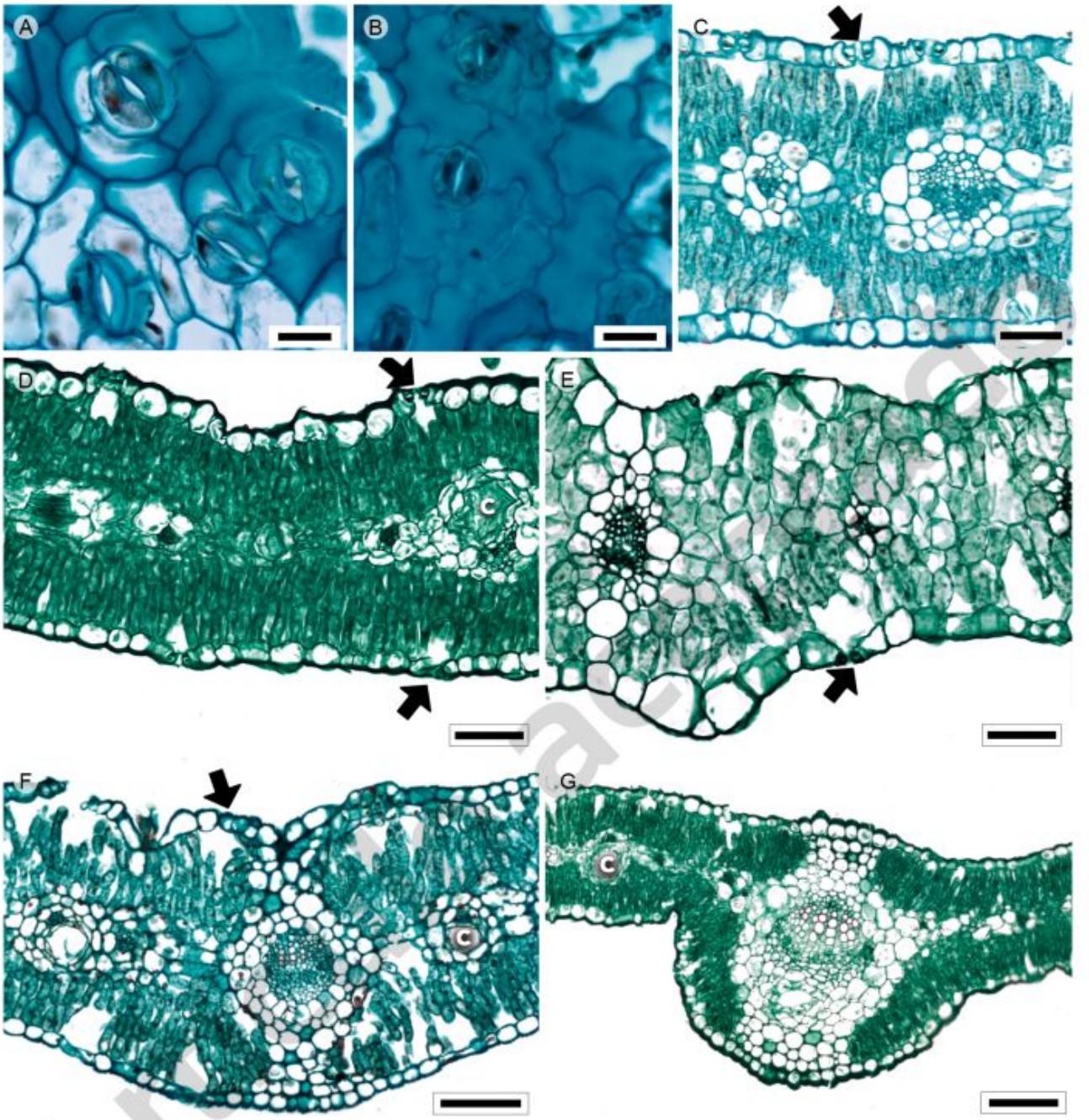

Figure 3: Lamina and midvein in Astereae. A, C. *Baccharis salicifolia* (Ruiz & Pav.) Pers.; B. *Conyza bonariensis* (L.) Cronquist; D, G. *Conyza canadensis* (L.) Cronquist; E. *Laennecia sophiifolia* (Kunth) G.L. Nesom; F. *Baccharis pteronioides* DC. Scale is 20 µm in A, B; 50 µm in C-E, 100 µm in F, G. c=canal.

Fig. 1C) or palmatisect (*Florestina* Cass.); lamina size microphyll, lamina shape linear (*Schkuhria*) or elliptic (*Florestina*), margin entire, apex straight, base concave or cuneate; primary vein framework pinnate (Fig. 4A), primary vein undulate (*Florestina*), weak, secondary venation brochidodromous, areole development moderate, veinlets simple, straight, unbranched (*Florestina*) or absent (*Schkuhria*), marginal ultimate venation looped; teeth absent; leaves amphistomatic; in surface view, cells tetragonal or polygonal elongated with S-undulated and U-undulated anticlines (Figs. 5A, B), both surfaces subglabrous, with short glandular trichomes and anomocytic stomata (*Florestina*,

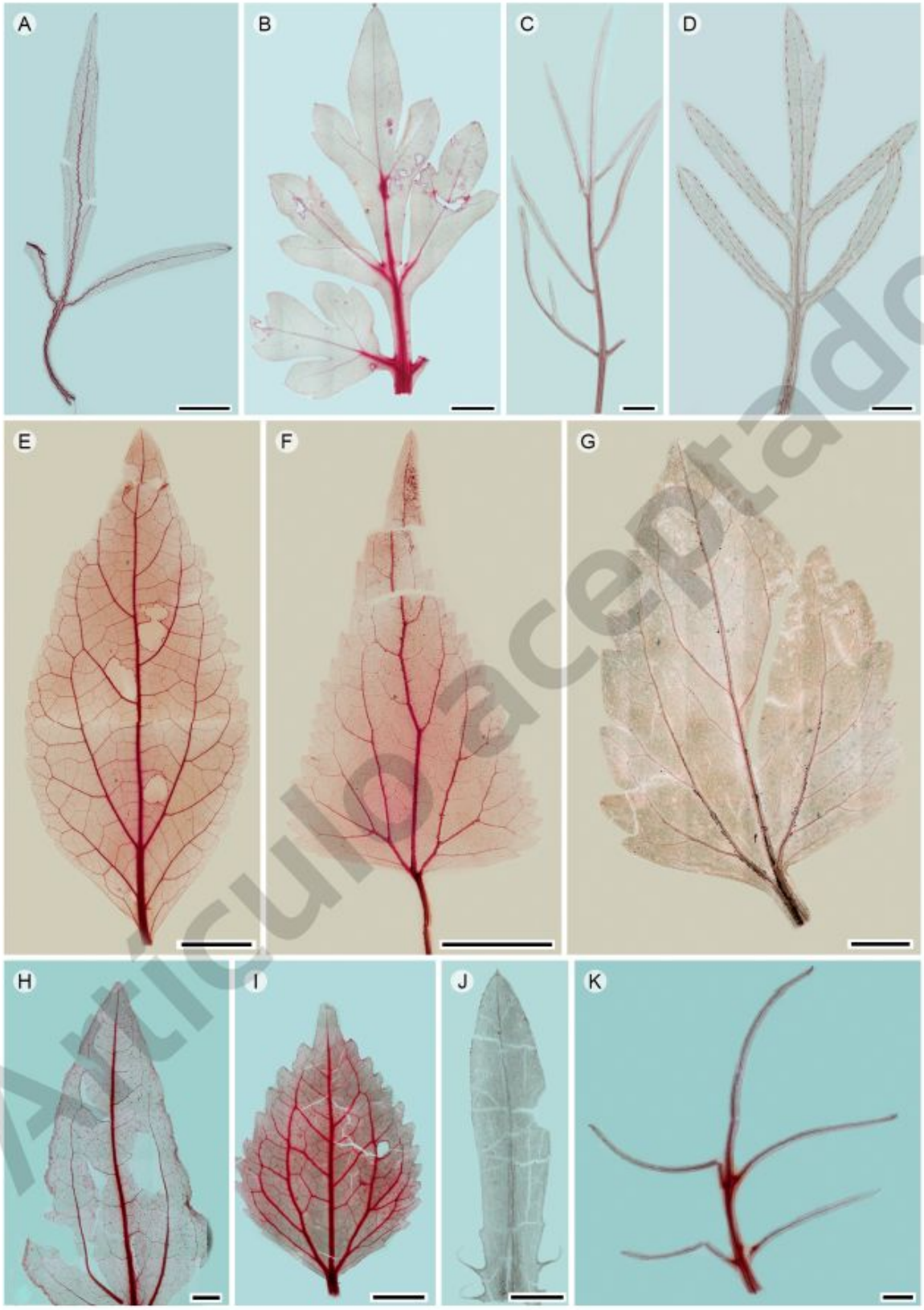

Figure 4: Cleared leaves. A. *Florestina pedata* (Cav.) Cass.; B. *Bidens odorata* Cav.; C. *Cosmos bipinnatus* Cav.; D. *Heterosperma pinnatum* Cav.; E. *Brickellia secundiflora* (Lag.) A. Gray; F. *Fleischmannia pycnocephala* (Less.) R.M. King & H. Rob.; G. *Stevia micrantha* Lag.; H. *Zinnia peruviana* (L.) L.; I. *Galinsoga parviflora* Cav.; J. *Pectis prostrata* Cav.; K. *Tagetes micrantha* Cav. Scale is 2 mm in A-D, G, H, J; 1 cm in E, F; 5 mm in I, 21 mm in K.

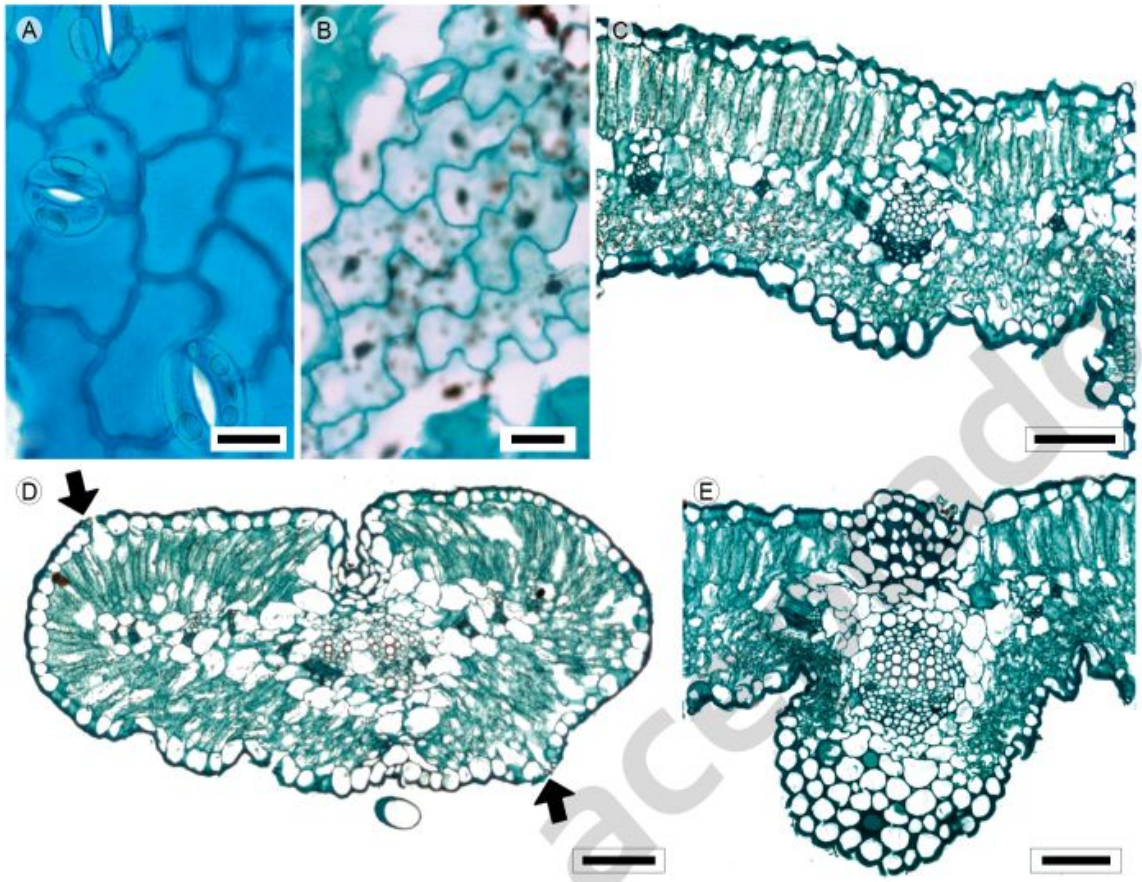

Figure 5: Lamina and midvein in Bahieae. A, C, E. *Florestina pedata* (Cav.) Cass.; B, D. *Schkuhria pinnata* (Lam.) Kuntze ex Thell. Scale is 20 µm in A, B; 100 µm in C-E.

Schkuhria) or anisocytic (*Schkuhria*), in transverse view, cuticle striate with a thickness between 0.33 to 0.38 µm, epidermises uniseriate, with rectangular to hemispherical cells and thicker outer periclinal walls (Figs. 5C, D), adaxial epidermis wider than abaxial (*Florestina*) or both epidermis the same width (*Schkuhria*), stomata at the same level as the epidermal cells; mesophyll heterogeneous (*Florestina*, Fig. 5C) or homogeneous (*Schkuhria*, Fig. 5D), palisade parenchyma occupying 54% of the leaf in *Florestina*; collateral vascular bundles with a parenchymatous bundle sheath, sheath extensions rare (Fig. 5C), canals associated with vascular bundles; midvein contour with a slight central depression adaxially and flat abaxially in *Schkuhria* (Fig. 5D), round and slightly projected adaxially and round and protruding abaxially in *Florestina* (Fig. 5E), cuticle similar to lamina, epidermises uniseriate, with cupola cells and thicker outer periclinal walls, immediately beneath the epidermises, four rows of annular collenchyma (*Schkuhria*) or two to four rows of angular collenchyma (*Florestina*) and parenchyma towards the vascular tissue, with palisade one third towards the abaxial surface, a single central collateral vascular bundle (Fig. 5E) or two bundles, within the bundle, xylem with radial rows of three to four vessels separated by one or two rows of parenchyma, phloem formed by three to five rows of cells, caps of sclereids with thin walls associated to xylem and phloem.

Tribe Cardueae

One species: *Cirsium vulgare* (Savi) Ten. (Fig. 6).

Leaves sessile, alternate, pinnately lobed; lamina size mesophyll, lamina shape triangular, margin toothed

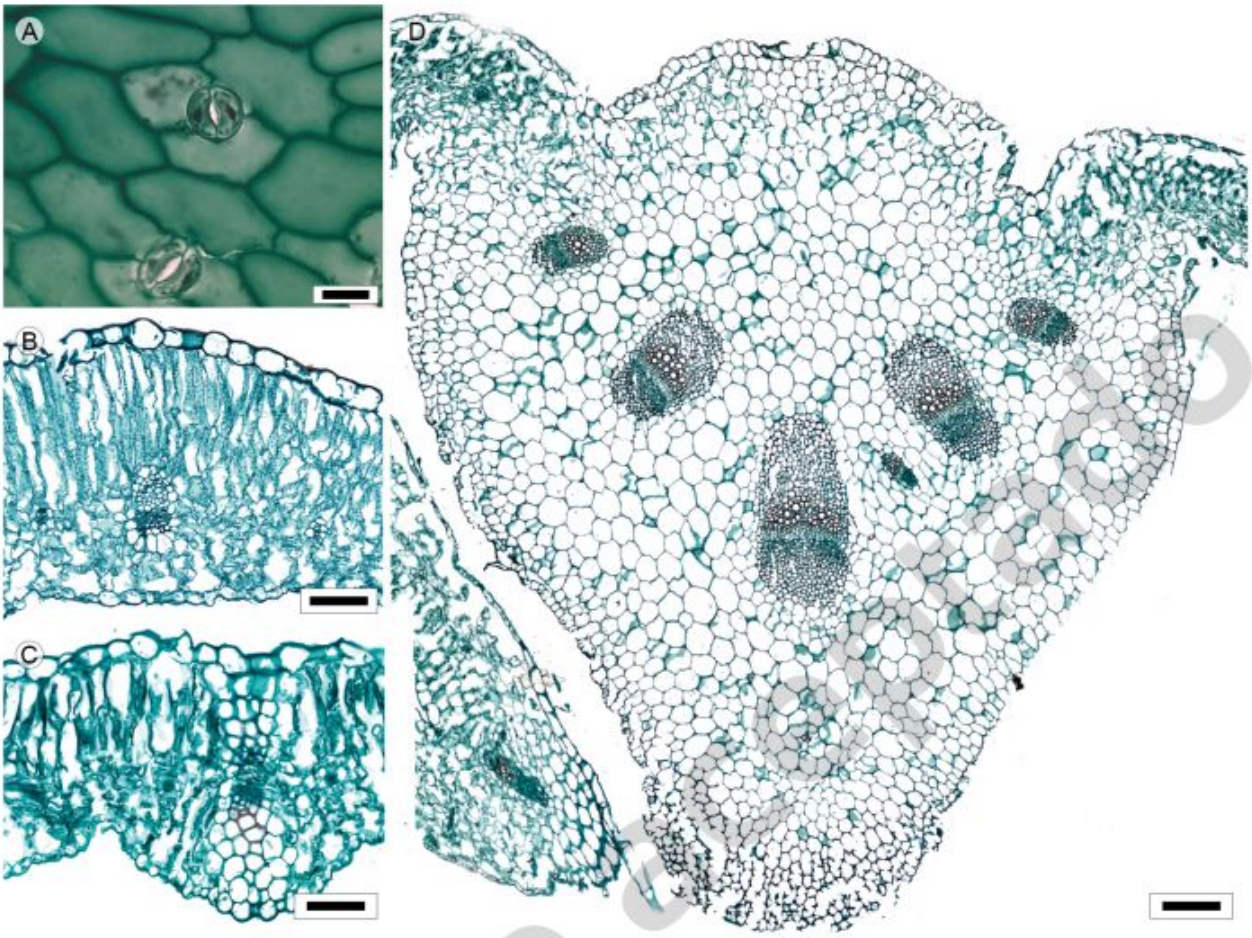

Figure 6: Lamina and midvein in Cardueae. A-D. *Cirsium vulgare* (Savi) Ten. Scale is 20 µm in A; 100 µm in B, C; 300 µm in D.

and ending in spines, apex acuminate and base cordate to amplexicaul; primary vein framework pinnate, primary vein straight, prominent, secondary venation brochidodromous, areole development moderate, veinlets simple, curved, unbranched, marginal ultimate venation looped; teeth lacking principal vein, with accessory vein straight, spinose; leaves amphistomatic; in surface view, cells elongated polygonal with straight anticlines (Fig. 6A), abaxial and adaxial surfaces pubescent, with multicellular trichomes and stiff hairs stomata anomocytic or anisocytic in the same plant, in transverse view, cuticle striate and 0.3 µm in thickness, epidermises uniseriate, with rectangular cells and thicker cells and outer periclinal walls, adaxial epidermis wider than abaxial epidermis, stomata at the same level as epidermal cells; mesophyll heterogeneous with two or three rows of palisade parenchyma (Fig. 6B), occupying 54% of the mesophyll; collateral vascular bundles with a parenchymatous bundle sheath, girders seldom in secondary veins, with some lignified cells (Fig. 6C), canals absent; midvein contour protruded adaxially and sharply projected abaxially triangular (Fig. 6D), cuticle similar to lamina, epidermises uniseriate, with squared cells, immediately beneath the abaxial epidermis annular collenchyma (1-3 layers) and abundant parenchyma towards the vascular tissue, no palisade in the midvein, five to six collateral vascular bundles, the smaller ones towards the adaxial surface (Fig. 6D), within the bundles, xylem in clusters of two to nine vessels or radial rows of three to five vessels and parenchyma, phloem abundant and formed by five to seven rows of cells, caps of sclereids wide (10-12 layers) on each side of the vascular tissue.

Tribe Cichorieae

Four species: *Helminthotheca echioides* (L.) Holub, *Lactuca serriola* L., *Sonchus oleraceus* L. and *Taraxacum officinale* F.H. Wigg. (Figs. 1, 7).

Leaves sessile, although at the lamina base narrow looking like a petiole, leaves opposite or alternate, simple (*Helminthotheca* Vaill., *Lactuca* L., Fig. 1D) to pinnately lobed (*Sonchus* L., *Taraxacum* F.H. Wigg., Figs. 1E, F); lamina size mesophyll to macrophyll, lamina shape variable, generally elliptic to obovate, margin dentate or serrate, frequently combining both characteristics, edge of the leaf blade appear sinuous (*Helminthotheca*), apex straight, base truncate or lobate to cordate; primary vein framework pinnate or palmate (*Sonchus*), primary vein straight, prominent, secondary venation brochidodromous (*Helminthotheca*, *Lactuca*, *Taraxacum*) or basal actinodromous (*Sonchus*), areole development moderate, veinlets simple, straight (*Lactuca*, *Taraxacum*) or once-branched (*Helminthotheca*, *Sonchus*), marginal ultimate venation looped (*Lactuca*, *Sonchus*, *Taraxacum*) or incomplete (*Helminthotheca*); teeth principal vein terminating in a tooth apex (*Helminthotheca*, *Taraxacum*) or accessory veins straight (*Sonchus*); leaves amphistomatic or hypostomatic (*Sonchus*); in surface view, cells tetragonal or tetragonal elongated with S-undulate or U-undulate anticlines (Figs. 7A-C), both surfaces glabrous (*Sonchus*, *Taraxacum*) or bristly and glandular (*Helminthotheca*) and anomocytic stomata, in transverse view, cuticle smooth and thickness between 0.31 and 0.45 µm, epidermises uniseriate, with conical cells and thicker outer periclinal walls, adaxial epidermis wider than the abaxial (*Sonchus*, *Taraxacum*), stomata at the same level as epidermal cells; mesophyll heterogeneous (*Helminthotheca*, *Sonchus*, Figs. 7D, E) or homogeneous (*Lactuca*, *Taraxacum*, Fig. 7F), palisade occupying 36 to 46% of the mesophyll, collateral vascular bundles with a parenchymatous bundle sheath (Figs. 7D-F), girders present in all species and running across the mesophyll, canals absent; midvein contour ample flat adaxially and round sharply projected abaxially (Fig. 7G) or protruded in both surfaces with a large lysogenic area in *Taraxacum* (Fig. 7H), cuticle similar to lamina, epidermises uniseriate, with squared cells, immediately below a hypodermis (single layer) and parenchyma towards the vascular tissue, with palisade parenchyma abaxially in *Taraxacum,* three to five collateral vascular bundles, within the bundles, xylem in radial rows of three to eight vessels separated by one or two rows of parenchyma, phloem formed by five to ten rows of cells, caps of sclereids (four to six layers) associated with xylem and phloem.

Tribe Coreopsideae

Six species: *Bidens odorata* Cav., *B. pilosa* L., *Cosmos bipinnatus* Cav., *C. parviflorus* (Jacq.) Pers., *Dahlia coccinea* Cav. and *Heterosperma pinnatum* Cav. (Figs. 1, 4, 8).

Leaves petiolate, rarely sessile (*C. bipinnatus*), opposite, generally pinnatisect or bipinnatisect; lamina size microphyll to mesophyll, lamina shape linear (*Cosmos* Cav., *Heterosperma* Cav.) or ovate to elliptic (*Bidens* L., *Dahlia* Cav., Figs. 1G, H), margin serrate (*Bidens*, *Dahlia*) or entire (*Cosmos*, *Heterosperma*), apex acuminate or straight, base truncate or concave; primary vein framework pinnate (Figs. 4B-D), primary vein straight or undulate (*Heterosperma*), prominent or weak (*Heterosperma*), secondary venation brochidodromous, areole development moderate (*Bidens*, *Dahlia*), poor (*Cosmos*) or lacking (*Heterosperma*), veinlets simple, curved (*Bidens*, *Heterosperma*), straight (*C. bipinnatus*) or once branched (*C. parviflorus*, *Dahlia*), marginal ultimate venation looped; teeth with principal vein and two accessory veins straight (*Bidens*), accessory veins convex (*Dahlia*) or absent (*Cosmos*, *Heterosperma*); leaves amphistomatic, in surface view, cells tetragonal elongated or polygonal elongated with straight (*Dahlia*) or S-undulated to U-undulated anticlines (Figs. 8A-C), both epidermises glabrous or glabrate (*Cosmos*, *Heterosperma*), with multicellular trichomes and glands (*Bidens*, *Dahlia*) and stomata anomocytic or anisocytic (*Dahlia*, *Heterosperma*), in transverse view, cuticle striate in all species except *C. parviflorus* and thickness between 0.23 and 0.42 µm, epidermises uniseriate, with cupola or conical cells and thicker outer periclinal walls, tannins occluding cell lumina in *Cosmos* (Fig. 8E), stomata at the same level as epidermal cells; mesophyll homogeneous in *C. bipinnatus* (Fig. 8D) and heterogeneous in *C. parviflorus* (Fig. 8E) and the rest of the species (Fig. 8F), palisade occupying 41 to 53% of the mesophyll; collateral vascular bundles with a parenchy-

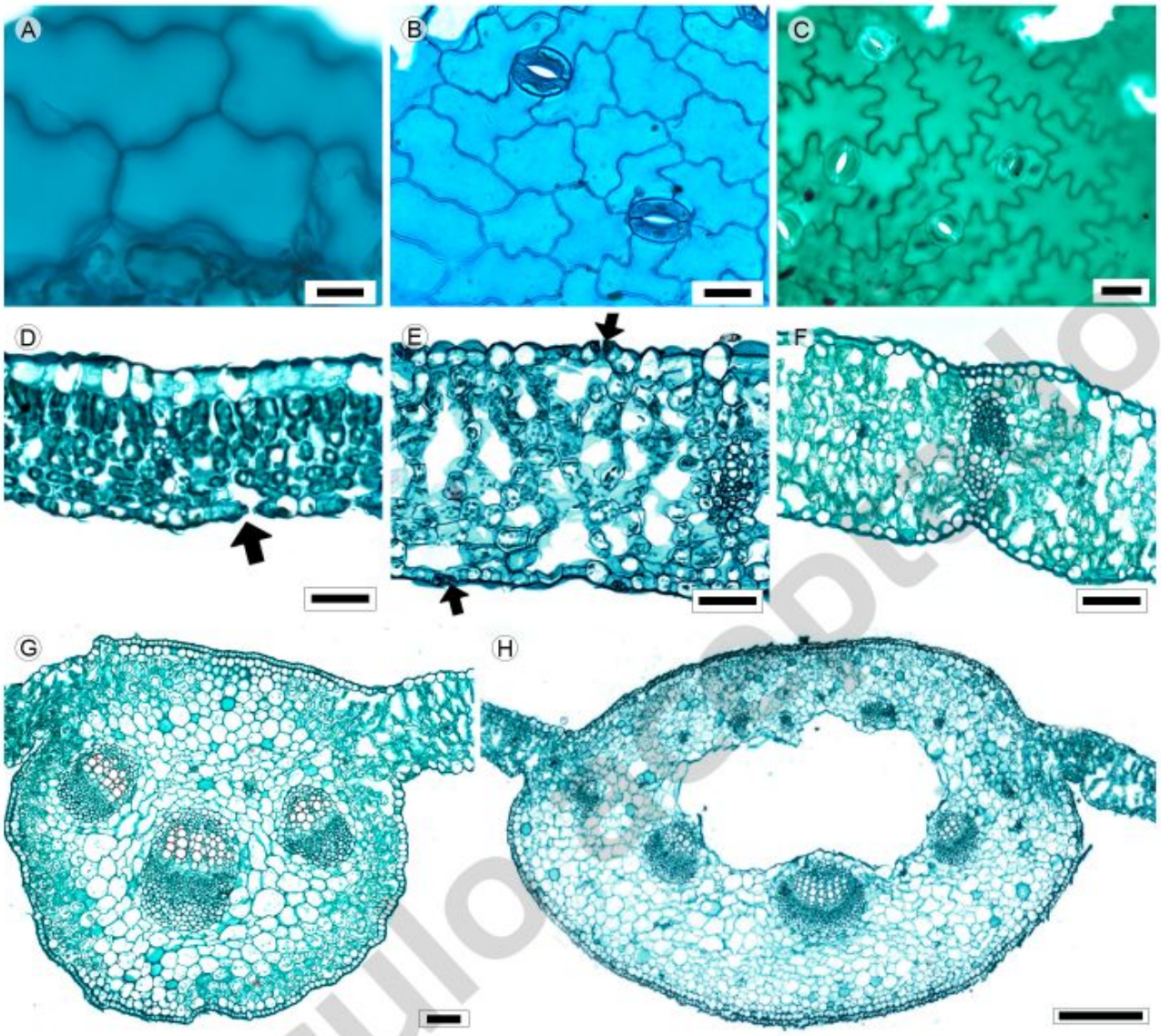

Figure 7: Lamina and midvein in Cichorieae. A, D. *Sonchus oleraceus* L.; B, E, H. *Taraxacum officinale* F.H. Wigg., C. *Lactuca serriola* L.; F, G. *Helminthotheca echioide* (L.) Holub. Scale is 20 µm in A-C; 50 µm in D, E, 100 µm in F, G; 300 µm in H.

matous bundle sheath (Figs. 8E, F), girders in *Dahlia* and *Heterosperma,* canals associated with vascular bundles or in the mesophyll (Fig. 8F); midvein contour projection on the adaxial surface and round and slightly protruding on the abaxial (*Bidens*, *Dahlia*, Fig. 8G) or convex adaxially and round abaxially (*Cosmos*, *Heterosperma* Figs. 8H, I), cuticle similar to lamina. epidermises uniseriate, with convex cells and thick-walled cells, below the adaxial epidermis one-three layers of annular collenchyma and palisade parenchyma and abaxially two layers of angular collenchyma, vascular tissue surrounded by parenchyma or a sheath (Fig. 8H), a single central collateral vascular bundle (*Cosmos*, *Heterosperma*) or three bundles (*Bidens*, *Dahlia*, Fig. 8G), within the bundles, xylem in radial rows of three to five vessels, phloem formed by three to five rows of cells, a cap of sclereids (three to four layers) external to xylem in *Bidens*.

Tribe Eupatorieae

Fourteen species: *Ageratina adenophora* (Spreng.) R.M. King & H. Rob., *A. choricephala* (B.L. Rob.) R.M. King & H. Rob., *A. cylindrica* (McVaugh) R.M. King & H. Rob., *A. deltoidea* (Jacq.) R.M. King & H. Rob., *Brickellia secundiflora*

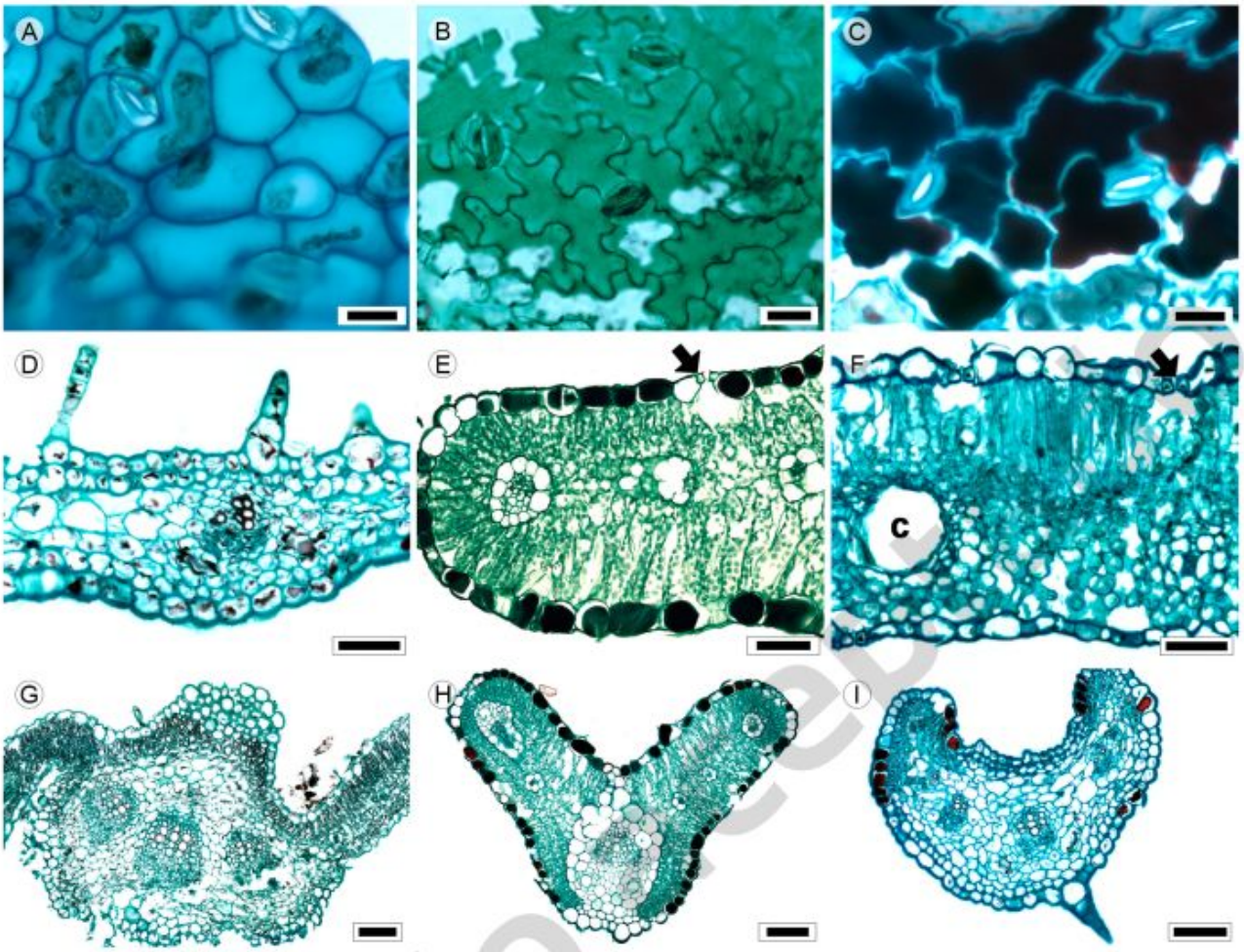

Figure 8: Lamina and midvein in Coreopsideae. A, F. *Dahlia coccinea* Cav.; B. *Bidens odorata* Cav.; C, E, G. *Cosmos parviflorus* (Jacq.) Pers.; D, I. *Cosmos bipinnatus* Cav.; H. *Cosmos parviflorus* (Jacq.) Pers. Scale is 20 µm in A-C; 50 µm in D-F, 100 µm in G-I. c=canal.

(Lag.) A. Gray, *B. veronicifolia* (Kunth) A. Gray, *Chromolaena pulchella* (Kunth) R.M. King & H. Rob., *Fleischmannia pycnocephala* (Less.) R.M. King & H. Rob., *Piqueria trinervia* Cav., *Stevia micrantha* Lag., *S. origanoides* Kunth, *S. salicifolia* Cav., *S. subpubescens* Lag. and *S. tomentosa* Kunth (Figs. 1, 4, 9).

Leaves petiolate rarely sessile (*S. salicifolia*), opposite, simple (Fig. 1I); lamina size variable from microphyll (*B. veronicifolia*) to macrophyll (*A. deltoidea*), lamina shape ovate to elliptic, linear (*S. salicifolia*) or triangular (*A. deltoidea*), margin crenate to serrate, sometimes both (*Stevia* Cav.), apex straight to rounded, base highly variable: truncate, concave, concavo-convex, cuneate or cordate; primary vein framework pinnate or palmate, primary vein straight, prominent or weak (*Ageratina* Spach, *Stevia*), secondary venation brochidodromous or actinodromous basal (*Fleischmannia* Sch. Bip., *Stevia*, Figs. 4F, G), areole development moderate or poor (*Brickellia* Elliott), veinlets simple, curved, linear (*Ageratina*) or once-branched (*Chromolaena* DC., *S. tomentosa*), marginal ultimate venation looped; teeth with accessory veins and simple or lacking teeth (*Chromolaena*); leaves hypostomatic in most species, just four species amphistomatic (*A. cylindrica*, *P. trinervia*, *S. origanoides*, *S. salicifolia*); in surface view, cells tetragonal-elongated or polygonal with straight, V-undulated, U-undulated or S-undulated anticlines (Figs. 9A-C), both surfaces tomentose, rarely glabrous (*Piqueria*), with multicellular trichomes and stomata anomocytic, anisocytic (*S. origanoides*) or staurocytic (*A. cylindrica*, *P. trinervia*, *S.*

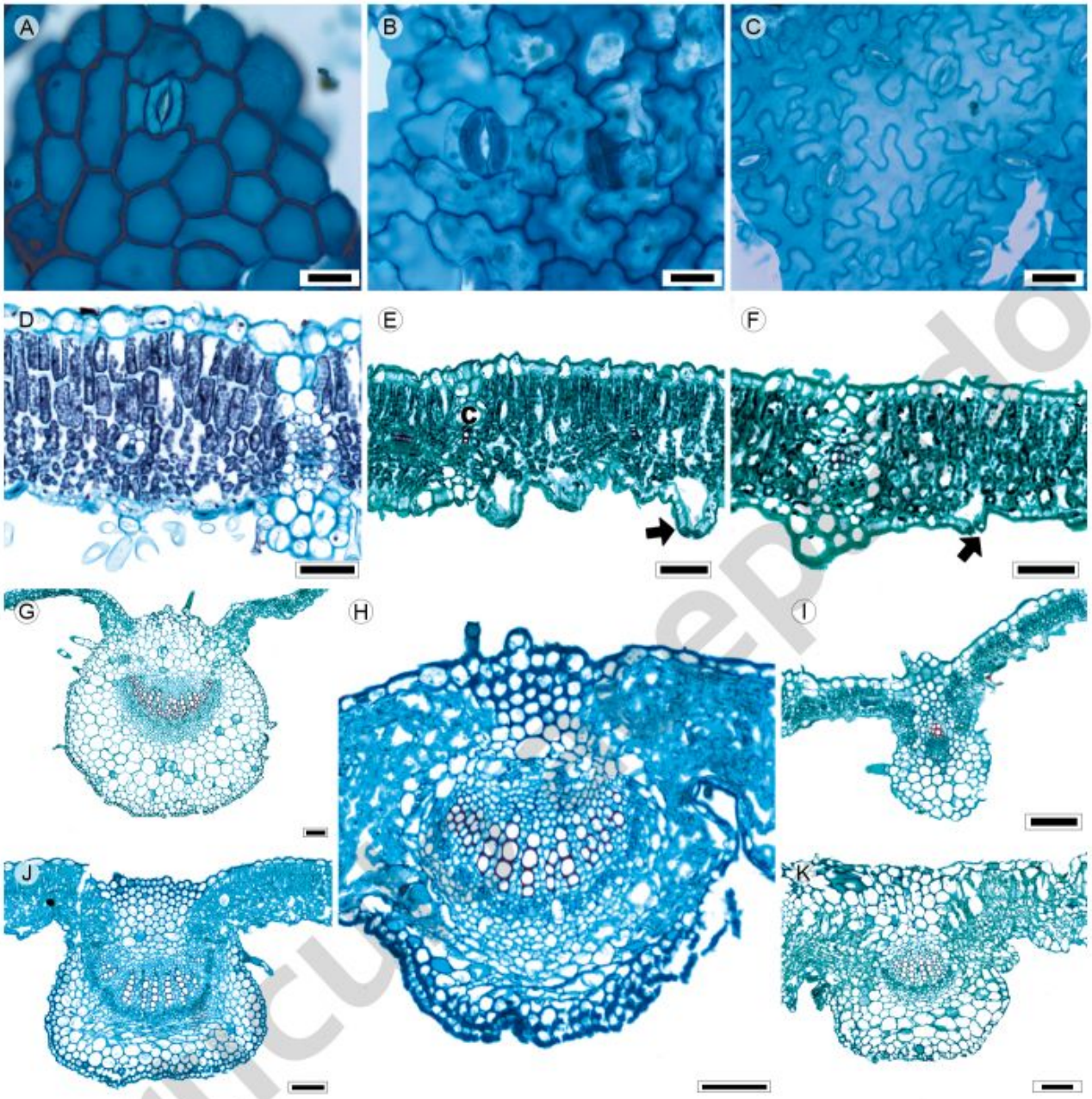

Figure 9: Lamina and midvein in Eupatorieae. A, K. *Stevia origanoides* Kunth; B. *Stevia salicifolia* Cav.; C, D. *Stevia tomentosa* Kunth, E. *Brickellia veronicifolia* (Kunth) A. Gray; F. *Fleischmannia pycnocephala* (Less.) R.M. King & H. Rob.; G. *Ageratina deltoidea* (Jacq.) R.M. King & H. Rob.; H. *Piqueria trinervia* Cav.; I. *Brickellia secundiflora* (Lag.) A. Gray; J. *Ageratina choricephala* (B.L. Rob.) R.M. King & H. Rob. Scale is 20 µm in A-C; 50 µm in D-F, 100 µm in H-K; 300 µm in G.

salicifolia); in transversal view, cuticle striate and 0.21 to 0.60 µm in thickness, epidermises uniseriate, with rectangular or convex cells and thicker outer periclinal walls (Figs. 9D-F), adaxial epidermis sometimes wider than the abaxial (*A. cylindrica*, *A. deltoidea*, *Brickellia*, *Chromolaena*, *Fleischmannia*, *Stevia*), stomata at the same level or above the epidermal cells (*A. deltoidea*, *Brickellia*, *C. pulchella*, *Fleischmannia*, *S. micrantha*, *S. origanoides*, Figs. 9E, F); mesophyll heterogeneous (Figs. 9D-F), except in *Piqueria*, homogeneous, palisade occupying 45 to 56% of the mesophyll, collateral vascular bundles with a parenchymatous bundle sheath or sclerenchyma (*S. salicifolia*), girders in secondary

veins (Fig. 9F), canals associated with vascular bundles or in the mesophyll; midvein contour flat or slightly projected adaxially and abaxially round or square protruded or highly protruded (Figs. 9G-K), cuticle conspicuously striate, epidermises uniseriate, with square and convex cells and thicker outer periclinal walls, below both epidermises, angular collenchyma (1 to 3 layers) limited by the mesophyll (*Piqueria*) or exclusively parenchyma (Fig. 9K), a central collateral vascular bundle forming an open arc, within the bundle, xylem in clusters of three to ten vessels or radial rows of three to five vessels separated by radial rows of parenchyma, phloem formed by one to five rows of cells, caps of parenchyma or sclerenchyma (three to seven layers) associated to xylem and phloem (Fig. 9H).

Tribe Gnaphalieae

Three species: *Pseudognaphalium canescens* (DC.) Anderb., *P. semilanatum* (DC.) Anderb. and *P. viscosum* (Kunth) Anderb. (Figs. 1, 10).

Leaves sessile, alternate, simple (Fig. 1J); lamina size microphyll, lamina shape elliptic to oblong, margin entire, revolute (*P. viscosum*), apex straight to acuminate, base truncate, sometimes cordate to sagittate (*P. viscosum*); primary vein framework pinnate, primary vein straight, weak, secondary venation brochidodromous, areole development moderate, veinlets simple, curved, marginal ultimate venation looped; teeth absent; leaves hypostomatic (*P. canescens*, *P. semilanatum*) or amphistomatic (*P. viscosum*); in surface view, cells tetragonal or elongated tetragonal with U-undulated to V-undulated anticlines (Figs. 10A-C), adaxial surface hirsute, abaxially densely tomentose, with glandular trichomes and anomocytic stomata, in transverse view, cuticle apparently smooth and between 0.20 and 0.42 µm in thickness, epidermises uniseriate, with rectangular and conical cells (Figs. 10D, E), adaxial epidermis wider than abaxial, stomata at the same level as epidermal cells; mesophyll heterogeneous with one to two rows of palisade parenchyma, palisade occupying 41 to 46% of the mesophyll; vascular bundles collateral with a parenchymatous bundle sheath in *P. canescens* and *P. semilanatum*, with girders (Figs. 10D, E), canals absent; midvein contour flat or slightly convex adaxially and abaxially round and protruded (Figs. 10F-H), cuticle similar to lamina, epidermises uniseriate with convex cells and thick outer periclinal walls, immediately beneath the abaxial epidermis a layer of angular collenchyma and mesophyll extending to most of the abaxial faces, abundant parenchyma surrounding the vascular tissue, a single central collateral vascular bundle, within the bundle, xylem in radial rows of two to five vessels, phloem formed by three to four rows of cells, a cap of parenchyma (two or three layers) associated with phloem.

Tribe Heliantheae

Eleven species: *Acmella repens* (Walter) Rich., *Aldama buddleiiformis* (DC.) E.E. Schill. & Panero, *A. excelsa* (Willd.) E.E. Schill. & Panero, *Ambrosia cumanensis* Kunth, *Lagascea rigida* (Cav.) Stuessy, *Montanoa grandiflora* Alamán ex DC., *M. tomentosa* Cerv., *Simsia amplexicaulis* (Cav.) Pers., *Tithonia tubiformis* (Jacq.) Cass., *Verbesina virgata* Cav. and *Zinnia peruviana* (L.) L. (Figs. 1, 4, 11).

Leaves petiolate or sessile, opposite, sometimes alternate near the apex, simple or pinnately lobed (*Ambrosia* L., Fig. 1K; *M. grandiflora*, *Simsia* Pers.); lamina size microphyll to megaphyll, lamina shape ovate to elliptic, margin entire (*Acmella* Rich. ex Pers., *Aldama* La Llave, *Zinnia* L.), crenate to serrate (*M. grandiflora*), dentate (*V. virgata*) and erose (*Simsia*), apex straight to acuminate, base truncate, concave, cuneate or cordate (Figs. 1L, M); primary vein framework pinnate or palmate, primary vein straight (Fig. 4H), prominent (*Lagascea* Cav.), secondary venation brochidodromous or actinodromous basal (*Aldama*, *M. tomentosa*, *Tithonia* Desf. ex Juss., *Zinnia*, Fig. 4H), areole development moderate or good (*Lagascea*), veinlets once-branched (*Acmella*, *M. tomentosa*), simple, straight (*Aldama*, *Ambrosia*, *Lagascea*, *Tithonia*, *Zinnia*) or simple curved in the other taxa, marginal ultimate venation looped or incomplete (*Simsia*, *Verbesina* L.); teeth with primary and accessory veins straight (*Ambrosia*), only accessory veins curved (*Acmella*, *Simsia*, *Tithonia*, *Verbesina*) or absent (*Aldama*, *Lagascea*, *Zinnia*); leaves amphistomatic, rarely hypostomatic; in surface view, cells tetragonal, tetragonal-elongated or polygonal with straight to S-undulate anticlines (Figs. 11A-C), both epidermises hispid to tomentose, with glands and unicellular or multicellular trichomes and

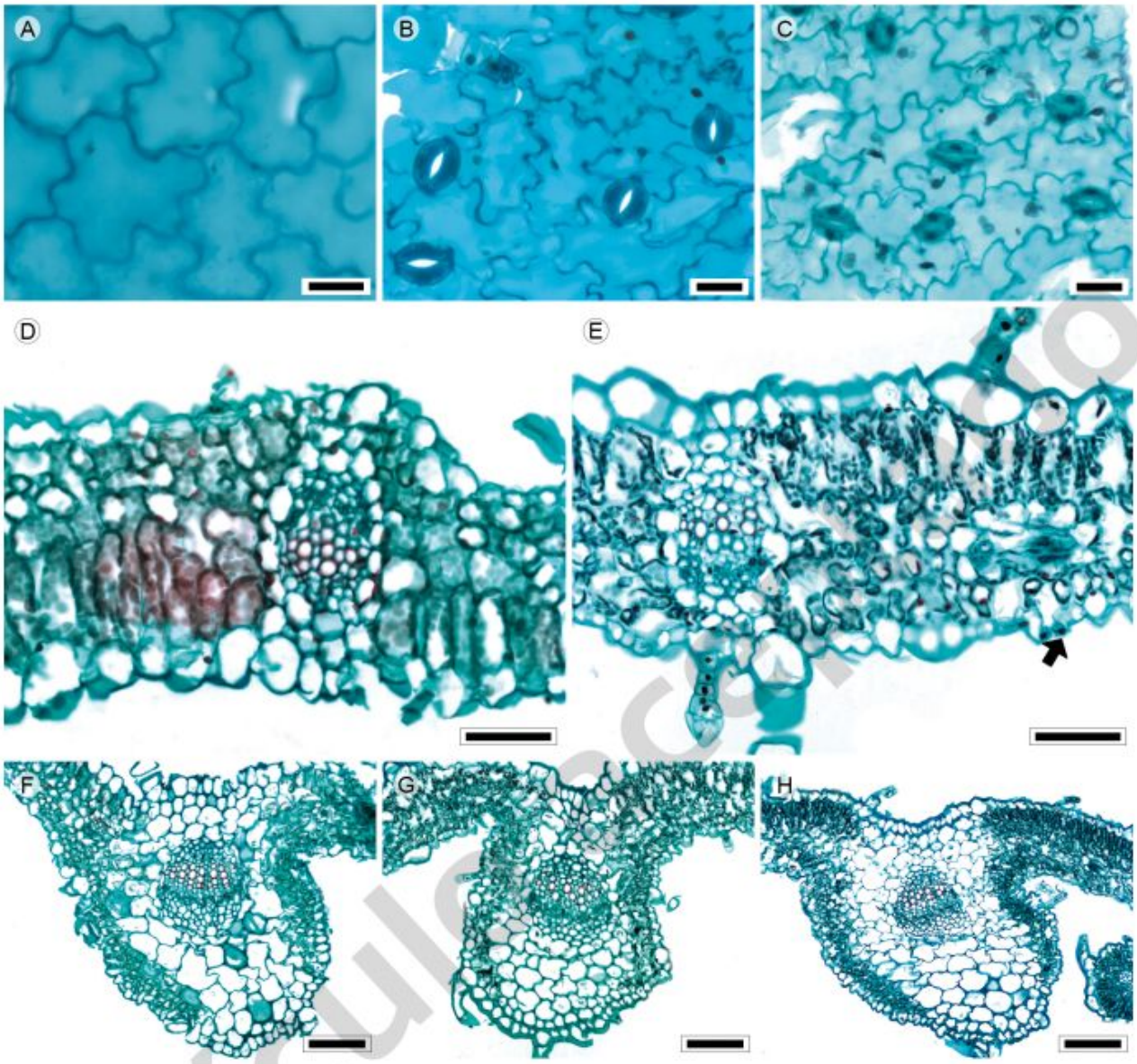

Figure 10: Lamina and midvein in Gnaphalieae. A, D, F. *Pseudognaphalium canescens* (DC.) Anderb.; B, E, G. *Pseudognaphalium semilanatum* (DC.) Anderb.; C, H. *Pseudognaphalium viscosum* (Kunth) Anderb. Scale is 20 µm in A-C; 50 µm in D, E, 100 µm in F-H.

stomata anomocytic, anisocytic or staurocytic (*Lagascea*), in transverse view, cuticle striate and between 0.21 and 1.15 µm in thickness, epidermises uniseriate, with cupola or rectangular cells and thicker outer periclinal walls, adaxial epidermis is wider than abaxial in most species or both are equally wide (*Acmella*, *Lagascea*, *Verbesina*), stomata at the same level as other epidermal cells or above (Fig. 11G); mesophyll heterogeneous (Figs. 11D-G), palisade occupying from 32 to 100% of the mesophyll (*Aldama*, *Lagascea*, *Simsia*); collateral vascular bundles with a parenchymatous bundle sheath, girders of parenchyma (Figs. 11D, E) or sclerenchyma (*Aldama*, *Lagascea*, Fig. 11F), secondary veins with angular collenchyma toward both surfaces, canals associated with vascular bundles; midvein contour flat or protruded on the adaxial surface and abaxially round and protruding (Figs. 11H-J) or a crest toward both surfaces (*Ambrosia*, Fig. 11G), cuticle similar to lamina, epidermises uniseriate with cupola cells and thicker outer periclinal walls, immediately beneath the epidermises, parenchyma (Fig. 11H) or annular or angular collenchyma, frequently

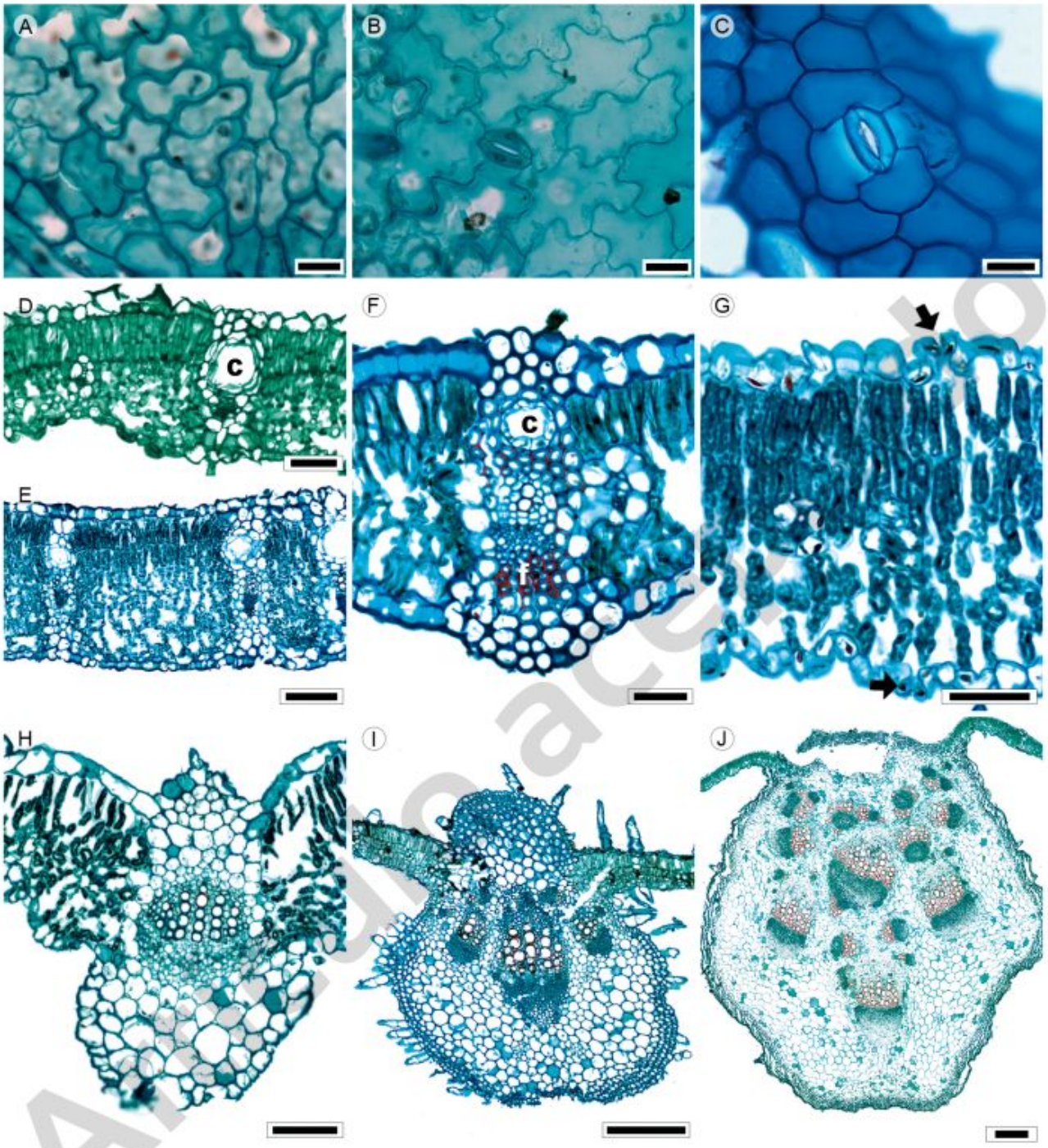

Figure 11: Lamina and midvein in Heliantheae. A, D. *Montanoa tomentosa* Cerv., B, J. *Montanoa grandiflora* Alamán ex DC.; C, F. *Lagascea rigida* (Cav.) Stuessy, E. *Verbesina virgata* Cav.; G. *Ambrosia cumanensis* Kunth, H. *Zinnia peruviana* (L.) L.; I. *Tithonia tubiformis* (Jacq.) Cass. Scale is 20 µm in A-C; 50 µm in D, F, G, 100 µm in E, H; 300 µm in I, J. c=canal.

continuous (four to five layers, Figs. 11I, J) or discontinuous (two to five layers), mesophyll or parenchyma towards the vascular tissue, a single central collateral vascular bundle or more than ten bundles (Figs. 11H-J), within the bundles, xylem in radial rows of two to ten vessels or in clusters of three to fifteen or more vessels, phloem formed by five to ten rows of cells, caps of parenchyma (*Simsia*, *Zinnia*) or sclerenchyma associated with xylem and phloem.

Tribe Millerieae

Four species: *Galinsoga parviflora* Cav., *Jaegeria hirta* (Lag.) Less., *Melampodium longifolium* Cerv. ex Cav. and *M. perfoliatum* (Cav.) Kunth (Figs. 1, 4, 12).

Leaves sessile (*Jaegeria* Kunth) or petiolate (*Galinsoga* Ruiz & Pav., *Melampodium* L.), opposite and simple; lamina size notophyll to mesophyll, lamina shape elliptic, obovate or ovate, margin crenate to serrate (Fig. 1N), apex acuminate to straight, base cuneate to rounded, sometimes concave (*Galinsoga*) or sagittate (*Melampodium*); primary vein framework palmate (Fig. 4I), primary veins straight, prominent, secondary venation actinodromous basal, areole development moderate, veinlets simple, curved (*Galinsoga*), straight (*Jaegeria*) or once-branched (*Melampodium*), marginal ultimate venation looped (*Jaegeria*, *Galinsoga*) or incomplete (*Melampodium*), teeth with accessory veins straight; leaves amphistomatic; in surface view, cells tetragonal-elongated with S-undulated, U-undulated or V-undulated anticlines (Figs. 12A-C), both epidermis strigose to hirsute, with multicellular trichomes and stomata anomocytic, in transverse view, the cuticle apparently smooth and between 0.23 and 0.24 µm in thickness, epidermises uniseriate, with cupola or rectangular cells and thicker outer periclinal walls, adaxial epidermis wider than the abaxial, stomata at the same level as the epidermal cells; mesophyll heterogeneous (Figs. 12C, D), palisade occupying 25 to 39% of the mesophyll, in *Jaegeria* most of the mesophyll occupied by aerenchyma (Fig. 12C); collateral vascular bundles with a parenchymatous bundle sheath and girders (*Galinsoga* and *Melampodium*), canals associated with vascular bundles; midvein contour with a projection on the adaxial surface and abaxially wide slightly protruding (*Jaegeria*) or round and protruding (Figs. 12G-I), cuticle similar to lamina, epidermises uniseriate, rectangular or convex cells with thicker outer periclinal walls, immediately beneath the epidermises parenchyma or annular collenchyma, one to three layers, restricted to the projections and parenchyma towards vascular tissue, a central arc of vascular tissue (Figs. 12G, I) and in *Galinsoga* opposite to the main arc phloem and some vessels evident (Fig. 12H), within the bundle, xylem in radial rows of two to five vessels or clusters of five to ten vessels, phloem formed by one to four rows of cells, caps associated to vascular tissue absent.

Tribe Nassauvieae

One species: *Acourtia cordata* (Cerv.) B.L. Turner (Fig. 13).

Leaves sessile, alternate and simple; lamina size macrophyll, lamina shape elliptic to oblong, margin dentate, apex convex, base cordate; primary vein framework pinnate, primary vein straight, prominent, secondary venation brochidodromous, areole development moderate, veinlets simple, curved, marginal ultimate venation looped; teeth more than one central plus two accessories straight; leaves amphistomatic; in superficial view, cells tetragonal-elongated with S-undulate anticlines (Fig. 13A), both surfaces pubescent, with multicellular trichomes and stomata anomocytic or anisocytic, in transverse view, the cuticle smooth and less than 0.06 µm in thickness, epidermises uniseriate, with convex cells and thicker outer periclinal walls, both epidermis of the same width, stomata at the same level as epidermal cells; mesophyll heterogeneous with one row of palisade parenchyma, occuping 26% of the mesophyll (Fig. 13B); collateral vascular bundles with a parenchymatous sheath, girders present in secondary veins and usually composed of sclerenchyma associated to phloem (Fig. 13C), canals absent; midvein contour adaxially flat and abaxially protruded triangular (Fig. 13D), cuticle similar to lamina, epidermises uniseriate with narrow convex cells and thick outer periclinal walls, immediately beneath the epidermises, angular collenchyma of one or two layers and parenchyma towards the vascular tissue, three collateral vascular bundles, within the bundles, xylem with more than twelve vessels, separated by parenchyma, phloem formed by six to ten rows of cells, caps of abundant sclerenchyma associated with xylem and phloem (Fig. 13D).

Tribe Senecioneae

Three species: *Barkleyanthus salicifolius* (Kunth) H. Rob. & Brettell, *Pittocaulon praecox* (Cav.) H. Rob. & Brettell and *Roldana lobata* La Llave (Figs. 1, 14).

Leaves petiolate, alternate and simple; lamina size mesophyll to macrophyll, lamina shape ovate-elliptic (*Pit-*

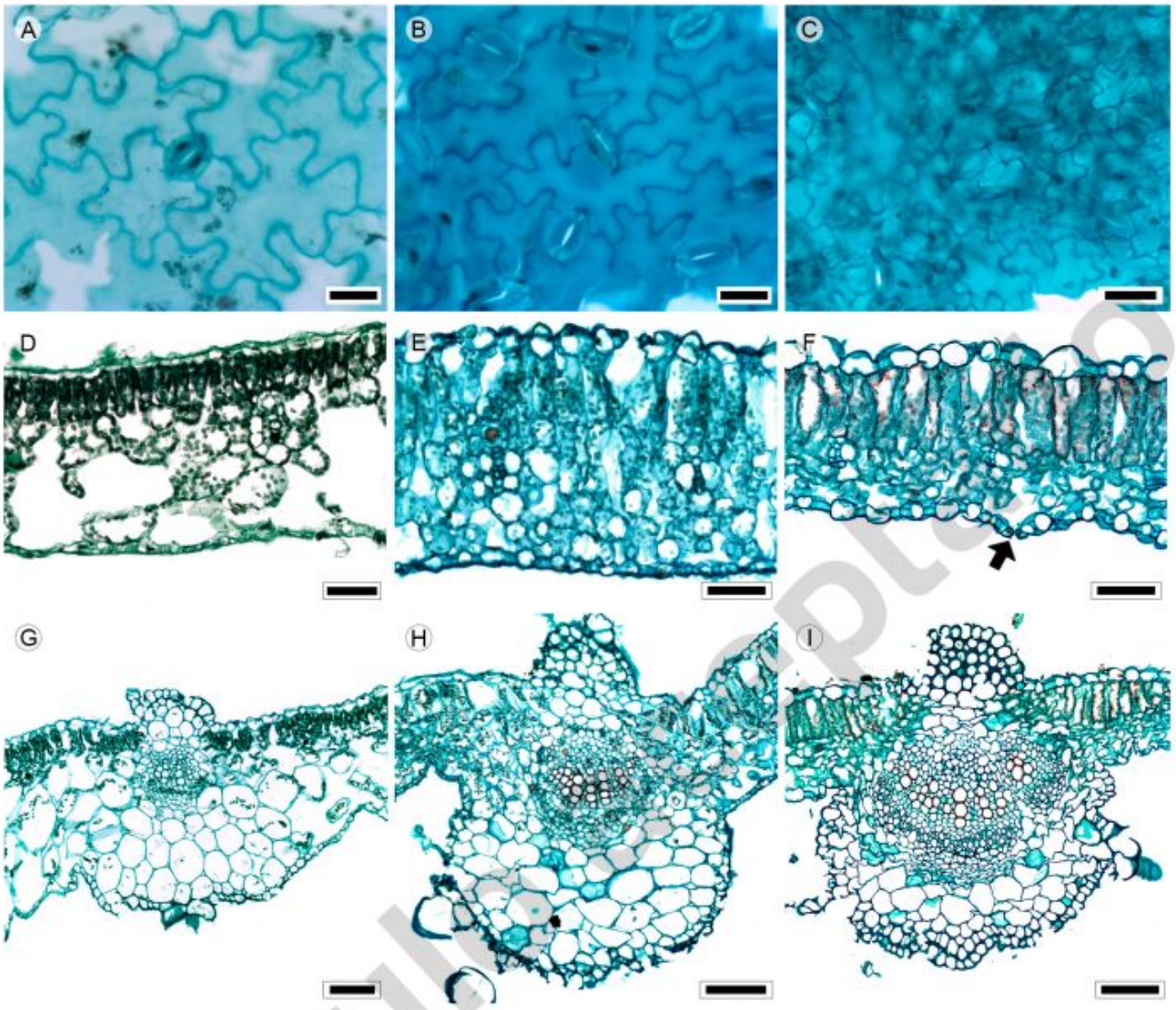

Figure 12: Lamina and midvein in Millerieae. A, D, G. *Jaegeria hirta* (Lag.) Less.; B, E, H. *Galinsoga parviflora* Cav.; C. *Melampodium longifolium* Cerv. ex Cav.; F, I. *Melampodium perfoliatum* (Cav.) Kunth. Scale is 20 µm in A-C; 50 µm in D-F, 100 µm in G-I.

tocaulon H. Rob. & Brettell) or linear-elliptic (*Barkleyanthus* H. Rob. & Brettell, *Roldana* La Llave), margin entire, serrate or dentate, apex acuminate acute (*Barkleyanthus*, Fig. 10; *Pittocaulon*, *Roldana*), base lobate (*Pittocaulon*, Fig. 1P), rounded to truncate (*Roldana*) or cuneate (*Barkleyanthus*); primary vein framework pinnate or actinodromous, primary vein straight, prominent, secondary venation is parallelodromous (*Barkleyanthus*), actinodromous suprabasal (*Pittocaulon*) or mixed craspedrodromous (*Roldana*), areole development moderate (*Barkleyanthus*, *Pittocaulon*) or good (*Roldana*), veinlets once-branched (*Barkleyanthus*, *Pittocaulon*) or absent (*Roldana*), marginal ultimate venation looped; teeth with accessory veins (*Barkleyanthus*, *Roldana*) or absent (*Pittocaulon*); leaves amphistomatic or hypostomatic, in surface view, cells polygonal-elongated with straight anticlines (Figs. 14A-C), both epidermises glabrous or glabrate and stomata anomocytic, in transverse view, the cuticle striate and between 0.36 and 0.46 µm in thickness, epidermises uniseriate, with rectangular or conical cells and thicker outer periclinal walls, both epidermis equally in width, stomata at the same level as the epidermal cells; mesophyll heterogeneous (Fig. 14D) or homogeneous (*Pittocaulon*, *Roldana*, Figs. 14E, F), palisade occupying 53% of the mesophyll (*Barkleyanthus*); collater-

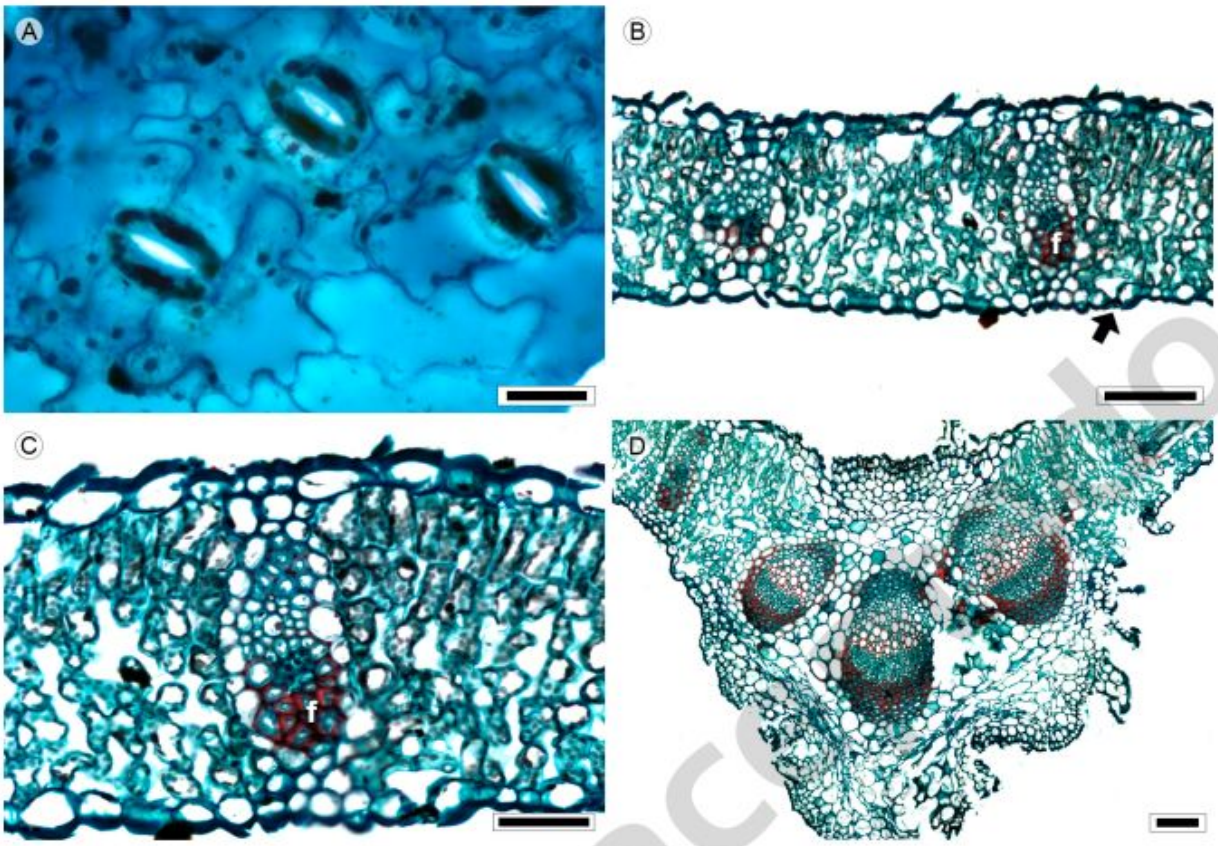

Figure 13: Lamina and midvein in Nassauvieae. A-D. *Acourtia cordata* (Cerv.) B.L. Turner. Scale is 20 µm in A; 100 µm in B, D; 50 µm in C. f=fibers.

al vascular bundles with a parenchymatous bundle sheath, girders in secondary veins, canals associated with vascular bundles or in the mesophyll (*Barkleyanthus*); midvein contour slightly convex or protruded adaxially and abaxially round and protruded (Figs. 14G, I) or gently protruded in both surfaces (Fig. 14H), cuticle similar to lamina, epidermises uniseriate with convex cells and thicker outer periclinal walls, immediately beneath the epidermis angular collenchyma, one to three layers surrounding almost all the midrib (*Pittocaulon*), restricted to a small region in either surface (*Barkleyanthus*) or lacking (*Roldana*), a single central collateral vascular bundle or three bundles forming an arc (Fig. 14E), within the bundle, xylem in radial rows of two to ten vessels, phloem formed by two to five rows of cells, cap of sclerenchyma associated with xylem.

Tribe Tageteae

Four species: *Dyssodia papposa* (Vent.) Hitchc., *Pectis prostrata* Cav., *Tagetes micrantha* Cav. and *T. tenuifolia* Cav. (Figs. 1, 4, 15).

Leaves sessile, opposite, simple (*Pectis* L.) or pinnatisect (*Dyssodia* Cav., *Tagetes* L., Figs. 1Q, R); lamina size nanophyll to mesophyll, lamina shape linear-elliptic to obovate, margin entire (*P. prostrata*, *T. micrantha*), toothed (*Dyssodia*) or serrate (*T. tenuifolia*), apex straight or acuminate, base cuneate or truncated; primary vein framework pinnate (Figs. 4J, K), primary vein straight, prominent, secondary venation brochidodromous, areole development moderate (*T. tenuifolia*), poor (*Dyssodia*, *Pectis*) or lacking (*T. micrantha*), veinlets simple, curved (*Dyssodia*, *Tagetes*), straight (*Pectis*) or absent (*Pectis*), marginal ultimate venation looped (*Pectis*, *Tagetes*) or incomplete (*Dyssodia*); teeth with primary and accessory veins straight, primary vein termination at the apex of tooth; leaves amphistomatic; in surface view, cells tetragonal, tetragonal-elongated or polygonal with straight to S-undulate anticlines (Figs. 15A-C), glabrate in both surfaces and stomata anomocytic or anisocytic, in transverse view, the cuticle striated or smooth and between 0.19 and 0.44 µm in thickness, epidermises uniseriate, with square

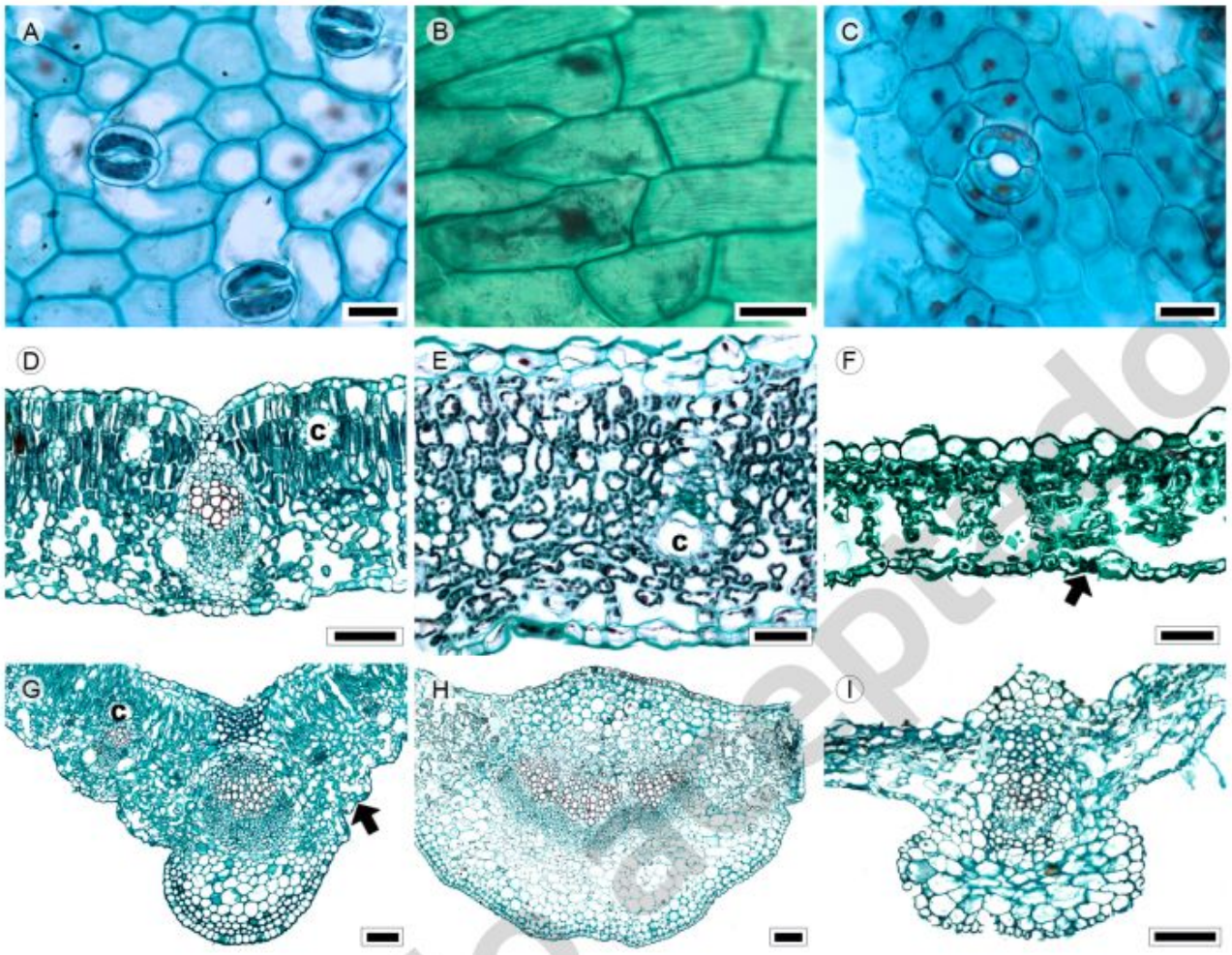

Figure 14: Lamina and midvein in Senecioneae. A, D, G. *Barkleyanthus salicifolius* (Kunth) H. Rob. & Brettell; B, E, H. *Pittocaulon praecox* (Cav.) H. Rob. & Brettell; C, F, I. *Roldana lobata* La Llave. Scale is 20 µm in A-C; 50 µm in E, F; 100 µm in D, G-I; 300 µm in I, J. c=canal.

or convex cells and thicker outer periclinal walls, both epidermises equall in width, tannins occluding cell lumina in *Dyssodia,* stomata at the same level as the epidermal cells; mesophyll heterogeneous (Figs. 15D-F), palisade occupying 27 to 42% of the mesophyll; collateral vascular bundles with a parenchymatous bundle sheath, paraveinal mesophyll between vascular bundles (Fig. 15D), canals associated with vascular bundles or in the mesophyll (Fig. 15E); midvein contour adaxially flat and abaxially gently protruded (*Pectis*, (Fig. 15H, *T. micrantha*) or adaxially flat and abaxially protruded round (*T. tenuifolia*) or triangular (*Dyssodia*) (Figs. 15G, I), cuticle conspicuously striate and thicker than the lamina, epidermises uniseriate with convex cells and thicker outer periclinal walls, immediately beneath the epidermises, a layer of annular collenchyma and parenchyma towards the vascular tissue, a single central collateral vascular bundle or three bundles (Fig. 15H), within the bundles, xylem in grouped in radial rows of two to four vessels, phloem formed of two to three rows of cells, caps of sclerenchyma (one to three layers) associated with phloem and mainly xylem.

Discussion

We find that most of the studied species are petiolate (61%), simple (68%) and microphyll (29%). The laminar shape is most often ovate (45%) with some type of vascularized margin projections in 62% of the species. The brochidodromous venation pattern predominates (62%), followed by actinodromous (33%); areole development is moderate in 86% of the species and the freely ending veinlets are simple and curved in 53% of these; 90% of the species have looped

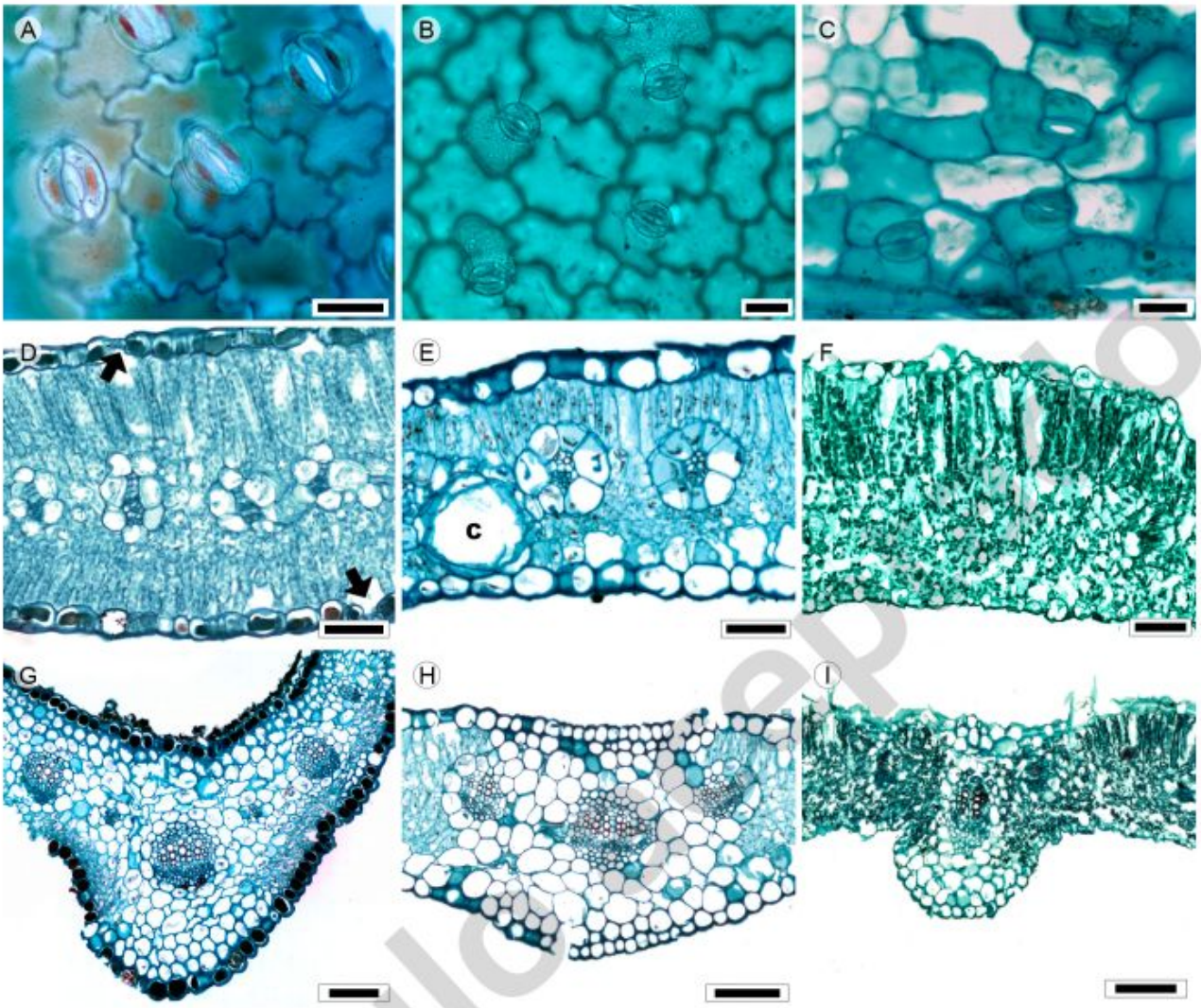

Figure 15: Lamina and midvein in Tageteae. A, D, G. *Dyssodia papposa* (Vent.) Hitchc.; B, E, H. *Pectis prostrata* Cav.; C. *Tagetes micrantha* Cav.; F, I. *Tagetes tenuifolia* Cav. Scale is 20 µm in A-C; 50 µm in D-F, 100 µm in G-I.

marginal ultimate venation. The leaves are amphistomatic (66%) with anomocytic stomata (62%). Seventy four percent of the species have striate cuticle. The surface of 75% of the species is pubescent. The stomata of 77% of the species are at the same level as the rest of the epidermal cells. Epidermal cells in superficial view are tetragonal-elongated with S-undulated anticlines (63%). Almost all species (98%) have a vascular bundle sheath, but only in 73% of these there are sheath extensions, whereas sclerified cells associated with bundle sheaths are rare in the species studied, as in other members of the family (Aytas-Akcin and Akcin, 2017). Bundle sheaths have also been recorded for other taxa of Asteraceae (Bombo et al., 2012; Chwil et al., 2015; Mendes et al., 2016; De Sousa et al., 2018; Lusa et al., 2018) and they appear to be one of the most common traits in the family.

Although we did not find a unique combination of characters for any of the tribes, some patterns exist. For instance, the brochidodromous venation pattern appears to be the most common trait in most tribes, except in Senecioneae which has three different types (parallelodromous, actinodromous suprabasal and mixed craspedrodromous); this is consistent with Rojas-Leal et al. (2018), who found four venation patterns for the Mexican genera of the Senecioneae tribe. Stomata raised above epidermal cells appear to be a trait more common in the Eupatorieae as well as a

particular type of midvein contour (adaxially flat or slightly projected and abaxially round or square protruded or highly protruded). This combination of characters has been recorded in other members of this tribe (Milan et al., 2006; Santos et al., 2016). The presence of paraveinal mesophyll and bundle sheaths without girders are common in the Tageteae tribe whereas the presence of bundle sheaths with extensions and canals associated with them is a common feature in the Heliantheae as recorded for other members (Bombo et al., 2012). The occurrence of abundant sclerified cells associated with vascular bundles in the lamina and the midvein in *A. cordata* is a distinctive feature of the Nassauvieae compared to the other tribes studied. The midvein contour which is flat or slightly convex adaxially and round and protruded abaxially, is a common trait of the *Pseudognaphalium* species studied (Gnaphalieae). In the Millerieae, the midvein contour (adaxially projected and abaxially slightly protruding or round and conspicuously protruded) is common to the three species studied, which also represent three genera. Sampling more species of all tribes of Asteraceae will allow improving not only the tribal descriptions, but also confirm the traits just mentioned for some tribes or find additional ones.

In the Asteraceae tribes studied there is a predominance of small leaf size, serrate margins, poorly developed areole development and the presence of bundle sheath extensions. This combination of characters has been related to efficiency in leaf hydraulic conductance (Sack and Scoffoni, 2013; Sack et al., 2015), light absorption (Nikolopoulos et al., 2002) and biomechanical support (Read and Stokes, 2006). Rivera et al. (2017) tried to explain leaf variation in this xerophytic scrub associating it with different drought-resistance strategies (Ferraro and Scremin-Dias, 2018). However, their results showed that leaf variation could not be solely explained by dry conditions promoted by the volcanic outcrop rocks. Rivera et al. (2017) suggest that characters as plant lifespan, growth form, genome size and species biogeographic history help explain the leaf variation since this attributes have been found related to different extents with anatomical characters in different plant species (Press, 1999; Santiago and Wright, 2007). Detailed anatomical and architectural descriptions are the first steps for any exhaustive study on the causes of leaf variation.

Conclusions

Here we present leaf characters occurring in the thirteen tribes of the Asteraceae present in the REPSA. There is significant variation in leaf architecture and anatomy in this area. The variation observed is consistent with the diversity reported for other authors for the family and for some Mexican tribes. The results of this descriptive study will allow testing evolutionary and ecological hypotheses about the effect of intrinsic and extrinsic factors of the species on the leaf diversity in this area. We suggest future avenues of research on the leaf anatomy of Asteraceae following the format and the terms used in our descriptions of the tribes. We consider that this model will allow standardization of leaf descriptions without losing useful information. Anatomical descriptions are a fundamental piece of the evolutionary, ecological and physiological study of the leaves in Asteraceae.

Author contributions

PR, TT and JLV designed the study. PR carried out the laboratory work and drafted the manuscript. TT and ARL revised and corrected the descriptions and discussion. JLV reviewed and approved the manuscript. All authors read and approved the final manuscript.

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Literature cited

Adedeji, O. and O. Jewoola. 2008. Importance of leaf epidermal characters in the Asteraceae family. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 36(2): 7-16.

Akinnubi, F. M., A. J. Akinloye, O. Olaleye-Otunla and A. Adenegan-Alakinde. 2014. Foliar anatomy of some species of Asteraceae in southwestern Nigeria. African Journal of Plant Sciences 8(9): 426-440. DOI: https://doi.org/10.5897/AJPS2014.1196

Al-Edany, T. Y. and S. A. A. M. Al-Saadi. 2012. Taxonomic significance of anatomical characters in some species of the family Myrtaceae. American Journal of Plant Sciences 3(5): 572-581. DOI: https://doi.org/10.4236/ajps.2012.35069

Anderberg, A. A., B. Baldwin, R. G. Bayer, J. Breitwieser, C. Jeffrey, M. O. Dillon, P. Eldenäs, V. Funk, N. Garcia-Jacas, D. J. N. Hind, P. O. Karis, H. W. Lack, G. L. Nesom, B. Nordenstam, C. Oberprieler, J. L. Panero, C. Puttock, H. Robinson, T. F. Stuessy, A. Susanna, E. Urtubey, R. Vogt, J. Ward and L. E. Watson. 2007. Compositae. In: Kadereit, J. W. and C. Jeffrey (eds.). Families and Genera of Vascular Plants. Flowering Plants, Eudicots, Asterales, vol. 8. Springer-Verlag. Berlin, Germany. Pp. 61-588.

Araújo, J. S., A. A. Alves, L. C. Silva and R. M. S. A. Meira. 2010. Leaf anatomy as an additional taxonomy tool for 16 species of Malpighiaceae found in the Cerrado area (Brazil). Plant Systematics and Evolution 286(1-2): 117-131. DOI: https://doi.org/10.1007/s00606-010-0268-3

Aytas-Akcin, T. and A. Akcin. 2017. Anatomy and micromorphology of *Inula helenium* subsp. *orgyalis* and *I. ensifolia* (Asteraceae) from Turkey. Notulae Scientia Biologicae 9(1): 104-109. DOI: https://doi.org/10.15835/nsb919950

Bercu, R. M., L. B. Făgăra and L. Broască. 2012. Anatomical features of *Aster tripolium* L. (Asteraceae) to saline environments. Annals of the Romanian Society for Cell Biology 17(1): 271-277.

Bombo, A. B., T. S. De Oliveira, A. D. S. S. De Oliveira, V. L. G. Rehder, M. A. G. Magenta and B. Appezzato-Da-Glória. 2012. Anatomy and essential oils from aerial organs in three species of *Aldama* (Asteraceae-Heliantheae) that have difficult delimitation. Australian Journal of Botany 60(7): 632-642. DOI: https://doi.org/10.1071/BT12160

Bondarev, N. I., M. A. Sukhanova, O. V. Reshetnyak and A. M. Nosov. 2003. Steviol glycoside content in different organs of *Stevia rebaudiana* and its dynamics during ontogeny. Biologia Plantarum 47(2): 261-264. DOI: https://doi.org/10.1023/B:BIOP.0000022261.35259.4f

Cambi, V., A. Bucciarelli, A. Flemmer and P. Hansen. 2006. Morfoanatomía de *Pluchea sagittalis* (Asteraceae), especie nativa de interés medicinal. Acta Farmacéutica Bonaerense 25(1): 43-49.

Castro, M. M., H. F. Leitão-Filho and W. R. Monteiro. 1997. Utilização de estruturas secretoras na identificação dos gêneros de Asteraceae de uma vegetação de cerrado. Brazilian Journal of Botany 20(2): 163-174. DOI: https://dx.doi.org/10.1590/S0100-84041997000200007

Céspedes, L., E. Ortiz and J. L. Villaseñor. 2018. La familia Asteraceae en la Reserva Ecológica del Pedregal de San Ángel, Ciudad de México, México. Revista Mexicana de Biodiversidad 89(1): 193-207. DOI: https://dx.doi.org/10.22201/ib.20078706e.2018.1.2203

Chwil, M., M. Krawiec, P. Krawiec and S. Chwil. 2015. Micromorphology of the epidermis and anatomical structure of the leaves of *Scorzonera hispanica* L. Acta Societatis Botanicorum Poloniae 84(3): 357-367. DOI: https://doi.org/10.5586/asbp.2015.033

De Faria, A. P. G., A. C. M. Vieira and T. Wendt. 2012. Leaf anatomy and its contribution to the systematics of *Aechmea* subgenus *Macrochordion* (de Vriese) Baker (Bromeliaceae). Anais da Academia Brasileira de Ciências 84(4): 961-971. DOI: https://dx.doi.org/10.1590/S0001-37652012005000053

De Sousa, D. M. F., R. D. Sá, E. L. Araújo and K. P. Randau. 2018. Anatomical, phytochemical and histochemical study of *Solidago chilensis* Meyen. Anais da Academia Brasileira de Ciências 90(2 suppl. 1): 2107-2120. DOI: https://dx.doi.org/10.1590/0001-3765201720160280

Dickison, W. C. 2000. Integrative Plant Anatomy. Academic Press. San Diego, USA. 533 pp.

Ellis, B., D. C. Daly, L. J. Hickey, J. D. Mitchell, K. R. Johnson, P. Wilf and S. L. Wing. 2009. Manual of Leaf Architecture. Cornell University Press. New York, USA. 190 pp.

Endress, P. K., P. Baas and M. Gregory. 2000. Systematic plant morphology and anatomy: 50 years of progress. Taxon 49(3): 401-434. DOI: https://dx.doi.org/10.2307/1224342

Ferraro, A. and E. Scremin-Dias. 2018. Structural features of species of Asteraceae that arouse discussions about adaptation to seasonally dry environments of the Neotropics. Acta Botanica Brasilica 32(1): 113-127. DOI: https://dx.doi.org/10.1590/0102-33062017abb0246

García-Sánchez, F., M. E. López-Villafranco, S. Aguilar-Rodríguez and A. Aguilar-Contreras. 2012. Etnobotánica y morfo-anatomía comparada de tres especies de *Tagetes* que se utilizan

en Nicolás Romero, Estado de México. Botanical Sciences 90(3): 221-232. DOI: https://doi.org/10.17129/botsci.388

Garnier, E., B. Shipley, C. Roumet and G. Laurent. 2001. A standardized protocol for the determination of specific leaf area and leaf dry matter content. Functional Ecology 15(5): 688-695. DOI: https://doi.org/10.1046/j.0269-8463.2001.00563.x

Grigore, M. and C. Toma. 2006. Ecological anatomy elements related to Asteraceae halophytes species. Studii Şil Comunicări, Complexul Muzeal de Ştiinţele Naturii "Ion Borcea" Bacău 21: 94-98.

Hulley, I. M., A. M. Viljoen, P. M. Tilney, S. F. Van Vuuren, G. P. P. Kamatou and B. E. Van Wyk. 2010. The ethnobotany, leaf anatomy, essential oil variation and biological activity of *Pteronia incana* (Asteraceae). South African Journal of Botany 76(4): 668-675. DOI: https://doi.org/10.1016/j.sajb.2010.08.007

Image Pro-plus. 2019. Image Pro-plus version 7.0. Media Cybernetics, Inc. Bethesda, USA.

Koch, K., B. Bhushan and W. Barthlott. 2009. Multifunctional surface structures of plants: An inspiration for biomimetics. Progress in Materials Science 54(2): 137-178. DOI: https://doi.org/10.1016/j.pmatsci.2008.07.003

Lin, C. Y. and D. Y. Tan. 2015. The taxonomic significance of leaf epidermal micromorphological characters in distinguishing 43 species of *Allium* L. (Amaryllidaceae) from central Asia. Pakistan Journal of Botany 47(5): 1979-1988.

Lot, A. and Z. Cano-Santana (eds.). 2009. Biodiversidad del ecosistema del Pedregal de San Ángel. Libro Conmemorativo del 25 aniversario de la Reserva Ecológica de Ciudad Universitaria (1983-2008). Reserva Ecológica del Pedregal de San Ángel. Coordinación de la Investigación Científica, Universidad Nacional Autónoma de México. México, D.F., México. 538 pp.

Lusa, M. G., B. F. P. Loeuille, D. Ciccarelli and B. Apezzato-da-Glória. 2018. Evolution of stem and leaf structural diversity: a case study in Lychnophorinae (Asteraceae). Botanical Review 84(3): 203-241. DOI: https://doi.org/10.1007/s12229-017-9191-4

Martínez-Cabrera, D., T. Terrazas and H. Ochotorena. 2007. Leaf architecture of Hamelieae (Rubiaceae). Feddes Repertorium 118(7-8): 286-310. DOI: https://doi.org/10.1002/fedr.200711140

McKown, A. D. and N. G. Dengler. 2007. Key innovations in the evolution of Kranz anatomy and C4 vein pattern in *Flaveria* (Asteraceae). American Journal of Botany 94(3): 382-399. DOI: https://dx.doi.org/10.3732/ajb.94.3.382

Mendes, K. R., S. R. Machado, A. C. E. Amaro, S. C. M. Silva, V. F. Júnior and T. M. Rodrigues. 2016. Distribution of homobaric and heterobaric leafed species in the Brazilian Cerrado and seasonal semideciduous forest. Flora 225: 52-59. DOI: https://doi.org/10.1016/j.flora.2016.10.005

Metcalfe, C. and L. Chalk. 1979. Anatomy of the Dicotyledons. 2nd edition. Oxford University Press. New York, USA. 276 pp.

Milan, P., A. H. Hayashi and B. Appezzato-da-Glória. 2006. Comparative leaf morphology and anatomy of three Asteraceae species. Brazilian Archives of Biology and Technology 49(1): 135-144. DOI: https://dx.doi.org/10.1590/S1516-89132006000100016

Moroney, J. R., P. W. Rundel and V. L. Sork. 2013. Phenotypic plasticity and differentiation in fitness-related traits in invasive populations of the Mediterranean forb *Centaurea melitensis* (Asteraceae). American Journal of Botany 100(10): 2040-2051. DOI: https://dx.doi.org/10.3732/ajb.1200543

Nikolopoulos, D., G. Liakopoulos, I. Drossopoulos and G. Karabourniotis. 2002. The relationship between anatomy and photosynthetic performance of heterobaric leaves. Plant Physiology 129(1): 235-243. DOI: https://dx.doi.org/10.1104/pp.010943

Noyes, R. D. and L. H. Rieseberg. 1999. ITS sequence data support a single origin for North American Astereae (Asteraceae) and reflect deep geographic divisions in *Aster* s.l. American Journal of Botany 86(3): 398-412. DOI: https://doi.org/10.2307/2656761

Press, M. C. 1999. The functional significance of leaf structure: a search for generalizations. New Phytologist 143(1): 213-219. DOI: https://doi.org/10.1046/j.1469-8137.1999.00432.x

Read, J. and A. Stokes. 2006. Plant biomechanics in an ecological context. American Journal of Botany 93(10): 1546-1565. DOI: https://dx.doi.org/10.3732/ajb.93.10.1546

Rivera, P., J. L. Villaseñor and T. Terrazas. 2017. Meso- or xeromorphic? Foliar characters of Asteraceae in a xeric scrub of Mexico. Botanical Studies 58(1): 12. DOI: https://doi.org/10.1186/s40529-017-0166-x

Rojas-Leal, A., T. Terrazas and J. L. Villaseñor. 2014. Desarrollo del patrón de venación en cuatro especies de la tribu Senecioneae (Asteraceae). Botanical Sciences 92(1): 23-36. DOI: https://dx.doi.org/10.17129/botsci.25

Rojas-Leal, A., J. L. Villaseñor and T. Terrazas. 2017. Tricomas foliares en *Senecio* sección *Mulgediifolii* (Senecioneae, Asteraceae). Acta Botanica Mexicana 119: 69-78. DOI: https://dx.doi.org/10.21829/abm119.2017.1232

Rojas-Leal, A., T. Terrazas and J. L. Villaseñor. 2018. Foliar architecture of some members of the tribe Senecioneae (Asteraceae) with a key for identification of the Mexican genera. Phytotaxa 364(2): 136-156. DOI: https://dx.doi.org/10.11646/phytotaxa.364.2.2

Rossatto, D. R. and R. M. Kolb. 2010. *Gochnatia polymorpha* (Less.) Cabrera (Asteraceae) changes in leaf structure due to differences in light and edaphic conditions. Acta Botanica Brasilica 24(3): 605-612. DOI: https://dx.doi.org/10.1590/S0102-33062010000300002

Ruzin, E. S. 1999. Plant microtechnique and microscopy. Oxford University Press. New York, USA. 322 pp.

Sack, L. and C. Scoffoni. 2013. Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future. New Phytologist 198(4): 983-1000. DOI: https://dx.doi.org/10.1111/nph.12253

Sack, L., C. Scoffoni, D. M. Johnson, T. N. Buckley and T. J. Brodribb. 2015. The Anatomical Determinants of Leaf Hydraulic Function. In: Hacke, U. (ed.). Functional and Ecological Xylem Anatomy. Springer. Cham, Switzerland. Pp. 255-271. DOI: https://doi.org/10.1007/978-3-319-15783-2_10

Santiago, L. S. and S. C. Kim. 2009. Correlated evolution of leaf shape and physiology in the woody *Sonchus* alliance (Asteraceae: Sonchinae) in Macaronesia. International Journal of Plant Sciences 170(1): 83-92. DOI: https://dx.doi.org/10.1086/593044

Santiago, L. S. and S. J. Wrigth. 2007. Leaf functional traits of tropical forest plants in relation to growth form. Functional Ecology 21(1): 19-27. DOI: https://doi.org/10.1111/j.1365-2435.2006.01218.x

Santos, R. F., B. M. Nunes, R. D. Sá, L. A. L. Soares and K. P. Randau. 2016. Morpho-anatomical study of *Ageratum conyzoides*. Revista Brasileira de Farmacognosia 26(6): 679-687. DOI: https://doi.org/10.1016/j.bjp.2016.07.002

Suárez-Mota, M. E. and J. L. Villaseñor. 2011. Las Compuestas endémicas de Oaxaca, México: diversidad y distribución. Botanical Sciences 88: 55-66. DOI: https://dx.doi.org/10.17129/botsci.308

UNAM. 2006. Reserva Ecológica del Pedregal de San Ángel de Ciudad Universitaria. Reglamento interno del Comité Técnico. Lineamientos para el desarrollo de actividades dentro de la Reserva Ecológica. Acuerdo 2005. Secretaría Ejecutiva de la Reserva Ecológica del Pedregal de San Ángel, Programas Universitarios, Circuito de la Investigación Científica, Universidad Nacional Autónoma de México. C.d. Mx., México. 29 pp.

Villaseñor, J. L. 2018. Diversidad y distribución de la familia Asteraceae en México. Botanical Sciences 96(2): 332-358. DOI: https://dx.doi.org/10.17129/botsci.1872

Appendix: Collectors and collection numbers of vouchers of the species studied of Asteraceae. All species are deposited in the Herbario Nacional de México (MEXU) Instituto de Biología, Universidad Nacional Autónoma de México.

Asteraceae

Tribe Anthemideae

Artemisia ludoviciana Nutt., *L. Céspedes 443*

Cotula australis (Sieber ex Spreng.) Hook. f., *L. Céspedes 476*

Tribe Astereae

Baccharis pteronioides DC., *O. Hinojosa 565*

Baccharis salicifolia (Ruiz & Pav.) Pers., *L. Céspedes 438*

Conyza bonariensis (L.) Cronquist; *F. Soto 71, L. Céspedes 477*

Conyza canadensis (L.) Cronquist, *O. Hinojosa 464*

Conyza coronopifolia Kunth, *O. Hinojosa 467*

Laennecia sophiifolia (Kunth) G.L. Nesom, *O. Hinojosa 583*

Tribe Bahieae

Florestina pedata (Cav.) Cass., *L. Céspedes 223, 470*

Schkuhria pinnata (Lam.) Kuntze ex Thell., *L. Céspedes 124*

Tribe Cardueae

Cirsium vulgare (Savi) Ten., *O. Hinojosa 506*

Tribe Cichorieae

Helminthotheca echioides (L.) Holub., *L. Céspedes 478*

Lactuca serriola L., *F. Soto 91*

Sonchus oleraceus L., *L. Céspedes 456*

Taraxacum officinale F.H. Wigg., *O. Hinojosa 525*

Tribe Coreopsideae

Bidens odorata Cav., *O. Hinojosa 518*

Bidens pilosa L., *O. Hinojosa 550*

Cosmos bipinnatus Cav., *L. Céspedes 440*

Cosmos parviflorus (Jacq.) Pers., *L. Céspedes 439*

Dahlia coccinea Cav., *O. Hinojosa 585*

Heterosperma pinnatum Cav., *O. Hinojosa 515*

Tribe Eupatorieae

Ageratina adenophora (Spreng.) R.M. King & H. Rob., *O. Hinojosa 563*

Ageratina choricephala (B.L. Rob.) R.M. King & H. Rob., *L. Céspedes 623*

Ageratina cylindrica (McVaugh) R.M. King & H. Rob., *L. Céspedes 280*

Ageratina deltoidea (Jacq.) R.M. King & H. Rob., *O. Hinojosa 549*

Brickellia secundiflora (Lag.) A. Gray, *O. Hinojosa 555*

Brickellia veronicifolia (Kunth) A. Gray, *O. Hinojosa 497*

Chromolaena pulchella (Kunth) R.M. King & H. Rob., *O. Hinojosa 559*

Fleischmannia pycnocephala (Less.) R.M. King & H. Rob., *O. Hinojosa 554*

Piqueria trinervia Cav., *O. Hinojosa 522*

Stevia micrantha Lag., *F. Soto 153*

Stevia origanoides Kunth, *L. Céspedes 668, O. Hinojosa 523*

Stevia salicifolia Cav., *L. Céspedes 638, O. Hinojosa 498*

Stevia subpubescens Lag., *O. Hinojosa 561*

Stevia tomentosa Kunth, *L. Céspedes 433*

Tribe Gnaphalieae

Pseudognaphalium canescens (DC.) Anderb., *L. Céspedes 484*

Pseudognaphalium semilanatum (DC.) Anderb., *L. Céspedes 516*

Pseudognaphalium viscosum (Kunth) Anderb., *O. Hinojosa 504*

Tribe Heliantheae

Acmella repens (Walter) Rich., *O. Hinojosa 519*

Aldama buddleiiformis (DC.) E.E. Schill. & Panero, *L. Céspedes 441*

Aldama excelsa (Willd.) E.E. Schill. & Panero, *L. Céspedes 370*

Ambrosia cumanensis Kunth, *O. Hinojosa 507*

Lagascea rigida (Cav.) Stuessy, *L. Céspedes 483*

Montanoa grandiflora Alamán ex DC., *L. Céspedes 436, 607*

Montanoa tomentosa Cerv., *L. Céspedes 369, F. Soto 15*

Simsia amplexicaulis (Cav.) Pers., *L. Céspedes 473*

Tithonia tubiformis (Jacq.) Cass., *L. Céspedes 444*

Verbesina virgata Cav., *L. Céspedes 437*

Zinnia peruviana (L.) L., *O. Hinojosa 508*

Tribe Millerieae

Galinsoga parviflora Cav., *L. Céspedes 475*

Jaegeria hirta (Lag.) Less., *L. Céspedes 372, F. Soto 176*

Melampodium longifolium Cerv. ex Cav., *F. Soto 14*

Melampodium perfoliatum (Cav.) Kunth, *F. Soto 19*

Tribe Nassauvieae

Acourtia cordata (Cerv.) B.L. Turner, *L. Céspedes 442*

Tribe Senecioneae

Barkleyanthus salicifolius (Kunth) H. Rob. & Brettell, *L. Céspedes 559, F. Soto 85*

Pittocaulon praecox (Cav.) H. Rob. & Brettell, *O. Hinojosa 505*

Roldana lobata La Llave, *L. Céspedes 448*

Tribe Tageteae

Dyssodia papposa (Vent.) Hitchc., *L. Céspedes 206, 471*

Pectis prostrata Cav., *O. Hinojosa 524*

Tagetes micrantha Cav., *L. Céspedes 434*

Tagetes tenuifolia Cav., *L. Céspedes 474*

Capítulo 2.

Variación foliar asociada a la adaptación a un matorral xerófilo.

Rivera P., Terrazas T., Villaseñor J.L. 2017. Meso- or xeromorphic? Foliar characters of Asteraceae in a xeric scrub of México. Botanical Studies 58:12

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Botanical Studies

ORIGINAL ARTICLE

Open Access

Meso- or xeromorphic? Foliar characters of Asteraceae in a xeric scrub of Mexico

Patricia Rivera[1,2*], José Luis Villaseñor[1] and Teresa Terrazas[1]

Abstract

Background: The anatomical traits associated with water deficit are also observed in plants growing in poor soils. The species may resist water deficit through three main strategies: escape, avoid or tolerate. The Pedregal de San Ángel Ecological Reserve (REPSA), Mexico, is an environment with low nutrient soil and low water availability. It is set on the basalt formation derived from the Xitle volcano eruption. The main vegetation type is characterized as xerophytic shrub. Thus we expect that species growing in this community will show leaf xeromorphic traits and may have any of the three response strategies. We analyzed the foliar anatomy of 52 species of the Asteraceae family at the REPSA because it is the most abundant angiosperm family in the site, showing a wide variety of growth forms and anatomical variation.

Results: The foliar anatomies of the studied Asteraceae were highly variable as well as their quantitative traits as revealed by principal component analysis. This agrees with previous studies that found great anatomical variation within the family. Leaves have multiple layered palisade parenchyma and parenchyma bundle sheaths and could not be categorized as xeromorphic because they possess mesomorphic leaf features as simple lamina, single-layered epidermis, and soft large-size glabrous leaves with high specific leaf area.

Conclusions: The combination of mesomorphic and few xeromorphic foliar traits with other characters at the genus and tribal level probably has been essential in Asteraceae to colonize various environments, including those with low water and nutrient availability.

Keywords: Astereae, Eupatorieae, Heliantheae, Foliar anatomy, Poor soils, Water stress

Background

Plants growing under xeric conditions can have a set of anatomical, physiological or phenological adaptations allowing them to escape, avoid or tolerate water stress (Santos and Ochoa 1990; Fahn and Cutler 1992; Dickison 2000; Valladares et al. 2004). Drought-escaping species generally are annual or biannual herbs that develop their life-cycle in short time periods when conditions are favorable (Kramer 1983; Santos and Ochoa 1990; Fahn and Cutler 1992; Valladares et al. 2004) or they usually possess perennation structures, such as bulbs, rhizomes or runners that remain latent until conditions are favorable again. Plants showing the avoidance-of-stress strategy possess some features enabling them to minimize or compensate for water loss or to increase water uptake and avoid desiccation. Indicator traits of the drought avoidance mechanism include deep roots, small or strongly lobulated leaves, mesophyll cells smaller in size, thick cell-walls and cuticles, multiseriate epidermis, strongly developed palisade parenchyma, stomata sunken or in crypts and abundant trichomes (Esau 1976; Kramer 1983; Santos and Ochoa 1990; Gibson 1996; Dickison 2000; Valladares et al. 2004; Fang and Xiong 2015). Drought tolerating plants can sustain a certain level of physiological activities under stress conditions. Tolerating plants show tissues resistant to dehydration and characters such as stress-induced leaf abscission, succulent leaves, lignified cell-walls, mucilage accumulation and the occurrence of buliform cells (Kramer 1983; Santos

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and Ochoa 1990; Dickison 2000; Valladares et al. 2004; Fang and Xiong 2015).

Traits of stress-avoiding and stress-tolerating plants have also been related to soil nutrient deficit (Dickison 2000; Fonseca et al. 2000; Wright et al. 2002). In fact, soil nutrient availability explains better the distribution of foliar characters in plants around the world than mean annual temperature, mean annual precipitation and irradiance together (Ordoñez et al. 2009). It has been hypothesized that xeromorphism has evolved from the adaptation of mesomorphic plants to low nutrient soil (Fahn and Cutler 1992) or to both, the lack of water and poor soils. These two conditions are found at the Pedregal de San Ángel Ecological Reserve in the Basin of Mexico.

The Pedregal de San Ángel Ecological Reserve (REPSA) is a protected area for the vegetation at the southern edge of the Mexico basin (Fig. 1a). The REPSA is located in the southwest region of the Mexico City basin (19°18′21″ to 19°20′11″N and 99°10′15″ to 99°12′4″W), at an elevation interval of 2292–2365 m, and it encompasses an area of 237.3 ha (Fig. 1a). The vegetation type has been characterized as xerophilous scrub (Rzedowski 2006). This ecosystem is on a set of basaltic formations produced by the solidification of lava flow during the eruption of the Xitle volcano about 1670 years ago (Siebe 2000). Although mean annual precipitation is 883 mm, the bedrock does not allow water retention and the soil is shallow. So aridity in this site is more edaphic than climatic. It is a unique system in the world because it is within the biogeographic province of the Transmexican Volcanic Belt (Morrone 2006) and the flora composition has Neotropical and Nearctic affinities (Rzedowski 1998; Rzedowski and Rzedowski 2005). The vegetation surrounding the REPSA includes various types of forests, including oak forest, fir forest, and cloud forest (Rzedowski and Rzedowski 2005; Lot and Cano-Santana 2009), which is consistent with the vegetation type expected given the elevation (over 2240 masl), the mean annual rainfall (770–1200 mm), and the mean temperature (11–15 °C) of the site (Fig. 1b). Therefore, it is assumed that species hat colonized after the eruption came mainly from the surrounding forests; however, the current vegetation type is xerophilous scrub (Castillo-Argüero et al. 2007; Lot and Cano-Santana 2009). At the REPSA, the Asteraceae family shows the highest number of species compared to the 73 other vascular plant families found there (Castillo-Argüero

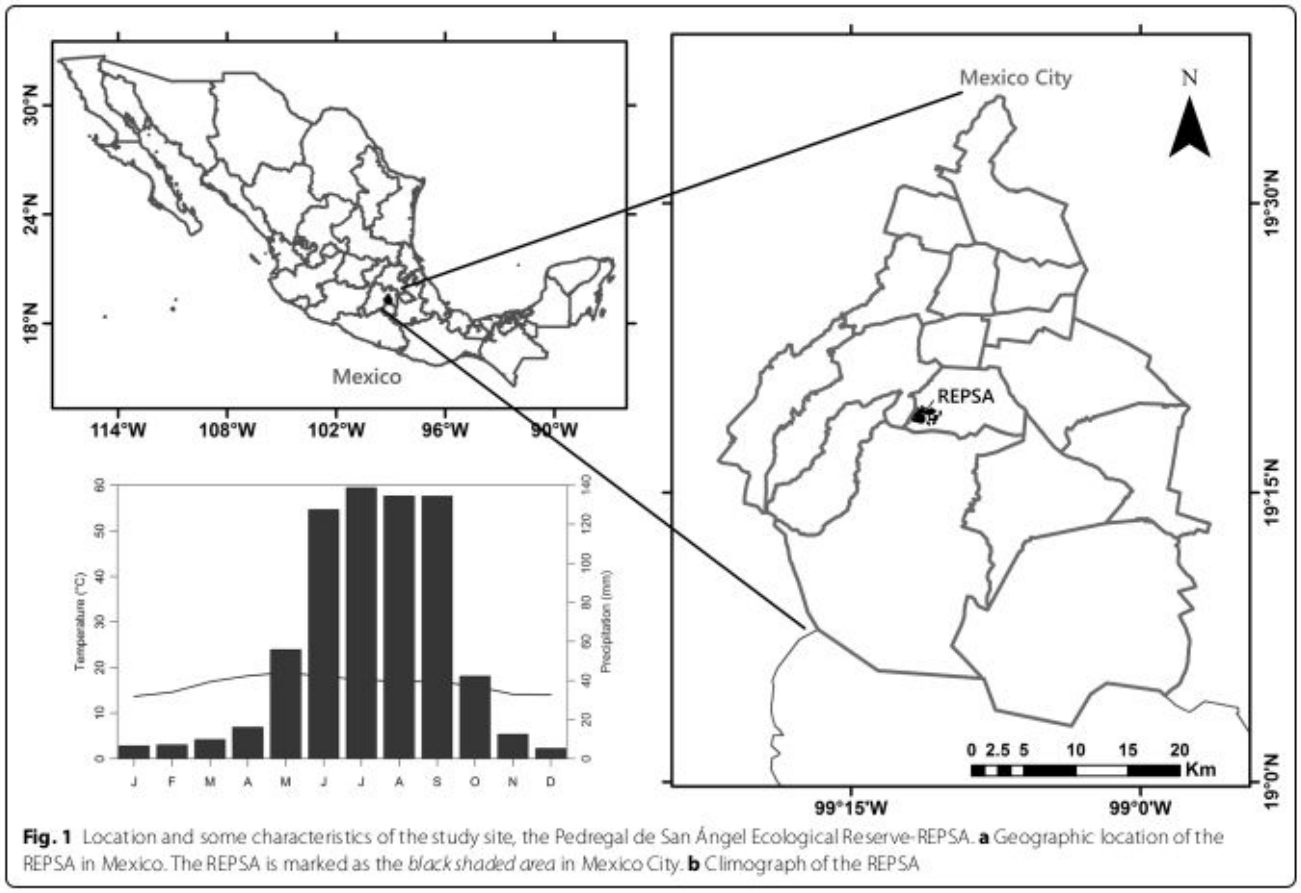

Fig. 1 Location and some characteristics of the study site, the Pedregal de San Ángel Ecological Reserve-REPSA. **a** Geographic location of the REPSA in Mexico. The REPSA is marked as the *black shaded area* in Mexico City. **b** Climograph of the REPSA

et al. 2004, 2007; Lot and Cano-Santana 2009). Céspedes (2010) reported 93 species belonging to 51 genera classified in 13 tribes of Asteraceae in this site. Moreover, the morphological diversity of the family is well represented, as there is a wide variety of growth forms, polyploid species and species with high chromosomic numbers (Soto-Trejo et al. 2011).

The foliar anatomy of Asteraceae has been studied mainly in species with economical value (Ragonese 1988; Ferreira et al. 2002; Freire et al. 2005, 2007; Milán et al. 2006). The leaves are usually dorsiventral, hypo- or amphistomatic and have anomocytic stomata. However, it is difficult to assert generalizations regarding the foliar anatomy of Asteraceae because there is so much variation in characters such as stomata distribution, trichome density and type, hypodermis development, presence of secretory structures and parenchymatic vascular bundles sheaths (Anderson and Creech 1975; Metcalfe and Chalk 1979; Freire et al. 2005). The usefulness of foliar characters for taxonomic classification at the genus or species level (Ferreira and De Oliveira 1989; Castro et al. 1997; Luque et al. 1999; Delbón et al. 2007; Adedeji and Jewoola 2008) or for phylogenetic and ecological studies (Boeger and Wisniewski 2003; Horn et al. 2009) has been overlooked because most studies have focused exclusively on examining the epidermal surface (Ferreira et al. 2002; Freire et al. 2005, 2007; Adedeji and Jewoola 2008; Gil et al. 2012; Redonda-Martínez et al. 2016) and ecological or leaf evolution traits are missing for Asteraceae as they have been generated for other plant families (Luckow 2002; Schmerler et al. 2012; Brodribb et al. 2013).

We hypothesize that Asteraceae species at this ecological protected area (REPSA) will show anatomical traits for avoiding or tolerating stress, the aim of this study was to characterize the foliar anatomy of a sample of 52 species belonging to 41 genera and 13 tribes of Asteraceae living in the xeric environment of this reserve.

Methods

The present study was conducted in the REPSA, within the central campus of the National Autonomous University of Mexico (UNAM, Fig. 1a). Samples were collected during two rainy seasons from August 2008 to December 2009. Three to six fully developed leaves showing no evidence of injury were removed from three individuals of each selected species. All the leaves were photographed with a digital camera. Leaf area was determined using an image analyzer according to the procedure described by Garnier et al. (2001). After being photographed, two to three the leaf laminas per species were dried in oven at 60 °C for at least 48 h to constant weight and then weighed on an analytical balance to determine the dry mass of the leaf. Specific leaf area (SLA) was calculated as the ratio between the leaf area and leaf dry mass [SLA = leaf area (cm^2)/dry leaf mass (g)]. The remaining leaves (1–4 per species) were fixed with a formaldehyde-glacial acetic acid-ethyl alcohol solution (Ruzin 1999). They were then rinsed with tap water to remove fixative residues and stored in a glycerin-ethyl-alcohol-water solution (1:1:1) until sectioning. Voucher specimens information is given in Additional file 1.

Portions of the middle region of the leaf, including the intercostal area from the midvein to the margin were cut, rinsed and dehydrated in increasing concentrations of tert-butanol (10–100%) with a Leica automatic tissue processor (TP1020) remaining for 24 h in each concentration. The tissues were embedded in paraffin (melting point: 56 °C) and transverse and paradermal sections 10–12 μm in thickness were cut with a Leica rotatory microtome (RM2125). The resulting sections were stained with safranin-fast green (Johansen 1940) and mounted on synthetic resin. In the paradermal sections, the cuticle, guard cell length and epidermal cell shapes were examined. In the transverse sections, the thickness of the following features was measured: the total leaf, mesophyll, palisade parenchyma, spongy parenchyma, cuticle, and height of the abaxial and adaxial epidermis cells. The measurements were obtained through an Olympus microscope BX-51 attached to an image analyzer (Image Pro-plus version 6.1, Media Cybernetics 2006). For each variable, 25 measures were recorded. Other features, such as the number of palisade parenchyma layers or the spongy parenchyma type (open or compact), were also determined. Laminar size classification followed Webb (1959), detecting 6 categories: nanophyll (0.25–2.25 cm^2), microphyll (2.25–20.25 cm^2), notophyll (20.25–45 cm^2), mesophyll (45–182.25 cm^2), macrophyll (182.25–1640.25 cm^2) and megaphylly (>1640.25 cm^2). Sclerophylly index (SI) was calculated following Boeger and Wisniewski (2003) where SI = leaf dry mass (g)/2 leaf area (cm^2), considering SI > 0.6 as sclerophylly and SI < 0.6 as mesophylly). Stomatal pore area index (SPI) was calculated following Tian et al. (2016) where SPI = stomatal density*stomatal length*10^{-4}. Leaf lamina and midvein descriptions followed Dickison (2000) and tribe classification followed Funk et al. (2009).

Characters were square root and log10-transformed prior to statistical analyses. Pearson and Sperman correlations were calculated between pairs of leaf traits. In addition we produce a principal component analysis (PCA) to evaluate which leaf traits explained the variation in this site. Analyses were performed with R (R Core Team 2015) and SAS (Statistical Analysis System software version 9, SAS Institute 2002).

Results

Leaf morphology

The leaves were simple in all the species. However, 29% of species, including most of the studied species of the Tageteae tribe, were deeply lobed. Based on the laminar size, most species were classified in four categories: microphyll (29% of the species), mesophyll (29%), notophyll (23%) and macrophyll (13%). Only two species (*Montanoa grandiflora* and *Tithonia tubiformis*) were classified as megaphyll and one species (*Tagetes micrantha*) as nanophyll leaves. Laminar shape was variable, from ovate, obovate to elliptic. Toothed or untoothed leaf margins were observed. Serrate margins were common and crenate margins were present in some species. Leaf morphological characters by species are given in Additional file 2.

Epidermis

On the surface, the abaxial and adaxial epidermises were glabrous in 95% of the examined species. The remaining 5% were glandular, tector or pluricellular uniseriate or possessed multiseriate trichomes. Trichomes could be found on one or both leaf surfaces, but they were more frequent on the abaxial surface. The epidermal cell shape of both surfaces was elongated polygonal or isodiametrical polygonal, with straight (Fig. 2a–c) or wavy anticlinal walls (Fig. 2d–f). Both amphistomatic and hypostomatic leaves were observed. When amphistomatic, the stomata were more abundant on the abaxial surface. The stomata were anomocytic and rarely anisocytic. The average stomatal density was 362 stomata/mm^2, and the average per tribe is given in Table 1.

On the transverse section, the examined species had cuticle thicknesses varying between 0.19 and 1.15 µm. In 25% of the species, the cuticle appeared smooth. Cuticular striae (Fig. 3a–c) were found in greater abundance in cells of the veins and around trichomes. The epidermises were simple in all taxa. Abaxial (7.48–35.79 µm in height) and adaxial (6.22–40.69 µm in height) epidermises were variable in size. The epidermal cells were generally rectangular in shape and rarely square. In some species, the epidermal cells appeared ovoidal in the periclinal walls due to the elevation of the outer periclinal wall. The outer periclinal wall was often thicker than the internal one,

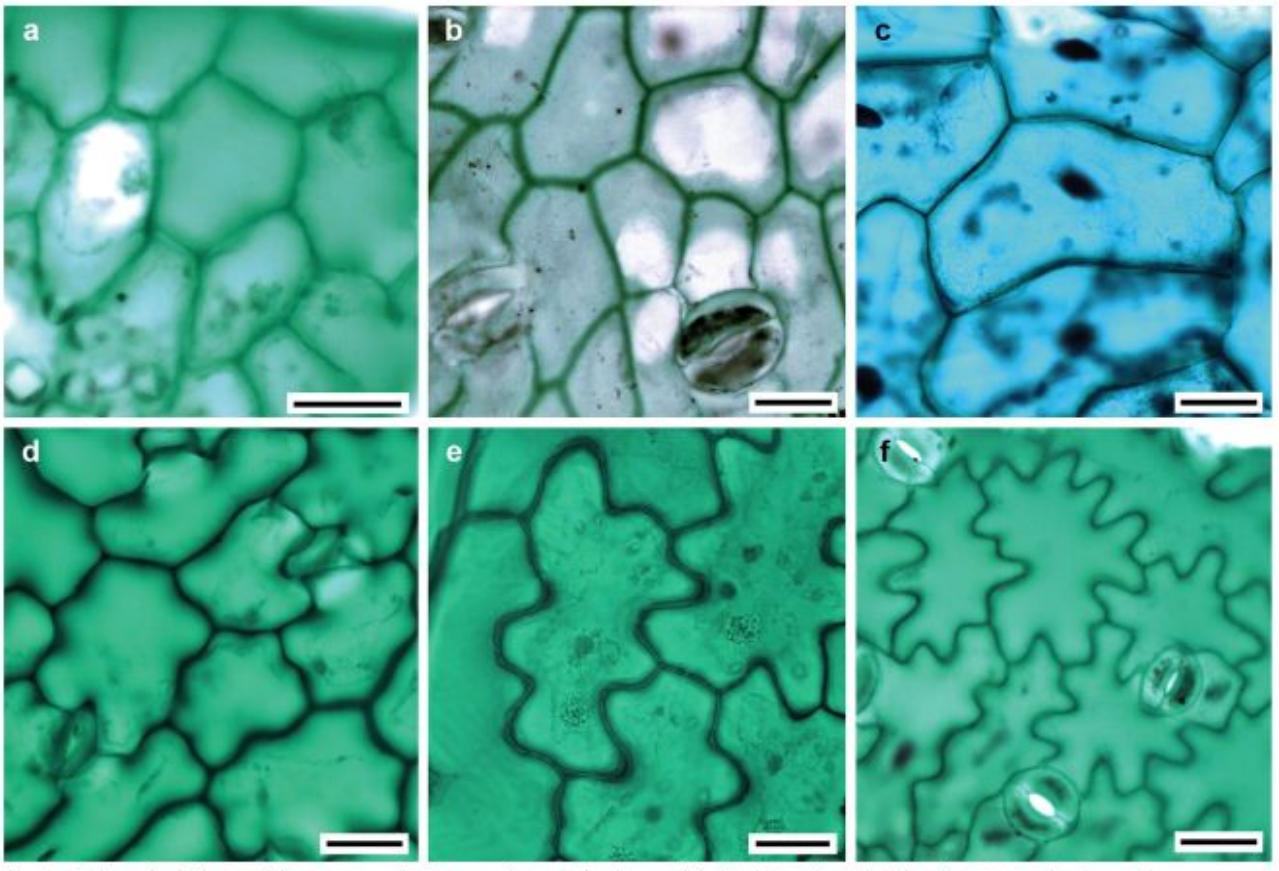

Fig. 2 Epidermal cells forms of Asteraceae. **a** *Conyza canadensis* (Tribe Astereae). **b** *Barckleyanthus salicifolius* (Senecioneae). **c** *Pittocaulon praecox* (Senecioneae). **d** *Pectis prostrata* (Tageteae). **e** *Ageratina adenophora* (Eupatorieae). **f** *Lactuca serriola* (Cichorieae). *Bar* is 20 µm

Table 1 Leaf characters by tribe in Asteraceae of the xeric scrub at the REPSA, Mexico

Tribe	Anthemidae	Astereae	Bahieae	Carduae	Cichorieae	Coreopsidae	Eupatorieae	Gnaphalieae	Heliantheae	Millerieae	Nassauvieae	Senecioneae	Tageteae
No. species/character	2	5	2	1	3	5	12	2	10	2	1	3	4
Leaf size classification	Micro*	Noto (3), micro (2)	Micro	Macro	Macro (2), meso	Meso (2), micro (2), noto (1)	Noto (5), meso (4), micro (2), macro (1)	Micro	Meso (4), noto (2), mega (2), macro (1), micro (1)	Meso, noto	Meso	Macro (2), meso	micro (2), meso (1), nano (1)
Lobation	Lobed	Unlobed (4), lobed	Lobed	Lobed	Lobed (2), unlobed	Lobed	Unlobed	Unlobed	Unlobed (7), lobed	Unlobed	Unlobed	Unlobed (2), lobed	Unlobed (2), lobed
Margin type	Untoothed	Untoothed (3), toothed	Untoothed	Tooted	Toothed (2), untoothed	toothed (3), untoothed	Toothed (11spp), untoothed	Untoothed	Toothed (8) or untoothed	Toothed	Toothed	Toothed (2spp) or untoothed	Toothed (2spp) or untoothed
Tooth type	NA	Serrated (2 spp)	NA	Serrated	Eentate	Serrated (3)	Serrated (11)	NA	Serrated (6), crenated (2)	Serrated	Serrated	Serrated (1) or dentate (1)	Serrated (2)
Leaf type by the position of stomata	Amphi*	Amphi	Amphi	Amphi	Amphi (2), hypos	Amphi (3), hypos	Hypos (8), amphi	Amphi (1), hypos	Amphi (7), hypos	Amphi	Amphi	Hypos (2), amphi	Amphi
	Bifacial or equifacial	Bifacial	Bifacial or equifacial	Bifacial	Bifacial	Bifacial or equifacial	Bifacial	Bifacial	Bifacial	Bifacial	Bifacial	Bifacial	Bifacial or equifacial
Total lamina thickness, mean µm	102.8	217.3	326.7	413.1	182.8	246.4	148.5	126.8	200.5	197.07	231.7	238.2	274.9
Cuticle surface	Striated (1), smooth	Striated	Striated	Striated	Striated (2), smooth	Striated	Striated (11), smooth	Smooth	Striated (7), smooth	Smooth	Striated	Striated	Striated (3), smooth
Cuticle thickness, range µm	0.31–0.43	0.27–0.56	0.32–0.37	0.32	0.30–0.45	0.22–0.41	0.20–0.60	0.33–0.42	0.21–1.15	0.23–0.24	<0.1	0.36–0.46	0.19–0.44
Epidermal cell walls (surface view)	Straight to wavy	Straight to wavy	Wavy	Straight	Straight to wavy	Straight to wavy	Wavy	Wavy	Straight to wavy	Wavy	Wavy	Straight to wavy	Straight to wavy
Position of stomata regarding ordinary epidermal cells	Same level	Same level	Same level	Same level	Same level	Same level	Same level (10), higher	Same level	Same level (10), higher	Same level	Same level	Same level	Same level

Table 1 continued

Tribe	Anthemidae	Astereae	Bahieae	Carduae	Cichorieae	Coreopsidae	Eupatorieae	Gnaphalieae	Heliantheae	Millerieae	Nassauvieae	Senecioneae	Tageteae
Stomatal density (abaxial epidermis, mean stomata/mm^2)	244	369	366	349	322	383	421	349	394	279	174	256	349
Guard cell length, mean µm	21.36	21.04	23.8	24.38	18.7	21.61	19.64	17.89	20.31	20.18	25.09	23.09	19.39
Palisade parenchyma thickness, mean µm	22.48	69.99	117.65	195.58	46.2	98.03	47.29	44.43	76.27	54.34	52.40	45.47	72.68
Spongy parenchyma thickness, mean µm	53.38	48.20	58.08	165.68	90.94	92.89	71.48	52.97	81.54	113.32	140.46	157.6	93.12
Stomatal pore area index (SPI) %	10.37	15.53	15.70	20.75	15.06	17.27	14.56	11.13	15.09	11.39	10.98	13.57	13.80

In parenthesis number of species for each tribe

* See text for complete names

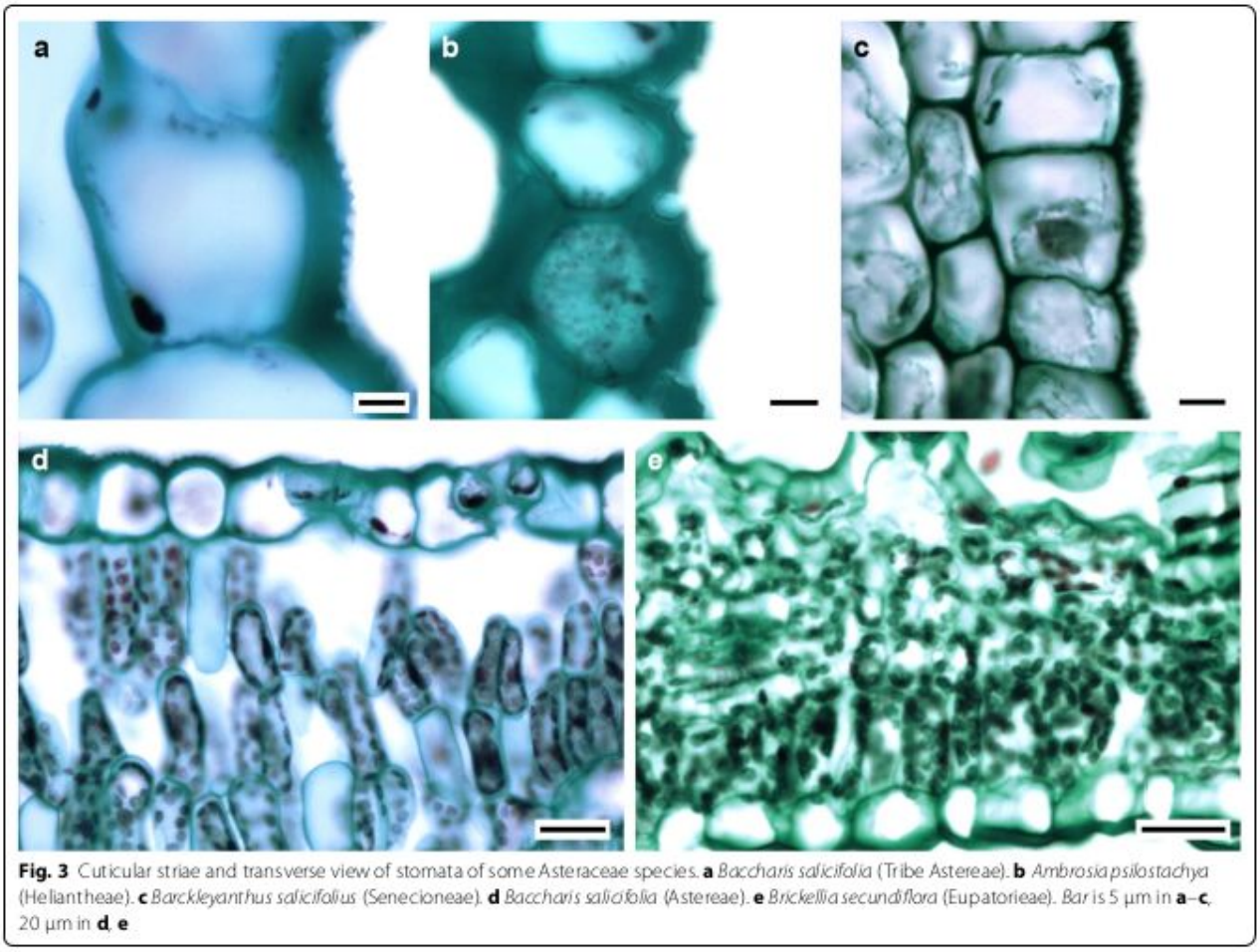

Fig. 3 Cuticular striae and transverse view of stomata of some Asteraceae species. **a** *Baccharis salicifolia* (Tribe Astereae). **b** *Ambrosia psilostachya* (Heliantheae). **c** *Barckleyanthus salicifolius* (Senecioneae). **d** *Baccharis salicifolia* (Astereae). **e** *Brickellia secundiflora* (Eupatorieae). *Bar* is 5 μm in **a**–**c**, 20 μm in **d**, **e**

as observed in *Dyssodia papposa*, *Laennecia sophiifolia* and *Schkuhria pinnata*. In some species belonging to the Coreopsideae, Heliantheae and Tageteae tribes, some contents were stained a dark color in the cell lumen of the epidermis. The stomata were located at the same level as other epidermal cells (Fig. 3d) except for some species of the Eupatorieae and Heliantheae tribes, for which they were localized at a higher level than other epidermal ordinary cells (Fig. 3e).

Mesophyll

A hypodermis was only observed in *Pectis prostrata* from the Tageteae tribe, where the hypodermis was one cell thick. The mesophyll was dorsiventral in most species and only isofacial in five species (Fig. 4a–d). The palisade parenchyma was formed by one to five cell strata, and, in most cases, it occupied more than half of the total mesophyll thickness. In some species of Astereae and Heliantheae, the palisade parenchyma occupied the most of the mesophyll (Fig. 4). The spongy parenchyma was usually compact in 65% of the species and loose in the rest. In some species from Anthemidae, Eupatorieae, Senecioneae and Tageteae tribes, it constituted the whole mesophyll (Fig. 5). There was an aerenchyma in *Jaegeria hirta* (Millerieae). No mineral contents in the mesophyll cells were observed.

Vascular bundles

Closed collateral vascular bundles were observed (Figs. 4, 5). In most species, they were surrounded by a sheath of one to three parenchyma layers, which were more conspicuous in species from the Astereae and Tageteae tribes. In the Eupatorieae, Heliantheae, Cichorieae and Senecioneae tribes, sheath extensions towards both surfaces were found (Fig. 5c, d). Secretory canals in some species of Astereae, Eupatorieae and Senecioneae were observed within the sheath (Fig. 4b).

Midvein

The midvein occupies the central position of the leaf. The epidermis of the midvein in all species was the same as in the lamina, except for *Acourtia cordata* (Nassauvieae),

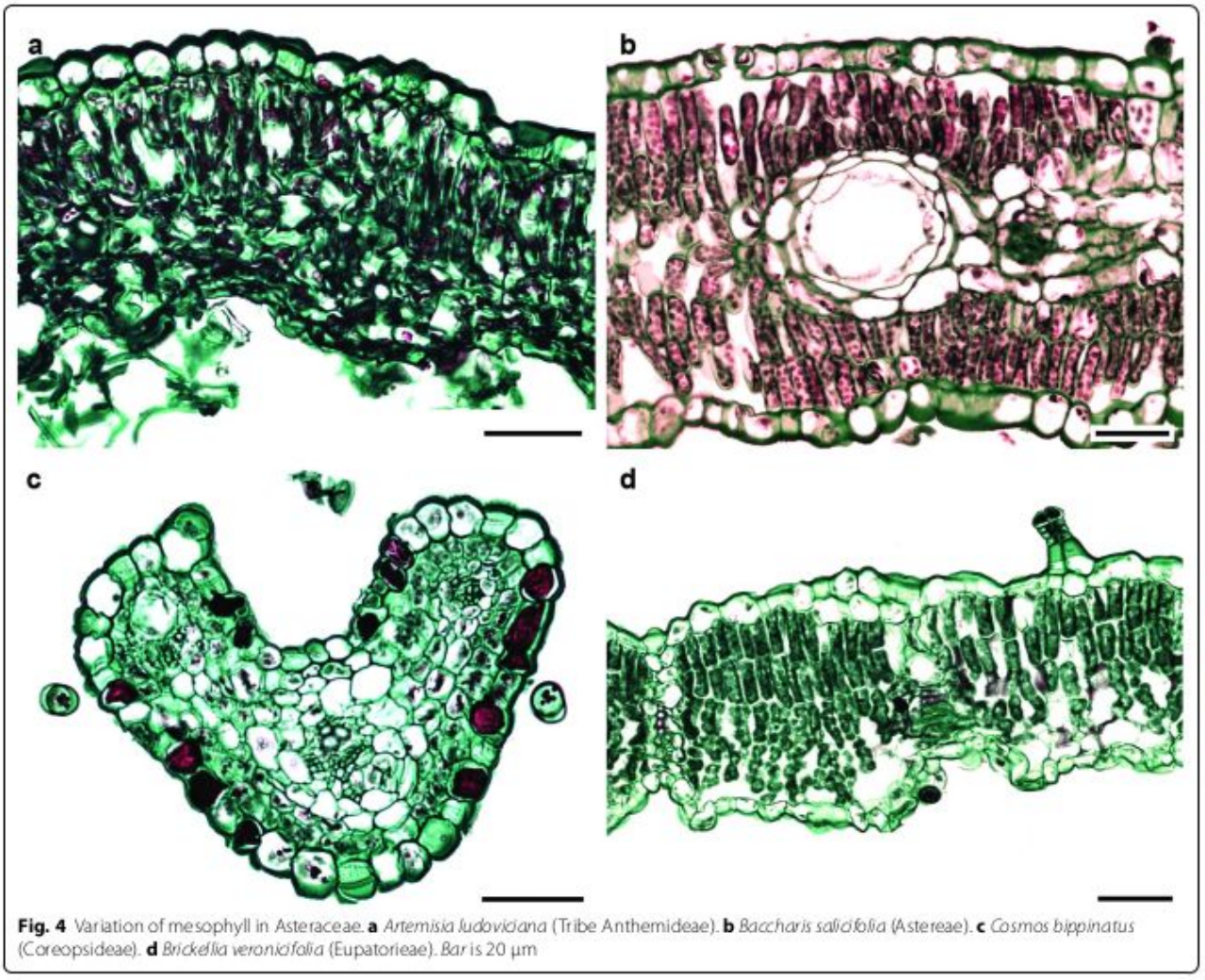

Fig. 4 Variation of mesophyll in Asteraceae. **a** *Artemisia ludoviciana* (Tribe Anthemideae). **b** *Baccharis salicifolia* (Astereae). **c** *Cosmos bippinatus* (Coreopsideae). **d** *Brickellia veronicifolia* (Eupatorieae). *Bar* is 20 µm

which had a thicker outer epidermal wall. In this area, the mesophyll and the palisade parenchyma were interrupted by angular collenchyma with 9–16 cell layers. The number of bundles composing the midvein was variable (Fig. 6). The vascular tissue generally formed an arch, with parenchyma at both ends of the vascular bundles, except for *A. cordata*, where fibers were observed (Fig. 6a, c–e). No foliar characters were unique to any tribe, and most character states were shared by most tribe members as seen in Table 1.

Statistical analyses

We found significant correlations between some of the studied characters. For example, there is a negative and significant relationship between the guard cell length and stomatal density (Fig. 7). We also found positive significant correlations between leaf thickness and the thickness of palisade and spongy parenchyma ($r = 0.66$, $P < 0.001$) and between guard cell length and the thickness of the leaves ($r = 0.43$, $P = 0.003$), other correlations are shown in Fig. 7. PCA explained in four axes 69% of the total variance (Table 2). The first axis explains the leaf thickness whereas the second axis explains the positive values for stomatal density and palisade thickness and negative values for guard cell length (Fig. 8).

Discussion

The foliar anatomy of Asteraceae was variable. In the epidermis, differences were found in the shape of epidermal cells, seen in surface view, and in stomata location, as well as epidermis and cuticle thickness. The cell types constituting the mesophyll were variable as well as their abundance. These observations were consistent with the high foliar variation reported for the family (Anderson and Creech 1975; Metcalfe and Chalk 1979; Ferreira et al. 2002; Freire et al. 2005, 2007; Milán et al. 2006; Adedeji and Jewoola 2008). The striae in the leaf cuticle have been previously observed by other authors (Adedeji and

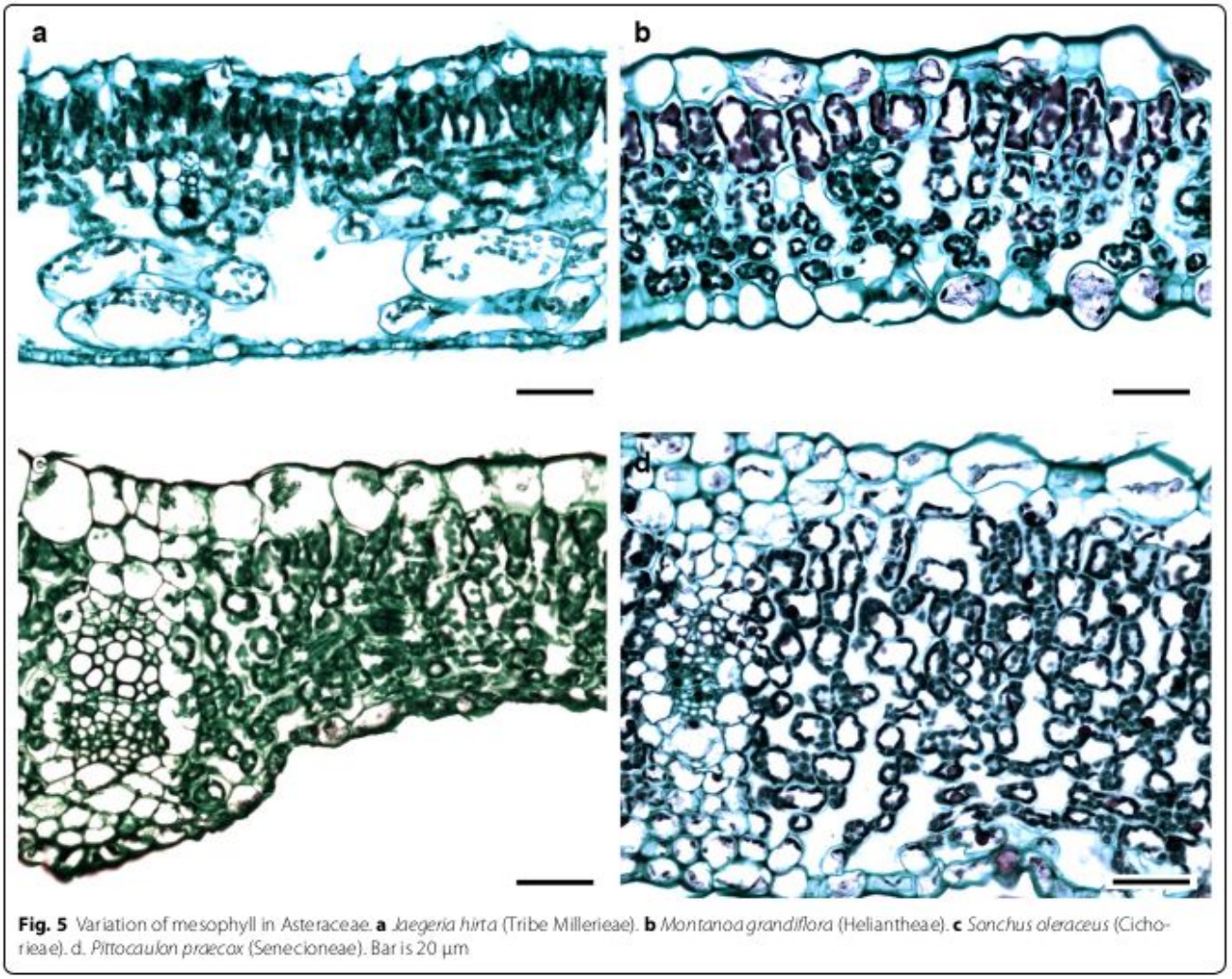

Fig. 5 Variation of mesophyll in Asteraceae. **a** *Jaegeria hirta* (Tribe Millerieae). **b** *Montanoa grandiflora* (Heliantheae). **c** *Sonchus oleraceus* (Cichorieae). d. *Pittocaulon praecox* (Senecioneae). Bar is 20 µm

Jewoola 2008), who have suggested that the striae could be used to separate species. The observed stomatal types and the stomata being preferably distributed on the abaxial surface agrees with previous reports for the family in other works (Metcalfe and Chalk 1979; Adedeji and Jewoola 2008).

It was difficult to find a defined anatomical pattern for the family. However, we found that the combination of anatomical features is usually more homogenous within the tribes than the family level. For example, most examined species show bifacial leaves, except for some members of the Bahieae, Coreopsidae and Tageteae tribes, which had equifacial leaves. It should also be stressed that all the members of Astereae had amphistomatic bifacial leaves with striated cuticles, while striae were absent in all members of Gnaphalieae. These observations should be confirmed by examining more Astereae and Gnaphalieae members.

Relationship between the leaves and the habitat

Given that the Asteraceae examined here were found in a habitat with low water availability and poor soils, it was expected that the foliar anatomy would display xerophytic features (Shields 1951; Kramer 1983; Esau 1976; Santos and Ochoa 1990; Fahn and Cutler 1992; Dickison 2000; Cutler et al. 2007). This assertion was not supported by the anatomical features found. The leaves of the Asteraceae studied are better classified as mesomorphic and do not correspond with the vegetation community or the water deficit or poor soils of the volcanic outcrop they leave.

Leaf size

It was suggested that a small leaf size is a xeromorphic trait because it is correlated with a reduction in transpiration rate (Fahn and Cutler 1992), so we expected to observe leptophyll, nanophyll or microphyll leaves.

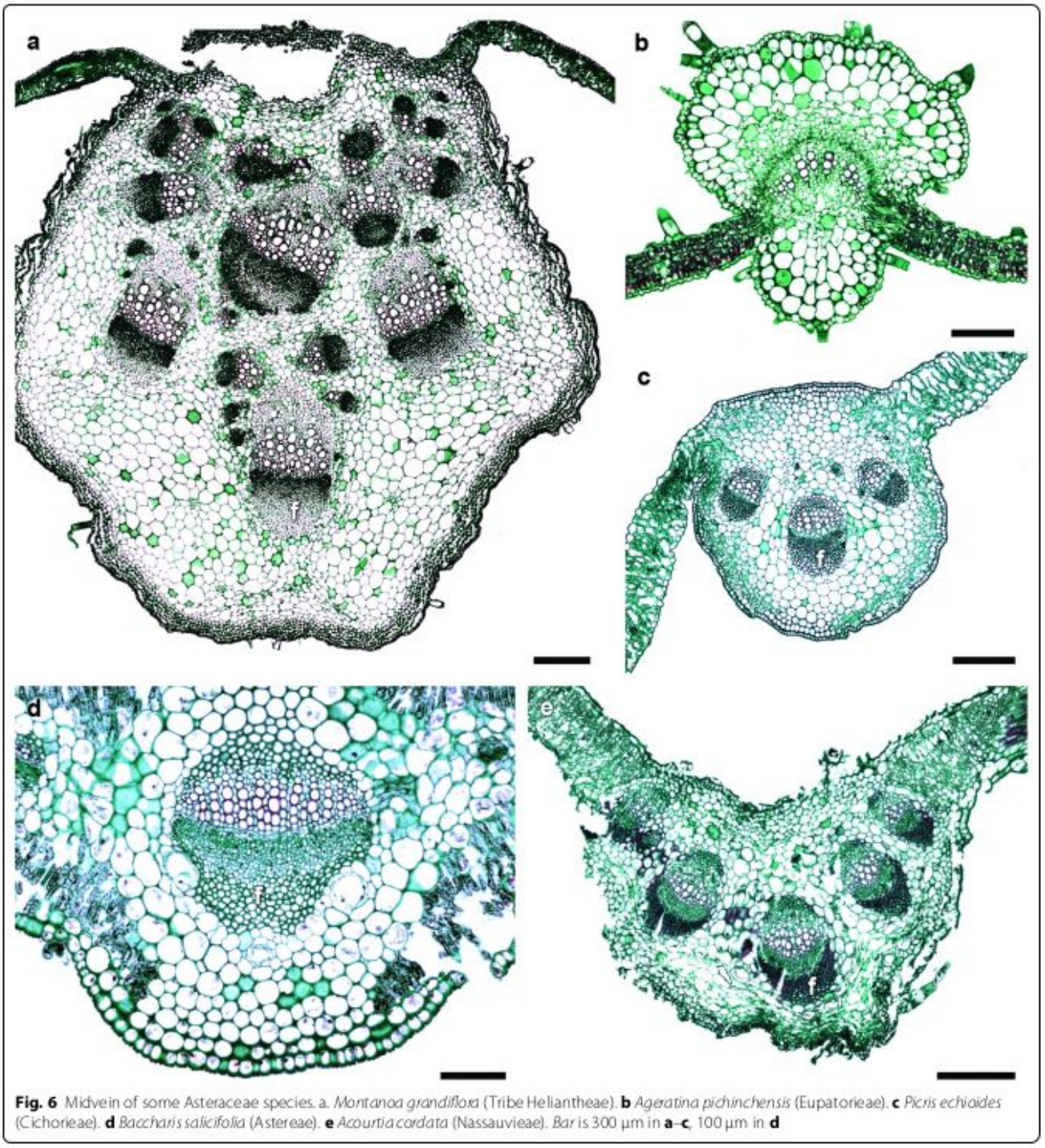

Fig. 6 Midvein of some Asteraceae species. a. *Montanoa grandiflora* (Tribe Heliantheae). **b** *Ageratina pichinchensis* (Eupatorieae). **c** *Picris echioides* (Cichorieae). **d** *Baccharis salicifolia* (Astereae). **e** *Acourtia cordata* (Nassauvieae). *Bar* is 300 μm in **a**–**c**, 100 μm in **d**

However, most of the studied species (46%) have large-sized laminae (mesophyll, macrophyll and rarely megaphyll). Twenty-three percent of the studied species had medium-sized laminae (notophyll), and only 31% of the studied species had small leaves. From the species with larger leaves, 29% had deeply lobed margins, such as *Dahlia coccinea* and *Tagetes tenuifolia*. Half of the species with the larger leaves were ephemeral herbs that only grow during the rainy season, such as *Tithonia tubiformis* (megaphyll) and *Cirsium vulgare* (macrophyll). The other 21% of the species with larger leaves were deciduous that usually respond rapidly to water stress by producing

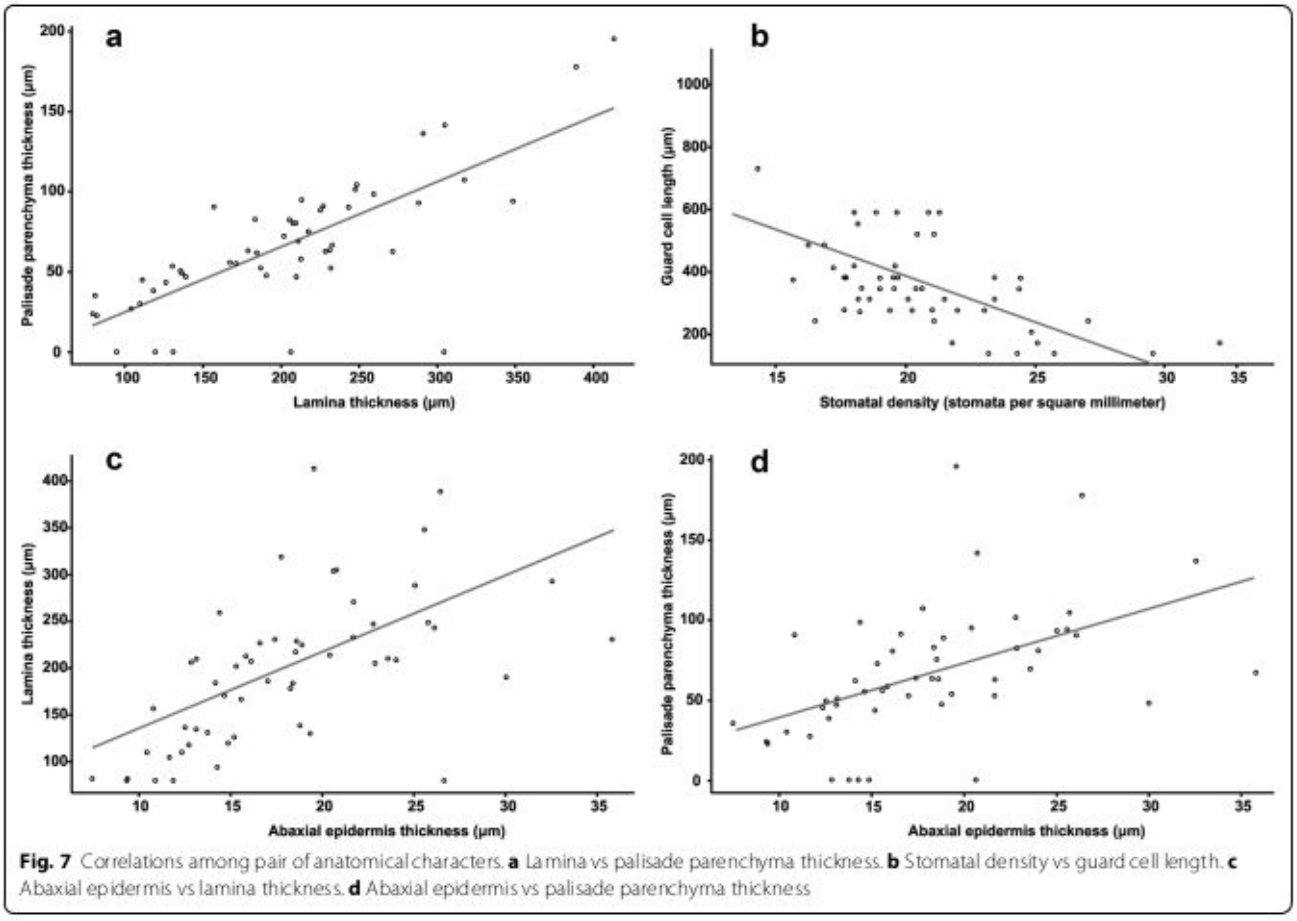

Fig. 7 Correlations among pair of anatomical characters. **a** Lamina vs palisade parenchyma thickness. **b** Stomatal density vs guard cell length. **c** Abaxial epidermis vs lamina thickness. **d** Abaxial epidermis vs palisade parenchyma thickness

leaves exclusively during the rainy season and losing them when the rain stops, such as *Pittocaulon praecox* (macrophyll) and *Roldana lobata* (macrophyll) that is a perennial herb. Laminar area was expressed as types and this trait was not revealed by our PCA as a variable that contributes to explain the variance in the first four components. The projections of the margin of the lamina (i.e., the lobes and teeth) are interpreted as being related to heat loss morphology (Freire et al. 2005, 2007; Fahn and Cutler 1992; Adedeji and Jewoola 2008; Gil et al. 2012; Schmerler et al. 2012; Redonda-Martínez et al. 2016). Eighty-three percent of the studied species had toothed or lobed margins, which is a common combination in Asteraceae (Bailey and Sinnott 1916; Rojas et al. in review).

Table 2 Eigenvector for principal component analysis for the 11 variables in the first four components analyzed for the leaf traits of the 52 species of Asteraceae at the REPSA, Mexico

Characters	Prin 1	Prin 2	Prin 3	Prin 4
Variation explained (%)	28.48	17.11	13.15	10.28
Eigenvalue	3.42	2.05	1.48	1.23
Lamina size	−0.127	0.332	0.455	−0.664
Specific leaf area	−0.216	−0.176	0.029	0.489
Sclerophyll index	−0.197	0.332	0.455	−0.066
Lamina thickness	−0.144	−0.134	0.575	−0.024
Cuticle thickness	−0.016	0.294	−0.058	−0.430
Abaxial epidermis height	0.426	0.059	0.278	0.127
Stomatal density	−0.159	0.518	−0.137	0.214
Guard cell length	0.281	−0.487	0.133	−0.195
Mesophyll thickness	0.510	0.075	0.054	0.142
Palisade thickness	0.216	0.455	0.067	0.204
Spongy thickness	0.138	−0.105	−0.524	0.269

Palisade parenchyma

Gibson (1996) mentioned that non-succulent xerophytes have thicker leaves than mesophytes because they have well developed multi-layered palisade parenchyma. This agrees with our findings because the palisade parenchyma was well developed in most tribes (Table 1), and in some species as *Baccharis salicifolia* (Astereae) and *Lagascea rigida* (Heliantheae) the mesophyll is composed exclusively of palisade parenchyma. The palisade thickness was within the range (94–355 µm) found in other plant families growing in dry environments (Nevo et al.

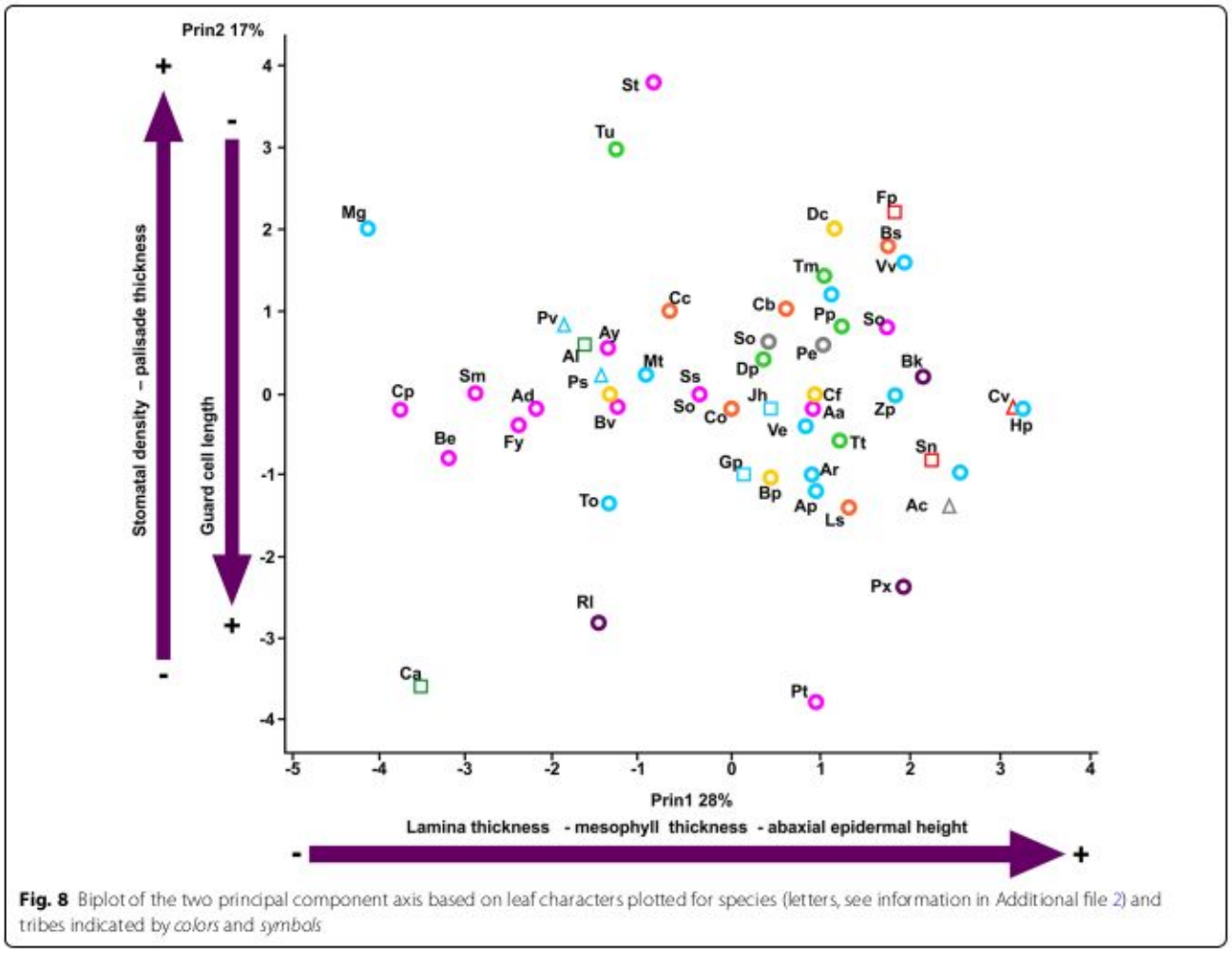

Fig. 8 Biplot of the two principal component axis based on leaf characters plotted for species (letters, see information in Additional file 2) and tribes indicated by *colors* and *symbols*

2000; Rotondi et al. 2003; Bacelar et al. 2004; Gratani and Varone 2004). Palisade thickness was one of the variables with high loading to explain the variation found in the species studied, this agrees with the results of Tian et al. (2016) who found that leaf thickness is closely related to palisade and spongy thickness. This close relationship appears to be a strategy together with phenology to be efficient during the rainy season that is short.

SLA, SI and SPI

Specific leaf area is positive correlated with moisture and nutrient availability (Ackerly et al. 2002) and is mainly determined by leaf density and thickness (Ackerly et al. 2002; Meziane and Shipley 2001). SLA was expected to be low (less than 10 mm^2/mg, Ackerly 2004) because it enhances the photosynthetic rates and the resistance to cell wall collapse under water-stress (Turner 1994; Ackerly 2004). Most of the studied species (78%) have high values of SLA compared with other species of semi-arid environments and are more similar to values found by other authors in temperate and cold forests (15.4–36.3 mm^2/mg, Chen et al. 2011; Tian et al. 2016). SLA have been negatively correlated with leaf lifespan (Shipley et al. 2006; Tian et al. 2016) and this relationship have been explained as the result of increased photosynthetic capacity per unit of leaf dry mass in plants with shorter leaf lifespan. Since the most of the studied species by us are herbs, the high SLA found can be explained by this correlation. Also, compared to the tree species studied by Tian et al. (2016) SLA of the studied species was significantly higher. This can be interpreted as evidence of the correlation between growth form and SLA.

The SI was expected to be higher than 0.6 which is indicative of sclerophyllous leaves. Sclerophylls are typically associated to low nutrient concentration (Turner 1994). However in all the studied species the SI values was lower than expected (Additional file 2). Together these two variables indicate that the studied leaves can be considered mallacophylls (Turner 1994) and mesomorphic. The SPI found for the plants in this study was

higher than that found for Tian et al. (2016) for some tree species within different ecosystems across China. They discuss that a higher SPI may be an adaptive strategy of leaf stomatal traits that results from higher stomatal conductance and therefore increased photosynthetic capacity which maximize carbon gain during the short growing season (Tian et al. 2016).

Cuticle and epidermis

We expected to see thick cuticles and a multiseriate epidermis on the leaf surface because those adaptations minimize transpiration rates. However, cuticle thickness was thin compared to other xerophytic plants, which are 2–22 µm thick (Rotondi et al. 2003; Bacelar et al. 2004; Gratani and Varone 2004). Moreover, the thin cuticles found in the REPSA are similar to those reported for species of *Ambrosia*, *Artemisia* and *Encelia* growing in a desert of North America (Gibson 1996). We also expected to see the leaf surface covered with abundant trichomes, but most of the observed species (97%) were glabrous or had trichomes that were scarcely distributed, except for *Pseudognaphalium*, *Artemisia* and *Conyza* which are herbs growing in open spaces. Most authors have based their taxonomic discussions on trichome morphology (Freire et al. 2005, 2007; Adedeji and Jewoola 2008; Gil et al. 2012; Redonda-Martínez et al. 2016). However in the studied species they were not abundant.

Stomata

Fahn and Cutler (1992) have hypothesized that amphistomatic leaves may have evolved in response to increasing aridity during the Tertiary period because they increase leaf conductance to CO_2. Therefore, amphistomatic leaves favored high maximum leaf conductances and are present in species growing in arid environments (Camargo and Marenco 2011), especially in herbs and shrubs from different environments such as successional forest, deserts or swamps (Mott et al. 1982; Mott and Michaelson 1991). In this study, 60% of the observed species had amphistomatic leaves and agrees with Mott and Michaelson (1991) findings in *Ambrosia cordifolia* because most of these species live in full sun during the short rainy season. The stomata in the studied species were at the same level as other epidermal cells. This pattern is different from the one observed in xeromorphic plants, where stomata are usually sunken or protected in crypts. The mean stomatal density found in this study (362 stomata/mm^2) was within the range found for other plants growing in arid environments (133–537 stomata/mm^2. Fahn and Cutler 1992; Bacelar et al. 2004; Yiotis et al. 2006; Gil et al. 2012). However, the stomatal densities observed in this environment (Table 1) are lower but not significantly different than those found in mesic environments (462–846 stomata/mm^2, Popma et al. 1992; Camargo and Marenco 2011). Wood (1934) suggested that although stomatal density increases with aridity, variations in stomatal density in sclerophyllous forests of South Australia were more related to intra-family characteristics rather than environmental conditions. It is possible that the observed variation in stomatal density has a phylogenetic signal, thus further analysis with a larger Asteraceae sampling growing in different communities is needed to support this assertion. The observed stomatal lengths are similar to the values reported by other authors for Asteraceae growing in arid and temperate environments (16–24 µm, Bacelar et al. 2004; Yiotis et al. 2006; Gil et al. 2012). Both stomatal density and length showed a significant negative scaling as found for other species of different families (Hetherington and Woodward 2003; Pearce et al. 2006; Camargo and Marenco 2011) and were important traits that explained part of the variance according to PCA suggesting that they adjust with palisade thickness and spongy parenchyma to maintain efficient photosynthesis.

Dark staining deposits and oils

It has been suggested that the presence of specialized cell types, such as oil containing cells or tannin cells, may be advantageous in dry environments because they protect the mesophyll cells against excess radiation or against herbivores and help to reduce the evaporation rate by interfering with water movement through the leaves (Fahn and Cutler 1992; Jordaan and Theunissen 1992; Turner 1994). Although we did not perform histochemical tests for tannins, the dark staining deposits that we described may be these contents. Thirty percent of the studied species had oil glands associated to vascular bundles, including *B. salicifolia* and *Barkleyanthus salicifolius*. Dark staining deposits were found only in two species (*Cosmos parviflorus* and *D. papposa*).

Allometric relationships within the leaf

The correlation between abaxial epidermis thickness and palisade thickness can be viewed from a functional perspective since it can be interpreted as a relationship between the gas exchange mechanism and the photosynthetic tissue resulting in greater efficiency of the leaf (Shields 1951; Rotondi et al. 2003). Our results of the PCA confirms the close allometric relationships between lamina thickness, palisade and spongy parenchyma and abaxial epidermis found by John et al. (2013) in a sample including various angiosperm families. This relationship has been interpreted as evidence of coordinated changes of the tissues composing the leaf (Brodribb et al. 2013).

Particularly the correlation between guard cell length and leaf thickness may be showing that the changes in cell size are key to the coordinated variation of leaf size.

Leaves classification

Some of the studied species showed indicator traits of each of three types of response strategies to water stress and absence of soil. In species of the Eupatorieae (2 spp: *Ageratina adenophora* and *Stevia tomentosa*), Tageteae (3 spp: *D. papposa*, *T. micrantha*, *Pectis prostrata*), and Heliantheae (*L. rigida*) tribes, some foliar features corresponding to the avoidance strategy—water loss minimization—were observed. Those characteristics were hypostomatic or bifacial leaves with well developed, many-layers-thick palisade parenchyma, generally striated cuticles, thick epidermises, and vascular bundles surrounded by a well differentiated parenchyma sheath. In accordance with the foliar anatomy some other plant features indicate that these species prefer the avoidance strategy: Eupatorieae species that are generally shrubs or perennial herbs, there are rhizomes allowing them to store water and all leaves from the examined species of Tageteae were lobulate. The tolerance strategy was observed in all the species from the Senecioneae tribe. They showed traits such as leaf abscission during the higher stress season (November–May), mucilage accumulation (in the shoot), and perennation structures that allow regrowth. Some species from the Astereae tribe (4 spp, including *Conyza canadensis* and *L. sophiifolia*), and some members of Coreopsidae (e.g. *C. parviflorus*) and Heliantheae (e.g. *Tithonia tubiformis*) tribes, showed an escape strategy because they usually developed their life cycle during the rainy season (June–October) and produced seeds before water stress conditions arrived.

Most of the species showed a combination of characters that made their classification difficult. For example, species from the Bahieae and Carduae tribes are annual herbs that grow mainly from June to October; thus, they could be considered escapists. However, these species showed some avoidance features, such as lobulate leaves with striated cuticles and a well-developed palisade parenchyma. This agrees with the asseveration from some authors (Jones 1992; Chaves et al. 2003) about the three strategies not being mutually exclusive but instead we can find a combination of indicator traits of all three strategies within the same plant. The different character combinations for each species could be explained as a result of a fast evolutionary process as proposed by Stebbins (1952) for some members of Cichorieae (Asteraceae).

Conclusions

The foliar anatomy of Asteraceae was variable, even among members of the same tribe growing in the same locality. If the observed variations were the result of adaptation to a xeric and poor-soil environment, then the observed features would correspond to non-succulent xeromorphic leaves but it was not the case. The majority of the studied species possess mesomorphic leaf features as simple lamina, single-layered epidermis, and soft (mallacophyllous) large-size leaves with high SLA. Although some characters of drought resistance can be observed as for well-developed palisade and parenchyma bundle sheaths. The aforementioned character combination made difficult to classify the species studied within one of the three main response strategies to water stress. We suggest that the occurrence of specific character combinations in each species is due to a very fast evolutionary process involving the growth form, the adaptation to the environment and the phylogeny of the family. A character evolution analysis in an Asteraceae phylogeny including genera endemic to Mexico is needed in order to support this hypothesis (Terrazas, on going research).

The combined study of morphological and anatomical traits and its correlations is important to understand the constraints imposed over the diversity generated by phylogeny and adaptation. For example, although leaf anatomy is not xeromorphic in the studied species, it is advantageous for the species living in the REPSA to have the combination of traits mentioned. Those traits allow them to survive in full sun during the short rainy season having efficient photosynthetic capacity. Assuming that species from the forests around the Xitle colonized the REPSA, it is not surprising that they retained some of their mesic characters and also adapted to the low water environment. There were also species with Neotropical and Nearctic affinities that probably had to modify their leaves to colonize new environments. These species probably retained the capacity to adapt to dry environments, and that is one of the reasons for their success. It is known that in ecological succession, pioneer species need to have low nutritional requirements and efficient metabolisms to survive. The invasiveness of Asteraceae species is probably related to their capacity to grow in this poor-soil environment.

Additional files

Additional file 1. Voucher information for species used in this study. All specimens deposited in Herbario Nacional de México (MEXU), Instituto de Biología, Universidad Nacional Autónoma de México.

Additional file 2. Leaf characters by species in Asteraceae.

Abbreviations
REPSA: Pedregal de San Ángel Ecological Reserve; UNAM: National Autonomous University of Mexico; SLA: specific leaf area; SI: sclerophylly index; SPI: stomatal pore area index; PCA: principal component analysis.

Authors' contributions
PR and TT designed the study, PR carried out the laboratory work, analyzed the data and drafted the manuscript. TT analyzed the data, revised and corrected the manuscript. JLV approved the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The dataset supporting the conclusions of this article is included within the article and its Additional files 1, 2.

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References
Ackerly DD (2004) Adaptation, niche conservatism and convergence: comparative studies of leaf evolution in the Californian chaparral. Amer Nat 163:654–671
Ackerly DD, Knight CA, Weiss SB, Barton K, Starmer KP (2002) Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses. Oecologia 130:449–457
Adedeji O, Jewoola OA (2008) Importance of leaf epidermal characters in the Asteraceae family. Not Bot Horti Agrobot Cluj Napoca 36:7–16
Anderson LC, Creech JB (1975) Comparative leaf anatomy of *Solidago* and related Asteraceae. Am J Bot 62:486–493
Bacelar EA, Correia CM, Moutinho-Pereira JM, Goncalves BC, Lopes JI, Torres-Pereria JMG (2004) Sclerophylly and leaf anatomical traits of five field-grown olive cultivars growing under drought conditions. Tree Physiol 24:233–239
Bailey IW, Sinnott EW (1916) The climatic distribution of certain types of angiosperms leaves. Am J Bot 3:24–39
Boeger MRT, Wisniewski C (2003) Comparacao da morfologia foliar de espécies arbóreas de tres estádios sucessionais distintos de floresta ombrófila densa (Floresta Atlántica) no Sul do Brasil. Rev Brasil Bot 26:61–72
Brodribb TJ, Jordan GJ, Carpenter RJ (2013) Unified changes in cell size permit coordinated leaf evolution. New Phytol 199:559–570
Camargo MAB, Marenco RA (2011) Density, size and distribution of stomata in 35 rainforest tree species in Central Amazonia. Acta Amazonica 41:205–212
Castillo-Argüero S, Montes-Cartas G, Romero-Romero MA, Martínez-Orea Y, Guadarrama-Chávez P, Sánchez-Gallén I, Núñez-Castillo O (2004) Dinámica y conservación de la flora del matorral xerófilo de la Reserva Ecológica del Pedregal de San Angel (D.F.) México. Bol Soc Bot México 74:51–75
Castillo-Argüero S, Martínez-Orea Y, Romero-Romero MA, Guadarrama-Chávez P, Núñez-Castillo O, Sánchez-Gallén I, Meave JA (2007) La reserva ecológica del Pedregal de San Ángel: Aspectos floristicos y ecológicos. México: Secretaria Ejecutiva de la Reserva Ecológica del Pedregal de San Ángel de Ciudad Universitaria, Universidad Nacional Autónoma de México
Castro MM, Leitão-Filho HF, Monteiro WR (1997) Utilização de estruturas secretoras na identificação dos gêneros de Asteraceae de uma vegetação de cerrado. Rev Brasil Bot 20:163–174
Céspedes CLA (2010) Riqueza floristica de Asteraceae en los fragmentos de vegetación de la Reserva Ecológica del Pedregal de San Ángel, México D.F. M. Sc. Thesis. México: Facultad de Ciencias, Universidad Nacional Autónoma de México
Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought-from genes to the whole plant. Funct Plant Biol 30:239–264
Chen FS, Niklas KJ, Zeng DH (2011) Important foliar traits depend on species-grouping: analysis of a remnant temperate forest at the Keerqin Sandy Lands, China. Plant Soil 340:337–345
Cutler DF, Botha T, Stevenson DW (2007) Plant anatomy: an applied approach. Blackwell Publishing, Massachusetts
Delbón NM, Cosa T, Dottori N, Stiefkens L (2007) Análisis comparativo de los caracteres epidérmicos en *Flourensia campestris* y *F. oolepis* (Asteraceae). Bol Soc Argent Bot 42:245–250
Dickison WC (2000) Integrative plant anatomy. Academic Press, San Diego
Esau K (1976) Anatomía vegetal. Ediciones Omega, España. English edition: Esau K (1976) Plant anatomy (trans: Pons J). John Willey & Sons Inc., New York
Fahn A, Cutler DF (1992) Xerophytes. Gebrüder Borntraege, Berlin
Fang Y, Xiong L (2015) General mechanisms of drought response and their application in drought resistance improvement in plants. Cell Mol Life Sci 72(673):689
Ferreira JL, De Oliveira FV (1989) Identificaçao de *Sonchus oleraceus* L. (serralha): Principais caracteristicas estruturais e quimicas. Rev Bras Farmacogn 2/4:67–77
Ferreira EA, Procópio SO, Silva EAM, Silva AA, Rufino RJN (2002) Estudos anatômicos de folhas de espécies de plantas daninhas II. Planta Daninha 20:327–335
Fonseca CR, Overton JM, Collins B, Westoby M (2000) Shifts in trait-combinations along rainfall and phosphorus gradients. J Ecol 88:954–977
Freire S, Arambarri AM, Bayón N, Sancho G, Urtubey E, Monti C, Novoa MC, Colares MN (2005) Epidermal characteristics of toxic plants for cattle from the Salado river basin (Buenos Aires, Argentina). Bol Soc Argent Bot 40:241–281
Freire S, Urtubey E, Guiliano DA (2007) Epidermal characters of *Baccharis* (Asteraceae) species uses in traditional medicine. Caldasia 29:23–38
Funk VA, Susanna A, Stuessy TF, Robinson H (2009) Classification of Compositae. In: Funk VA, Susanna A, Stuessy TF, Bayer RJ (eds) Systematics, evolution, and biogeography of Compositae. Sheridan Books Inc, Ann Arbor
Garnier E, Shipley B, Roumet C, Laurent G (2001) A standarized protocol for the determination of specific leaf area and leaf dry matter content. Func Ecol 15:688–695
Gibson AC (1996) Structure—function relations of warm desert plants. Springer Verlag, Berlin
Gil SP, Seisdedos L, Reyna ME, Cerana MM (2012) Epidermis foliar de tres especies de asteráceas nativas de Argentina con potencial ornamental. Phyton (B. Aires) 81:205–210
Gratani L, Varone L (2004) Leaf key traits of *Erica arborea* L., *Erica multiflora* L. and *Rosmarinus officinalis* L. co-occurring in the Mediterranean maquis. Flora 199:58–69
Hetherington AM, Woodward FI (2003) The role of stomata in sensing and driving environmental change. Nature 424:901–908
Horn JW, Fisher JB, Tomlinson PB, Lewis CE, Laubengayer K (2009) Evolution of lamina anatomy in the palm family (Arecaceae). Am J Bot 96:1462–1486
Johansen DA (1940) Plant microtechnique. MacGraw Hill, New York
John GP, Scoffoni C, Sack L (2013) Allometry of cells and tissues within leaves. Am J Bot 100:1936–1948
Jones HG (1992) Plants and microclimate. Cambridge University Press, Cambridge

Jordaan A, Theunissen JD (1992) Phenolic deposits and tannin in the leaves of five xerophytic species from southern Africa. Bot Bull Academia Sinica 33:55–61
Kramer PJ (1983) Water relations of plants. Academic Press, New York
Lot A, Cano-Santana Z (2009) Biodiversidad del ecosistema del pedregal de San Ángel. Universidad Nacional Autónoma de México, México
Luckow M (2002) Anatomical features of the leaves in the *Dichrostachys* group (Leguminosae: Mimosoideae) and their utility for Phylogenetic studies. Syst Bot 27:29–40
Luque R, De Menezes NL, Semir J (1999) Anatomia foliar de *Lychnophora* Mart. (Vernonieae: Asteraceae). Plantula 2:141–152
Metcalfe C, Chalk L (1979) Anatomy of the dicotyledons, 2nd edn. Oxford University Press, New York
Meziane D, Shipley B (2001) Direct and indirect relationships between specific leaf area, leaf nitrogen and leaf gas exchange. Effects of irradiance and nutrient supply. Ann Bot 88:915–927
Milán P, Hayashi AH, Appezzato-da-Glóra B (2006) Comparative leaf morphology and anatomy of three Asteraceae species. Braz Arch Biol Technol 49:135–144
Morrone JJ (2006) Biogeographic areas and transition zones of Latin America and the Caribbean islands based on panbiogeographic and cladistic analyses of the entomofauna. Annu Rev Entomol 51:467–494
Matt KA, Michaelson O (1991) Amphistomy as an adaptation to high light-intensity in *Ambrosia cordifolia* (Compositae). Am J Bot 78:76–79
Matt KA, Gibson AC, I'Leary JW (1982) The adaptive significance of amphistomatic leaves. Plant Cell Environ 5:455–460
Nevo E, Pavliek T, Beharav A, Bolshakova MA, Martyn GI, Musatenko LI Sytnik KM (2000) Drought and light anatomical adaptive leaf strategies in three woody species caused by microclimatic selection at "Evolution Canyon", Israel. Israel J Plant Sci 48:33–46
Ordoñez JC, Van Bodegom PM, Witte JPM, Wright IJ, Reich PB, Aerts R (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Global Ecol Biogeogr 18:137–149
Pearce DW, Millard S, Bray DF, Rood SB (2006) Stomatal characteristics of riparian poplar species in a semi-arid environment. Tree Physiol 26:211–218
Popma J, Bongers F, Werger MJA (1992) Gap-dependence and leaf characteristics of trees in a tropical lowland rain forest in Mexico. Oikos 63:207–214
R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
Ragonese AM (1988) Canales secretores en los órganos vegetativos de *Eupatorium inulaefolium* H.B.K. (Compositae). Acta Farm Bonaer 7:161–168
Redonda-Martínez R, Villaseñor JL, Terrazas T (2016) Trichome diversity in the subtribe Leiboldiinae (Vernonieae, Asteraceae). J Torrey Bot Soc 143:298–310
Rojas A, Terrazas T, Villaseñor JL (in review). Foliar architecture in members of Senecioneae (Asteraceae). Rev Mex Biodivers
Rotondi A, Rossi F, Asunis C, Cesaraccio C (2003) Leaf xeromorphic adaptations of some plants of a coastal Mediterranean macchia ecosystem. J Mediterr Ecol 4:25–35
Ruzin ES (1999) Plant microtechnique and microscopy. Oxford University Press, New York
Rzedowski J (1998) Diversidad y orígenes de la flora fanerogámica de México. In: Ramamoorthy TP, Bye R, Lot A, Fa J (eds) Diversidad biológica de México: Orígenes y distribución. Instituto de Biología, Universidad Nacional Autónoma de México, México, D.F
Rzedowski J (2006) Vegetación de México. 1st digital edition. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México
Rzedowski GC, Rzedowski J (2005) Flora fanerogámica del Valle de México. 2nd ed., 1st reimp., Instituto de Ecología, A.C. y Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Pátzcuaro
Santos MS, Ochoa N (1990) Adaptación de las plantas al déficit hídrico. Ciencia 41:333–344
Schmerler SB, Clement WL, Beaulieu JM, Chatelet DS, Sack L, Donoghue MJ, Edwards EJ (2012) Evolution of leaf form correlates with tropical-temperate transitions in *Viburnum* (Adoxaceae). Proc R Soc Lond B Bio 279:3905–3913
Shields LM (1951) Leaf xeromorphy in dicotyledon species from a gypsum sand deposit. Am J Bot 38:175–190
Shipley B, Lechowicz MJ, Wright I, Reich PB (2006) Fundamental trade-offs generating the worldwide leaf economics spectrum. Ecology 87:535–541
Siebe C (2000) Age and archaeological implications of Xitle volcano, southwestern Basin of Mexico-City. J Volcanol Geotherm Res 104:45–64
Sota-Trejo F, Palomino G, Villaseñor JL (2011) Números cromosómicos de Asteraceae de la Reserva Ecológica del Pedregal de San Ángel (REPSA), México, Distrito Federal. Rev Mex Biodivers 82:383–393
Stebbins GL (1952) Aridity as a stimulus to plant evolution. Amer Nat 86:33–44
Tian M, Yu G, He N, Hou J (2016) Leaf morphological and anatomical traits from tropical to temperate coniferous forests: mechanisms and influencing factors. Sci Rep 6:19703
Turner IM (1994) Sclerophylly: primary protective? Funct Ecol 8:669–675
Valladares F, Vilagrosa A, Peñuelas J, Ogaya R, Camarero JJ, Corcuera L, Sisó S, Gil-Pelegrín E (2004) Estrés hídrico: ecofisiología y escalas de la sequía. In: Valladares F (ed) Ecología del bosque mediterráneo en un mundo cambiante. Ministerio de Medio Ambiente, EGRAF, S. A, Madrid
Webb LJ (1959) A physiognomic classification of Australian rain forests. J Ecol 47:551–570
Wood JG (1934) The physiology of xerophytism in Australian plants. The stomatal frequencies, transpiration and osmotic pressures of sclerophyll and tomentose-succulent leaved plants. J Ecol 22:69–87
Wright IJ, Westoby M, Reich PB (2002) Convergence towards higher leaf mass per area in dry and nutrient poor habitats has different consequences for leaf life span. J Ecol 90:534–543
Yiotis C, Manetas Y, Psaras GK (2006) Leaf and green stem anatomy of the drought deciduous Mediterranean shrub *Calicotome villosa* (Poiret) Link. (Leguminosae). Flora 201:102–107

Capítulo 3

La importancia de los taxones de Asteraceae mexicanos en la filogenia de la familia

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Rivera et al., Mexican taxa of Asteraceae in the phylogeny of the family

The importance of the Mexican taxa of Asteraceae on the family phylogeny

La importancia de los taxones de Asteraceae mexicanos en la filogenia de la familia

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Abstract

Asteraceae is the largest plant family in México with about 417 genera and 3,113 species, more than 60% of them endemic. Phylogenetic relationships at subfamily and tribal levels have been previously resolved employing both nuclear and plastid molecular markers. However, Asteraceae species native to Mexico have been underrepresented in such phylogenies. To tackle this issue, the taxon sampling of this study included 94 Asteraceae species native to México, 101 previously sequenced species and six outgroups. With this sampling, all the Asteraceae subfamilies and 85% of the tribes recognized to date are represented. The analyzed data set consisted of eleven chloroplast markers (*atpB, matK, ndhC, ndhD, ndhF, ndhI, ndhJ, ndhK, rbcL, trnL-trnF,* 23*S-trnA*). We present two phylogenetic reconstructions obtained by maximum likelihood and Bayesian inference methods. The results are mostly congruent with previous studies carried out with different

sequencing methods, including next-generation sequencing. The Mexican species are distributed mainly in the subfamily Asteroideae (85 species), followed by Cichorioideae (6 species), Carduoideae (2 species) and Mutisioideae (2 species). The trees generated in this work are to date the phylogenetic hypotheses of the family with the best representation of native species of Mexico.

Resumen

Asteraceae es la familia más grande de plantas con flores en México, con alrededor de 417 géneros y 3113 especies, de las cuales más del 60% son endémicas. Las relaciones filogenéticas a nivel de subfamilias y tribus han sido resueltas mediante el uso de marcadores moleculares nucleares y de cloroplasto. Sin embargo, las especies de Asteraceae nativas de México han sido poco representadas en las filogenias recientes. Para abordar este problema, el muestreo taxonómico de este estudio incluyó 94 especies de Asteraceae nativas de México, 101 especias previamente secuenciadas y seis grupos externos. Con este muestreo, todas las subfamilias de Asteraceae y el 85% de las tribus reconocidas actualmente están representadas. La base de datos analizados consiste de once marcadores del cloroplasto (*atpB, matK, ndhC, ndhD, ndhF, ndhI, ndhJ, ndhK, rbcL, trnL–trnF,* 23*S-trnA*). Presentamos dos reconstrucciones filogenéticas obtenidas mediante los métodos de máxima verosimilitud e inferencia bayesiana. Los resultados son congruentes, en su mayoría, con estudios previos realizados con distintos métodos de secuenciación, incluyendo secuenciación de nueva generación. Las especies mexicanas se distribuyen principalmente en la subfamilia Asteroideae (85 especies), seguida por Cichorioideae (6 especies), Carduoideae (2 especies) y Mutisioideae (2 especies). Los análisis filogenéticos generados en este trabajo son hasta la fecha

las hipótesis filogenéticas de la familia con la mejor representación de especies nativas de México.

Introduction

Asteraceae is one of the most diverse angiosperm families, it includes ca. 23, 600 species (Stevens, 2001; Panero and Crozier, 2016). The Asteraceae family is cosmopolitan and represented in a variety of ecosystems (Mandel et al., 2017). Its species display a great diversity of growth forms, vegetative morphology and anatomy, chromosome numbers, floral structure, and pollen form and structure (Anderberg et al., 2007; Funk et al., 2009). The family is recognized as a monophyletic group because of various synapomorphies, including the flowers arranged in an inflorescence called "capitulum", the modification of the calyx on a pappus, the syngenesious stamen in which the pollen is exposed by the bifid style, the ovaries with a single basal egg and the production of sesquiterpene lactones (Bremer, 1994; Anderberg et al., 2007; Funk et al., 2009; Mandel et al., 2017).

Phylogenetic relationships within the family are still under study. The number of subfamilies and tribes within the Asteraceae continues to change mainly due to the use of data from massive dna-sequencing in phylogenetic reconstruction. Processes like whole genome duplication and hybridization are common in Asteraceae (Ellstrand et al., 1996; Barker et al., 2008; Symonds et al., 2010; Soto-Trejo et al., 2013; Huang et al., 2016) and can obscure evolutionary patterns and hinder the recognition and delimitation of clades. Currently, 12 to 13 families and between 35 and 45 tribes are recognized (Anderberg et al., 2007; Funk et al., 2009; Panero et al., 2014; Fu et al., 2016; Panero and Crozier, 2016). The efforts to resolve relationships within the family have resulted in robust phylogenetic

hypothesis (Funk et al., 2009; Panero et al., 2014; Huang et al., 2016; Panero and Crozier, 2016; Mandel et al., 2017, 2019). The incorporation of nuclear and chloroplast data, obtained from next-generation sequencing (NSG) methods, has allowed resolving problematic branches and increase the support of clades (Mandel et al., 2014, 2015, 2017, 2019; Huang et al., 2016). However, the Mexican genera of Asteraceae are still poorly represented in recent phylogenetic hypotheses.

It has been suggested that the family originated in southern South America, approximately 50 to 70 million years ago, at the end of the Cretaceous (Funk et al., 2009; Stuessy, 2010; Torices, 2010; Panero and Crozier, 2016; Mandel et al., 2019). Its diversification most likely coincides with the Early Eocene Climatic Optimum (Torices, 2010; Huang et al.,, 2016). Later the family emigrated to Africa, where it expanded and colonized the rest of the continents and experienced several diversification events. This complex biogeographic history manifests in the uneven species richness of Asteraceae species between continents; South America is the area with the highest richness followed by Asia and North America (Panero and Crozier, 2016). There is also a species richness disparity within the family, i.e., that the oldest lineages are less diverse than those that originated later in the history of the family (Panero and Crozier, 2016). This is a pattern that has been observed in several eukaryotic clades and the processes that can cause this imbalance are currently under study (Purvis et al., 2011; McGuire et al., 2014; Tank et al., 2015).

North America, particularly Mexico, is an area of interest for the study of Asteraceae, because it represents an important site for the diversification of some of the most diverse lineages, such as the "Heliantheae alliance" clade within the subfamily Asteroideae (Noyes and Rieseberg, 1999; Suárez-Mota and Villaseñor,

2011; Villaseñor, 2018). Evidence suggests that this clade is the result of a single colonization event from Africa or Asia to North America 22 to 35 million years ago (Panero, 2007; Funk et al., 2009; Panero et al., 2014; Panero and Crozier, 2016). The Heliantheae alliance is composed of 13 tribes, including: Athroismeae, Bahieae, Chaenactideae, Coreopsideae, Eupatorieae Helenieae, Heliantheae, Madieae, Millerieae, Neurolaeneae, Perityleae, Polymnieae and Tageteae (Anderberg et al., 2007), and contains approximately 26% of the total number of species in the family. Although its current distribution is worldwide, North America is the site with the largest number of species, around 3200 species representing 12% of the total family diversity (Anderberg et al., 2007; Funk et al., 2009; Panero et al., 2014; Fu et al., 2016; Panero and Crozier, 2016).

According to Villaseñor (2016, 2018) Asteraceae is the largest family of the Mexican flora. Recent estimates indicate that there are more than 3000 species distributed in 417 genera (Villaseñor, 2018) that represent 26 of the 45 tribes recognized by Funk et al., (2009). This richness is greater than that reported for other Neotropical countries with territories similar to or greater in size than México, and places Mexico as an important diversification center for the family (Villaseñor et al., 1998; Villaseñor, 2018). Recent phylogenetic hypotheses for the family have a narrow sampling of the tribes that make up the subfamily Asteroideae and the Heliantheae alliance (Panero and Crozier, 2016), which influences the evolutionary inferences that can be made about the subfamily. It is therefore important to increase the sampling of the species distributed in Mexico in the phylogenetic hypotheses of Asteraceae. Thus, the aim this work was to generate a phylogeny of Asteraceae

representing the lineages that diversified in Mexico, including seven endemic genera (Villaseñor, 1990, Villaseñor et al., 1998).

Materials and methods

Tissue sampling. Leaves of herbarium specimens or from plants collected in the field of 94 native species of Mexico that include seven endemic genera were collected (Appendix 1, Villaseñor, 1990; Villaseñor et al., 1998). The voucher specimens are deposited in the National Herbarium of Mexico (MEXU, Appendix 1).

Extraction, amplification, and sequencing. Total DNA was extracted using the CTAB method (Doyle and Doyle, 1987) modified by Salazar et al., (2003). Eleven chloroplast markers were selected including the genes *atpB* (1381 sites), *matK* (2006 sites), *ndhD* (714 sites), *ndhF* (2243 sites), *ndhI* (504) sites), *rbcL* (1440 sites), exons *ndhJ*, *ndh*K and *ndhC* including the intergenic spacers between these three genes (1735 sites), as well as the intergenic spacers *trnL-trnF* (1091 sites) and 23*S-trnA* (648 sites). DNA fragments were amplified using the Polymerase Chain Reaction (PCR) in 25 µl reactions following the method described by Panero and Crozier (2003), with temperatures modified according to the Polymerase used (Invitrogen™ Platinum™ Taq DNA Polymerase). The primers described in Panero and Crozier (2003), Panero and Funk (2008) and Panero et al., (2014) were used. The PCR products were purified using the PureLink™ PCR Micro Kit (Invitrogen, USA) and sequenced at the “Laboratorio Nacional de Biodiversidad” (LaNaBio) of the Biology Institute of the National Autonomous University of Mexico. The sequencing reactions were run with the POP-7 polymer in a 96-capillary DNA Analyzer 3730xL (Thermo Scientific).

Editing and alignment of sequences. Data used in this study include data from 107 species retrieved from GenBank from previous studies (Panero and Funk, 2008; Panero et al., 2014, Panero and Crozier, 2016): 101 species of Asteraceae and 6 species as outgroups (4 species of the family Calyceraceae, one species of Goodeniaceae and one species of Menyanthaceae). Sequences for 94 species native to México were generated in this study. With this sampling, the 13 subfamilies recognized by Panero and Crozier (2016) are represented. The sequences generated in this study were assembled using Sequencher® version 4.7 (Gene Codes Corporation, Ann Arbor, USA). Individual matrices for each marker were exported as fasta format files and aligned using MAFFT Version 7 online (Kuraku et al., 2013; Katoh et al., 2017). The aligned matrices were revised with PhyDE-1 (version 0.9971, Müller et al., 2010) and minimal manual adjustments were made. For some species, there was no data available in GenBank or it was not possible to amplify some markers, and were coded as missing data and represent 10.7% of the positions in the concatenated matrix.

Individual gene analyses. We conducted Bayesian inference and Maximum likelihood analyses, employing MrBayes version 3.2.6 (Ronquist et al., 2012) and RAxML-HPC version 8.2.9 (Stamatakis, 2014), respectively. Analyses were performed for all individual markers using a modified version of the TICR pipeline (Stenz et al., 2015) in the “Laboratorio Nacional de Análisis y Síntesis Ecológica” (LANASE) hybrid cluster. Results were visually inspected, the resulting trees were topologically congruent at subfamily and tribe level. Because of this congruence and also because chloroplast DNA is largely maternally inherited (McCauley et al., 2007)

we decided to concatenate matrices for each gene in Mesquite (Maddison and Maddison, 2018).

Phylogenetic analysis of the concatenated matrix. Maximum likelihood and Bayesian inference analyses were performed on the concatenated matrix. The maximum likelihood analysis was performed with the IQ-TREE algorithm (Nguyen et al., 2015) in the W-IQ-TREE portal (Trifinopoulos et al., 2016). The "Model Finder" option was implemented to select the evolutionary model of each partition (Chernomor et al., 2016; Kalyaanamoorthy et al., 2017). The models with the best fit for each partition according to the Bayesian information criterion are presented in Table 1. Branch support was calculated using the ultrafast bootstrap option (Hoang et al., 2018) with 1000 replicas. The resulting tree was visualized using iTOL version 4.4.2 (Letunic and Bork, 2019) and was rooted with *Menyanthes trifoliata*, a member of the Menyanthaceae family which is the sister group of the clade formed by Goodeniaceae, Calyceraceae and Asteraceae (Kadereit, 2007; Chase et al., 2016). PartitionFinder 2 (Lanfear et al., 2016) with PhyML (Guindon et al., 2010) to define the partitions and evolutionary models for the Bayesian analysis of the same concatenated matrix. First, a greedy algorithm (Lanfear et al., 2012) was implemented in The best partition scheme found includes all the markers in one partition and the TVM+I+G model was selected as the best fit according to the Bayesian information criterion. The Bayesian Inference analysis was conducted on MrBayes version 3.2.6 (Ronquist et al., 2012) through the CIPRES Science Gateway portal (Miller et al., 2010). Four simultaneous Markov chains were run for 35 million generations and two independent runs were made. Chains were sampled every 1000 generations. Twenty five percent of the total trees sampled were discarded as

burning after examination of the runs with Tracer version v1.7 (Rambaut et al., 2018). The resulting tree was visualized using iTOL version 4.4.2 (Letunic and Bork, 2019) and was rooted with *Menyanthes trifoliata*. Topological congruence between the resulting phylogenetic trees was visually evaluated.

Results

The concatenated matrix had a total length of 11215 bp and included 201 species. The characteristics of each individual alignment comprised in the concatenated matrix are presented in Table 1. The resulting tree from the maximum likelihood analysis is presented in figures 1 and 2. The final likelihood was -lnL = 102824.5. The majority rule consensus tree resulting from the Bayesian analysis is presented in figure 3 and 4. Overall, the trees resulting from both Bayesian inference and Maximum Likelihood analysis are topologically congruent and well-resolved. All the 13 subfamilies and 34 of the 40 tribes recognized by Panero and Crozier (2016) are recovered by our analysis as monophyletic groups and most of them are strongly supported (Posterior probabilities= 1, Bootstrap support >85%; Figures 1, 2 and 3). More than 89 % of the species native of Mexico sampled for this work are placed within the Asteroideae subfamily. The rest of the Mexican species were placed in the subfamilies Cichorioideae (6 species), Carduoideae (2 species) and Mutisioideae (2 species). The tribes conforming the “Heliantheae alliance” are the most represented ones and correspond to the 28.3 % of the total of species included in this study. At the subfamilies level, the tree obtained by maximum likelihood is consistent with the topology obtained from the Bayesian inference analysis with the exception of the relationships between the subfamilies Hecastocleidoideae, Stifftioideae, Gochnatioideae, and Wunderlichioideae that are not resolved in the Bayesian

inference tree (Figure 3) and the subfamily Wunderlichioideae is not recognized as a monophyletic group, although the tribes that compose that subfamily: Wunderlichieae and Hyalideae are recovered as monophyletic groups.

Discussion

Subfamilies relationships. The topologies generated in this work differs with the proposals of Fu et al. (2016) and Panero and Crozier (2016) in three points: 1) The position of the subfamily Gymnarrhenoideae as a sister group of the clade formed by Asteroideae and Corymbioideae. 2) In the position of the subfamily Wunderlichioideae as a sister group of Stiftioideae and 3) These two subfamilies as a sister group of the rest of the Asteraceae, except Gochnatioideae, Mutisioideae, Famantinoideae and Barnadesioideae (Figure 1). The lack of resolution of the relationships between Hecastocleidoideae, Stifftioideae, Gochnatioideae, and Wunderlichioideae in the Bayesian inference analysis, is consistent with the results of previous studies (Panero and Funk, 2008; Funk et al., 2014; Panero et al., 2014; Mandel et al., 2017). The relationships between the four subfamilies mentioned have proved difficult to reconstruct even with extensive genus-level sampling and with the use of large-scale molecular data obtained from next-generation sequencing methods. In the trees resulting of both methods (Figures 2, 4), we can observe relatively short branches at the base of the conflicting subfamilies and tribes. It has been argued that short branches may lead to errors in the phylogenetic reconstructions because it has not been enough time to accumulate informative substitutions and because short branches are prone to gene and species trees discordance (Wiens et al., 2008). Some authors have proposed that the difficulty in resolving relationships between these four subfamilies is due to the short period of

time where cladogenesis and subsequent diversification of these lineages occurred (Panero and Funk, 2008).

Tribes relationships. Our extended sampling of species of the subfamily Asteroideae allowed us to obtain a phylogeny with high values of bootstrap support (> 85%) for most tribes. In both analyses, all tribes are recovered as monophyletic groups, except Gochnatieae which is divided into *Cyclolepis* and the rest of Gochatieae in the Bayesian tree (Figure 3). In the maximum likelihood tree Gochnatieae appears as a monophyletic group, however the support for the inclusion of *Cyclolepis* in the tribe is low (<85 % of bootstrap support). This segregation of *Cyclolepis* from the Gochnatieae tribe has been previously reported and its position within the tribe is uncertain (Funk et al., 2014). The main differences between our results are observed in the relationships found among the tribes of the subfamily Asteroideae. The sister group of Anthemideae is Gnaphalieae in the maximum likelihood phylogeny and Astereae in the Bayesian tree. The maximum likelihood phylogeny includes Senecioneae as the sister group of the clade formed by Astereae, Gnaphalieae, and Anthemideae, while in the Bayesian analysis the position of Senecioneae is not resolved. The most recent family phylogenies (Fu et al., 2016; Huang et al., 2016; Panero and Crozier, 2016; Mandel et al., 2017) usually present different relationships between the tribes of the subfamily Asteroideae. In particular, the relationships between Heliantheae+Eupatorieae+Millerieae and Astereae+Anthemideae+Gnaphalieae are variable in the different hypotheses (Fu et al., 2016; Huang et al., 2016; Panero and Crozier, 2016; Mandel et al., 2017). The differences between the phylogenies generated in this work and the previous works may be due to the number and type of markers used (Fehrer et al., 2007; Álvarez et

al., 2008; Yu et al., 2013). The incongruence between phylogenies based on chloroplast markers and nuclear markers has been previously reported for several plant groups (Soltis and Kuzoff, 1995; Yu et al., 2013) and for several members of the Asteraceae family (Fehrer et al., 2007; Barker et al., 2009; Pelser et al., 2010). The numerous nested duplications of the genome in this family, particularly in the members of the clade Heliantheae alliance and in the tribes Gnaphalieae and Senecioneae, may contribute to the inconsistency between the different hypotheses (Barker et al., 2008; Smissen et al., 2011; Huang et al., 2016). Incomplete lineage sorting or ancestral hybridization events at the beginning of the diversification of several Asteraceae lineages may be the cause of the incongruence between phylogenies generated by different markers (Pelser et al., 2010; Wang et al., 2014). Several studies have reported a large number of hybrids in Asteraceae compared to other families of vascular plants (Ellstrand et al., 1996; Guo et al., 2005; Fehrer et al., 2007; Schilling, 2011; Smissen et al., 2011; Chester et al., 2012; Mráz et al., 2012; Hovick and Whitney, 2014) and it has been proposed that this propensity to hybridization is associated with the great diversity and invasiveness of the family (Ellstrand and Schierenbeck, 2000; Hovick and Whitney, 2014). Therefore, for future reconstructions, it is important to employ phylogenetic methods that are capable of incorporating hybridization events. We propose the use of phylogenetic network methods that allow the incorporation of hybridization events and calculate support for hybrid branches, for example, the SNaQ method of phylogenetic network reconstruction proposed by Solís-Lemus and Ané (2016).

The phylogeny generated in this work is, to date, the phylogenetic hypothesis of the Asteraceae family that best represents the clades of the subfamily

Asteroideae. Our sampling included mostly species that are distributed in Mexico of the subfamilies Asteroideae, Carduoideae, and Mutisioideae. The majority (97%) of the species sequenced in this work are grouped within the tribes to which they have previously been assigned. The exception is two species: *Tridax rosea* and *Dyssodia papposa* that are grouped with individuals from other tribes within the subfamily Asteroideae in both phylogenies. For these species, there are no sequences of 5 of the 11 markers, so this anomalous group is probably due to a lack of data.

Conclusions

Despite the limitations of the sequencing method, compared to the new generation strategies, we obtained a resolved phylogeny that is consistent with those generated by Huang et al., (2016) and Mandel et al., (2017) through strategies of new generation sequencing. The 13 previously recognized subfamilies are recovered in this work as monophyletic groups with high support values. This phylogeny is the first phylogenetic hypothesis of the family in which the taxa of the subfamily Asteroideae are proportionally represented and which includes Mexican representatives. The Mexican species sequenced are grouped within their corresponding tribes, which indicates that despite the differences between the phylogenies of different authors, our hypothesis is robust. Therefore, this phylogeny of the family can be used for other analysis regarding variation patterns on vegetative and reproductive structures. It is necessary to include complete plastome data and nuclear gene data to improve the support of the nodes and contrast uni- and biparental inherited data.

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Literature

Álvarez I., Costa, A. and Feliner, G.N. (2008). Selecting Single-Copy Nuclear Genes for Plant Phylogenetics: A Preliminary Analysis for the Senecioneae (Asteraceae). Journal of Molecular Evolution, 66, 276-291.

Anderberg, A.A., Baldwin, B.G., Bayer, R.J., Breitwieser, I., Jeffrey, C., Dillon, M.O., Eldenäs, P., Funk, V., Garcia-Jacas, N., Hind, D.J.N., Karis, P.O., Lack, H.W., Nesom, G., Nordenstam, B., Oberprieler, C., Panero, J.L., Puttock, C., Robinson, H., Stuessy, T.F., Susanna, A., Urtubey, E., Vogt, R., Ward, J. and Watson, L.E. (2007). Compositae. In: Kadereit, J.W., Jeffrey C. (eds.). The Families and Genera of Vascular Plants, Volume 8, Flowering Plants. Eudicots. Asterales (pp 61-588) Berlin: Springer.

Barker, M.S., Kane, N.C., Matvienko, M., Kozik, A., Michelmore, R.W., Knapp, S.J. and Rieseberg, L.H. (2008). Multiple paleopolyploidizations during the evolution of the compositae reveal parallel patterns of duplicate gene retention after millions of years. Molecular Biology and Evolution, 25, 2445-2455.

Barker, N.P., Howis, S., Nordenstam, B., Källersjö, M., Eldenäs, P., Griffioen, C. and Linder, H.P. (2009). Nuclear and chloroplast DNA-based phylogenies of Chrysanthemoides Tourn. ex Medik. (Calenduleae; Asteraceae) reveal extensive incongruence and generic paraphyly, but support the recognition of infraspecific taxa in C. monilifera. South African Journal of Botany, 75, 560-572.

Bremer, K. (1994). Asteraceae: Cladistics and classification. Portland: Timber Press.

Chase, M.W., Christenhusz, M.J.M., Fay, M.F., Byng, J.W., Judd, W.S., Soltis, D.E., Mabberley, D.J., Sennikov, A.N., Soltis, P.S. and Stevenset, P.F. (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society, 181, 1-20.

Chernomor, O., von Haeseler, A. and Minh, B.Q. (2016). Terrace aware data structure for phylogenomic inference from supermatrices. Systematic Biology, 65, 997-1008.

Chester, M., Gallagher, J.P., Symonds, Veruska Cruz da Silva, A.E., Mavrodiev, V., Leitch, A.R., Soltis, P.S. and Soltis, D.E. (2012). Extensive chromosomal variation in a recently formed natural allopolyploid species, *Tragopogon miscellus* (Asteraceae). Proceedings of the National Academy of Sciences U.S.A. 109, 1176-1181.

Doyle, J.J. and Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19, 11-15.

Ellstrand, N.C., Whitkus, R.W. and Rieseberg, L.H.(1996). Distribution of spontaneous plant hybrids. Proceedings of the National Academy of Sciences U.S.A. 93, 5090-5093.

Ellstrand, N.C. and Schierenbeck, K. (2000). Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences U.S.A. 97, 7043-7050.

Fehrer, J., Gemeinholzer, B., Chrtek, J. and Bräutigam, S. Jr. (2007). Incongruent plastid and nuclear DNA phylogenies reveal ancient intergeneric hybridization in *Pilosella hawkweeds* (Hieracium, Cichorieae, Asteraceae) Molecular Phylogenetics and Evolution, 42, 347-361.

Fu, Z., Jiao, B., Nie, B., Zhang, G., and Gao, T. (2016). A comprehensive generic-level phylogeny of the sunflower family: Implications for the systematics of Chinese Asteraceae. Journal of Systematics and Evolution, 54, 416-437.

Funk, V.A., Anderberg, A.A., Baldwin, B.G., Bayer, R.J., Bonifacino, J.M., Breitwieser, I., Brouillet, L., Carbajal, R., Chan, R., Coutinho, A.X.P., Crawford, D.J., Crisci, J.V., Dillon, M.O., Freire, S.E., Galbany-Casals, M., Garcia-Jacas, N., Gemeinholzer, B., Gruenstaeudl, M., Hansen, H.V., Himmelreich, S., Kadereit, J.W., Källersjö, M., Karaman-Castro, V., Karis, P.O., Katinas, L., Keeley, S.C., Kilian, N., Kimball, R.T., Lowrey, T.K., Lundberg, J., McKenzie, R.J., Tadesse, M., Mort, M.E., Nordenstam, B., Oberprieler, C., Ortiz, S., Pelser, P.B., Randle, C.P., Robinson, H., Roque, N., Sancho, G., Semple, J.C., Serrano, M., Stuessy, T.F., Susanna, A., Unwin, M., Urbatsch, L., Urtubey, E., Vallès, J., Vogt, R., Wagstaff, S., Ward, J. and Watson, L.E. (2009). Compositae metatrees: The next generation. In: Funk, V.A., Susanna, A., Stuessy, T. and Bayer, R.J. (eds.), Systematics, evolution, and biogeography of Compositae. (pp 747-777). Vienna: IAPT.

Funk, V.A., Sancho, G., Roque, N., Kelloff, C.L., Ventosa-Rodríguez, I., Diazgranados, M., Bonifacino, J.M. and Chan, R. (2014). A phylogeny of the

Gochnatieae: Understanding a critically placed tribe in the Compositae. Taxon 63: 859-882.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. and Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic biology, 59, 307-321.

Guo, Y.P., Saukel, J., Mittermayr, R. and Ehrendorfer, F. (2005). AFLP analyses demonstrate genetic divergence, hybridization, and multiple polyploidization in the evolution of Achillea (Asteraceae-Anthemideae). New Phytologist, 166, 273-289.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q. and Vinh, L.S. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution, 35, 518-522.

Hovick, S.M. and Whitney, K.D. (2014). Hybridisation is associated with increased fecundity and size in invasive taxa: meta-analytic support for the hybridisation-invasion hypothesis. Ecology Letters, 17, 1464-1477.

Huang, C.H., Zhang, C., Liu, M., Hu, Y., Gao, T., Qi, J. and Ma, H. (2016). Multiple polyploidization events across Asteraceae with two nested events in the early history revealed by nuclear phylogenomics. Molecular Biology and Evolution, 33, 2820-2835.

Kadereit, J.W. (2007). Asterales: Introduction and Conspectus. In: Kadereit, J.W. and Jeffrey, C. (eds.), The Families and Genera of Vascular Plants, Volume 8, Flowering Plants. Eudicots. Asterales. (pp 1-6). Berlin: Springer.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.F.K., von Haeseler, A. and Jermiin, L.S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods, 14, 587-589.

Katoh, K., Rozewicki, J. and Yamada, K.D. (2017). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics, bbx108.

Kuraku, S., Zmasek, C.M., Nishimura, O. and Katoh, K. (2013). aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. Nucleic Acids Research, 41, W22-W28.

Lanfear, R., Calcott, B., Ho, S.Y.W., and Guindon, S. (2012). Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution, 29, 1695-1701.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T. and Calcott, B. (2016). PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution, 34, 772-773.

Letunic, I. and Bork, P. (2019). Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Research, 47, W256-W259.

Maddison, W. P. and Maddison, D.R. (2018). Mesquite: a modular system for evolutionary analysis. Version 3.51 http://www.mesquiteproject.org

Mandel, J.R., Dikow, R.B., Funk, V.A., Masalia, R.R., Staton, S.E., Kozik, A., Michelmore, R.W., Rieseberg, L.H. and Burke, J.M. (2014). A target enrichment method for gathering phylogenetic information from hundreds of loci: An example from the Compositae. Applications in Plant Sciences, 2, 1300085.

Mandel, J.R., Dikow, R.B. and Funk, V.A. (2015). Using phylogenomics to resolve mega-families: An example from Compositae. Journal of Systematics and Evolution, 53, 391-402.

Mandel, J.R., Barker, M.S., Bayer, R.J., Dikow, R.B., Gao, T., Jones, K.E., Keeley, S., Kilian, N., Ma, H., Siniscalchi, C.M., Susanna, A., Thapa, R., Watson, L. and Funk, V.A. (2017). The Compositae Tree of Life in the age of phylogenomics. Journal of Systematics and Evolution, 55, 405-410.

Mandel, J.R., Dikow, R.B., Siniscalchi, C.M., Thapa, R., Watson, L.E. and Funk, V.A. (2019). A fully resolved backbone phylogeny reveals numerous dispersals and explosive diversifications throughout the history of Asteraceae. Proceedings of the National Academy of Sciences U.S.A., 116, 14083-14088.

McCauley, D.E., Sundby, A.K., Bailey, M.F. and Welch, M.E. (2007). Inheritance of chloroplast DNA is not strictly maternal in *Silene vulgaris* (Caryophyllaceae): evidence from experimental crosses and natural populations. American Journal of Botany, 94, 1333-1337.

McGuire, J.M., Witt, C.C, Remsen, J.V., Corl, A., Rabosky, D.L., Altshuler, D.L. and Dudley, R. (2014). Molecular phylogenetics and the diversification of hummingbirds. Current Biology, 24, 910-916.

Miller, M.A., Pfeiffer, W. and Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 1-8.

Mraz, P., Garcia-Jacas, N., Gex-Fabry, E., Susanna, A., Barres, L. and Muller-Scharer, H. (2012). Allopolyploid origin of highly invasive *Centaurea stoebe* s.l. (Asteraceae). Molecular Phylogenetics and Evolution, 62, 612- 623.

Müller, J., Müller, K., Neinhuis, C. and Quandt, D. (2010). PhyDE: Phylogenetic Data Editor. Version 0.9971 www.phyde.de

Nguyen, L.T., Schmidt, H.A., von Haeseler, A. and Minh, B.Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution, 32, 268-274.

Noyes, R.D. and Rieseberg, L.H. (1999). ITS sequence data support a single origin for North American Astereae (Asteraceae) and reflect deep geographic divisions in Aster s.l. American Journal of Botany, 86, 398-412.

Panero, J.L. (2007). Key to the tribes of the Heliantheae alliance. In: Kadereit, J.W. and Jeffrey, C. (eds.). The Families and Genera of Vascular Plants. Volumen 8, Flowering Plants. Eudicots. Asterales. (pp 391-395). Berlin: Springer.

Panero, J.L. and Crozier, B.S. (2003). Primers for PCR amplification of Asteraceae chloroplast DNA. Lundellia, 6, 1-9.

Panero, J.L and Crozier, B.S. (2016). Macroevolutionary dynamics in the early diversification of Asteraceae. Molecular Phylogenetics and Evolution, 99, 116-132.

Panero, J.L. and Funk, V.A. (2008). The value of sampling anomalous taxa in phylogenetic studies: major clades of the Asteraceae revealed. Molecular Phylogenetics and Evolution, 47, 757-782.

Panero, J.L., Freire, S.E., Ariza, E.L., Crozier, B.S., Barboza, G.E. and Cantero, J.J. (2014). Resolution of deep nodes yields an improved backbone phylogeny and a new basal lineage to study early evolution of Asteraceae. Molecular Phylogenetics and Evolution, 80, 43-53.

Pelser, P.B., Kennedy, A.H., Tepe, E.J., Shidler, J.B., Nordenstam, B., Kadereit, J.W. and Watson, L.E. (2010). Patterns and causes of incongruence between plastid and nuclear Senecioneae (Asteraceae) phylogenies. American Journal of Botany, 97, 856-873.

Purvis, A., Fritz, S.A., Rodríguez, J., Harvey, P. H. and Grenyer, R. (2011). The shape of mammalian phylogeny: patterns, processes and scales. Philosophical Transactions of the Royal Society B: Biological Sciences, 366, 2462-2477.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. and Suchard, M.A. (2018). Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7, Systematic Biology, 67, 901-904.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. and Huelsenbeck, J.P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61, 539-542.

Salazar, G.A., Chase, M.W., Soto Arenas, M.A. and Ingrouille, M. (2003). Phylogenetics of Cranichideae with emphasis on Spiranthinae (Orchidaceae, Orchidoideae): evidence from plastid and nuclear DNA sequences. American Journal of Botany, 90, 777-795.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30, 1312-1313.

Schilling, E.E. (2011). Hybrid genera in Liatrinae (Asteraceae: Eupatorieae). Molecular Phylogenetics and Evolution, 59, 158-167.

Sequencher® version 4.7 DNA sequence analysis software, Gene Codes Corporation, Ann Arbor, MI USA http://www.genecodes.com

Smissen, R.D., Galbany-Casals, M. and Breitwieser, I. (2011). Ancient allopolyploidy in the everlasting daisies (Asteraceae: Gnaphalieae): Complex relationships among extant clades. Taxon, 60, 649-662.

Solís-Lemus, C. and Ané, C. (2016). Inferring Phylogenetic Networks with Maximum Pseudolikelihood under Incomplete Lineage Sorting. PLOS Genetics, 12, e1005896.

Soltis, D. and Kuzoff, R. (1995). Discordance between Nuclear and Chloroplast Phylogenies in the Heuchera group (Saxifragaceae). Evolution, 49, 727-742.

Soto-Trejo, F., Palomino, G., Villaseñor, J.L. and Crawford, D.J. (2013). Polyploidy in Asteraceae of the xerophytic scrub of the Ecological Reserve of the Pedregal of San Angel, Mexico City. Botanical Journal of the Linnean Society, 173, 211-229.

Stenz, N.W.M., Larget, B., Baum, D.A. and Ané, C. (2015). Exploring tree-like and non-tree-like patterns using genome sequences: An example using the inbreeding plant species *Arabidopsis thaliana* (L.) Heynh. Systematic Biology, 64, 809-823.

Stevens, P.F., 2001. Angiosperm Phylogeny Website. Version 14, July 2017. http://www.mobot.org/MOBOT/research/APweb/>

Stuessy, T. (2010). The rise of Sunflowers. Science, 329, 1605-1606.

Suárez-Mota, M.E. and Villaseñor, J.L. (2011). Las compuestas endémicas de Oaxaca, México: diversidad y distribución. Boletín de la Sociedad Botánica de México, 88, 55-61.

Symonds, V.V., Soltis, P.S. and Soltis, D.E. (2010). Dynamics of polyploid formation in Tragopogon (Asteraceae): recurrent formation, gene flow, and population structure. Evolution, 64, 1984-2003.

Tank, D.C., Eastman, J.M., Pennell, M.W., Soltis, P.S., Soltis, D.E., Hinchliff, C.E., Brown, J.W., Sessa, E.B. and Harmon, L.J. (2015). Nested radiations and the pulse of angiosperm diversification: increased diversification rates often follow whole genome duplications. New Phytologist, 207, 454-467.

Torices, R. (2010). Adding time-calibrated branch lengths to the Asteraceae supertree. Journal of Systematics and Evolution, 48, 271-278.

Trifinopoulos, J., Nguyen, L.T., von Haeseler, A. and Minh, B.Q. (2016). W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research, 44, W232–W235.

Villaseñor, J.L. (1990). The genera of Asteraceae endemic to Mexico and adjacent areas. Aliso, 12, 685-692.

Villaseñor, J.L. (2016). Checklist of the native vascular plants of México. Revista Mexicana de Biodiversidad, 87, 559-902.

Villaseñor, J.L. (2018). Diversidad y distribución de la familia Asteraceae en México. Botanical Sciences, 96, 332-358.

Villaseñor, J.L., Ibarra-Manríquez, G. and Ocaña, D. (1998). Strategies for the conservation of Asteraceae in México. Conservation Biology, 12, 1066-1075.

Wang, G.Y., Meng, Y., Deng, T. and Yang, Y.P. (2014). Molecular phylogeny of Faberia (Asteraceae: Cichorieae) based on nuclear and chloroplast sequences. Phytotaxa, 167, 223-234.

Wiens, J.J., Kuczynski, C.A., Smith, S.A., Mulcahy, D.G., Sites, J.W., Townsend, T.M. and Reeder, T.W. (2008). Branch Lengths, Support, and Congruence: Testing the Phylogenomic Approach with 20 Nuclear Loci in Snakes. Systematic Biology, 57, 420-431.

Yu, W.B., Huang, P.H., Li, D.Z. and Wang, H. (2013). Incongruence between nuclear and chloroplast DNA phylogenies in Pedicularis section Cyathophora (Orobanchaceae). PLOS ONE, 8, e74828.

Table 1. Characteristics of the chloroplast markers used for the phylogenetic reconstruction of Asteraceae.

Marker	Length (pb)	Number of species	New sequences/ Genbank sequences	Best-fit model according to BIC
23S-trnA	648	173	86/87	K2P+G4
trnL-trnF	1091	189	83/106	TVM+F+I+G4
atpB	1381	162	95/67	HKY+F+I+G4
matK	2006	197	93/104	TVM+F+I+G4
ndhJKC	1735	156	95/61	TVM+F+I+G4
ndhD	714	183	81/102	TVM+F+I+G4
ndhF	2243	170	69/101	TVM+F+I+G4
ndhI	504	200	95/105	TIM+F+I+G4
rbcL	1440	191	92/99	K3P+I+G4

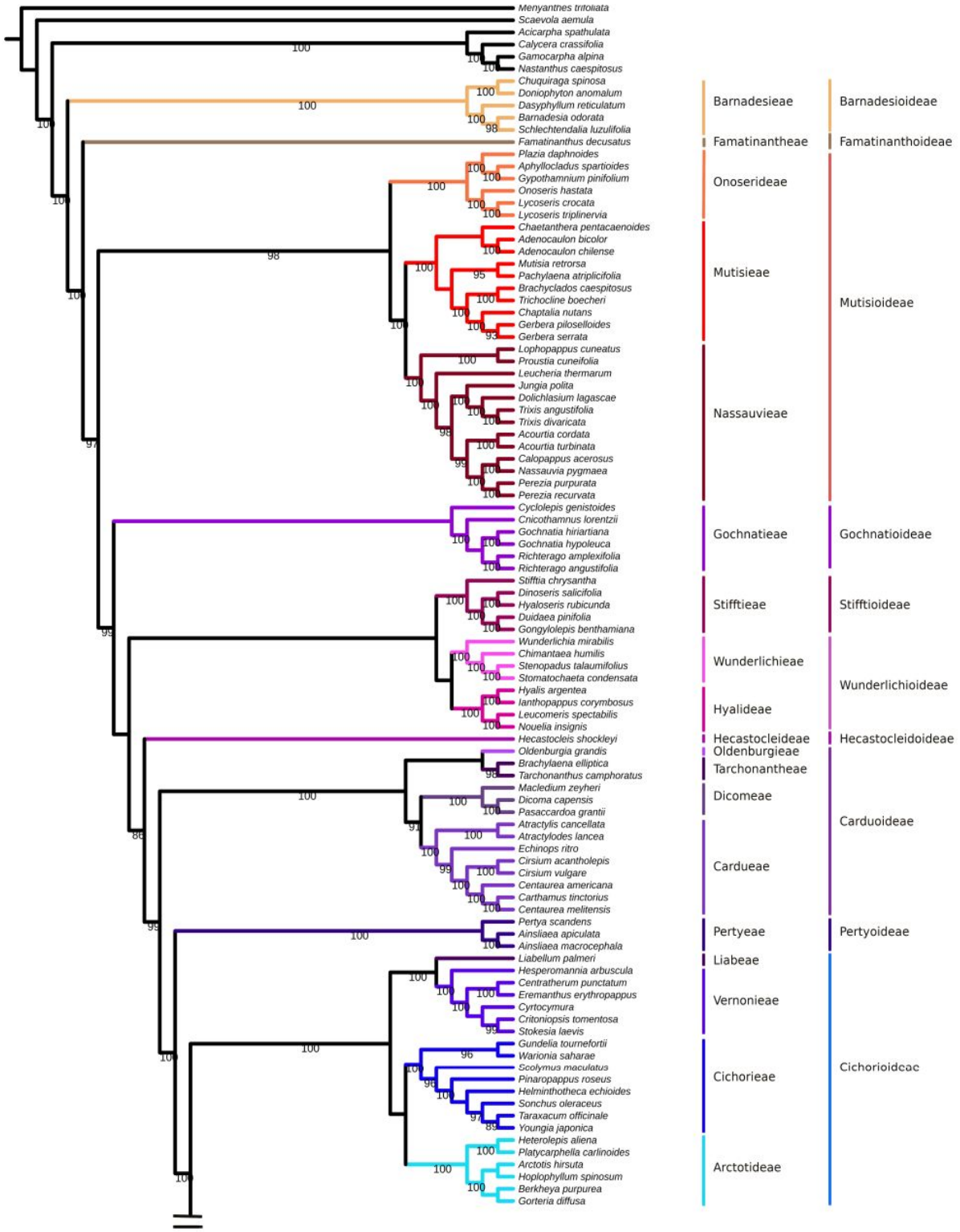


Figure 1. Overview of the phylogenetic relationships of 201 species of Asteraceae based on the concatenated matrix of eleven chloroplast markers (*atpB*, *matK*, *ndhD*, *ndhF*, *ndhI*, *rbcL*, *ndhJ*, *ndhK*, *ndhC*, *trnL-trnF*, *23S-trnA*). Branches were colored by

tribe. The names of the tribes are presented next to each clade, subfamily names are shown on the far right column. Bootstrap values ≥85% are shown below the branches.


Gymnarrhena micrantha
Corymbium glabrum
Psacalium paucicapitatum
Barkleyanthus salicifolius
Psacalium silphiifolium
Pittocaulon praecox
Roldana lobata
Phaneroglossa bolusii
Senecio polypodioides
Emilia
Ekmaniopappus
Elekmania
Dimorphotheca sinuata
Chrysanthemoides monilifera
Osteospermum asperulum
Cotula australis
Ursinia speciosa
Achillea millefolium
Artemisia ludoviciana
Oncosiphon grandiflorus
Relhania calycina
Chionolaena salicifolia
Gamochaeta pensylvanica
Syncarpha vestita
Pseudognaphalium semilanatum
Pseudognaphalium viscosum
Felicia heterophylla
Ixiochlamys cuneifolia
Aphanostephus ramosissimus
Erigeron janivultus
Erigeron tenuis
Conyza canadensis
Conyza bonariensis
Conyza coronopifolia
Laennecia sophiifolia
Baccharis neglecta
Baccharis salicifolia
Heterotheca chrysopsidis
Gymnosperma glutinosum
Psilactis brevilingulata
Arida blepharophylla
Grindelia oxylepis
Pluchea carolinensis
Helenium scorzonerifolium
Tetraneuris scaposa
Dahlia coccinea
Hidalgoa pentamera
Dyssodia papposa
Electranthera mutica
Bidens odorata
Bidens pilosa
Cosmos diversifolius
Cosmos parviflorus
Loxothysanus sinuatus
Florestina pedata
Heterosperma pinnatum
Schkuhria pinnata
Chrysactinia mexicana
Bajacalia crassifolia
Pectis prostrata
Thymophylla pentachaeta
Tagetes micrantha
Tagetes tenuifolia
Jaegeria hirta
Trigonospermum melampodioides
Schistocarpha bicolor
Alloispermum scabrum
Galinsoga parviflora
Stevia origanoides
Stevia salicifolia
Ageratina cylindrica
Oxylobus adscenens
Ageratina adenophora
Ageratina deltoidea
Piqueria trinervia
Brickellia secundiflora
Brickellia veronicifolia
Peteravenia schultzii
Tamaulipa azurea
Fleishmannia pycnophephala
Kyrsteniopsis spinaciifolia
Tridax rosea
Montanoa grandiflora
Montanoa tomentosa
Lasianthaea crocea
Jefea lantanifolia
Perymenium buphthalmoides
Ratibida columnifera
Tetrachyron websteri
Verbesina virgata
Ambrosia cumanensis
Acmella repens
Zinnia peruvianna
Dugesia mexicana
Zaluzania parthenioides
Sclerocarpus uniserialis
Hymenostephium cordatum
Dendroviguiera quinqueradiata
Aldama excelsa
Tithonia tubiformis
Lagascea rigida
Simsia amplexicaulis
Gymnarrheneae
Corymbieae
Senecioneae
Calenduleae
Anthemideae
Gnaphalieae
Astereae
Inuleae
Helenieae
Coreopsideae
Bahieae
Tageteae
Millerieae
Eupatorieae
Heliantheae
Gymnarrhenoideae
Corymbioideae
Asteroideae

Figure 1 (Continuation). Overview of the phylogenetic relationships of 201 species of Asteraceae based on the concatenated matrix of eleven chloroplast markers (*atpB*, *matK*, *ndhD*, *ndhF*, *ndhI*, *rbcL*, *ndhJ*, *ndhK*, *ndhC*, *trnL-trnF*, *23S-trnA*). Branches were colored by tribe. The names of the tribes are presented next to each clade, subfamily names are shown on the far right column. Bootstrap values ≥85% are shown below the branches.

Figure 2. Detail of the maximum likelihood phylogeny of Asteraceae estimated from eleven chloroplast markers. Branch lengths were drawn.

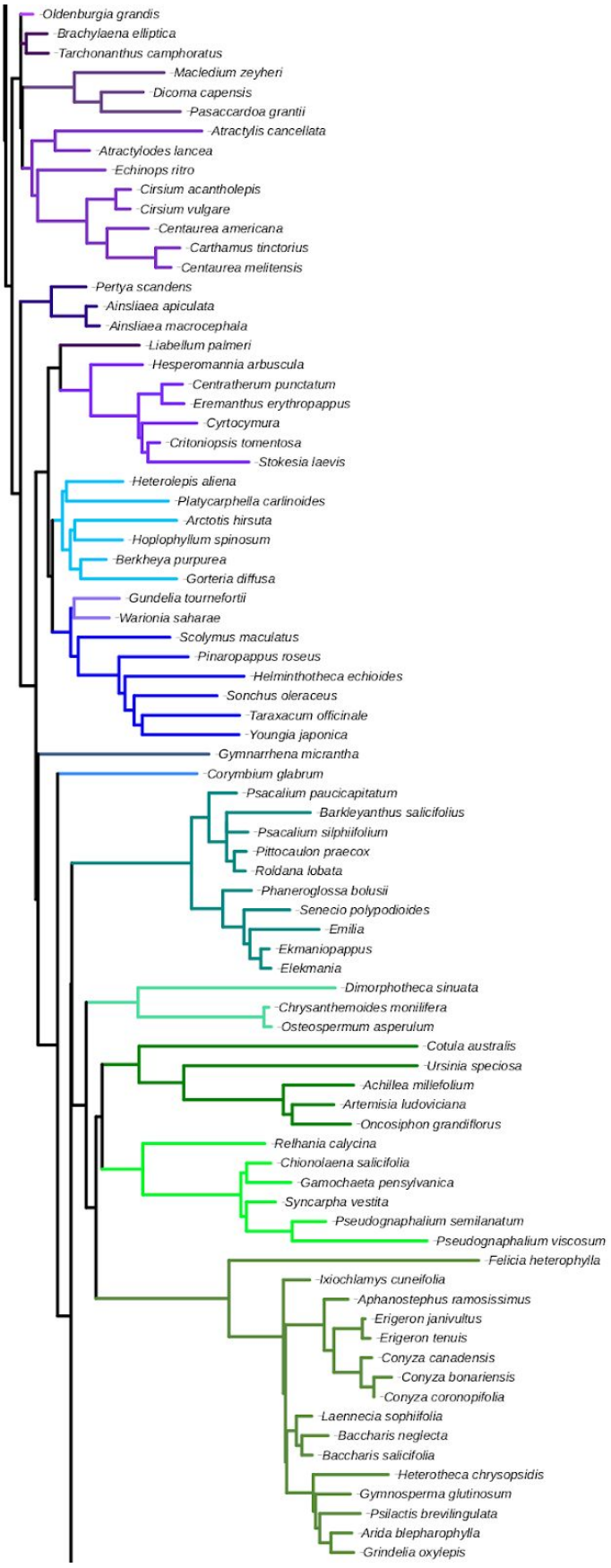

Oldenburgia grandis
Brachylaena elliptica
Tarchonanthus camphoratus
Macledium zeyheri
Dicoma capensis
Pasaccardoa grantii
Atractylis cancellata
Atractylodes lancea
Echinops ritro
Cirsium acantholepis
Cirsium vulgare
Centaurea americana
Carthamus tinctorius
Centaurea melitensis
Pertya scandens
Ainsliaea apiculata
Ainsliaea macrocephala
Liabellum palmeri
Hesperomannia arbuscula
Centratherum punctatum
Eremanthus erythropappus
Cyrtocymura
Critoniopsis tomentosa
Stokesia laevis
Heterolepis aliena
Platycarphella carlinoides
Arctotis hirsuta
Hoplophyllum spinosum
Berkheya purpurea
Gorteria diffusa
Gundelia tournefortii
Warionia saharae
Scolymus maculatus
Pinaropappus roseus
Helminthotheca echioides
Sonchus oleraceus
Taraxacum officinale
Youngia japonica
Gymnarrhena micrantha
Corymbium glabrum
Psacalium paucicapitatum
Barkleyanthus salicifolius
Psacalium silphiifolium
Pittocaulon praecox
Roldana lobata
Phaneroglossa bolusii
Senecio polypodioides
Emilia
Ekmaniopappus
Elekmania
Dimorphotheca sinuata
Chrysanthemoides monilifera
Osteospermum asperulum
Cotula australis
Ursinia speciosa
Achillea millefolium
Artemisia ludoviciana
Oncosiphon grandiflorus
Relhania calycina
Chionolaena salicifolia
Gamochaeta pensylvanica
Syncarpha vestita
Pseudognaphalium semilanatum
Pseudognaphalium viscosum
Felicia heterophylla
Ixiochlamys cuneifolia
Aphanostephus ramosissimus
Erigeron janivultus
Erigeron tenuis
Conyza canadensis
Conyza bonariensis
Conyza coronopifolia
Laennecia sophiifolia
Baccharis neglecta
Baccharis salicifolia
Heterotheca chrysopsidis
Gymnosperma glutinosum
Psilactis brevilingulata
Arida blepharophylla
Grindelia oxylepis

Figure 2 (Continuation). Maximum likelihood phylogeny of Asteraceae estimated from eleven chloroplast markers. Branch lengths are shown.

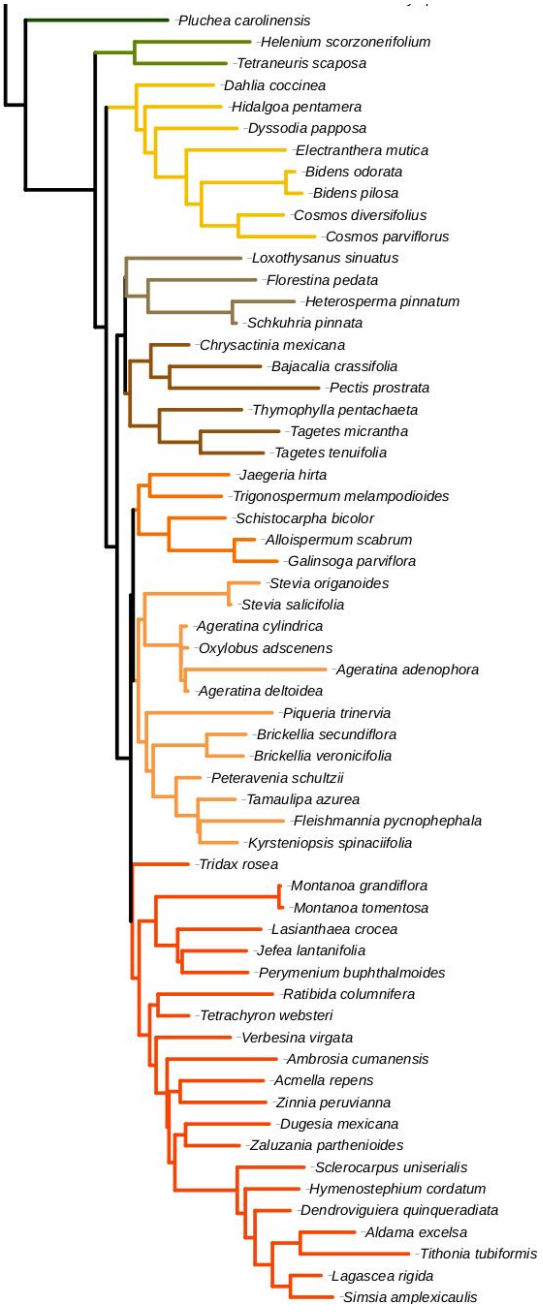


Figure 2 (Continuation). Maximum likelihood phylogeny of Asteraceae estimated from eleven chloroplast markers. Branch lengths are shown.

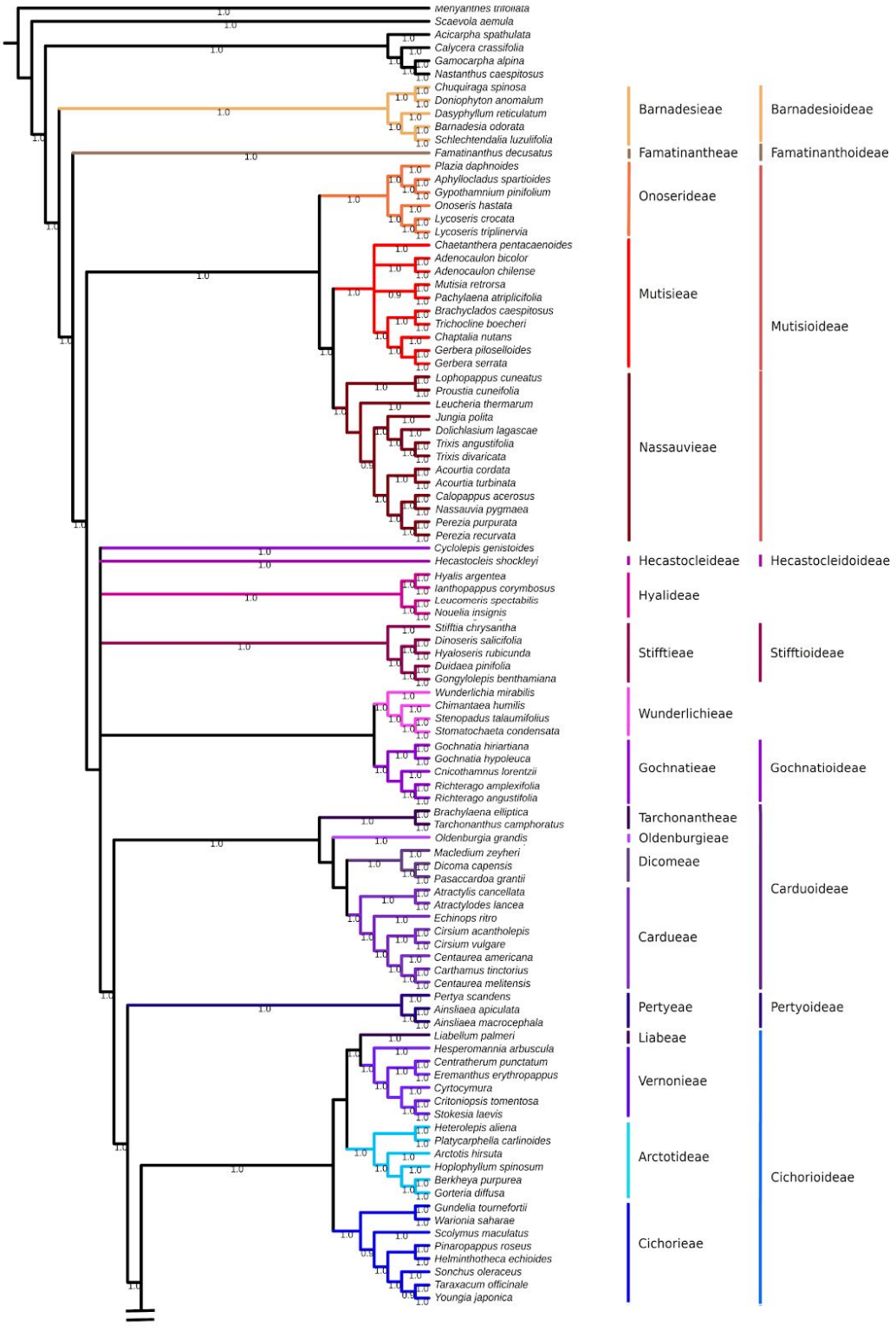
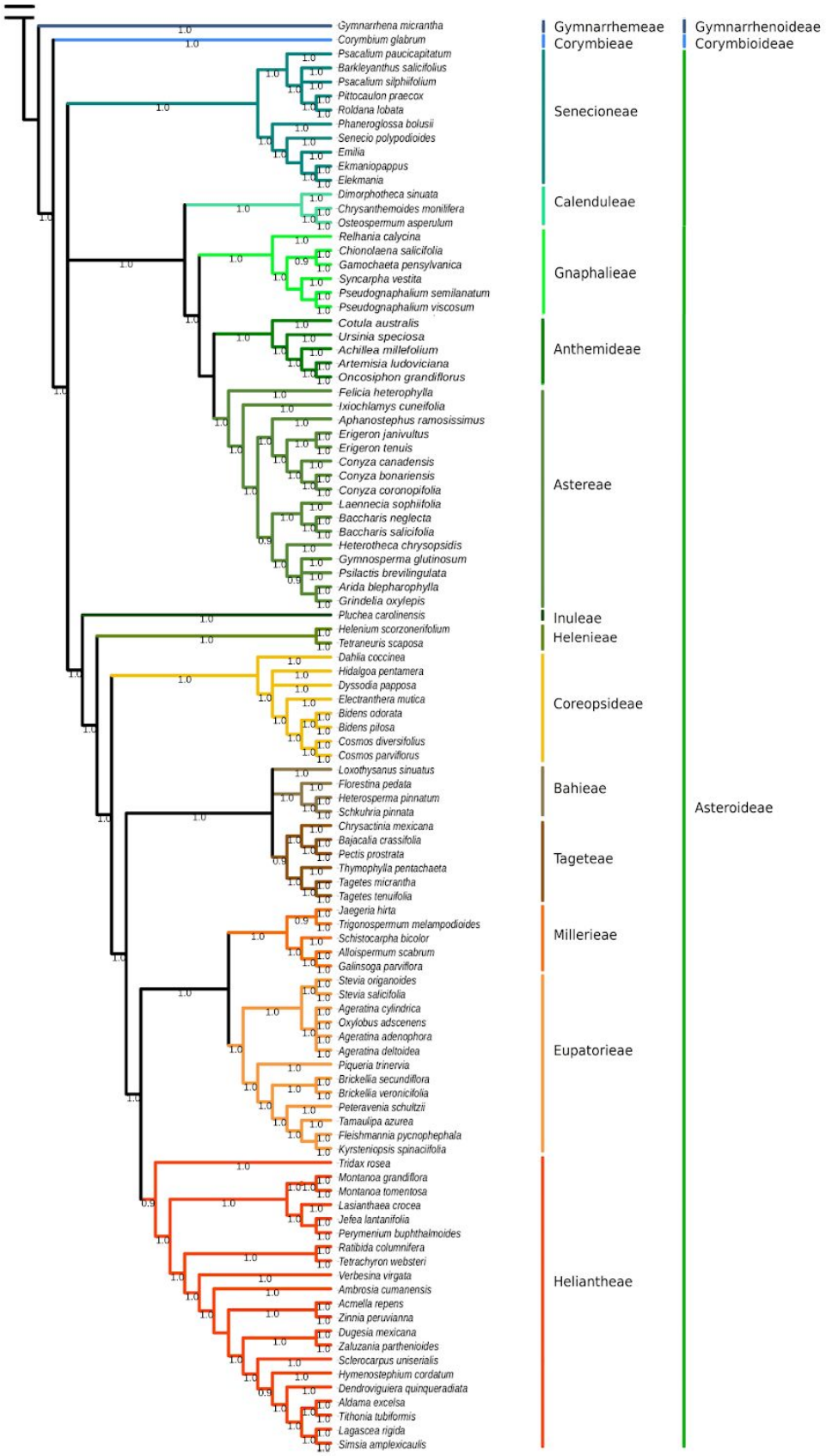


Figure 3. Majority consensus rule tree resulting from the Bayesian inference analysis of eleven chloroplast markers. Posterior probabilities higher than 0.85 are shown

below the branches. Branches were colored by tribe. The names of the tribes are presented next to each clade, subfamily names are shown on the far right column.


Gymnarrhena micrantha
Corymbium glabrum
Psacalium paucicapitatum
Barkleyanthus salicifolius
Psacalium silphiifolium
Pittocaulon praecox
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Phaneroglossa bolusii
Senecio polypodioides
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Ekmaniopappus
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Dimorphotheca sinuata
Chrysanthemoides monilifera
Osteospermum asperulum
Relhania calycina
Chionolaena salicifolia
Gamochaeta pensylvanica
Syncarpha vestita
Pseudognaphalium semilanatum
Pseudognaphalium viscosum
Cotula australis
Ursinia speciosa
Achillea millefolium
Artemisia ludoviciana
Oncosiphon grandiflorus
Felicia heterophylla
Ixiochlamys cuneifolia
Aphanostephus ramosissimus
Erigeron janivultus
Erigeron tenuis
Conyza canadensis
Conyza bonariensis
Conyza coronopifolia
Laennecia sophiifolia
Baccharis neglecta
Baccharis salicifolia
Heterotheca chrysopsidis
Gymnosperma glutinosum
Psilactis brevilingulata
Arida blepharophylla
Grindelia oxylepis
Pluchea carolinensis
Helenium scorzonerifolium
Tetraneuris scaposa
Dahlia coccinea
Hidalgoa pentamera
Dyssodia papposa
Electranthera mutica
Bidens odorata
Bidens pilosa
Cosmos diversifolius
Cosmos parviflorus
Loxothysanus sinuatus
Florestina pedata
Heterosperma pinnatum
Schkuhria pinnata
Chrysactinia mexicana
Bajacalia crassifolia
Pectis prostrata
Thymophylla pentachaeta
Tagetes micrantha
Tagetes tenuifolia
Jaegeria hirta
Trigonospermum melampodioides
Schistocarpha bicolor
Alloispermum scabrum
Galinsoga parviflora
Stevia origanoides
Stevia salicifolia
Ageratina cylindrica
Oxylobus adscenens
Ageratina adenophora
Ageratina deltoidea
Piqueria trinervia
Brickellia secundiflora
Brickellia veronicifolia
Peteravenia schultzii
Tamaulipa azurea
Fleishmannia pycnophephala
Kyrsteniopsis spinaciifolia
Tridax rosea
Montanoa grandiflora
Montanoa tomentosa
Lasianthaea crocea
Jefea lantanifolia
Perymenium buphthalmoides
Ratibida columnifera
Tetrachyron websteri
Verbesina virgata
Ambrosia cumanensis
Acmella repens
Zinnia peruvianna
Dugesia mexicana
Zaluzania parthenioides
Sclerocarpus uniserialis
Hymenostephium cordatum
Dendroviguiera quinqueradiata
Aldama excelsa
Tithonia tubiformis
Lagascea rigida
Simsia amplexicaulis
Gymnarrhemeae
Corymbieae
Senecioneae
Calenduleae
Gnaphalieae
Anthemideae
Astereae
Inuleae
Helenieae
Coreopsideae
Bahieae
Tageteae
Millerieae
Eupatorieae
Heliantheae
Gymnarrhenoideae
Corymbioideae
Asteroideae

Figure 3 (Continuation). Majority consensus rule tree resulting from the Bayesian inference analysis of eleven chloroplast markers. Posterior probabilities higher than 0.85 are shown below the branches. Branches were colored by tribe. The names of the tribes are presented next to each clade, subfamily names are shown on the far right column.

Figure 4. Detail of the majority consensus rule tree resulting from the Bayesian inference analysis of eleven chloroplast markers. Branches were drawn according to their length.

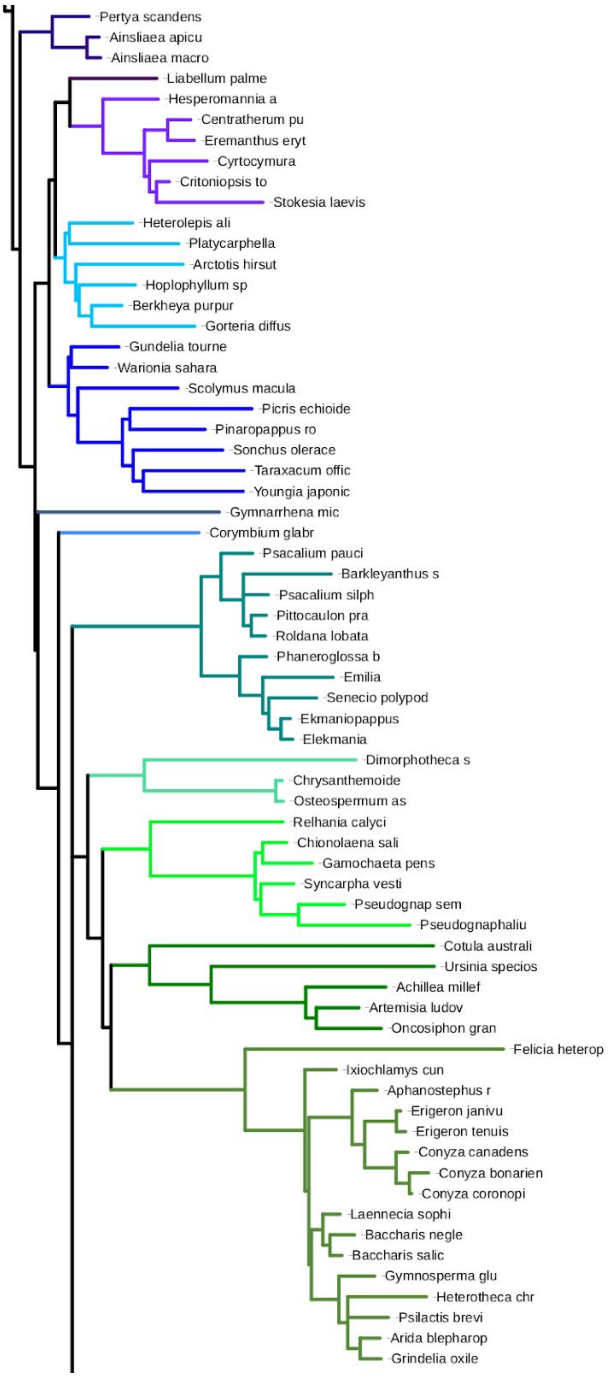

Figure 4 (Continuation). Detail of the majority consensus rule tree resulting from the Bayesian inference analysis of eleven chloroplast markers. Branches were drawn according to their length.

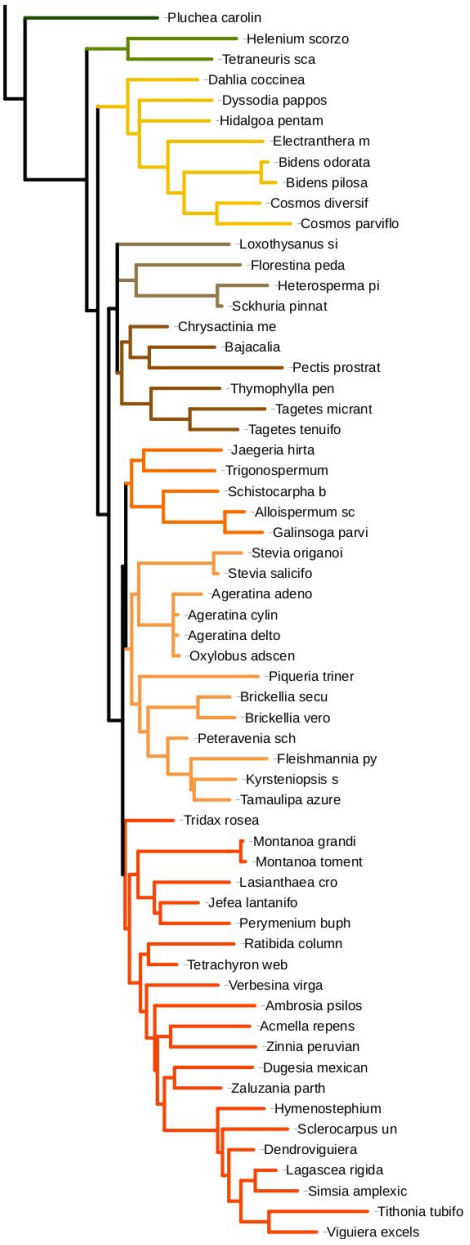

Figure 4 (Continuation). Detail of the majority consensus rule tree resulting from the Bayesian inference analysis of eleven chloroplast markers. Branches were drawn according to their length.

Appendix 1: Collectors and collection numbers of the voucher specimens for the studied species. All specimens are deposited in the Herbario Nacional de México (MEXU) Biology Institute, Universidad Nacional Autónoma de México.

Acmella repens; O. Hinojosa 519

Acourtia cordata; O. Hinojosa 442

Ageratina adenophora; O. Hinojosa 563

Ageratina cylindrica; O. Hinojosa 500

Ageratina deltoidea; O. Hinojosa 499

Aldama excelsa; L. Céspedes 370

Alloispermum scabrum; J.L. Villaseñor 2170

Ambrosia psylostachia; O. Hinojosa 507

Artemisia ludoviciana; O. Hinojosa 443

Aphanostephus ramosissimus; R. Torres 14721

Arida blepharophylla; Nesom 6541

Baccharis salicifolia; O. Hinojosa 438

Barkleyanthus salicifolius; F. Soto 85

Bidens odorata; O. Hinojosa 518

Bidens pilosa; O. Hinojosa 503

Brickellia secundiflora; O. Hinojosa 555

Brickellia veronicifolia; O. Hinojosa 497

Centaurea americana; R. Torres 15924

Chionolaena salicifolia; D. Alvarez 14834

Chrysactinia mexicana; R. Torres 17160

Cirsium acantholepis; J.L. Villaseñor 2161

Cirsium vulgare; O. Hinojosa 506

Conyza bonariensis; L. Céspedes 477

Conyza canadensis; O. Hinojosa 464

Conyza coronophifolia; O. Hinojosa 467

Cosmos diversifolius; J.L. Villaseñor 2164

Cosmos parviflorus; O. Hinojosa 439

Cotula australis; L. Céspedes 476

Critoniopsis tomentosa; R. Redonda et al., 722

Cyrtocymura sp.; I. Fragoso 459

Dahlia coccinea; O. Hinojosa 585

Dendroviguiera quinqueradiata; O. Hinojosa 623

Dugesia mexicana; J.L. Villaseñor 2168

Dyssodia papposa; L. Céspedes 206

Ekmaniopappus sp.; I. Fragoso 513

Electranthera mutica; D. Alvarez 15147

Elekmania sp.; I. Fragoso 577

Emilia sp.; I. Fragoso 536

Erigeron janivultus; J.L. Villaseñor 2166

Fleischmannia pycnocephala; O. Hinojosa 554

Florestina pedata; L. Céspedes 470

Galinsoga parviflora; L. Céspedes 475

Gochnatia smithii; R. Redonda et al., 826

Gymnosperma glutinosum; R. Torres 15090

Helenium scorzonerifolium; D. Alvarez 15480

Helminthotheca echioides; L. Céspedes 478

Heterosperma pinnatum; O. Hinojosa 515

Heterotheca chrysopsidis; R. Torres 14598

Hidalgoa pentamera; J.L. Villaseñor 2243

Hymenostephium cordatum; D. Alvarez 15232

Jaegeria hirta; L. Céspedes 372

Jefea lantanifolia; J.L. Villaseñor 2203

Kyrsteniopsis spinaciifolia; R. Torres 15603

Laennecia sophiifolia; O. Hinojosa 583

Lagascea rigida; L. Céspedes 483

Loxothysanus sinuatus; Pruski 4201

Montanoa grandiflora; L. Céspedes 607

Oxylobus adscenens; D. Alvarez 15508

Pectis prostrata; O. Hinojosa 524

Perymenium buphthalmoides; J.L. Villaseñor 2225

Perymenium cornutum; R. Torres 15437

Peteravenia schultzii; J.L. Villaseñor 2247

Pinaropappus roseus; D. Alvarez 15162

Piqueria trinervia; O. Hinojosa 522

Pittocaulon praecox; O. Hinojosa 505

Psacalium silphiifolium; J.L. Villaseñor 2173

Pseudognaphalium semilanatum; L. Céspedes 516

Pseudognaphalium viscosum; O. Hinojosa 504

Psilactis brevilingulata; J.L. Villaseñor 2174

Ratibida columnifera; R. Torres 15763

Roldana lobata; L. Céspedes 448

Schistocarpha bicolor; J.L. Villaseñor 2248

Sclerocarpus uniserialis; J.L. Villaseñor 2197

Simsia amplexicaulis; L. Céspedes 473

Schkuhria pinnata; L. Céspedes 124

Sonchus oleraceus; L. Céspedes 456

Stevia origanoides; O. Hinojosa 523

Stevia salicifolia; O. Hinojosa 498

Tagetes micrantha; L. Céspedes 434

Tagetes tenuifolia; L. Céspedes 474

Tamaulipa azurea; R. Torres 14997

Taraxacum officinale; O. Hinojosa 525

Tetrachyron websteri; R. Torres 15505

Tetraneuris scaposa; R. Torres 15793

Thelesperma longipes; R. Torres 14912

Thymophylla pentachaeta; R. Torres 14629

Tithonia tubiformis; L. Céspedes 444

Trixis angustifolia; R. Torres 15174

Tridax rosea; J.L. Villaseñor 2179

Trigonospermum melampodioides; D. Alvarez 15047

Verbesina virgata; L. Céspedes 437

Zaluzania parthenioides; J.L. Villaseñor 2190

Zinnia peruviana; O. Hinojosa 508

Capítulo 4.

Variación foliar asociada a la poliploidía y la forma de crecimiento en Asteraceae desde la perspectiva de la biología comparada.

Resumen

La variación foliar en las plantas de la familia Asteraceae se ha relacionado con factores como la variación ambiental, la forma de crecimiento y la variación en los números cromosómicos y la poliploidía. En este trabajo se analiza la relación entre los caracteres foliares, los números cromosómicos, la poliploidía y la forma de crecimiento de las plantas utilizando métodos comparados filogenéticos que tomen en cuenta las relaciones filogenéticas. Encontramos que caracteres como el número cromosómico, la densidad estomática y la longitud de las células guarda presentan señal filogenética. La distribución de caracteres como las vainas de los haces vasculares y el tipo de margen de las hojas es diferente en distintas partes del árbol filogenético de las Asteráceas. En las subfamilias Barnadesioideae y Mutisieae, que se divirgieron tempranamente en la historia de la familia, se observa una tendencia a presentar hojas relativamente pequeñas con márgenes enteros en las especies de las subfamilias. En la subfamilia Asteroideae, que se diversificó más recientemente se observan hojas de mayor tamaño con márgenes aserrados o dentados u hojas muy divididas con márgenes enteros. La tendencia a ser malezas, que se observado en algunas especies de la familia, suele ser más frecuente en miembros de Asteroideae. Sugerimos que la invasividad de las especies y su resistencia a ambientes perturbados puede estar relacionada con las características de las hojas, la forma de crecimiento y la poliploidía.

Abstract

Leaf variation in members of the Asteraceae family has been related to environmental variation, growth form, chromosome number variation, and polyploidy. In this study we analyze the relationship between leaf characters, chromosome numbers,polyploidy and growth form using phylogenetic comparative methods that take into account the phylogenetic relationships between species. We found that characters as chromosome number, stomatal density, and guard cell length show phylogenetic signal. The distribution of characters such as vascular bundle sheaths and leaf margin type differ within the Asteraceae phylogenetic tree. In species of the Barnadesioideae and Mutisieae subfamilies, that diverge early in the history of the family, we observe a tendency to present relatively small leaves with entire margins. Whereas in the Asteroideae subfamily that diversified more recently in the family history we observe bigger leaves with serrate or dentate margins or highly dissected leaves with entire margins. The weediness traits, observed in some species of the family, is more common in members of the Asteroideae subfamily. We suggest that species invasiveness and their resistance to disturbed environments can be related to some leaf characters, and to the growth form, and the polyploidy.

Introducción

Las hojas son uno de los principales órganos de las plantas. Son una parte esencial de diversas cadenas alimenticias y guían los ciclos biogeoquímicos del nitrógeno y el carbono, por lo cual son sumamente importantes para el funcionamiento y mantenimiento de los ecosistemas terrestres (Wright et al., 2004; Mason et al., 2015). Su estructura es muy variable y esta variación se ha encontrado

asociada a aspectos del funcionamiento de la planta, como las distintas estrategias metabólicas, la tasa de crecimiento y la defensa contra herbívoros (Lambers y Poorter 1992; Chapin et al., 1993; Westoby 1998; Westoby et al., 2002, Díaz et al., 2004; Mason et al., 2015). Aunque la variación en algunas características de las hojas suele asociarse con la variación del ambiente (Parkhurst y Loucks 1972; Peñuelas y Matamala 1990; Woodward y Kelly 1995; Poorter et al., 2009; Abdala-Roberts et al., 2018), algunos trabajos han demostrado que en realidad, la variación ambiental tiene un efecto moderado sobre los caracteres foliares (Díaz et al., 2004; Wright et al., 2004, 2005; Poorter et al., 2009) y que, factores como la forma de crecimiento (Holbrook y Putz 1996; Wright et al., 2004; Rossatto et al., 2015), las relaciones alométricas entre los tejidos foliares (Poorter et al., 2009; Brodribb et al., 2013; John et al., 2013) y la poliploidía (Balao et al., 2011; Baker et al., 2017), pueden explicar un alto porcentaje de la variación interespecífica en las hojas.

La poliploidía puede definirse como “el incremento heredable del número de copias completas del genoma” (Wood et al., 2009). Sus efectos en las hojas varían dependiendo del número de copias del genoma y del conjunto particular de caracteres que se estén considerando (Anssour et al., 2009; Hodgson et al., 2010; Balao et al., 2011; Baker et al., 2017). En plantas, la poliploidía se ha reconocido y estudiado extensivamente desde el siglo XIX. Aunque su papel evolutivo ha sido debatido (Stebbins 1940, 1950; Arnold y Martin 2010; Soltis et al., 2014), los métodos moleculares modernos han demostrado que varios linajes de eucariontes, incluyendo las angiospermas, descienden de ancestros poliploides y que algunos clados han experimentado varias rondas de duplicaciones del genoma completo

(Otto y Whitton 2000; Dehal y Boore 2005; Soltis 2005; Aury et al., 2006; Otto 2007). La duplicación del genoma completo de las plantas tiene efectos directos sobre su fenotipo. Se ha demostrado que propicia cambios en el volumen nuclear y celular, completamente independientes de factores genéticos (Sax y Sax 1937; Cavalier-Smith 1978; Masterson 1994; Sugiyama 2005). Este aumento en el tamaño celular frecuentemente tiene como consecuencia, un incremento general del tamaño de la planta y sus órganos (Stebbins 1950, 1971; Bretagnole y Lumaret 1995; Otto y Whitton 2000; Balao et al., 2011; Beyaz et al., 2013; Zhang et al., 2019). Las alteraciones anatómicas y morfológicas provocadas por las duplicaciones del genoma alteran también los procesos metabólicos, especialmente aquellos procesos celulares que involucran membranas, debido a que se modifica la proporción celular de superficie/volumen (Weiss et al., 1975; Otto y Whitton 2000). Los cambios en el metabolismo celular desencadenan cambios en las tasas de crecimiento (Cavalier-Smith 1978; Otto y Whitton 2000), en el intercambio gaseoso de la hoja (Li et al., 1996), la transpiración, las concentraciones de metabolitos secundarios (Hull-Sanders et al., 2009), los niveles hormonales, la tasa fotosintética y el balance hídrico (Warner et al., 1987; Levin 2002; Coate et al., 2012; Wang et al., 2012). En conjunto estas modificaciones tienen efectos directos en la adecuación de la planta. Por ejemplo, se ha demostrado que la poliploidía puede resultar en un incremento en el diámetro de los vasos del xilema secundario (Maherali et al., 2009), lo cual puede influenciar la conductividad hidráulica y la tolerancia al estrés hídrico y a la cavitación (Zimmermann 1983; Maherali et al., 2009). Se ha sugerido que después de la modificación de los órganos de la planta por efecto de la duplicación del genoma, algunos conjuntos de caracteres experimentan regímenes

de selección natural direccional con distintos óptimos (Balao et al., 2011) y que las relaciones filogenéticas entre los taxones influyen en la evolución de los caracteres foliares, incluso en aquellos que suelen relacionarse con variaciones ambientales (Balao et al., 2011; Glade-Vargas et al., 2018). Por ello para los estudios de poliploidía/fenotipo, se debe tomar en cuenta las relaciones de parentesco entre los individuos estudiados (Balao et al., 2011).

La familia Asteraceae es un grupo ideal para estudiar las causas de la variación en las hojas debido a su gran diversidad morfo-anatómica y de formas de crecimiento, a la abundancia de poliploides que se refleja en una diversidad de números cromosómicos y al reconocimiento de distintos eventos anidados de duplicaciones del genoma completo (Barker et al., 2008; Huang et al., 2016). Varias especies de Asteraceae son malezas, es decir que toleran vivir en sitios perturbados, por lo cual pueden invadir con facilidad nuevos ambientes. La diversidad de las hojas de Asteraceae de México ha sido descrita previamente (Rivera et al., 2019) y se ha estudiado con relación un ambiente xérico (Rivera et al., 2017); sin embargo estos trabajos previos han demostrado que las variables ambientales por sí solas no explican la variación foliar observada en esta familia cosmopolita. En este trabajo se aborda la relación entre la variación de las hojas y la poliploidía y la forma de crecimiento en la familia Asteraceae mediante el uso de métodos comparados filogenéticos. Para ello se plantean las siguientes preguntas de investigación: ¿Cómo es la variación de las hojas en Asteraceae con distintos niveles de ploidía y con distintas formas de crecimiento? ¿Hay relaciones significativas entre caracteres foliares y los números cromosómicos, el nivel de ploidía y la forma de crecimiento? ¿La variación en los caracteres foliares está

asociada a la historia filogenética de las especies? Con base en estas preguntas planteamos los siguientes objetivos de investigación:

- Generar una base de datos comparativos de la variación foliar, la variación en números cromosómicos y en niveles de ploidía y, las distintas formas de crecimiento de representantes de los diversos linajes (subfamilias y tribus) que componen a la familia Asteraceae.
- Corroborar si las correlaciones entre los caracteres foliares y los números cromosómicos, el nivel de ploidía y la forma de crecimiento, encontradas en otros grupos de plantas, son constantes en los diversos linajes de la familia.
- Analizar la variación en los caracteres foliares, incluyendo los asociados a variables ambientales, para descubrir si está relacionada a la historia filogenética de las especies estudiadas.

Nuestras hipótesis son las siguientes: 1) Hay una gran variación en los caracteres foliares, los números cromosómicos y el nivel de ploidía dentro de la familia. 2) La poliploidía propiciará un aumento del tamaño general de la hoja y sus tejidos. La forma de crecimiento se relaciona con el número cromosómico, de tal forma que las plantas herbáceas tendrán números cromosómicos más grandes que las leñosas. 3) Los caracteres foliares estudiados, incluyendo aquellos asociados a variables ambientales, presentan señal filogenética. Para probar nuestras hipótesis consideramos veinticinco caracteres foliares. Se incluyeron caracteres que han probado ser sensibles a los cambios en el nivel de ploidía y que también se han encontrado relacionados con la forma de crecimiento, por ejemplo la longitud de las células guarda, la densidad estomática, el área foliar, el área foliar específica, el tipo

de margen en las hojas y la presencia de vainas en los haces vasculares (Ackerly et al., 2002; Vile et al 2005; Santiago y Wright 2007; Anssour et al., 2009; Schmerler et al., 2012; Baker et al., 2017; Lukács et al., 2017)

Material y Método

Base de datos comparativos. Se obtuvieron y procesaron muestras de hojas de 150 especies de Asteraceae. Las muestras incluyen especies colectadas en México y hojas tomadas de ejemplares de herbario de subfamilias de África, Europa y Sudamérica. Los herbarios consultados fueron el Herbario Nacional de México (MEXU) y el Herbario de la Universidad de Texas en Austin (TEX). Las muestras fueron procesadas como se indica en Rivera et al. (2017). Se construyó una base de veinticinco caracteres comparativos para 150 géneros de Asteraceae, incluyendo veinte caracteres foliares, tres caracteres citogenéticos (número cromosómico haploide (n), número cromosómico diploide (2n) y el nivel de ploidía de la especie), la forma de crecimiento y la condición de maleza de las especies. Los caracteres foliares medidos se enlistan en el cuadro 1. La codificación de los caracteres foliares se realizó siguiendo a Ellis et al. (2009). La medición de caracteres y el cálculo de los índices se describen en Rivera et al. (2017). Los números cromosómicos se obtuvieron de la base de datos de conteos cromosómicos de Rice et al. (2015) y de los trabajos de Soto-Trejo et al. (2011, 2013) sobre especies mexicanas de Asteraceae. La categorización en malezas y no malezas se realizó con base en su prevalencia en ambientes perturbados e invadidos (MacKinnon et al., 2014). Los caracteres cuantitativos presentaron una distribución log-normal, por lo cual se transformaron utilizando el Log_{10} antes de analizarlos. De la misma forma, los

conteos fueron transformados mediante raíz cuadrada para conseguir que su distribución fuera aproximadamente normal.

Cuadro 1. Caracteres foliares medidos para 150 géneros de Asteraceae. Los caracteres cualitativos de codificaron siguiendo a Ellis et al., (2009)

Caracteres	Unidades
Longitud estomática	Micrómetros(μm)
Densidad estomática	Células/ mm^2
Área foliar	Milímetros cuadrados (mm^2)
Masa seca foliar	Miligramos (mg)
Área foliar específica (AFE)	mm^2/ mg
Índice de esclerofilia (IE)	mg/ mm^2
Grosor de la cutícula	μm
Grosor de la hoja	μm
Tipo de unión a la hoja	Peciolada/sésil
Tamaño de la lámina	Nanofila, microfila, notofila, leptofila, macrofila, megafila
Tipo de lobulación de la hoja	Pinnatisecta, palmatisecta, sin lóbulos
Forma de las hojas	Elíptica, ovada, oblonga, ovobada, linear
Tipo de margen	Entero, aserrado, dentado
Tipo de areolación	Ausente, incompleta, imperfecta
Terminaciones de las vénulas	Ninguna, simple linear, simple curvada, ramificada una vez
Estrías en cutícula	Presencia/ ausencia
Posición de los estomas en la hoja	Anfiestomáticas/ hipoestomáticas
Vainas en los haces vasculares	Presencia/ ausencia
Extensiones de las vainas	Presencia/ ausencia
Tipo de extensiones de las vainas	Parenquimatosas / Esclerénquimatosas

Pruebas estadísticas no filogenéticas. Para evaluar cómo se correlacionan entre sí los caracteres foliares se obtuvieron matrices de correlación de Pearson y de Spearman usando la función *corrplot* del paquete "corrplot" (Wei y Simko 2017) en R (versión 3.4.4, R Core Team 2015). Se realizaron regresiones lineales para las variables cuantitativas y regresiones logísticas para evaluar la relación entre las mediciones y los caracteres discretos. También se hizo un Análisis de Componentes Principales (PCA) para explorar los patrones multivariados de variación en los caracteres foliares. Para evaluar la asociación entre los ejes principales de variación de los caracteres y el número cromosómico, la forma de crecimiento y la condición de malezas, se llevaron a cabo regresiones logísticas con la forma de crecimiento y la condición de maleza como variables predictoras de los valores z estandarizados de los primeros tres ejes del PCA. Todas las pruebas se efectuaron en R.

Señal filogenética. Se realizaron pruebas de señal filogenética para comprobar el supuesto de independencia filogenética, es decir si existe una dependencia estadística entre los valores de los caracteres de distintas especies debido a sus relaciones de parentesco. Se calcularon los índices *C* de Abouheif (Abouheif 1999), *λ* de Pagel (Pagel 1999) y *K* de Blomberg (Blomberg y Garland 2002; Blomberg et al., 2003) con la función *phyloSignal* del paquete "phylosignal" (Keck et al., 2016) en R. El índice *C* de Abouheif es un método basado en autocorrelación y no considera un modelo evolutivo particular. Se calcula a partir de la topología del árbol e ignora la longitud de las ramas. Para determinar la presencia de señal filogenética se compara el coeficiente C obtenido de los caracteres

observados con un valor crítico tabulado de C (Abouheif 1999; Münkenmüller et al., 2012). El índice λ de Pagel es una aproximación basada en un modelo evolutivo de movimiento browniano y ha probado ser muy útil para discriminar entre patrones de distribución de caracteres aleatorios y de movimiento browniano. Es ligeramente sensible a la incertidumbre en la longitud de ramas y es constante ante politomias y longitudes de ramas faltantes. Valores de λ cercanos a cero indican que las especies se parecen entre sí menos a lo predicho por el modelo evolutivo (no hay señal filogenética) y valores cercanos a uno indican que la estructura de la filogenia explica los cambios en los caracteres (hay señal filogenética). Por último, el índice K de Blomberg también es un método basado en un modelo evolutivo explícito. K es constante ante politomias y es muy sensible a la falta de información de longitudes de ramas. Si K es igual a uno, la evolución de los caracteres sigue un modelo evolutivo de movimiento browniano (hay señal filogenética), valores mayores a uno indican que los taxones son más similares de lo esperado (hay un mayor efecto de la filogenia) y valores menores a uno indican que las especies cercanas se parecen menos de lo predicho bajo el modelo evolutivo (no hay señal filogenética).

Reconstrucción de estados ancestrales. Antes de realizar la reconstrucción de estados ancestrales de los caracteres se comparó el ajuste de distintos modelos evolutivos para los caracteres estudiados usando el criterio de información de Akaike, corregido por el tamaño de la muestra (AICc, Sugihara 1978). Para los caracteres continuos se evaluaron los modelos de Movimiento browniano (MB), Ornstein-Uhlenbeck (OU, Hansen 1997; Butler y King 2004), “Explosión temprana” (Early Burst, EB) y un modelo no filogenético (“White noise”,WN). El modelo MB es útil para describir el cambio en los caracteres que resulta de la combinación de un

gran número de fuerzas débiles independientes. Bajo MB, después de un evento de especiación, las especies descendientes se desvían una de otra siguiendo una "caminata aleatoria", en la cual el valor del carácter cambia aleatoriamente tanto en dirección como en distancia. Este modelo predice que las especies cercanamente relacionadas deberían ser más similares entre sí y que los cambios después del evento de especiación no son direccionales (Harvey y Pagel 1991; Freckleton y Harvey 2006; Baum y Smith 2013). El modelo OU describe un proceso en el cual la deriva browniana es suplementada por atracción hacia uno o más "óptimos evolutivos". Esta atracción puede verse como una "liga" ya que la fuerza de atracción se hace más fuerte mientras más se aleja del atractor. OU es útil para modelar escenarios en los que el carácter está sujeto a selección estabilizadora alrededor de cierto valor (Butler y King 2004; Beaulieu et al., 2012; Harmon 2019). El modelo EB describe procesos en los cuales la tasa neta de evolución disminuye exponencialmente a través del tiempo (Blomberg et al., 2003; Ingram et al., 2012). Bajo el modelo de ruido blanco (WN) los caracteres de cada especie son modelados como una muestra independiente de una distribución normal (Cressler et al., 2015). Para los caracteres discretos se evaluaron los modelos de tasas iguales (ER) y de todas las tasas diferentes (ARD). En el primer caso se asume que todas las transiciones entre estados de carácter tienen la misma tasa *q*, sin importar su estado inicial ni final. En el modelo ARD para caracteres discretos se permiten diferentes transiciones con diferentes tasas dependiendo de los estados iniciales y finales. Los modelos se compararon utilizando el criterio de información de Akaike corregido (AICc). Las reconstrucciones de estados ancestrales se hicieron tomando en cuenta el mejor modelo evolutivo para cada carácter. Las reconstrucciones se

realizaron mediante métodos de verosimilitud y mapeo estocástico de caracteres. La reconstrucción de verosimilitud y el mapeo estocastico de caracteres se realizaron con las funciones *fastAnc* y *make.simmap* del paquete "phytools" (Revell 2012) en R. Las gráficas se generaron con la función "densityMap".

Relaciones entre caracteres tomando en cuenta la filogenia. Se calcularon "Mínimos cuadrados generalizados filogenéticos" (PGLS) para remover el efecto de las relaciones filogenéticas al estudiar las relaciones entre los caracteres. Además, se realizaron ANOVAs filogenéticas para comparar los valores entre grupos tomando en cuenta las relaciones entre las especies con la función *gls* del paquete "ape" (Paradis y Schliep 2018) en R.

Resultados

Correlaciones entre caracteres. Las correlaciones entre los caracteres estudiados se presentan en la figura 1. El área y la masa seca foliar presentan una correlación positiva y significativa. El número cromosómico se encontró correlacionado positivamente con la longitud de los estomas, el área foliar y la masa seca de la hoja y negativamente con la densidad estomática. Los resultados del PCA indican que los primeros cuatro ejes explican el 93% de la variación en los caracteres foliares medidos (Cuadro 2). El componente principal uno (CP1) se encuentra relacionado positivamente al número cromosómico y la longitud de los estomas y negativamente a la densidad estomática. El componente dos (CP2) está asociado positivamente al área foliar específica y al nivel de ploidía y negativamente con el índice de esclerofilia. El componente tres (CP3) se encontró relacionado negativamente con el área y la masa seca foliar y el componente cuatro (CP4) está

relacionado positivamente con el nivel de ploidía y la densidad estomática (Cuadro 2; Figura 2).

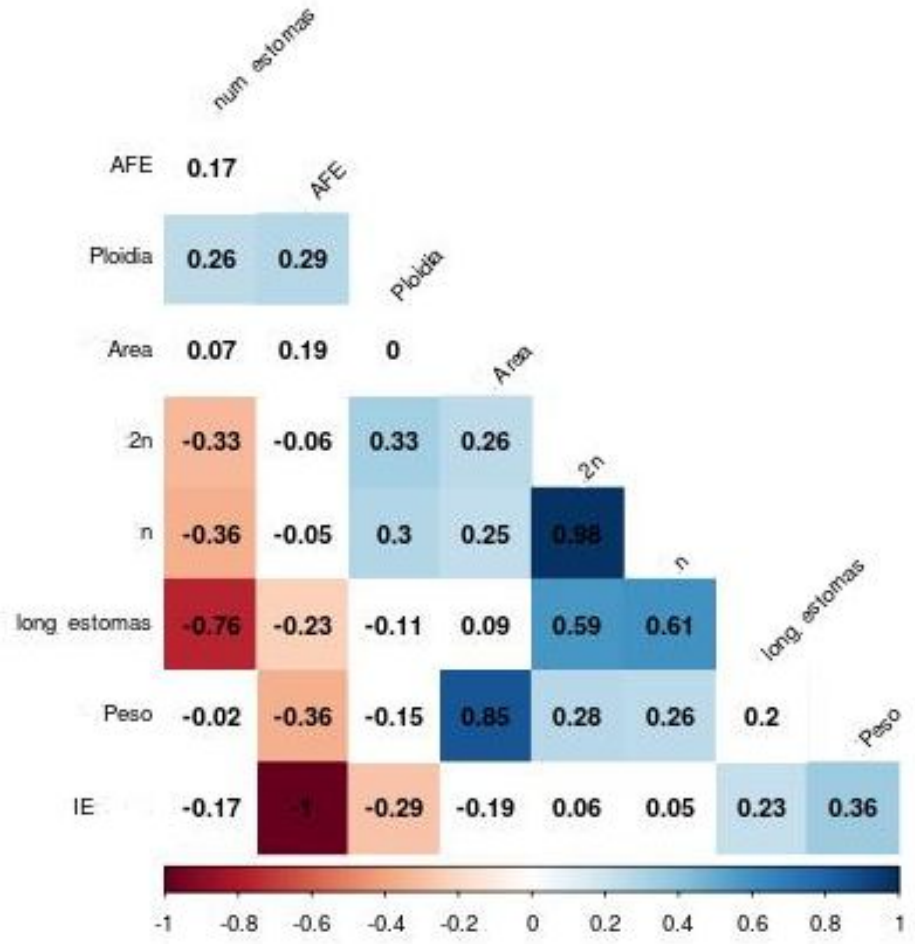

Figura 1. Correlaciones entre caracteres foliares. Correlaciones positivas se muestran en azul, correlaciones negativas en rojo. La intensidad de los colores es proporcional a los coeficientes de correlación. Celdas en color blanco indican correlaciones no significativas. Los coeficientes de correlación se muestran al interior de cada cuadro. AFE=Área foliar específica. IE= Índice de esclerofilia.

Cuadro 2. Eigenvectores de los cuatro primeros componentes principales de variación en los caracteres foliares calculados de las variables medidas para 147 especies de Asteraceae.

Caracteres	CP1	CP2	CP3	CP4
Variación explicada (%)	35.43	25.50	19.32	13.11
Eigenvalue	1.7857	1.5147	1.3185	1.0864
Número cromosómico	0.4543	0.3038	0.1116	0.2298
Nivel de ploidía	-0.0188	0.4085	0.0760	0.5927
Densidad estomática	-0.3413	0.0729	-0.3613	0.4692
Longitud estomática	0.4580	0.0132	0.2613	-0.2693
Área	0.1920	0.2370	-0.6265	-0.2324
Masa seca foliar	0.3162	-0.0629	-0.6156	-0.0492
AFE	-0.2510	0.5413	0.0373	-0.3225
IE	0.2510	-0.5413	-0.0373	0.3225

Las regresiones logísticas indicaron que el CP1 está asociado significativamente de forma positiva con la forma de crecimiento ($Z=3.61$, $p=0.003$, Figura 2A) y negativa con la condición de maleza ($Z=-3.984$, $p<0.0001$, Figura 2B). El componente CP2 está significativamente relacionado con la condición de maleza ($Z=3.232$, $p= 0.00123$).

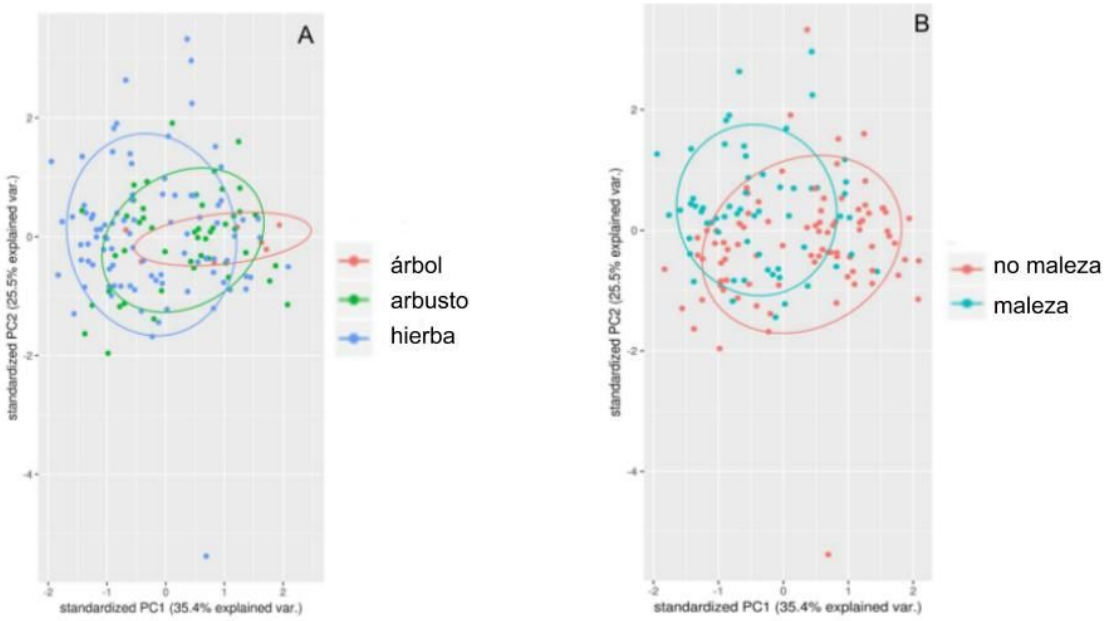

Figura 2. Representación bidimensional de los dos primeros componentes principales de la variación foliar en 147 especies de Asteraceae. A. Datos agrupados por forma de crecimiento. B. Datos agrupados por condición de malezas.

Señal filogenética. Los coeficientes de señal filogenética calculados para los datos obtenidos se presentan en el cuadro 3. Todos los coeficientes coinciden en que hay una señal filogenética estadísticamente significativa en los números cromosómicos, la longitud y densidad estomáticas y la masa seca foliar. Solo lambda detecta señal en el área foliar. En el cuadro 4 se presentan los resultados de la evaluación de los diferentes modelos evolutivos para los caracteres estudiados. Para longitud estomática, área y masa seca de la hoja el modelo con mejor ajuste de acuerdo con AICc fue el OU. Para los caracteres discretos el modelo con el mejor ajuste fue el ARD, excepto para la presencia de estrías en la cutícula (Cuadro 4). La reconstrucción de estados ancestrales de los caracteres cuantitativos muestran que la longitud de las células guarda tiende a disminuir en los taxones

recientemente divergentes (Figura 3). La masa seca (Figura 4A) y el área foliar (Figura 4B), a pesar de presentar señal filogenética, no se observa una tendencia particular en la reconstrucción de estados ancestrales. En general, las especies tienden hacia un valor medio tanto de área como de masa seca y los valores extremos se distribuyen a lo largo del árbol. Se observa que algunas tribus de la subfamilia Asteroideae como Anthemideae, Bahieae y Tageteae, tienen hojas más pequeñas y ligeras.

Cuadro 3. Índices de señal filogenética calculados para caracteres foliares continuos de 147 especies de Asteraceae.

Caracteres	C de Abouheif		K de Blomberg		λ de Pagel	
	C	*P*	K	*P*	λ	*P*
número cromosómico (n)	0.35	0.001	0.39	0.007	1.28	0.001
número cromosómico (2n)	0.32	0.001	0.37	0.006	1.16	0.001
Ploidía	-0.028	0.648	0.09	0.876	0	1
Densidad estomática	0.33	0.001	0.48	0.007	0.60	0.001
Longitud estomática	0.42	0.001	0.44	0.001	0.76	0.001
Área foliar	0.23	0.010	0.39	0.015	1.03	0.001
Masa seca foliar	0.27	0.001	0.65	0.001	0.85	0.001

AFE	0.22	0.002	0.35	0.023	0.42	0.012
IE	0.22	0.083	0.35	0.022	0.42	0.012

Cuadro 4. Valores de verosimilitud y AICc de los distintos modelos evolutivos evaluados para los caracteres estudiados. Para los caracteres continuos los modelos evaluados fueron: Movimiento Browniano (MB), Ornstein-Uhlenbeck (OU), Explosión temprana (EB) y White noise (WN). Para los caracteres discretos se evaluaron los modelos de tasas iguales (ER) y de tasas diferentes (ARD).

Caracteres	Modelos			
Continuos	MB	OU	EB	WN
Longitud estomática	lnL=96.51 AICc=-188.93	lnL=147.90 AICc=-289.63	lnL=96.50 AICc=-187.01	lnL=139.73 AICc=-275.38
Área foliar	lnL=-189.78 AICc=383.64	lnL=-139.84 AICc=285.84	lnL=-189.78 AICc=385.73	lnL=-149.32 AICc=302.72
Masa seca foliar	lnL=-196.99 AICc=398.065	lnL=-147.13 AICc=300.44	lnL=-196.99 AICc=400.15	lnL=-156.71 AICc=317.51
Discretos/ Categóricos	ARD	ER		
Número cromosómico	lnL=-505.35 AICc=1014.79	lnL=-447.55 AICc=897.14		
Forma de crecimiento	lnL=-211.22 AICc=605.37	lnL=-292.18 AICc=586.393		
Margen	lnL=-166.71 AICc=359.76	lnL=185.56 AICc=373.16		
Estrías	lnL=-63.08 AICc=130.262	lnL=-63.82 AICc=129.68		
Maleza	lnL=-95.201 AICc=194.48	lnL=-96.89 AICc=195.82		
Extensiones	lnL=-132.73	lnL=-145.26		

de la vaina	AICc=278.06	AICc=292.55		

Mientras que lo opuesto sucede en Senecioneae, Heliantheae y Wunderlichieae. Para los caracteres discretos el número cromosómico va de números altos entre 22 a 28 cromosomas a números bajos de entre 5 a 11 cromosomas en los taxones recientemente diversificados (Figura 5). La densidad estomática presenta la tendencia opuesta: disminuye hacia los taxones más recientemente diversificados. (Figura 6). La forma de crecimiento (Figura 7) leñosa es frecuente en los taxones tempranamente divergentes y mientras que en miembros de la subfamilia Asteroideae es más común el hábito herbáceo. El tipo de margen (Figura 8) es entero en los taxones tempranamente divergentes, y aserrado o dentado en algunos taxones tardíamente divergentes. En la subfamilia Asteroideae, se observan todos los tipos de margen, pero los márgenes enteros se encuentran con mayor frecuencia en hojas muy divididas y/o de tamaño pequeño. La condición de malezas (Figura 9) es poco frecuente en las especies tempranamente diversificadas y muy frecuente en miembros de la subfamilia Asteroideae. La presencia de extensiones en las vainas (Figura 10) es rara en los taxones tempranamente diversificados y cuando se presentan, suelen ser vainas de esclerénquima. La frecuencia de las vainas aumenta en los taxones de reciente diversificación y las vainas suelen estar conformadas por parénquima.

Análisis comparativos filogenéticos. La correlación encontrada entre masa seca y área foliar no es significativamente diferente de la encontrada con métodos estadísticos tradicionales. Las anovas filogenéticas arrojaron correlaciones significativas entre el número cromosómico y la longitud (r^2=0.54, p=2.34x10^{-15}) y

densidad estomáticas (r^2=0.41, p=7.49x10^{-10}) y la forma de crecimiento (r^2=0.08, p=0.0001).

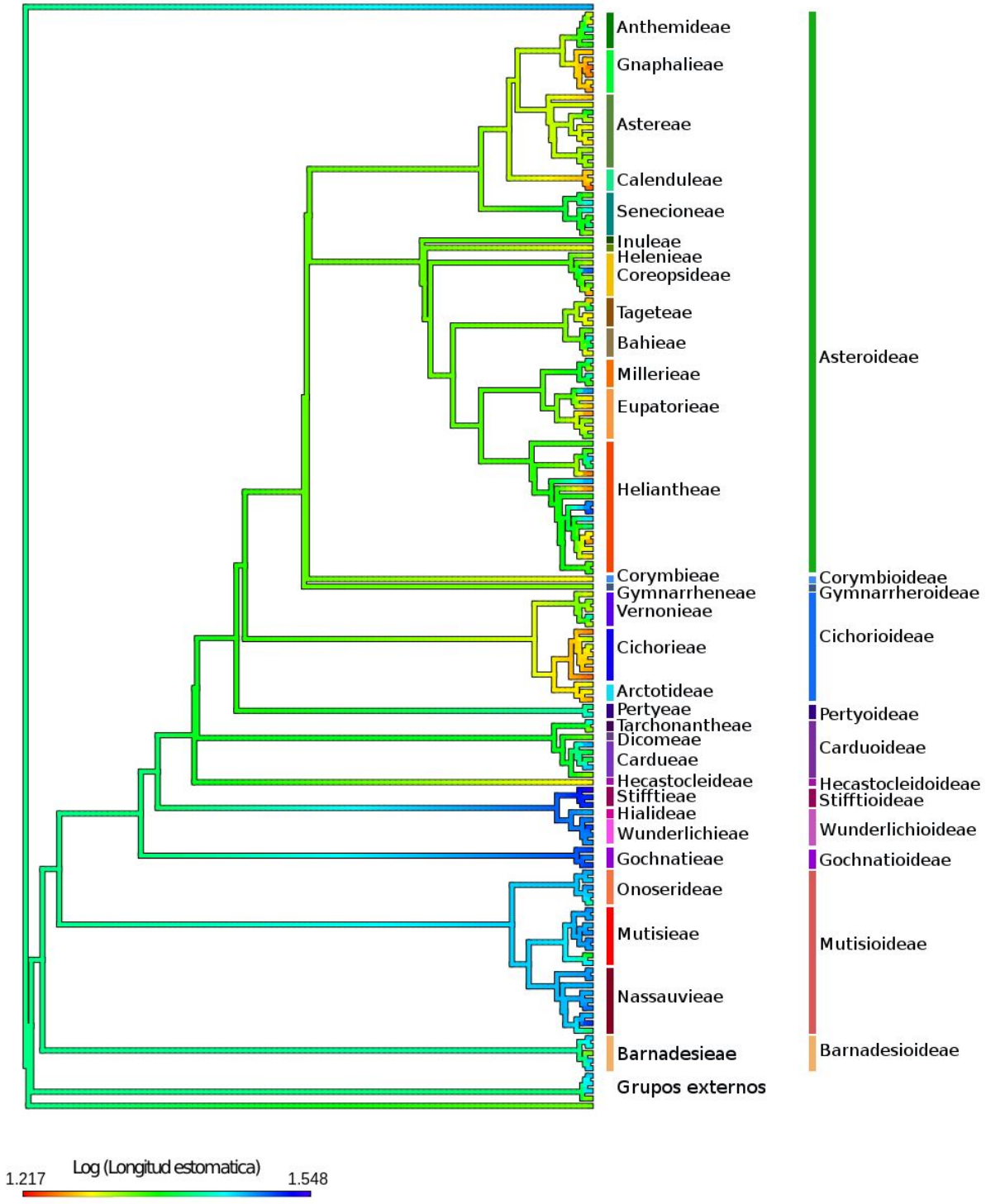

Figura 3. Reconstrucción de estados ancestrales mediante mapeo estocástico de caracteres de la longitud de células guarda para 147 especies de Asteraceae. Los nombres de las tribus se muestran junto a las terminales del árbol. Los nombres de las subfamilias se muestran en el extremo derecho.

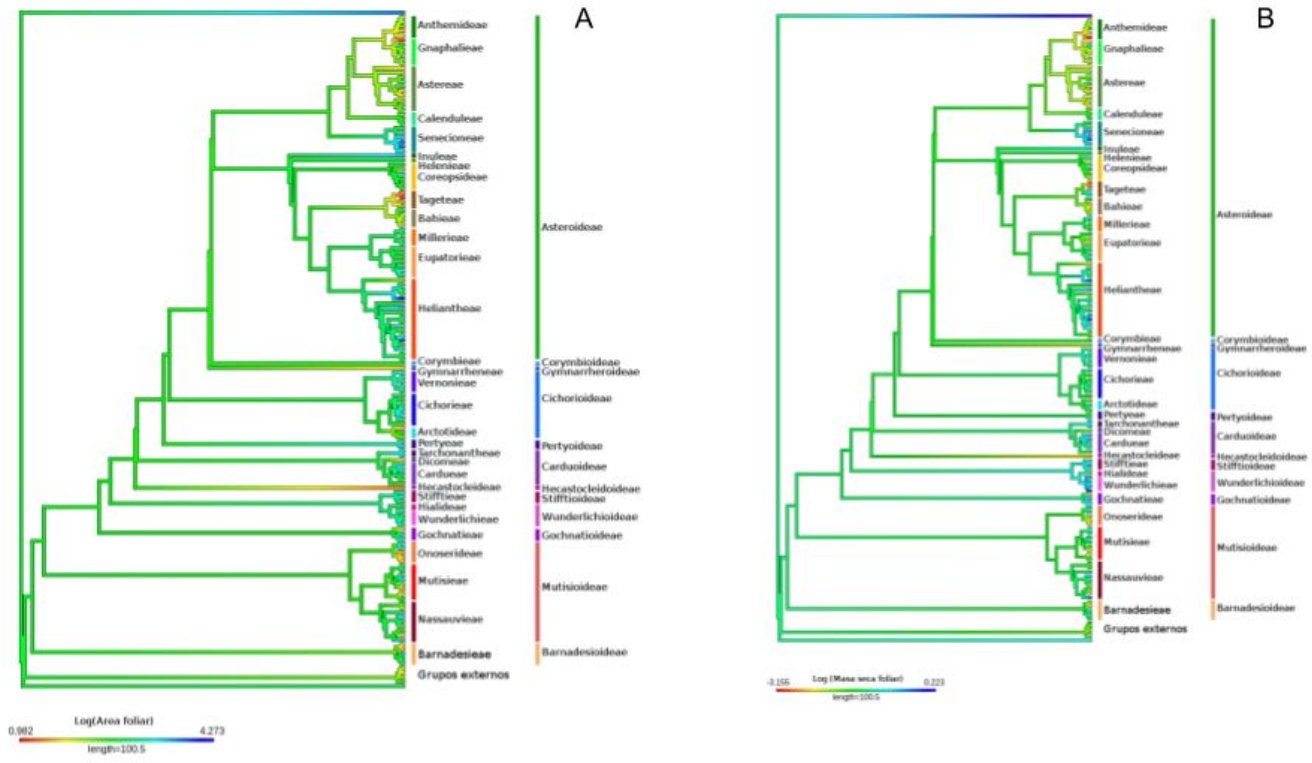

Figura 4. Reconstrucción de estados ancestrales mediante mapeo estocástico de caracteres para 147 especies de Asteraceae. A. Reconstrucción de la masa seca foliar. B. Reconstrucción de estados ancestrales del área foliar. Los nombres de las tribus se muestran junto a las terminales del árbol. Los nombres de las subfamilias se muestran en el extremo derecho.

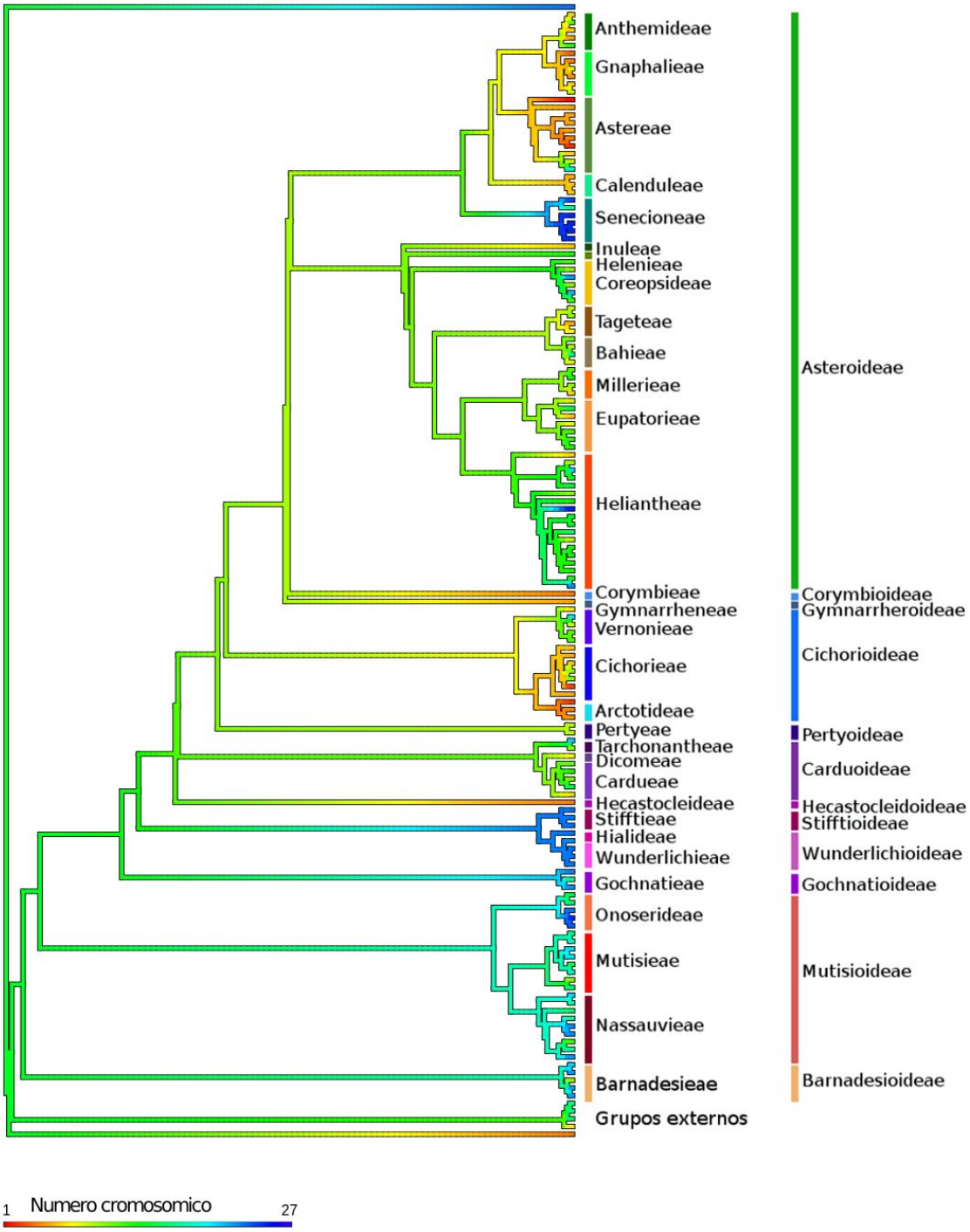

Figura 5. Reconstrucción de estados ancestrales mediante mapeo estocástico de caracteres del número cromosómico para 147 especies de Asteraceae. Los

nombres de las tribus se muestran junto a las terminales del árbol. Los nombres de las subfamilias se muestran en el extremo derecho.

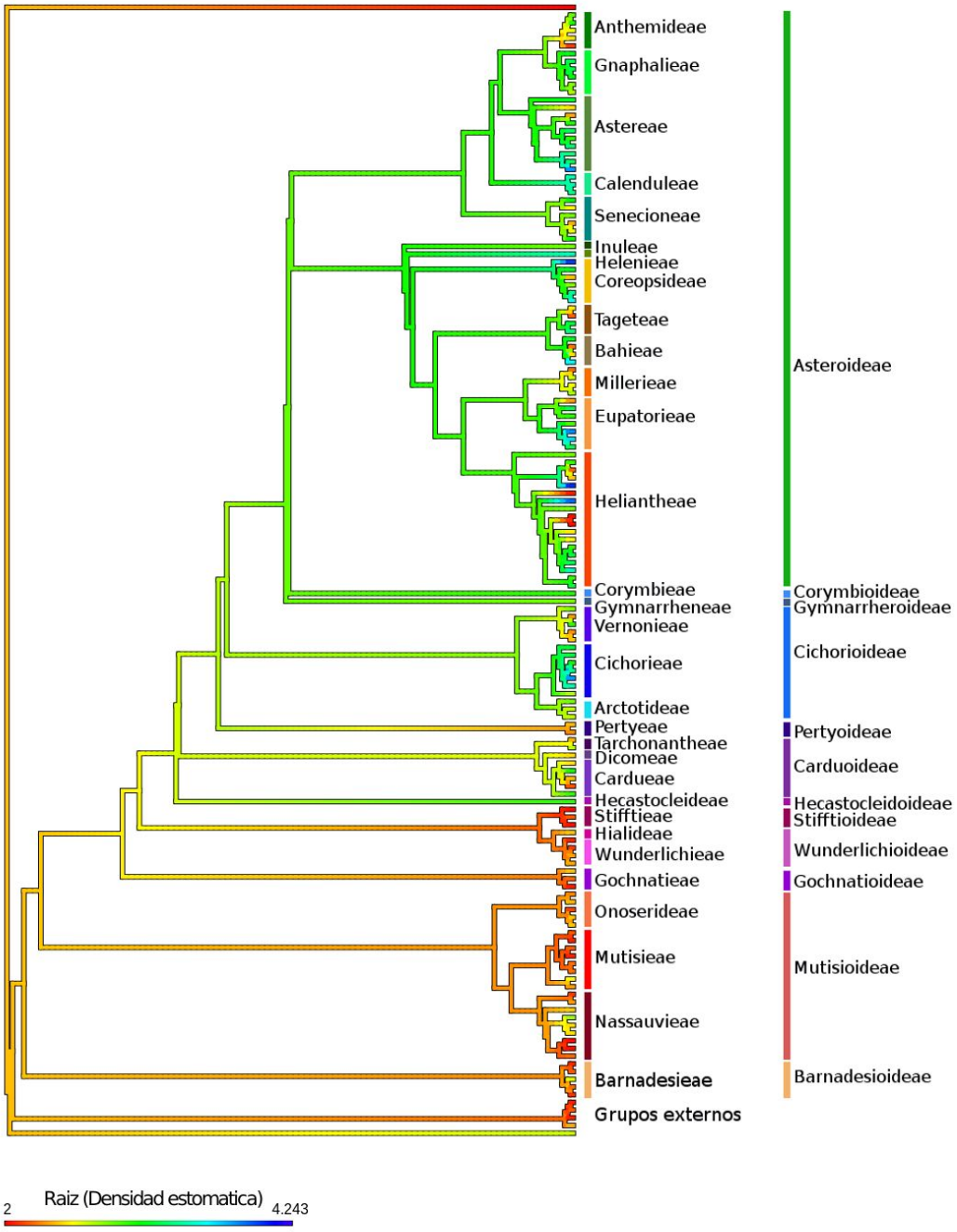

Figura 6. Reconstrucción de estados ancestrales mediante mapeo estocástico de caracteres de la densidad estomática. Los nombres de las tribus se muestran junto

a las terminales del árbol. Los nombres de las subfamilias se muestran en el extremo derecho.

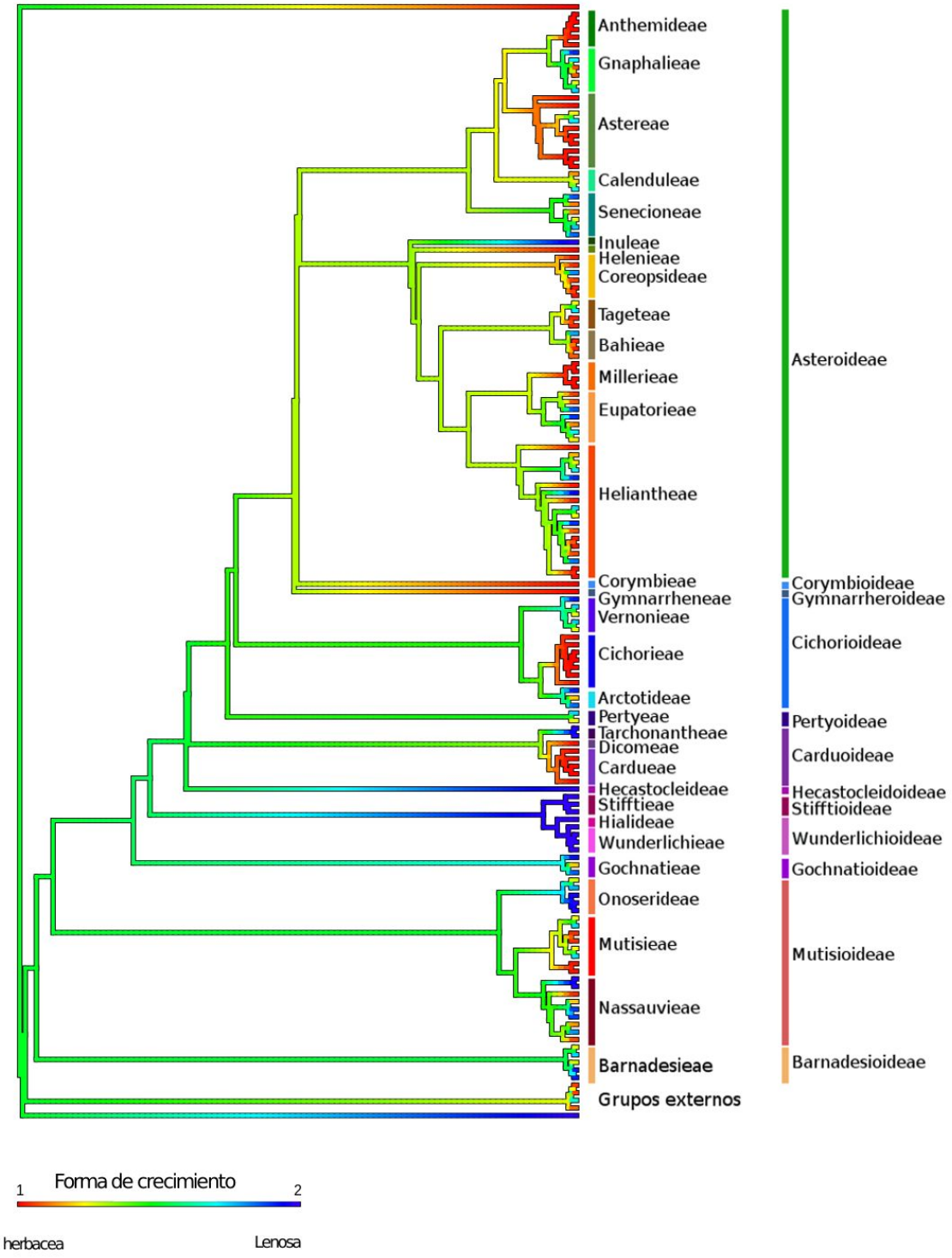

Figura 7. Reconstrucción de estados ancestrales mediante mapeo estocástico de caracteres de la forma de crecimiento.

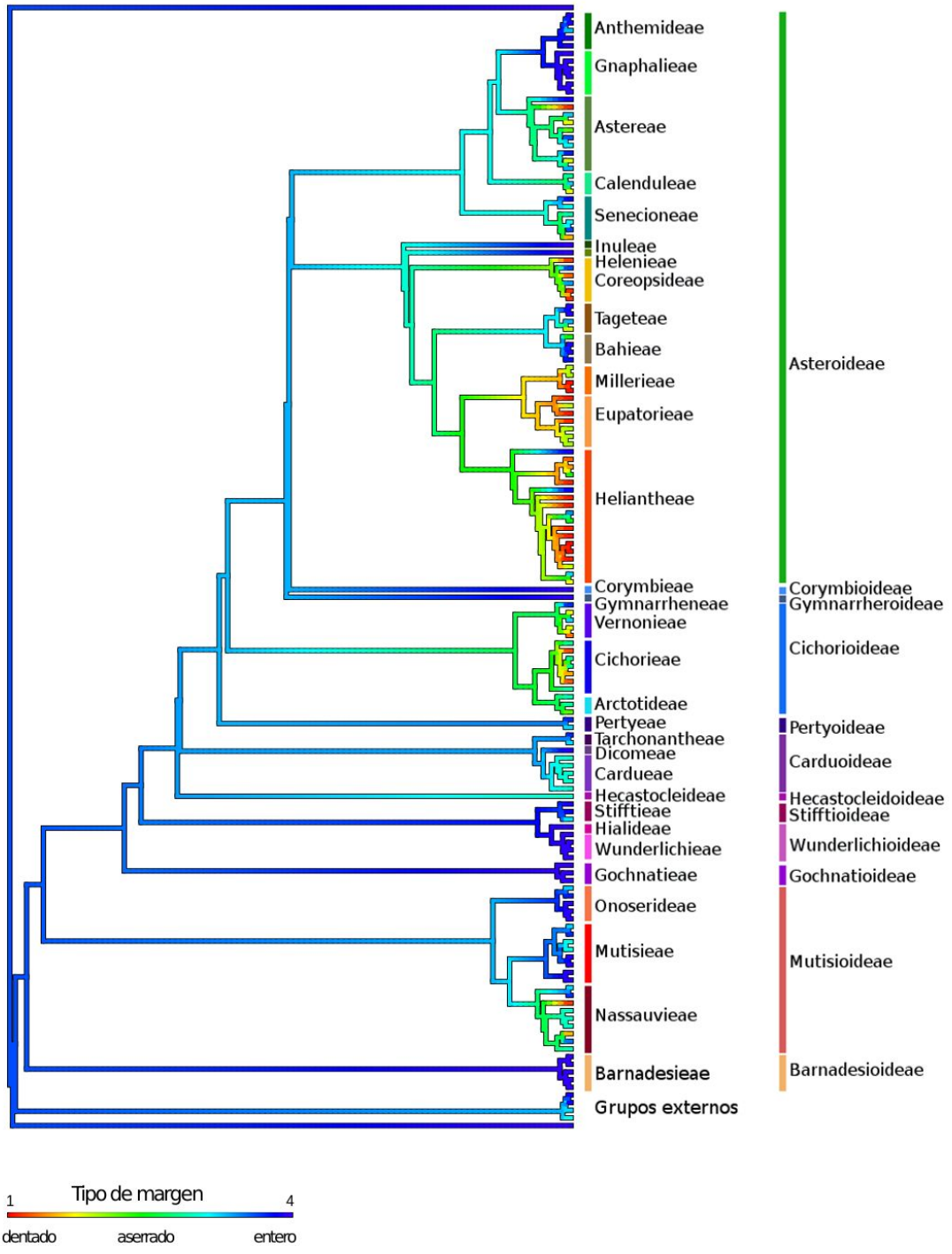

Figura 8. Reconstrucción de estados ancestrales mediante mapeo estocástico de caracteres del tipo de margen de la hoja.

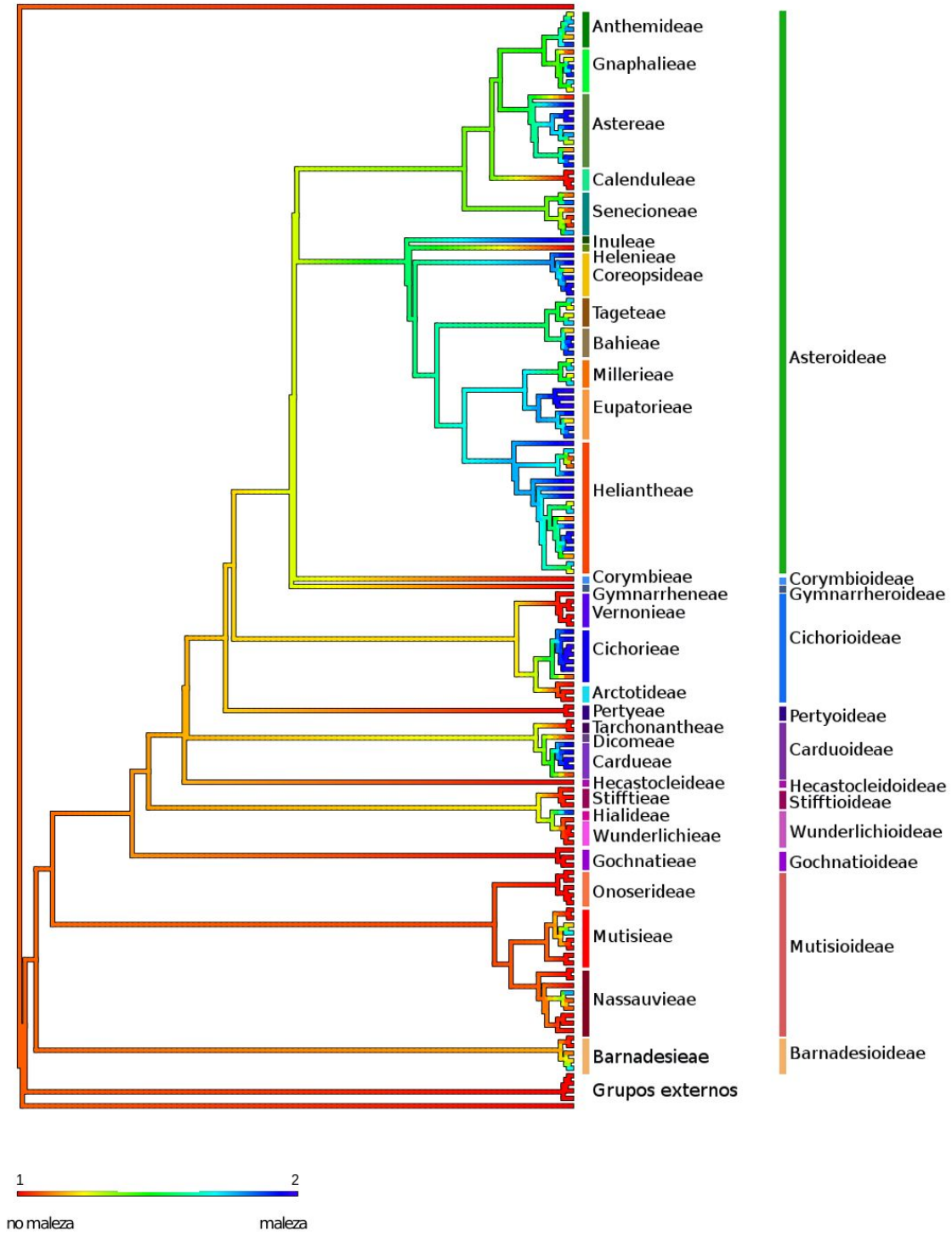

Figura 9. Reconstrucción de estados ancestrales mediante mapeo estocástico de caracteres de la condición de maleza para 147 especies de Asteraceae.

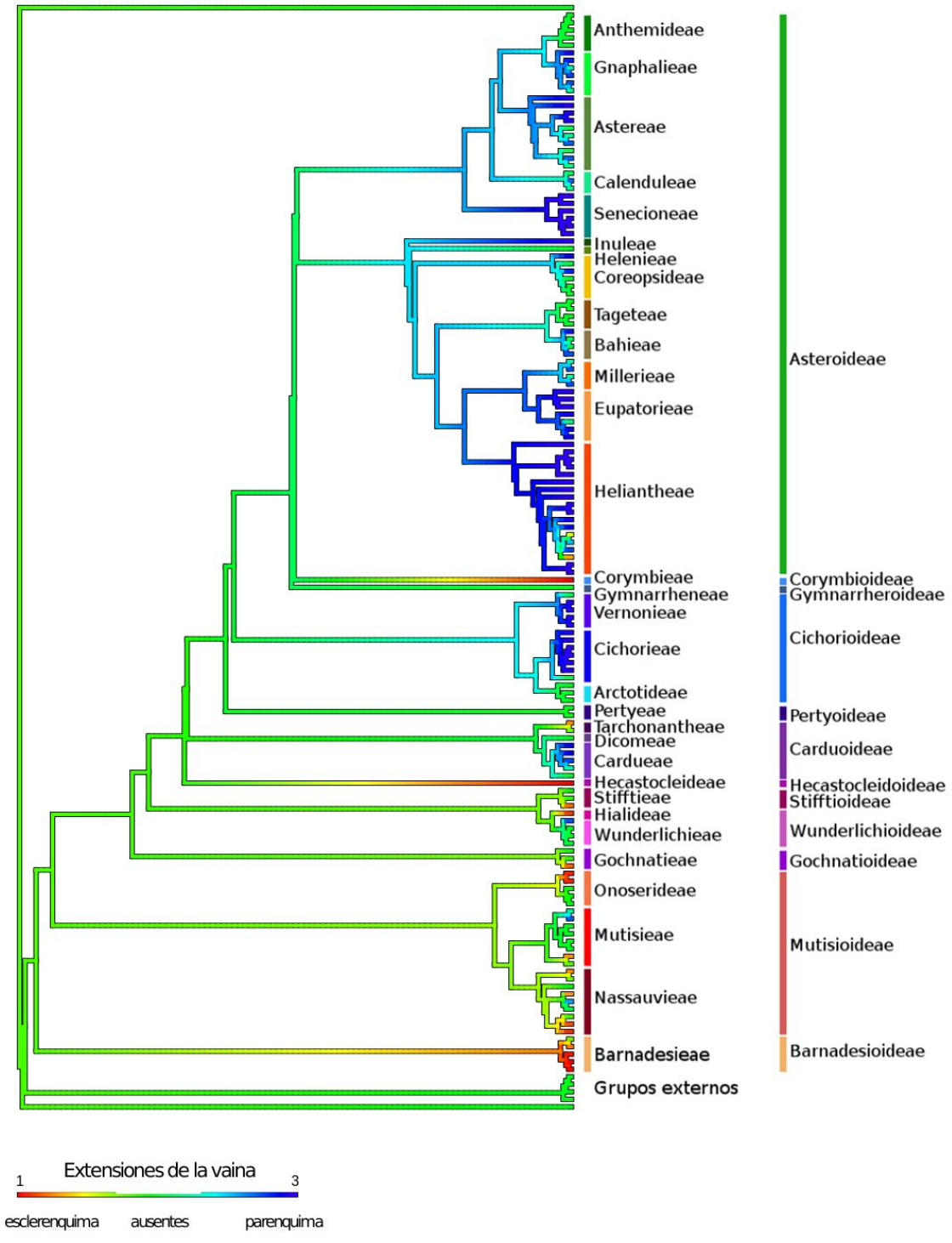

Figura 10. Reconstrucción de estados ancestrales mediante mapeo estocástico de caracteres del tipo de extensiones de la vaina de los haces vasculares para 147 especies de Asteraceae.

Discusión

Correlaciones entre caracteres foliares. Las correlaciones encontradas entre la longitud y la densidad estomática, y área foliar y masa seca coinciden con las reportadas previamente para una muestra de cincuenta y dos especies de Asteraceae de un matorral xerófilo en México (Rivera et al., 2017). Lo cual indica que a pesar de encontrarse en ambientes diversos, las proporciones de las hojas y las relaciones entre los tejidos se mantienen constantes tal y como reportan John et al., (2013) para catorce especies de angiospermas de diferentes ambientes y con distintas formas de crecimiento. Los análisis indican que la relación entre área y masa seca foliar es constante entre las distintas formas de crecimiento y entre los distintos linajes de la familia. Estos resultados apoyan observaciones previas que sugieren que las proporciones de la hoja son determinadas tempranamente en el desarrollo y probablemente están relacionadas a la eficiencia hidráulica de la hoja (Cookson et al., 2005). Las regresiones logísticas realizadas con los componentes principales indican que estas proporciones foliares podrían estar influidas por la forma de crecimiento y la condición de malezas. Aunque las categorías de ambos se encuentran sobrelapadas, las regresiones fueron estadísticamente significativas. Proponemos que la relación entre las formas geométricas de las hojas y sus propiedades mecánicas restringen la variación en las proporciones foliares (MacKinnon et al., 2014; Louf et al., 2018)

La correlación entre el aparato estomático y el sistema de conducción hidráulico de la hoja ha sido reportada en estudios previos y es importante para mantener el balance hídrico de la hoja (Buckley et al., 2011; Brodribb et al., 2013; Cardoso et al.,

2018). Por ello esperábamos encontrar correlaciones significativas entre el aparato estomático y el tipo de terminaciones de las vénulas y la presencia de extensiones de las vainas de los haces vasculares. Sin embargo, nuestros resultados no apoyaron esta hipótesis. Es posible que la caracterización cuantitativa del sistema hidráulico de la hoja (por ejemplo el cálculo de la densidad de vénulas) sea una mejor aproximación al estudio detallado de esta relación funcional que la tipificación que se realizó en este estudio. Por lo cual en el futuro, es necesario realizar mediciones detalladas de caracteres asociados a las venas para cada especie.

Correlaciones entre caracteres foliares y números cromosómicos. Los resultados de las correlaciones indican que hay relaciones estadísticamente significativas entre los números cromosómicos y el tamaño y número de estomas. Estos resultados coinciden con los reportados por otros autores para diversos grupos de plantas (Masterson 1994; Beaulieu et al., 2008; Marciniuk et al., 2010). Varios trabajos también han descrito diferencias significativas entre plantas con diferentes niveles de ploidía (Masterson 1994; Mishra 1997; Marciniuk et al., 2010); sin embargo en este trabajo no se encontraron correlaciones significativas entre el nivel de ploidía y el aparato estomático. Probablemente esta falta de correlación sea un efecto del nivel de estudio, ya que los trabajos anteriores se han enfocado en las diferencias entre especies del mismo género o dentro de series poliploides de la misma especie. Las correlaciones encontradas también indican relaciones significativas entre los números cromosómicos con el área y la masa seca foliar. Los resultados aquí presentados indican que las plantas con números cromosómicos más altos tienden a tener hojas de mayor tamaño. Esta correlación ha sido ampliamente

estudiada y reportada en la literatura para otras especies de plantas (Anssour et al., 2009; Balao et al., 2011; Beyaz et al., 2013; Zhang et al., 2019). Las correlaciones mencionadas son corroboradas en el análisis de componentes principales. El componente principal uno describe las proporciones entre el número cromosómico y el tamaño y número de células guarda. Mientras que el componente principal dos relaciona el número de copias del genoma con las proporciones de la hoja expresadas como cocientes entre el área y la masa seca foliar. El componente tres se relaciona con las proporciones generales de la hoja y el componente cuatro indica que hay una relación entre el número de copias del genoma y con el número de estomas de la hoja. En conjunto estos análisis indican que los números cromosómicos y el nivel de ploidía tienen efectos visibles en caracteres a nivel celular (en el tamaño y número de células guarda) y a nivel de órganos (en las proporciones de la hoja). Brodribb et al. (2013) postulan que la capacidad de adaptación a distintas condiciones ambientales de las hojas está dada por cambios coordinados en tamaños celulares de tejidos independientes y que los cambios en el tamaño del genoma pueden estar relacionados con el mantenimiento de estas relaciones funcionales al interior de la hoja.

Los números cromosómicos y los caracteres correlacionados a estos presentaron señal filogenética significativa. Esto quiere decir que las especies cercanamente emparentadas se parecen entre sí más que a cualquier otra especie muestreada al azar de la filogenia. La señal filogenética en el número cromosómico ha sido reportada previamente para la familia (Panero et al., 2014, Mota et al., 2016) y se ha interpretado en términos evolutivos. Los cambios en los números cromosómicos debidos a duplicaciones completas del genoma suelen asociarse con el éxito de la

familia para colonizar nuevos ambientes y con la aparición de “novedades evolutivas” como la inflorescencia de las compuestas y los frutos con fitomelaninas (Panero y Crozier 2016). Nuestros resultados indican que los caracteres foliares también son afectados por los cambios en el número de cromosomas, por lo cual sugerimos que otros caracteres vegetativos de la planta pueden estar siendo afectados por las duplicaciones del genoma. Las relaciones encontradas mediante métodos comparados filogenéticos indican que el número cromosómico está correlacionado con el número y longitud de las células guarda y que esta relación no es independiente de la filogenia de las especies.

La poliploidía como un atributo de especies “invasivas” o “malezoides” se ha discutido previamente en diversos trabajos (Pandit et al., 2011; te Beest et al., 2012). Nuestros resultados muestran que existe una tendencia de las malezas a presentar mayores niveles de ploidía, mayor área foliar específica y número de estomas por unidad de área foliar que las plantas que no prefieren sitios perturbados (Figura 2B). Las proporciones foliares modificadas por los cambios en el nivel de ploidía, podrían conferir una mayor resistencia a los ambientes perturbados a las malezas.

El ajuste de modelos arrojó que la longitud estomática, el área y masa seca de la hoja siguen un modelo evolutivo con tendencia hacia un valor óptimo del carácter. En términos de funcionalidad de la hoja, este modelo es el más probable, ya que se ha comprobado que existe una tendencia a minimizar la pérdida de agua a través de los estomas (Sack et al., 2003) y un balance entre la superficie foliar disponible para realizar la fotosíntesis y el costo de mantenimiento de los tejidos de la hoja (Wright et al., 2004). Las reconstrucciones de estados ancestrales del

número cromosómico y la forma de crecimiento coinciden con las de trabajos previos para la familia (Panero et al., 2014; Mota et al., 2016). El ancestro de las Asteráceas era probablemente una planta leñosa con número cromosómico básico (n) entre 8 y 9. Hay una tendencia a cambiar de leñosas a herbáceas en los taxones más recientemente diversificados y hacia números cromosómicos más altos (Figuras 5, 7). Soltis et al. (2013) encontraron tendencias similares en plantas del orden Saxifragales, en el cual han ocurrido al menos dos grandes cambios de leñosas a herbáceas, seguidos de múltiples reversiones en diversos linajes. Las reconstrucciones de los caracteres foliares son una aportación de este trabajo y destacan algunas tendencias en la evolución de las hojas en la familia. En las reconstrucciones del área foliar y la masa seca, que presentaron señal filogenética (Cuadro 3), no se observan tendencias marcadas en las subfamilias en particular hacia un valor del carácter. Esto puede deberse a que los valores de estos caracteres están sujetos a una selección estabilizadora como la que simula el modelo OU, en la cual los valores tienden hacia un óptimo. Un caso similar puede estar ocurriendo con el número cromosómico, que presenta una gran variación en la subfamilia Asteroideae. Los cambios en el número cromosómico y la estabilización del tamaño del genoma después de eventos de duplicación del genoma son frecuentes (Otto y Whitton 2000; Barker et al., 2016) y pueden explicar la amplitud de variación observada en esta subfamilia. Los resultados obtenidos de este trabajo además indican ciertas tendencias evolutivas en las plantas de la familia Asteraceae: Las tribus tempranamente divergentes de la familia son plantas leñosas. Raramente son malezas. Tienen números cromosómicos altos y hojas con márgenes enteros, longitudes estomáticas grandes que se encuentran

significativamente correlacionadas con densidades estomáticas bajas. Presentan extensiones de la vaina de esclerénquima o no presentan extensiones. Por su parte las tribus que divergieron más recientemente suelen ser hierbas. Frecuentemente se encuentran en sitios perturbados. Tienen números cromosómicos bajos y hojas con márgenes aserrados u hojas con márgenes enteros, pero las láminas se encuentran muy divididas. Las longitudes estomáticas son pequeñas y se encuentran relacionadas con densidades estomáticas altas. Presentan extensiones de la vaina de parénquima. Particularmente la subfamilia Asteroideae destaca por la proporción de especies que son malezas. La señal filogenética encontrada en la condición de maleza y su aparente correlación con varios atributos foliares, como la presencia y tipo de extensiones de las vainas y el tipo de margen de las hojas puede estar indicando que la habilidad para invadir y permanecer en ambientes perturbados está determinada por factores relacionados con la eficiencia fotosintética e hídrica y la presencia de tejidos de resistencia en las hojas.

Conclusiones

Los resultados de los análisis realizados indican que hay un efecto de los números cromosómicos sobre caracteres foliares que incluyen el tamaño y número de estomas y las proporciones foliares. Las reconstrucciones de estados ancestrales y los resultados de mínimos cuadrados generalizados filogenéticos indican que la variación en los caracteres foliares no es independiente de la historia evolutiva de las plantas, y que caracteres como el tipo de margen de la hoja y la presencia de extensiones de la vaina, que suelen relacionarse con variaciones ambientales, presentan señal filogenética. Por ello el estudio de las hojas de esta familia debe

tomar en cuenta las relaciones entre las especies. No se encontraron características exclusivas de las distintas formas de crecimiento, pero los análisis de componentes principales indican que hay una mayor amplitud de variación en las plantas herbáceas que en las leñosas. La propensión a habitar sitios perturbados (condición de malezas) también presentó señal filogenética y parece estar asociado a una forma de crecimiento herbácea.

Literatura citada

Abdala-Roberts L., Galmán A., Petry W.K., Covelo F., de la Fuente M., Glauser G., Moreira X. 2018. Interspecific variation in leaf functional and defensive traits in oak species and its underlying climatic drivers. PLOS ONE 13(8): e0202548.

Abouheif E. 1999. A method for testing the assumption of phylogenetic independence in comparative data. Evol. Ecol. Res. 1: 895-909.

Ackerly D.D, Knight C., Weiss S., Barton K., Starmer P. 2002. Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses. Oecologia 130: 449-457.

Anssour S., Krügel T., Sharbel T.F., Saluz H.P., Bonaventure G., Baldwin I.T. 2009. Phenotypic, genetic and genomic consequences of natural and synthetic polyploidization of *Nicotiana attenuata* and *Nicotiana obtusifolia*. Ann. Bot. 103: 1207-1217.

Arnold M.L., Martin N. 2010. Hybrid fitness across time and habitats. Trends Ecol. Evol. 25: 530-536.

Aury J.M., Jaillon O., Duret L., Noel B., Jubin C., Porcel B.M., Ségurens B., Daubin V., Anthouard V, Aiach N., Arnaiz O., Billaut A., Beisson J., Blanc I., Bouhouche K.,

Câmara F., Duharcourt S., Guigo R., Gogendeau D., Katinka M., Keller A.M., Kissmehl R., Klotz C., Koll F., Le Mouël A., Lepère G., Malinsky S., Nowacki M., Nowak J.K., Plattner H., Poulain J., Ruiz F., Serrano V., Zagulski M., Dessen P., Bétermier M., Weissenbach J., Scarpelli C., Schächter V., Sperling L., Meyer E., Cohen J., Wincker P. 2006. Global trends of whole-genome duplications revealed by the ciliate *Paramecium tetraurelia*. Nature 444: 171-178.

Balao F., Herrera J., Talavera S. 2011. Phenotypic consequences of polyploidy and genome size at the microevolutionary scale: a multivariate morphological approach. New Phytol. 192: 256-265.

Baker R.L., Yarkhunova Y, Vidal K., Ewers B.E., Weinig C. 2017. Polyploidy and the relationship between leaf structure and function: implications for correlated evolution of anatomy, morphology, and physiology in *Brassica*. BMC Plant Biol. 17: 3.

Barker M.S., Kane N.C., Matvienko M., Kozik A., Michelmore R.W., Knapp S.J., Rieseberg L.H. 2008. Multiple paleopolyploidizations during the evolution of the compositae reveal parallel patterns of duplicate gene retention after millions of years. Mol. Biol. Evol. 25: 2445-2455.

Barker M.S., Li Z., Kidder T.I., Reardon C.R., Lai Z., Oliveira L.O., Scascitelli M., Rieseberg L.H. 2016. Most Compositae (Asteraceae) are descendants of a paleohexaploid and all share a paleotetraploid ancestor with the Calyceraceae. Am. J. Bot. 103: 1203-1211.

Baum D., Smith S. 2013. Tree thinking: an introduction to phylogenetic biology. W.H.Freeman & Co Ltd. Greenwood Village, USA. 496 pp.

Beaulieu J.M., Leitch I.J., Patel S., Pendharkar A., Knight C.A. 2008. Genome size is a strong predictor of cell size and stomatal density in angiosperms. New Phytol. 179: 975-986.

Beaulieu J.M., Jhwueng D.C., Boettiger C., O'Meara B.C. 2012. Modeling stabilizing selection: expanding the Ornstein-Uhlenbeck model of adaptive evolution. Evolution 66: 2369-2383.

Beyaz R., Alizadeh B., Gürel S., Özcan S.F., Yildiz M. 2013. Sugar beet (*Beta vulgaris* L.) growth at different ploidy levels. Caryologia 66: 90-95.

Blomberg S.P., Garland T. 2002. Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. J. Evol. Biol. 15: 899-910.

Blomberg S.P., Garland T., Ives A.R. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution 57: 717-745.

Bretagnolle F., Lumaret R. 1995. Bilateral polyploidization in *Dactylis glomerata* L. subsp. *lusitanica*: occurrence, morphological and genetic characteristics of first polyploids. Euphytica 84: 197–207.

Brodribb T.J., Jordan G.J., Carpenter R.J. 2013. Unified changes in cell size permit coordinated leaf evolution. New Phytol. 199: 559-570.

Buckley T.N., Sack L., Gilbert M.E., 2011. The role of bundle sheath extensions and life form in stomatal responses to leaf water status. Plant Physiol. 156: 962-973.

Butler M.A., King A.A. 2004. Phylogenetic Comparative Analysis: A Modeling Approach for Adaptive Evolution. Amer. Naturalist 164: 683-695.

Cardoso A.A., Randall J.M., Jordan G.J., McAdam S.A.M. 2018. Extended differentiation of veins and stomata is essential for the expansion of large leaves in *Rheum rhabarbarum*. Am. J. Bot. 105:1967-1974.

Cavalier-Smith T. 1978. Nuclear volume control by nucleoskeletal dna, selection for cell volume and cell growth rate, and the solution of the DNA c-value paradox. J. Cell Sci. 34: 247-278.

Chapin F., Stuart I.I., Rincón E., Huante P. 1993. Environmental responses of plants and ecosystems as predictors of the impact of global change. J. Biosci. 18: 515- 524.

Coate J.E., Luciano A.K., Seralathan V., Minchew K.J., Owens T.G., Doyle J.J. 2012. Anatomical, biochemical, and photosynthetic responses to recent allopolyploidy in *Glycine dolichocarpa* (Fabaceae). Am. J. Bot. 99:55-67.

Cookson S.J., Van Lijsebettens M., Granier C. 2005. Correlation between leaf growth variables suggest intrinsic and early controls of leaf size in *Arabidopsis thaliana*. Plant. Cell Environ. 28: 1355-1366.

Cressler C.E., Butler M.A., King A.A. 2015. Detecting Adaptive Evolution in Phylogenetic Comparative Analysis Using the Ornstein-Uhlenbeck model. Syst. Biol. 64: 953-968.

Dehal P., Boore J.L. 2005. Two Rounds of Whole Genome Duplication in the Ancestral Vertebrate. PLOS Biol. 10: e314.

Díaz S., Hodgson J.G., Thompson K., Cabido M., Cornelissen J.H.C., Jalili A., Montserrat-Martí G., Grime J.P., Zarrinkamar F., Asri Y., Band S.R., Basconcelo S., Castro-Díez P., Funes G., Hamzehee B., Khoshnevi M., Pérez-Harguindeguy N., Pérez-Rontomé M.C., Shirvany F.A., Vendramini F., Yazdani S., Abbas-Azimi R., Bogaard A., Boustani S., Charles M., Dehghan M., de Torres-Espuny L., Falczuk V.,

Guerrero-Campo J., Hynd A., Jones G., Kowsary E., Kazemi-Saeed F., Maestro-Martínez M., Romo-Díez A., Shaw S., Siavash B., Villar-Salvador P., Zak M.R. 2004. The plant traits that drive ecosystems: evidence from three continents. J. Veg. Sci. 15: 295-304.

Ellis B., Daly D.C., Hickey L.J., Mitchell J.D., Johnson K.R., Wilf P., Wing S.L. 2009. Manual of Leaf Architecture. Cornell University Press. New York, USA. 190 pp.

Freckleton R.P., Harvey P.H. 2006. Detecting Non-Brownian Trait Evolution in Adaptive Radiations. PLoS Biol. 4: e373.

Glade-Vargas N., Hinojosa L.F., Leppe M. 2018. Evolution of Climatic Related Leaf Traits in the Family Nothofagaceae. Front. Plant. Sci. 9:1073.

Hansen T.F. 1997. Stabilizing selection and the comparative analysis of adaptation. Evolution 51:1341-1351.

Harvey P.H., Pagel M. 1991. The Comparative Method in Evolutionary Biology. Oxford University Press, Nueva York. 248 pp.

Harmon L.J. 2019. Phylogenetic Comparative Methods. Creative Commons Attribution 4.0 International License.

Hodgson J.G., Sharafi M., Jalili A., Díaz S., Montserrat-Martí G., Palmer C., Cerabolini B., Pierce S., Hamzehee B., Asri Y., Jamzad Z., Wilson P., Raven J.A., Band S.R., Basconcelo S., Bogard A., Carter G., Charles M., Castro-Díez P., Cornelissen J.H.C., Funes G., Jones G., Khoshnevis M., Pérez-Harguindeguy N., Pérez-Rontomé M.C., Shirvany F.C., Vendramini F., Yazdani S., Abbas-Azimi R., Boustani S., Dehghan M., Guerrero-Campo J., Hynd A., Kowsary E., Kazemi-Saeed F., Siavash B., Villar-Salvador P., Craigie R., Naqinezhad A., Romo-Díez A., de

Torres Espuny L., Simmons E. 2010. Stomatal vs. genome size in angiosperms: the somatic tail wagging the genomic dog?. Ann. Bot. 105: 573-584.

Holbrook N.M., Putz F.E. 1996. From epiphyte to tree: differences in leaf structure and leaf water relations associated with the transition in growth form in eight species of hemiepiphytes. Plant Cell Environ. 19: 631-642.

Hull-Sanders H.M., Johnson R.H., Owen H.A., Meyer G.A. 2009. Effects of polyploidy on secondary chemistry, physiology, and performance of native and invasive genotypes of *Solidago gigantea* (Asteraceae). Am. J. Bot. 96: 762-770.

Huang C.H., Zhang C., Liu M., Hu Y., Gao T., Qi J., Ma H. 2016. Multiple polyploidization events across Asteraceae with two nested events in the early history revealed by nuclear phylogenomics. Mol. Biol. Evol. 33: 2820-2835.

John G.P., Scoffoni C., Sack L. 2013. Allometry of cells and tissues within leaves. Am. J. Bot. 100: 1936-1948.

Ingram T., Harmon L.J., Shurin J.B. 2012. When should we expect early bursts of trait evolution in comparative data? Predictions from an evolutionary food web model. J. Evol. Biol. 25:1902-1910.

Keck F., Rimet F., Bouchez A., Franc A. 2016. Phylosignal: an R package to measure, test, and explore the phylogenetic signal. Ecol. Evol. 6: 2774-2780.

Lambers H., Poorter H. 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. Adv. Ecol. Res. 23: 187-261.

Levin D.A. 2002. The role of chromosomal change in plant evolution. Oxford University Press, Nueva York. 461 pp.

Li W.L., Berlyn G.P., Ashton P.M.S. 1996. Polyploids and their structural and physiological characteristics relative to water deficit in *Betula papyrifera* (Betulaceae). Am. J. Bot. 83: 15-20.

Louf J.F., Nelson L., Kang H., Song P.N., Zehnbauer T., Jung S. 2018. How wind drives the correlation between leaf shape and mechanical properties. Scientific Reports 8: 16314.

Lukács B.A., Vojtkó A.E., Mesterházy A., Molnár V.A., Süveges K., Végvári Z., Brusa G., Cerabolini B.E.L. 2017. Growth form and spatiality driving the functional difference of native and alien aquatic plants in Europe. Ecol. Evol. 7: 950-963.

Mackinnon E.D., Pratt R.B., Jacobsen A.L. 2014. Functional trait differences between weedy and non-weedy plants in southern California. Madroño 61: 328-338.

Maherali H., Walden A.E., Husband B.C. 2009. Genome duplication and the evolution of physiological responses to water stress. New Phytol. 184: 721-731.

Marciniuk J., Rerak J., Grabowska-Joachimiak A., Jastrząb I., Musiał K., Joachimiak A. 2010. Chromosome numbers and stomatal cell length in *Taraxacum* Sect. *Palustria* from Poland. Acta Biol. Cracov. Bot. 52: 117-121.

Mason C.M., Goolsby E.W., Humphreys D.P., Donovan L.A. 2015. Phylogenetic structural equation modelling reveals no need for an 'origin' of the leaf economics spectrum. Ecol. Lett. 19: 54-61.

Masterson J. 1994. Stomatal size in fossil plants: Evidence for polyploidy in majority of angiosperms. Science 264:421-423.

Mota L., Torices R., Loureiro J. 2016. The Evolution of Haploid Chromosome Numbers in the Sunflower family. Genome Biol. Evol. 8: 3516-3528.

Münkemüller T., Lavergne S., Bzeznik B., Dray S., Jombart T., Schiffers K., Thuiller W. 2012. How to measure and test phylogenetic signal. Met. Ecol. Evol. 3:743-756.

Otto S.P. 2007. The Evolutionary Consequences of Polyploidy. Cell 131:452-462.

Otto S.P., Whitton J. 2000. Polyploid incidence and evolution. Annu. Rev. Genet. 34: 401-437.

Pagel M. 1999. Inferring the historical patterns of biological evolution. Nature 401: 877-884.

Pandit M.K., Pocock M.J., Kunin W.E. 2011. Ploidy influences rarity and invasiveness in plants. J. Ecol. 99: 1108-1115.

Panero J.L., Crozier B.S. 2016. Macroevolutionary dynamics in the early diversification of Asteraceae. Mol. Phylogenet. Evol. 99: 116-132.

Panero J.L., Freire, S.E., Ariza, E.L., Crozier, B.S., Barboza, G.E., Cantero, J.J. 2014. Resolution of deep nodes yields an improved backbone phylogeny and a new basal lineage to study early evolution of Asteraceae. Mol. Phylogenet. Evol. 80: 43-53.

Paradis E., Schliep K. 2018. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35: 526-528.

Parkhurst D., Loucks O. 1972. Optimal leaf size in relation to environment. J. Ecol. 60: 505-537.

Peñuelas J., Matamala R. 1990. Changes in N and S Leaf Content, stomatal density and specific leaf area of 14 plant species during the last three centuries of CO_2 increase. J. Exp. Bot. 41: 1119-1124.

Poorter H., Niinemets Ü., Poorter L., Wright I.J., Villar R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol. 182: 565-588.

R Development Core Team. 2015. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Revell L.J. 2012. phytools: An R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3: 217-223.

Rice A., Glick L., Abadi S., Einhorn M., Kopelman N.M., Salman-Minkov A., Mayzel J., Chay O., Mayrose I. 2015. The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. New Phytol. 206: 19-26.

Rivera P., Villaseñor J.L., Terrazas T. 2017. Meso- or xeromorphic? Foliar characters of Asteraceae in a xeric scrub of Mexico. Bot. Stud. 58:12.

Rivera P., Terrazas T., Rojas-Leal A., Villaseñor J.L. 2019. Leaf architecture and anatomy of Asteraceae species in a xerophytic scrub in Mexico City, México. Acta Bot. Mex. 126: e1515.

Rossatto D.R., Kolb R.M., Franco A.C. 2015. Leaf anatomy is associated with the type of growth form in Neotropical savanna plants. Botany 93: 507-518.

Sack L., Cowan P.D., Jaikumar N., Holbrook N.M. 2003. The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. Plant Cell Environ. 26: 1343-1356.

Santiago L.S., Wrigth S.J. 2007. Leaf functional traits of tropical forest plants in relation to growth form. Funct. Ecol. 21: 19-27.

Sax K, Sax HJ.1937. Stomata size and distribution in diploid and polyploid plants. J. Arnold Arbor. 18: 164-172.

Schmerler S.B., Clement W.L., Beaulieu J.M., Chatelet D.S., Sack L., Donoghue M.J., Edwards E.J. 2012. Evolution of leaf form correlates with tropical-temperate transitions in *Viburnum* (Adoxaceae). Proc. Biol. Sci. 279: 3905-3913.

Soltis P.S. 2005. Ancient and recent polyploidy in angiosperms. New Phytol. 166: 5-8.

Soltis D.E., Visger C.J., Soltis P.S. 2014. The polyploidy revolution then… and now: Stebbins revisited. Am. J. Bot. 101:1-22.

Soto-Trejo F., Palomino G., Villaseñor J.L. 2011. Números cromosómicos de Asteraceae de la Reserva Ecológica del Pedregal de San Angel (REPSA), México, D.F. Rev. Mex. Biodiv. 82: 19-29.

Soto-Trejo F., Palomino G., Villaseñor J.L., Crawford D.J. 2013. Polyploidy in Asteraceae of the xerophytic scrub of the Ecological Reserve of the Pedregal of San Angel, Mexico City. Bot. J. Linn. Soc. 173: 211-229.

Stebbins G.L. 1940. The significance of polyploidy in plant evolution. Am. Nat. 74: 54-66.

Stebbins G.L. 1950. Variation and Evolution in Plants. Columbia University Press. New York, USA. 352 pp.

Stebbins G.L.1971. Chromosome Evolution in Higher Plants. Edward Arnold, London, UK. 220 pp.

Sugihara N. 1978. Further analysis of the data by Akaike's information criterion and the finite corrections. Comm. Stat. Theory Methods A7: 13-26.

Sugiyama S. 2005. Polyploidy and cellular mechanisms changing leaf size: comparison of diploid and autotetraploid populations in two species of *Lolium*. Ann. Bot. 96: 931-938.

Te Beest M., Le Roux J.J., Richardson D.M., Brysting A.K., Suda J., Kubesová M., Pysek P. 2011. The more the better? The role of polyploidy in facilitating plant invasions. Ann. Bot. 109: 19-45.

Vile D. Garnier E., Shipley B., Laurent G., Navas M.L., Roumet C., Lavorel S., Díaz S. M., Hodgson J.G., Lloret F., Midgley G. F., Poorter H., Rutherford M.C.,Wilson P.J., Wright I.J. 2005. Specific leaf area and dry matter content estimate thickness in laminar leaves. Ann. Bot. 96: 1129-1136.

Wang Y.P., Lu X.J., Wright I.J., Dai Y.J., Rayner P.J., Reich P.B. 2012. Correlations among leaf traits provide a significant constraint on the estimate of global gross primary production. Geophys. Res. Lett. 39: L19405.

Warner D., Ku M.S.B., Edwards G. 1987. Photosynthesis, leaf anatomy, and cellular constituents in the polyploid C_4 grass *Panicum virgatum*. Plant Physiol. 84: 461-466.

Wei T., Simko V. 2017. R package "corrplot": Visualization of a Correlation Matrix (Version 0.84). Disponible en https://github.com/taiyun/corrplot

Weiss R., Kukora J., Adams J. 1975. The Relationship between Enzyme Activity, Cell Geometry, and Fitness in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA. 72: 794-798.

Westoby M. 1998. A leaf-height-seed (LHS) plant ecology strategy scheme. Plant Soil 199: 213- 227.

Westoby M., Falster D.S., Moles A.T., Vesk P.A., Wright I.J. 2002. Plant ecological strategies: some leading dimensions of variation between species. Annu. Rev. Ecol. Evol. System. 33: 125-159.

Wood T.E., Takebayashi N., Barker M.S., Mayrose I., Greenspoon P.B., Rieseberg L.H. 2009. The frequency of polyploidy speciation in plants. Proc. Natl Acad. Sci. USA 106: 13875-13879.

Wright I.J., Reich P.B., Westoby M., Ackerly D.D., Baruch Z., Bongers F., Cavender-Bares J., Chapin T., Cornelissen J.H.C., Diemer M., Flexas J., Garnier E., Groom P.K., Gulias J., Hikosaka K., Lamont B.B., Lee T., Lee W., Lusk C., Midgley J.J., Navas M.L., Niinemets U., Oleksyn J., Osada N., Poorter H., Poot P., Prior L., Pyankov V.I., Roumet C., Thomas S.C., Tjoelker M.G., Veneklaas E.J., Villar R. 2004 The worldwide leaf economics spectrum. Nature 428: 821-827.

Wright I.J., Reich P.B, Cornelissen J.H.C, Falster D.S., Groom P.K, Hikosaka K., Lee W., Lusk C.H., Niinemets U., Oleksyn J., Osada N., Poorter H., Warton D.I., Westoby M.. 2005. Modulation of Leaf Economic Traits and Trait Relationships by Climate. Global Ecol. Biogeogr. 14: 411-421

Wood T.E., Takebayashi N., Barker M.S., Mayrose I., Greenspoon P.B., Rieseberg L.H. 2009. The frequency of polyploid speciation in vascular plants. Proc. Natl. Acad. Sci. USA. 106: 13875-13879.

Woodward I., Kelly C.K. 1995. The influence of CO_2 concentration on stomatal density. New Phytol. 131: 311-327.

Zhang Y., Wang B., Qi S., Dong M., Wang Z., Li Y., Chen S., Li B., Zhang J. 2019. Ploidy and hybridity effects on leaf size, cell size and related genes expression in triploids, diploids and their parents in *Populus*. Planta 249: 635-646.

Zimmermann M.H. 1983. Xylem structure and the ascent of sap. Springer, Nueva York.

Discusión general

El primer paso del estudio comparativo de los caracteres foliares implica la caracterización y comparación de los caracteres entre distintos grupos de plantas (Anderson y Creech 1975). El valor de las descripciones y bases de datos se extiende a varios niveles en estudios sistemáticos (De Faria et al., 2012; Lin y Tan 2015; Rojas-Leal et al., 2017; Lusa et al., 2018), ecológicos (Bercu et al., 2012; Rivera et al., 2017; Ferraro y Scremin-Dias 2018) y fisiológicos (Bondarev et al., 2003; Santiago y Kim 2009). En los estudios evolutivos este paso es crítico, ya que permite la identificación de homologías que serán la base para el estudio de la evolución de los organismos. La diversidad foliar encontrada en este estudio para la familia Asteraceae coincide con la reportada previamente para la familia (Milán et al., 2006; Adedeji y Jewoola 2008) y es, hasta ahora, el trabajo de anatomía foliar más exhaustivo para la familia.

Paralelamente, la disponibilidad de una hipótesis filogenética robusta es necesaria para cualquier estudio evolutivo. En este trabajo, obtuvimos una filogenia de Asteraceae en la que se incluyeron linajes que diversificaron en México. Las aproximaciones modernas a la resolución de las relaciones al interior de la familia utilizan con más frecuencia métodos de secuenciación de nueva generación (Mandel et al., 2015, 2017; Huang et al., 2016) que proveen cientos de marcadores . En este trabajo, a pesar de utilizar un número limitado de marcadores del cloroplasto, se obtuvieron filogenias resueltas y bien soportadas para la mayoría de

los nodos, que son congruentes con las propuestas más recientes para la familia. La inclusión de los taxones mexicanos es importante para el entendimiento de la historia evolutiva de esta familia tan diversa, y el uso de métodos de reconstrucción de historias filogenéticas reticuladas permitirá incorporar a las hipótesis procesos importantes en la historia de la familia como la hibridación.

La integración de la parte descriptiva de este trabajo con la información filogenética nos hizo posible postular hipótesis sobre la evolución de los caracteres foliares este grupo de plantas. Estos análisis hicieron posible el reconocimiento de patrones evolutivos importantes en caracteres que suelen considerarse adaptativos. La evaluación de diferentes modelos evolutivos permite ajustar el cambio observado en los caracteres a lo largo del árbol a distintas hipótesis biológicas. Esta visión evolutiva de la hoja es cada vez más frecuente en la literatura (Ackerly 2004; Mason y Donovan 2015; Glade-Vargas et al., 2018). Las relaciones entre caracteres citogenéticos y foliares en el contexto filogenético se describen por primera vez para la familia en este trabajo. Los resultados de los análisis comparativos indican que la variación foliar está asociada a la variación en los números cromosómicos y que estas relaciones son dependientes de la historia de la familia.

Conclusión general

El presente trabajo muestra distintas fases del trabajo comparativo. La descripción, la reconstrucción filogenética y la integración de ambos en análisis comparativos filogenéticos. La base de datos comparativos generada en este trabajo permitirá realizar análisis evolutivos y ecológicos más exhaustivos de la familia Asteraceae y particularmente de las especies distribuidas en México. Las filogenias generadas

permitirán analizar con más detalle la diversificación de los clados de Asteraceae nativos de México. Los análisis comparativos indican que es importante tomar en cuenta las relaciones filogenéticas entre las especies de Asteraceae al estudiar los caracteres foliares. Probablemente la gran diversidad foliar de la familia refleje distintos compromisos entre caracteres citogenéticos y foliares en respuesta a variaciones ambientales, y dichos compromisos suelen ser similares en especies cercanamente emparentadas.

Literatura general

Ackerly D. 2004. Adaptation, niche conservatism, and convergence: comparative studies of leaf evolution in the California chaparral. Amer. Nat. 163: 654-671.

Adedeji O., Jewoola O.A. 2008. Importance of leaf epidermal characters in the Asteraceae family. Not. Bot. Horti. Agrobot. Cluj-Na. 36: 7-16.

Anderson L.C., Creech J.B. 1975. Comparative leaf anatomy of *Solidago* and related Asteraceae. Am. J. Bot. 62: 486-493.

Balao F., Herrera J., Talavera S. 2011. Phenotypic consequences of polyploidy and genome size at the microevolutionary scale: a multivariate morphological approach. New Phytol. 192: 256-65.

Belnap J. 2012. Unexpected uptake. Nat. Geosci. 5: 443-444.

Bercu R.M., Fãgãra L.B., Broască L. 2012. Anatomical features of *Aster tripolium* L. (Asteraceae) to saline environments. Ann. Rom. Soc. Cell Biol. 17: 271-277.

Bondarev N.I., Sukhanova M.A., Reshetnyak O.V., Nosov A.M. 2003. Steviol glycoside content in different organs of *Stevia rebaudiana* and its dynamics during ontogeny. Biol. Plantarum 47: 261-264.

Brodribb T.J., Feild T.S. 2010. Leaf hydraulic evolution led a surge in leaf photosynthetic capacity during early angiosperm diversification. Ecol. Lett. 13: 175-183.

De Faria A. P. G., Vieira A. C. M., Wendt T. 2012. Leaf anatomy and its contribution to the systematics of *Aechmea* subgenus *Macrochordion* (de Vriese) Baker (Bromeliaceae). An. Acad. Bras. Ciênc. 84: 961-971.

Díaz S., Cabido M., Casanoves F. 1998. Plant functional traits and environmental filters at a regional scale. J. Veg. Sci. 9: 113-122.

Elbert W., Weber B., Burrows S., Steinkamp J., Budel B., Andreae M.O., Poschl U. 2012. Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. Nature Geosci. 5: 459-462.

Ferraro A., Scremin-Días E. 2018. Structural features of species of Asteraceae that arouse discussions about adaptation to seasonally dry environments of the Neotropics. Acta Bot. Bras. 32: 113-127.

Glade-Vargas N., Hinojosa L.F., Leppe M. 2018. Evolution of Climatic Related Leaf Traits in the Family Nothofagaceae. Front. Plant Sci. 9: 1073.

Harrison C.J., Morris J.L. 2017. The origin and early evolution of vascular plant shoots and leaves. Phil. Trans. R. Soc. B. 373: 20160496.

Herrera CM. 1992. Historical effects and sorting processes as explanations for contemporary ecological patterns: character syndromes in Mediterranean woody plants. Am. Nat. 140: 421-46.

Huang C.H., Zhang C., Liu M., Hu Y., Gao T., Qi J., Ma, H. 2016. Multiple polyploidization events across Asteraceae with two nested events in the early history revealed by nuclear phylogenomics. Mol. Biol. Evol. 33: 2820-2835.

John G.P., Scoffoni C., Sack L. 2013. Allometry of cells and tissues within leaves. Am. J. Bot. 100: 1936-1948.

Lin C.Y. Tan D.Y. 2015. The taxonomic significance of leaf epidermal micromorphological characters in distinguishing 43 species of *Allium* L. (Amaryllidaceae) from central Asia. Pak. J. Bot. 47: 1979-1988.

Lusa M.G., Loeuille B.F.P., Ciccarelli D., Apezzato-da-Glória B. 2018. Evolution of stem and leaf structural diversity: a case study in *Lychnophorinae* (Asteraceae). Bot. Rev. 84: 203-241.

Mandel J.R., Dikow R.B., Funk V.A. 2015. Using phylogenomics to resolve mega-families: An example from Compositae. J.Syst. Evol. 53: 391-402.

Mandel J.R., Barker M.S., Bayer R.J., Dikow R.B., Gao T., Jones K. E., Keeley S., Kilian N., Ma H., Siniscalchi C.M., Susanna A., Thapa R., Watson L., Funk V.A. 2017. The Compositae Tree of Life in the age of phylogenomics. J Syt. Evol. 55: 405-410.

Mason C.M., Donovan L.A. 2015. Evolution of the leaf economics spectrum in herbs: Evidence from environmental divergences in leaf physiology across *Helianthus* (Asteraceae). Evolution 69: 2705-2720.

Milán P., Hissae-Hayashi A., Appezzato-da-Glória B. 2006. Comparative leaf morphology and anatomy of three Asteraceae species. Braz. Arch. Biol. Technol. 49: 135-144.

Niklas K.J. 2016. Plant evolution: An introduction to the history of life. The University of Chicago Press. Chicago. 576 pp.

Osborn T.C., Pires J.C., Birchler J.A., Auger D.L., Chen Z.J., Lee H.S., Comai L., Madlung A., Doerge R.W., Colot V., Martienssen R.A. 2003. Understanding mechanisms of novel gene expression in polyploids. Trends Genet. 19: 141-147.

Panero J.L, Crozier B.S. 2016. Macroevolutionary dynamics in the early diversification of Asteraceae. Mol. Phylogenet. Evol. 99: 116-132.

Panero J.L., Funk V.A. 2008. The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed. Mol. Phylogenet. Evol. 47: 757-782.

Panero J.L., Freire S.E., Espinar L.A., Crozier B.S., Barbosa G.E., Cantero J.J. 2014. Resolution of deep nodes yields an improved backbone phylogeny and a new basal lineage to study early evolution of Asteraceae. Mol. Phylogenet. Evol. 80: 43-53.

Press M. 1999. The functional significance of leaf structure: A search for generalizations. New Phytol. 143: 213-219.

Rivera P., Villaseñor J.L., Terrazas T. 2017. Meso- or xeromorphic? Foliar characters of Asteraceae in a xeric scrub of Mexico. Bot. Stud. 58: 12.

Rojas-Leal A., Villaseñor J.L., Terrazas T. 2017. Tricomas foliares en *Senecio* sección *Mulgediifolii* (Senecioneae, Asteraceae). Acta Bot. Mex. 119: 69-78.

Santiago L.S., Kim S.C. 2009. Correlated evolution of leaf shape and physiology in the woody *Sonchus* alliance (Asteraceae: Sonchinae) in Macaronesia. Int. J. Plant Sci. 170: 83-92.

Santiago L.S., Wright S.J. 2007. Leaf functional traits of tropical forest plants in relation to growth form. Funct. Ecol. 21: 19-27.

Villaseñor J.L. 2003. Diversidad y distribución de las Magnoliophyta de México. Interciencia 28: 160-167.

Villaseñor J.L. 2004. Los géneros de plantas vasculares de la flora de México. Bol. Soc. Bot. Méx. 75: 105-135.

Westoby M., Falster D.S, Moles A.T., Vesk P.A., Wright I.J. 2002. Plant ecological strategies: some leading dimensions of variation between species. Annu. Rev. Ecol. Syst. 33: 125-159.

Woodward F.I., Smith T.M., Emanuel W.R. 1995. A global primary productivity and phytogeography model. Glob. Biogeochem. Cycles 9: 471-490

Wright I.J., Reich P.B., Westoby M., Ackerly D.D., Baruch Z., Bongers F., Cavender-Bares J., Chapin T., Cornelissen J.H.C., Diemer M., Flexas J., Garnier E., Groom P.K., Gulias J., Hikosaka K., Lamont B.B., Lee T., Lee W., Lusk C., Midgley J.J., Navas M-L., Niinemets U., Oleksyn J, Osada N., Poorter H., Poot P., Prior L., Pyankov V.I., Roumet C., Thomas S.C., Tjoelker M.G., Veneklaas E.J., Villar R. 2004. The worldwide leaf economics spectrum. Nature 428: 821-827.