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FILOGENIA Y DIVERSIFICACIÓN DE *FLORESTINA* (ASTERACEAE) EN REGIONES ÁRIDAS Y SEMIÁRIDAS DE MÉXICO

TESIS

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PRESENTA:

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

A T E N T A M E N T E
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INDICE

RESUMEN

*Florestin*a es un género de hierbas anuales que habitan principalmente en regiones áridas y semiáridas de México. Las especies son morfológicamente similares, lo que ha dificultado el establecimiento d e sus relaciones filogenéticas. En es te t rabajo, s e es tudiaron l as relaciones filogenéticas de *Florestina* con base e n caracteres morfológicos, s ecuencias d e ADN nuclear (ITS y ETS) y de cloroplasto (*rpl32-trnL* y *trnC-petN*). La filogenia obtenida a p artir d e los espaciadores ITS y ETS fue a ltamente r esuelta y permitió establecer l as siguientes conclusiones: 1) el género *Florestina* es un grupo monofilético y *Palafoxia* es el género más cer canamente r elacionado; 2) *F. latifolia* y *F. platyphylla* forman un c lado fuertemente apoyado; 3) cuatro taxones morfológicamente muy similares, *F. liebmannii*, *F. pedata*, *F. simplicifolia*, y *F. tripteris*, están muy cer canamente relacionados y l os datos moleculares sugieren que sólo dos especies deben ser reconocidas: una que comprende a *F. pedata* y *F. simplicifolia*, la cual muestra amplia variación mo rfológica a través de su área de distribución; y la otra que comprende a *F. liebmannii* y *F. tripteris*; y 4) *F. lobata* y *F. purpurea* son especies claramente distintas del resto. Además, los análisis filogenéticos de ADN de cloroplasto sugieren que procesos evolutivos como la hibridación e introgresión pueden es tar i nvolucrados en l a h istoria de *Florestina*. T ambién s e r ealizó u n a nálisis biogeográfico, el cual fue conducido a p artir de una filogenia datada de *Florestina* usando el pa quete B ioGeoBEARS. L os r esultados sugieren que : a) *Florestina* se originó probablemente a p artir d e u n a ncestro ampliamente di stribuido desde e l s ur de E stados Unidos hasta e l sur de M éxico; b) l a d ivergencia entre *Florestina* y *Palafoxia* fue probablemente influenciada po r e l desarrollo d el E je Neovolcánico Transversal; c) e l modelo biogeográfico más p robable mostró u n número r elativamente bajo de e ventos d e dispersión e ntre t odas la s áreas; y d) la d iversificación de *Florestina* fue probablemente resultado de los cambios climáticos ocurridos durante el Pleistoceno.

ABSTRACT

Florestina consists of annual herbs occurring mostly in arid and semiarid regions of Mexico. All of the species are morphologically similar and consequently phylogenetic relationships within the genus are poorly understood. We studied phylogenetic relationships based on morphological characters, DNA sequences of nuclear non-coding spacers (ETS and ITS), and chloroplast non-coding spacers (*rpl32-trnL* and *trnC-petN*). The ETS and ITS spacer-based phylogenies allowed several well-supported conclusions: 1) the genus *Florestina* is monophyletic and *Palafoxia* is its closest relative; 2) *Florestina latifolia* and *F. platyphylla* form a strongly supported clade; 3) four taxa that are morphologically very similar, *F. liebmannii*, *F. pedata*, *F. simplicifolia*, and *F. tripteris*, are phylogenetically closely related and the molecular data suggest that only two species should be recognized: one comprising *F. pedata* and *F. simplicifolia*, which shows wide morphological variation throughout its distributional range; and the other comprising *F. liebmannii* and *F. tripteris*; 4) *F. lobata* and *F. purpurea* are species very distinct from the remainder of the species in the genus. Furthermore, our phylogenetic analyses suggest that hybridization and introgression may be involved in the evolutionary history of *Florestina*. We also conducted a biogeographic analyses using a dated DNA phylogeny of *Florestina* estimated from nuclear ribosomal ITS and ETS regions and the software BioGeoBEARS. Our findings suggest that *Florestina* originated from a widespread ancestor that probably ranged from southern United States to southern Mexico. The divergence of *Florestina* and *Palafoxia* was probably due to the development of the Transvolcanic Belt. The most probable biogeographic model showed a relatively low number of dispersal events between all areas, suggesting that species evolved from one ancestral widespread taxon during Pleistocene, leading to the isolation of populations resulting in the diversification of *Florestina*.

INTRODUCCIÓN GENERAL

Florestina (Asteraceae: Bahieae) es un género pequeño de hierbas anuales que ocurren principalmente en regiones áridas y semiáridas de México. Las especies se caracterizan por presentar corolas zigomorfas y apéndices cuspidados en las ramas de estilo. El primer tratamiento taxonómico de *Florestina* fue realizado por Rydberg (1914), quien reconoció seis especies: *F. latifolia* (DC.) Rydb., *F. liebmannii* Sch.Bip. ex Greenm., *F. pedata* (Cav.) Cass., *F. platyphylla* B.L.Rob. & Greenm., *F. purpurea* (Brandegee) Rydb., y *F. tripteris* DC. Posteriormente, Turner (1963) realizó una revisión del género basado en datos morfológicos y citológicos, describiendo dos especies más: *Florestina simplicifolia* B.L.Turner y *F. lobata* B.L.Turner. A partir de este trabajo, Turner reconoció tres grupos basado principalmente en características morfológicas de hoja, tallo e inflorescencia. El primer grupo está formado por *Florestina purpurea* originalmente descrita como parte del género *Hymenothrix* por su similitud en características de la corola y del vilano, restringida a la región del Valle de Tehuacán-Cuicatlán. El segundo grupo, constituido por cinco especies, *Florestina liebmannii, F. lobata, F. pedata, F. simplicifolia* y *F. tripteris*, que son morfológicamente muy similares lo que ha complicado su reconocimiento y la reconstrucción de sus relaciones filogenéticas. Por ejemplo, Turner (1963) observó una aparente intergradación morfológica entre *Florestina liebmannii* y *F. tripteris*, así como entre *F. tripteris* y *F. pedata*. Además, *Florestina pedata* podría considerarse como un complejo de especies, debido a que es la especie morfológicamente más variable en el género y presenta una amplia distribución, desde el noreste, centro y sur de México hasta el oeste de Guatemala. Finalmente, el tercer grupo incluye a *Florestina latifolia* y *F. platyphylla*, las cuales presentan corolas púrpura, hojas simples y brácteas amplias del involucro. Por otro lado, las relaciones filogenéticas intergenéricas de *Florestina* han sido controversiales; el género ha sido cercanamente relacionado con

Schkuhria (Hoffman, 1894), *Hymenothrix* (Turner 1962, 1963) y *Palafoxia* (Shinners 1952; Baldwin et al. 2002). Además, algunos de las especies fueron descritas originalmente como pertenecientes a estos géneros.

La mayoría de las especies de *Florestina* son morfológicamente muy parecidas, por lo que pocos caracteres morfológicos han sido útiles en estudios taxonómicos. En este caso, los datos moleculares podrían ser útiles para esclarecer las relaciones filogenéticas entre las especies. Durante las últimas décadas, el uso de secuencias de ADN ha jugado un papel importante en la sistemática y en la inferencia filogenética de plantas (p.e. Taberlet et al., 1991; Baldwin 1992; Baldwin y Markos 1998). Los espaciadores ETS e ITS de ADN ribosomal han sido ampliamente utilizados para la inferencia filogenética en los niveles genérico e infragenérico, debido a que son heredados biparentalmente y presentan tasas altas de sustitución (Baldwin, 1992; Baldwin y Markos, 1998). Por otro lado, varias regiones espaciadoras intergénicas del cloroplasto han sido reportadas recientemente, tales como *trn*C-*pet*N, *rpl*32-*trn*L y *rps*16-*trn*Q, las cuales están siendo utilizadas con mayor frecuencia en estudios filogenéticos de plantas (Lee y Wen, 2004; Shaw et al., 2005; Mort et al., 2007; Shaw et al., 2007; Geleta et al., 2010). Por lo tanto, un estudio filogenético basado en secuencias de ADN nuclear y de cloroplasto podría ser útil para inferir la filogenia del género de *Florestina*.

Basado en su distribución geográfica, el género *Florestina* ofrece un caso interesante para elucidar patrones de diversificación de la flora de ambientes áridos y semiáridos de México, particularmente de la familia Asteraceae. Estos ambientes abarcan más de la mitad de la superficie del territorio mexicano y se caracterizan por la escasez de precipitación anual con periodos prolongados de sequía (8 meses o más). La diversidad

florística d e los a mbientes ár idos y s emiáridos es p articularmente al ta en t érminos d e endemismo, ap roximadamente e l 6 0% d e las especies d e su flora t otal s on e ndémicas (Mittermeier *et al.*, 2011). Estos hábitats fueron profundamente a fectados por los ca mbios climáticos o curridos d urante el P leistoceno, p romoviendo l a d iversificación d e l inajes d e plantas y animales co n u na p roporción a lta d e t axones e ndémicos (Hubbard, 1973; Ferrusquía-Villafranca y G onzález-Guzmán, 2005) . El d esarrollo d e las regiones ár idas y semiáridas de México está ligado a e ventos geológicos, tales como el origen y desarrollo de la S ierra M adre O ccidental y e l E je N eovolcánico T ransversal durante el Mioceno-Pleistoceno. Estos eventos aumentaron la tendencia a la aridez, reduciendo drásticamente la humedad y favoreciendo l a ev olución d e co munidades d e a mbientes á ridos y se miáridos (Ferrusquía-Villafranca y G onzález-Guzmán, 2005) . Recientemente, u n número creciente de estudios biogeográficos y filogeográficos abordan la e volución de o rganismos de zo nas áridas y semiáridas de México, tales como la Depresión del Balsas, el V alle de T ehuacán-Cuicatlán, e l I stmo d e Tehuantepec, el Altiplano M exicano y la Península d e B aja California (e.g. Sosa *et al.*, 2009; B ryson *et al.*, 2011, 2012; R uiz-Sánchez *et al.*, 201 2; Ruiz-Sánchez & Specht, 2013; Gándara & Sosa, 2014; Gándara *et al.*, 2014).

Los p rincipales o bjetivos d e esta tesis fueron 1) evaluar l a posición filogenética d e *Florestina*, 2) evaluar la m onofilia del género, 3) determinar las r elaciones filogenéticas entre l as es pecies, 4) determinar s i los t axones act ualmente c ircunscritos al g énero son distintos basado en d atos moleculares, 5) inferir p atrones de ev olución cromosómica a lo largo de la filogenia de *Florestina*, 6) estimar los tiempos de divergencia para el origen y la diversificación d el g énero *Florestina* y 7) evaluar l as h ipótesis concernientes al origen biogeográfico y la historia evolutiva de *Florestina*.

CAPÍTULO I

RELACIONES FILOGENÉTICAS EN EL GÉNERO *FLORESTINA*

(ASTERACEAE: BAHIEAE)

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ORIGINAL ARTICLE

Phylogenetic relationships in the genus *Florestina* (Asteraceae, Bahieae)

Fabiola Soto-Trejo[1,2] · Edward E. Schilling[3] · Sofía Solórzano[1] · Ken Oyama[4] · Rafael Lira[1] · Patricia Dávila[1]

Abstract *Florestina* is shown to consist of six annual species occurring mostly in arid and semiarid regions of Mexico. *Florestina* species are morphologically similar and consequently phylogenetic relationships within the genus are poorly understood. We present a phylogenetic study based on morphological characters, DNA sequences of nuclear non-coding spacers (ETS and ITS) and chloroplast non-coding spacers (*rpl32-trn*L and *trn*C-*pet*N). The ETS and ITS spacer-based phylogenies allowed several well-supported conclusions: (1) the genus *Florestina* is monophyletic and *Palafoxia* is its closest relative; (2) *Florestina latifolia* and *F. platyphylla* form a strongly supported clade; (3) four taxa that are morphologically very similar, *F. liebmannii*, *F. pedata*, *F. simplicifolia*, and *F. tripteris*, are phylogenetically closely related and based on the sequence data we suggest that these should be recognized as only two species, one comprising *F. pedata* and *F. simplicifolia*, which shows wide morphological variation throughout its distributional range; and the other comprising *F. liebmannii* and *F. tripteris*; (4) *F. lobata* and *F. purpurea* are species very distinct from the remainder of the species in *Florestina*. Our phylogenetic analyses suggest that hybridization and introgression may be involved in the evolutionary history of *Florestina*.

Keywords ETS · *Florestina* · ITS · Phylogeny · *rpl32-trn*L · *trn*C-*pet*N

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Introduction

Florestina Cass. (Asteraceae, Bahieae) is a small genus that includes annual herbs occurring mostly in arid and semiarid regions of Mexico (Turner 1963). The genus is characterized by having zygomorphic corollas and cuspidate appendages of the style branches. *Florestina* species are all morphologically similar and consequently, the relationships within the genus have been controversial and problematic. In the earliest taxonomic treatment of *Florestina*, Rydberg (1914) circumscribed six species in the genus: *F. latifolia* (DC.) Rydb., *F. liebmannii* Sch.Bip. ex Greenm., *F. pedata* Cass., *F. platyphylla* B.L.Rob. & Greenm., *F. purpurea* Rydb., and *F. tripteris* DC. Turner (1963) enlarged the genus by adding two newly described species: *F. simplicifolia* B.L.Turner and *F. lobata* B.L.Turner (Table 1). *Florestina* has been considered to be related to several genera by different authors, including *Schkuhria* Roth (Hoffmann 1894), *Hymenothrix* A.Gray (Turner 1962, 1963), and *Palafoxia* Lag. (Shinners 1952;

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Table 1 *Florestina* taxonomic comparison of Rydberg (1914), Turner (1963) and the present study

Rydberg (1914)	Turner (1963)	This study
F. latifolia	*F. latifolia*	*F. latifolia*
F. platyphylla	*F. platyphylla*	*F. platyphylla*
F. purpurea	*F. purpurea*	*F. purpurea*
	F. lobata	*F. lobata*
F. pedata	*F. pedata*	*F. pedata*
	F. simplicifolia	*F. pedata*
F. tripteris	*F. tripteris*	*F. tripteris*
F. liebmannii	*F. liebmannii*	*F. tripteris*

Baldwin et al. 2002). In addition, some taxa were originally described in other genera that are putatively near relatives, suggesting that the monophyly of the genus must be evaluated. The available data show that morphological information is inconsistent and does not help for resolving phylogenetic relationships among *Florestina* species.

In spite of the complex morphological variation, the current taxonomy of *Florestina* is based on morphological characters, complicating both the recognition of taxa and the unambiguous reconstruction of their phylogenetic relationships (Turner 1963). During the last few decades, however, the use of DNA sequences has played an increasing and significant role in plant systematics and in phylogenetic inference at different taxonomic levels (e.g., Taberlet et al. 1991; Baldwin 1992; Baldwin and Markos 1998). External and internal transcribed spacers (ETS, ITS) of nuclear ribosomal DNA have been widely used for phylogenetic inference at lower taxonomic levels, as they are biparentally inherited and have higher rates of base substitution than typically found in organellar genes (Baldwin 1992; Baldwin and Markos 1998). However, ribosomal genes may not adequately reconstruct phylogenetic history due to concerted evolution, which can mask past evolutionary events, such as hybridization or introgression (Álvarez and Wendel 2003). Recently, several fast-evolving regions of chloroplast have been reported, such as *trn*C-*pet*N, *rpl32-trn*L and *rps*16-*trn*Q intergenic spacer regions, which are being increasingly used for phylogenetic studies at lower taxonomic levels in flowering plants (Lee and Wen 2004; Shaw et al. 2005, 2007; Mort et al. 2007; Geleta et al. 2010).

Chromosome number is a useful tool in plant systematics and evolution, and can complement the information obtained from morphological and molecular data (Guerra 2008). Recently, a method was developed to infer of chromosome number evolution by means of formulated probabilistic models using either Maximum Likelihood (ML) or Bayesian methods (Mayrose et al. 2010). This analysis offers the ability to test hypotheses about the

patterns of chromosomal evolution along the phylogeny of *Florestina*.

For the current study, a comprehensive approach was taken which employed analyses of various data sets to evaluate the phylogenetic relationships of *Florestina*. Morphology was studied systematically using a large set of characters, supported by statistical methods. Studies of sequence variation of both nuclear and chloroplast spacer regions were conducted utilizing multiple samples from different geographic regions of each species. Furthermore, an analysis to infer chromosome number evolution along the phylogenetic tree of *Florestina* was performed using a probabilistic approach. The main goals of our study were to evaluate the phylogenetic placement of *Florestina*, to assess whether the genus as currently circumscribed is monophyletic, to elucidate the phylogenetic relationships among the species of the genus, and to ascertain whether they are all distinctive based on molecular data, and to infer patterns of chromosome evolution along the phylogeny of *Florestina*.

Materials and methods

Morphological analysis

Study species

Three hundred and fifty herbarium specimens from the National Herbarium of Mexico (MEXU) of the Universidad Nacional Autónoma de México (UNAM) were examined for morphological analyses. We included all species previously recognized by Turner (1963), (Table 1). Since the monophyly of *Florestina* is unclear, the species sampled from putative closely related genera are only tentatively considered to be "outgroups." The clear outgroup is *Chaenactis* DC. (Baldwin et al. 2002). Therefore, for the morphological analyses, we included seven species of *Palafoxia* (*P. arida* B.L.Turner & M.I.Morris, *P. callosa* (Nutt.) Torr. & A.Gray, *P. lindenii* A.Gray, *P. linearis* (Cav.) Lag., *P. riograndensis* Cory, *P. sphacelata* (Nutt. ex Torr.) Cory, and *P. texana* DC.); two of *Hymenothrix* (*H. wislizeni* A.Gray and *H. wrightii* A.Gray); two of *Bahia* Lag. (*B. absinthifolia* Benth., and *B. pringlei* Greenm.); two of *Schkuhria* (*S. pinnata* (Lam.) Kuntze ex Thell., and *S. schkuhrioides* Thell.); and one of *Chaenactis* (*C. macrantha* D.C.Eaton).

Data analyses

Twenty-six morphological characters from stem, leaf, inflorescence, floret, and achene were measured and scored (Table 2) from 350 herbarium specimens representing 23

species. These included the morphological characters originally used by Turner (1963), as well as newly scored characters obtained for this study. The extended morphological dataset was analyzed employing the Fitch parsimony criterion (unordered character states, equal weights), as implemented in WINCLADA 1.00.08 (Nixon 1999). Searches for the most parsimonious trees (mpt) in the analysis were performed with the heuristic algorithm, and tree construction was done via stepwise addition, using 1000 replicates with random addition of sequences, and tree-bisection reconnection (TBR) branch swapping. A strict consensus tree was constructed and clade support was estimated using bootstrap values (1000 bootstrap replicates with each 10 random taxon addition).

Additionally, we used a Principal Component Analysis (PCA) to estimate the contribution of each character in separating the species. The same 26 morphological characters as in parsimony analysis were used for the PCA (Table 2). Each one of the 326 specimens was taken as an operational taxonomic unit (OTU). The recovered groups were compared to those species recognized in the most recent taxonomic treatment (Turner 1963). This statistical analysis was performed using the software STATISTICA 8.0 (StatSoft Inc. 2007).

Molecular analyses

Plant material

Sampling included both field-collected and herbarium-sampled material. Seventy herbarium specimens of five species were obtained from National Herbarium of Mexico (MEXU) of the Universidad Nacional Autónoma de México (UNAM), as well as six samples of six species of Herbarium of the University of Texas, Austin, Texas, USA (LL, TEX). In total, 42 samples from the eight putative taxa in *Florestina* and nine samples of species of *Bahia*, *Hymenothrix*, *Palafoxia, Schkuhria*, and *Chaenactis* were sequenced for nuclear and chloroplast DNA (Table 3). The nuclear ribosomal ITS region and a portion of the ETS region, and the plastid regions *trn*C-*pet*N and *rpl32-trnL* were sequenced for all of the samples.

DNA isolation, PCR amplification, and sequencing

DNA was extracted from leaves taken from herbarium specimens or from fresh material stored in liquid nitrogen. DNA was extracted using the DNeasy plant DNA extraction kit (Qiagen, Germantown, Maryland, USA), following

Table 2 Twenty and six morphological characters used for the analysis of taxa of *Florestina* and related genera

1. Covering of the stem: 0-indumented, 1-glabrescent
2. Glandular hairs on vegetative portion of stem: 0-absent, 1-present
3. Glandular hairs on branches of the inflorescence: 0-absent, 1-present
4. Leaf type: 0-simple, 1-compound, 2-simple and compound
5. Covering of the leaf: 0-indumented, 1-glabrescent
6. Glandular hairs on leaves: 0-absent, 1-present
7. Inflorescence type: 0-simple cyme, 1-dichotomous cyme, 2-corymbiform cyme
8. Capitulum type: 0-homogamous, 1-heterogamous
9. Disposition of the bracts of the involucre: 0-two or three seriate 1-a single series
10. Accessory external bracts of the involucre: 0-absent, 1-present
11. Covering of the involucral bracts: 0-indumented, 1-glabrescent
12. Shape of the involucral bracts: 0-lanceolate or linear, 1-obovate or ovate
13. Glandular hairs on involucral bracts: 0-absent, 1-present
14. Length of the involucre: 0-short (3.5–5.5 mm), 1-medium (5.6–8.5 mm), 2-large (9.0–15.0 mm)
15. Corolla type of disc florets: 0-actinomorphic, 1-zygomorphic
16. Corolla color of disc florets: 0-yellow, 1-white–pink, 2-purple
17. Number of disc florets: 0–2–40, 1–60–120
18. Number of ray florets: 0–0, 1–2–4, 2–6–15
19. Apex of the stigmatic lines: 0-without cuspidate appendage, 1-with cuspidate appendage
20. Shape of the achene: 0-linear, 1-obpyramidal, 2-cuneate
21. Hairs along achene ribs: 0-absent, 1-present
22. Length of the achene: 0-short (2.5–3.8 mm), 1-medium (3.9–5.5 mm), 2-large (6.0–11.0 mm)
23. Number of pappus scales: 0–8 scales, 1–4–8 scales, 2–8–12 scales, 3–14–18 scales
24. Type of pappus scales: 0-homomorphic, 1-heteromorphic
25. Shape of pappus scales: 0-obovate or ovate, 1-lanceolate or linear
26. Pappus length: 0-short (0.8–2.8 mm), 1-medium (3.0–6.4 mm), 2-large (6.5–8.0 mm)

Table 3 Taxa examined in the molecular analysis of the genera *Florestina*, *Bahia*, *Chaenactis*, *Hymenothrix*, *Palafoxia* and *Schkuhria*

Taxon	Collector (Herbarium)	Locality	Genbank accession no.			
			ETS	ITS	*trn*C-*pet*N	*rpl*32-*trn*L
Ingroup						
Florestina latifolia	Guzmán & Soto-Trejo 3685 (MEXU)	SE Oaxaca	KP972222	KP972273	KP972324	KP972375
F. latifolia	Guzmán & Soto-Trejo 3686 (MEXU)	SE Oaxaca	KP972223	KP972274	KP972325	KP972376
F. latifolia	Guzmán & Soto-Trejo 3687 (MEXU)	SE Oaxaca	KP972224	KP972275	KP972326	KP972377
F. latifolia	Guzmán & Soto-Trejo 3688 (MEXU)	SE Oaxaca	KP972225	KP972276	KP972327	KP972378
F. liebmannii	Dorantes 774 (MEXU)	C Veracruz (coast)	KP972226	KP972277	KP972328	KP972379
F. liebmannii	Yahara 1364 (MEXU)	C Veracruz	KP972227	KP972278	KP972329	KP972380
F. liebmannii	Lot 1853 (MEXU)	S Veracruz (coast)	KP972228	KP972279	KP972330	KP972381
F. liebmannii	Guzmán & Soto-Trejo 3681 (MEXU)	C Veracruz (coast)	KP972229	KP972280	KP972331	KP972382
F. lobata	Soto-Trejo 245 (MEXU)	SO Estado de México	KP972230	KP972281	KP972332	KP972383
F. lobata	Guzmán & Soto-Trejo 3694 (MEXU)	SO Estado de México	KP972231	KP972282	KP972333	KP972384
F. pedata	Castañeda 152 (MEXU)	S Guanajuato	KP972232	KP972283	KP972334	KP972385
F. pedata	Soto-Trejo 232 (MEXU)	Distrito Federal	KP972233	KP972284	KP972335	KP972386
F. pedata	Soto-Trejo 233 (MEXU)	S Puebla	KP972234	KP972285	KP972336	KP972387
F. pedata	Soto-Trejo 234 (MEXU)	NW Oaxaca	KP972235	KP972286	KP972337	KP972388
F. pedata	Soto-Trejo 236 (MEXU)	NW Oaxaca	KP972236	KP972287	KP972338	KP972389
F. pedata	Soto-Trejo 243 (MEXU)	C Oaxaca	KP972237	KP972288	KP972339	KP972390
F. pedata	Spooner 2554 (MEXU)	W Michoacán	KP972238	KP972289	KP972340	KP972391
F. pedata	King 3655 (MEXU)	C Jalisco	KP972239	KP972290	KP972341	KP972392
F. pedata	Guzmán & Soto-Trejo 3693 (MEXU)	N Guerrero	KP972240	KP972291	KP972342	KP972393
F. pedata	Fernández 4657 (MEXU)	W Hidalgo	KP972241	KP972292	KP972343	KP972394
F. pedata	Martínez 4868 (MEXU)	S Guerrero	KP972242	KP972293	KP972344	KP972395
F. pedata	Calzada 19343 (MEXU)	S Oaxaca	KP972243	KP972294	KP972345	KP972396
F. platyphylla	Soto-Trejo 239 (MEXU)	S Puebla	KP972244	KP972295	KP972346	KP972397
F. platyphylla	Soto-Trejo 242 (MEXU)	C Oaxaca	KP972245	KP972296	KP972347	KP972398
F. platyphylla	Guzmán & Soto-Trejo 3689 (MEXU)	S Oaxaca	KP972246	KP972297	KP972348	KP972399
F. platyphylla	Guzmán & Soto-Trejo 3690 (MEXU)	S Oaxaca	KP972247	KP972298	KP972349	KP972400
F. purpurea	Soto-Trejo 237 (MEXU)	N Oaxaca	KP972248	KP972299	KP972350	KP972401
F. purpurea	Soto-Trejo 238 (MEXU)	S Puebla	KP972249	KP972300	KP972351	KP972402
F. simplicifolia	Victorino 37 (MEXU)	S Puebla	KP972250	KP972301	KP972352	KP972403
F. simplicifolia	Soto-Trejo 240 (MEXU)	C Oaxaca	KP972251	KP972302	KP972353	KP972404
F. simplicifolia	Soto-Trejo 241 (MEXU)	N Oaxaca	KP972252	KP972303	KP972354	KP972405
F. simplicifolia	Soto-Trejo 244 (MEXU)	SE Puebla	KP972253	KP972304	KP972355	KP972406
F. simplicifolia	Guzmán & Soto-Trejo 3691 (MEXU)	S Puebla	KP972254	KP972305	KP972356	KP972407
F. simplicifolia	Guzmán & Soto-Trejo 3692 (MEXU)	S Puebla	KP972255	KP972306	KP972357	KP972408
F. simplicifolia	Tenorio 6735 (MEXU)	S Puebla	KP972256	KP972307	KP972358	KP972409
F. tripteris	Ellison 129 (MEXU)	C Durango	KP972257	KP972308	KP972359	KP972410
F. tripteris	Prather 1294 (MEXU)	N Nuevo León	KP972258	KP972309	KP972360	KP972411
F. tripteris	Yahara 1403 (MEXU)	C San Luis Potosí	KP972259	KP972310	KP972361	KP972412
F. tripteris	Yahara 1460 (MEXU)	Nuevo León	KP972260	KP972311	KP972362	KP972413
F. tripteris	Guzmán & Soto-Trejo 3778 (MEXU)	C San Luis Potosí	KP972261	KP972312	KP972363	KP972414
F. tripteris	Nesom 6537 (MEXU)	S Chihuahua	KP972262	KP972313	KP972364	KP972415
F. tripteris	Henrickson 7572 (MEXU)	C Chihuahua	KP972263	KP972314	KP972365	KP972416
Outgroup						
Bahia pringlei	Guzmán & Soto-Trejo 3772 (MEXU)	Querétaro	KP972220	KP972271	KP972322	KP972372
Chaenactis macrantha	Panero & Crozier 8612 (LL, TEX)	S Nevada	KP972221	KP972272	KP972323	KP972373
Hymenothrix wislizeni	Felger 536 (LL, TEX)	S Arizona	KP972264	KP972315	KP972366	KP972417

Table 3 continued

Taxon	Collector (Herbarium)	Locality	Genbank accession no.			
			ETS	ITS	*trn*C-*pet*N	*rpl*32-*trn*L
H. wrightii	Hammond 10576 (LL, TEX)	N Arizona	KP972265	KP972316	KP972367	KP972418
Palafoxia arida	Reina 511 (MEXU)	N Sonora	KP972266	KP972317	KP972368	KP972419
P. feayi	Merello 563 (LL, TEX)	C Florida	KP972267	KP972318	KP972369	KP972420
P. sphacelata	Carr 27084 (LL, TEX)	S Texas	KP972268	KP972319	KP972370	KP972421
P. texana	Guzmán & Soto-Trejo 3682 (MEXU)	C Veracruz	KP972269	KP972320	KP972371	KP972422
Schkuhria pinnata	Carr 26340 (LL, TEX)	W Texas	KP972270	KP972321	KP972372	KP972423

manufacturer's protocol. Fragments were amplified by PCR in a 25 μL reaction volume containing 1–2 μL of total genomic DNA, 10× PCR buffer, 5 mM MgCl$_2$, 0.4 mM each dNTP, 0.5 μM each primer, 0.2–1.5 μL of 0.1 % bovine serum albumen and 1 U Taq DNA polymerase. Primers used were "ITS-4" and "ITS-5" (White et al. 1990) for ITS region, "18-S-ETS" (Baldwin and Markos 1998) and "Ast-1" (Markos and Baldwin 2001) for ETS region, "trnL" and "rpl32-F" (Shaw et al. 2007) for *rpl32-trnL* spacer region, and "9351F" and "10175R" (Geleta et al. 2010) for *trnC-petN* spacer region. Amplifications were achieved using a Veriti® 384 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) with the following conditions: denaturation at 94 °C for 3 min, followed by 35 cycles of 40 s denaturing at 94 °C for 40 s, primer annealing at 56 °C and primer extension for 1 min 20 s at 72 °C; and a final 5-min extension at 72 °C. Successfully amplified samples were purified using the QIAquick PCR purification kit (Qiagen, Germantown, MD, USA) and microcentrifuged following manufacturer's instructions. In other cases, the PCR products were cleaned with ExoSAP-IT (USB, Cleveland, Ohio). Sequencing reactions were performed using the BigDye® Terminator, v. 3.1, cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and carried out at 96 °C for 10 s, 55 °C for 10 s and 60 °C for 5 min for 25 cycles. The products of the sequencing reaction were purified using Sephadex G-50 and electrophoresed and detected on an ABI Prism 3100 automated sequencer in the Biology Institute, Universidad Nacional Autónoma de México and University of Tennessee Molecular Biology Resource Facility, Knoxville, TN.

Sequence alignment and data analyses

Sequences were edited using BIOEDIT version 7.1.5.0 (Hall 1999) and Geneious version 7. Alignments were carried out in MAFFT v.6 (Katoh and Toh 2008) using default parameters, and followed by manual adjustment when necessary. The aligned sequences are given as Online Resources 1 and 2. Maximum likelihood (ML), and Bayesian inference (BI) methods were used to reconstruct the phylogeny of *Florestina*. MrModeltest version 2.3 (Nylander 2004) was used for fitting the best nucleotide substitution model for each locus according to the Akaike information criterion. The SYM+G model was selected for ITS region; GTR+G was assigned for ETS and *trnC-petN* regions; and GTR+I+G was applied for rpl32-trnL spacer. BI was conducted in MrBayes version 3.1.1 (Ronquist and Huelsenbeck 2005), using the substitution models mentioned above and a random starting tree. Four Markov Chain Monte Carlo chains (MCMC) were run simultaneously, each for 1,000,000 generations and sampled every 1000 generations. The first 250,000 generations were deleted as the "burn in" after assessment for convergence in TRACER 1.5.0 (Rambaut and Drummond 2009). ML analyses were performed in RAxML (Stamatakis 2006) using the GTR+I+G model. Support values for nodes on the ML tree were estimated with 1000 bootstrap replicates (Felsenstein 1985).

Congruence among data sets

To assess the congruence between morphological, nuclear and chloroplast DNA data sets for estimating phylogenetic relationships within *Florestina*, we performed a partition homogeneity test (PHT) that is based on the incongruence length difference of Mickevich and Farris (Farris et al. 1995). PHT were computed in PAUP v.40b10 using 1000 replicates, a full heuristic search, simple taxon addition and TBR branch swapping. PHTs were conducted on each of the combined matrices (ETS–ITS, *rpl32-trnL–trnC-petN*, ETS/ITS–*rpl32-trnL/trnC-petN*, ETS/ITS–morphology, *rpl32-trnL/trnC-petN*–morphology).

Chromosome numbers

Chromosome numbers for five *Florestina* taxa were previously reported: $n = 12$ for *F. latifolia* (Turner 1963; Turner and Flyr 1966; Powell et al. 1975) and $n = 10$ for

F. liebmannii (Turner [1963]; Keil and Stuessy [1977]); *F. pedata* (Turner [1963]; Turner and Johnston [1961]; Keil et al.[1988]); *F. simplicifolia* (Turner [1963]; Sundberg et al. [1986]); and *F. tripteris* (Turner [1963]; Keil and Stuessy [1975]; Powell et al. [1975]). Moreover, previously reported chromosome numbers were also included in the present study for species of *Bahia* (Turner and Johnston [1961]), *Hymenothrix* (Turner [1962]), *Palafoxia* (Turner and Morris [1976]), *Schkuhria* (Keil et al. [1988]) and *Chaenactis* (Keil and Pinkava [1976]).

Chromosome counts for *F. platyphylla*, *F. purpurea* and *F. lobata* were obtained from cells in meiotic metaphase using squash technique. Flower buds were fixed in modified Carnoy's solution (6:1:1 absolute ethanol:chloroform:glacial acetic acid). Squash preparations were stained in 1.8 % propio-orcein solution and permanent preparations were made by mounting in Canada balsam. Meiotic counts were determined from at least three florets of each species. Slides were analyzed with a Zeiss FOMI II microscope equipped with a digital camera.

Reconstruction of basic chromosome numbers within clades

Based on the phylogenetic relationships within *Florestina* resulting from the BI analysis of the ETS/ETS dataset, the most likely ancestral chromosome numbers for clades were inferred by ML and BI frameworks using ChromEvol v.2.0 (Mayrose et al. [2010]). The eight chromosome evolution models in this software were analyzed with 10,000 simulations and the best fitting model was selected under the Akaike information criterion.

Results

### Phylogenetic and statistical analyses of the morphological data

Parsimony analyses recovered four equally most parsimonious trees with a length $(L) = 85$, a consistency index $(CI) = 0.52$, and a retention index $(RI) = 0.75$. Resolution was very poor, and the strict consensus $(L = 112$, a $CI = 0.40$, and a $RI = 0.51)$ recovered only two well-supported clades within the ingroup, with bootstrap values of 75 and 95 % (Fig. [1]). The samples of *Florestina* did not collectively form a clade.

PCA analysis showed that the first three PCs (PC 1, PC 2 and PC 3) accounted for 69.1 % of the total variance (Table [4]). PC 1 explained 31.7 % of the variance, with the characters related to the shape of the achene, length of involucre, number of ray florets, length of the pappus and length of the achene being the most important variables;

PC2 explained 26.9 % of the variance in which corolla type of disc florets and apex of the stigmatic lines bearing the higher load. Finally, PC 3 accounted for 10.6 % of the variance, with the glandular hairs on leaves being the most important character. The scatterplot of PC1 and PC2 showed that outgroups *Schkuhria* and *Bahia* are morphologically well-differentiated; however, separation between *Hymenothrix*, *Palafoxia* and *Florestina* is less clear (Fig. [2]). Within *Florestina*, PCA recovered three groups along PC1: the first one included *F. latifolia*, the second one included *F. platyphylla*, and the third one contained all of the remaining species (Fig. [3]). The achene and pappus morphology of *Florestina* taxa are illustrated in Figs. [4] and [5].

Phylogenetic analyses of DNA

DNA sequences of nuclear non-coding spacers (ETS and ITS) and chloroplast non-coding spacers (*rpl32-trn*L and *trn*C-*pet*N) were obtained for 51 individuals, representing all recognized taxa of *Florestina* and outgroup taxa (*Bahia, Chaenactis, Hymenothrix, Palafoxia* and *Schkuhria*). The total aligned length of the ITS sequence, including the 5.8S gene, was 668 positions and ETS was 452. The topology of the ML and BI trees from the analysis of the nDNA was very similar. The ETS and ITS spacer-based phylogenetic
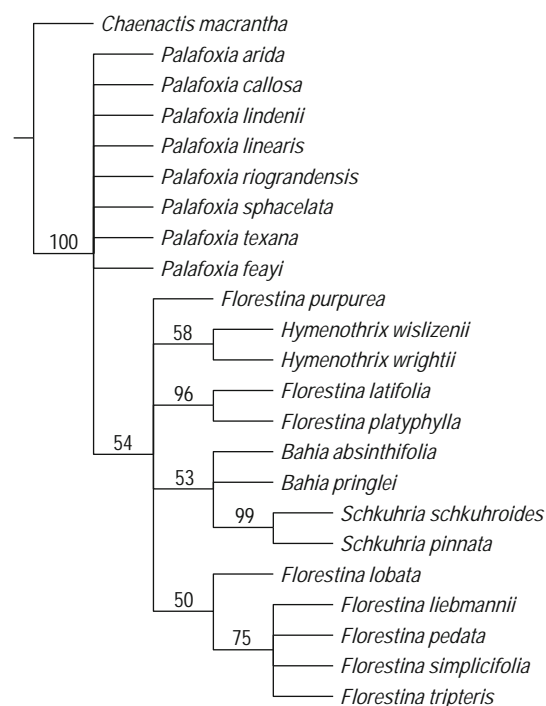
Fig. 1 Strict consensus tree from maximum parsimony analysis of 26 morphological characters in *Florestina* and related genera. *Numbers above* the branches indicate bootstrap values. $L = 112$, $CI = 40$, $RI = 59$
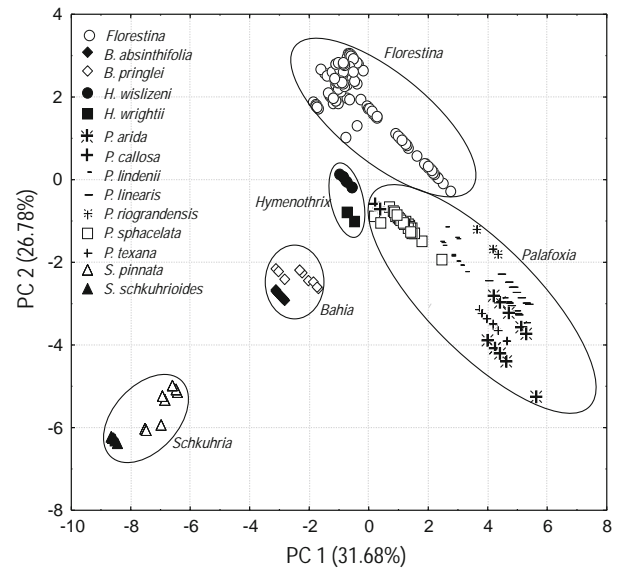
Table 4 Loadings of the first three principal components (PCs) for the twenty-six characters of *Florestina* taxa

Character	PC 1	PC 2	PC 3
1	0.318717	0.518124	0.130188
2	0.070216	0.617938	−0.519259
3	0.604252	0.232889	−0.053254
4	0.317119	0.368406	0.028459
5	0.646931	0.541842	0.248787
6	0.415397	−0.053512	−0.714755
7	0.547983	0.543666	0.305939
8	0.433576	0.623687	−0.057365
9	−0.492050	0.627033	−0.500538
10	0.673528	0.559342	0.273333
11	0.509645	0.149235	0.068617
12	0.666005	−0.516517	0.359733
13	−0.717549	0.426037	0.318851
14	0.739017	−0.590023	−0.043636
15	−0.093839	0.825371	−0.360163
16	0.620854	0.153702	−0.490459
17	−0.483221	−0.418459	0.100929
18	0.732453	0.635268	0.105662
19	0.138061	−0.873294	0.378601
20	0.870312	−0.075055	0.137145
21	0.673528	0.559342	0.273333
22	0.731845	−0.581905	0.019746
23	0.010915	0.493476	0.375629
24	0.673015	−0.429875	−0.199965
25	−0.456840	0.483957	0.630465
26	0.731171	−0.501497	−0.194472
Proportion of variance	31.7	26.8	10.6
Cumulative proportion	31.7	58.5	69.1

Fig. 2 *Scatterplot* of the first two components from principal component analysis (PCA) based on 26 of *Bahia*, *Florestina*, *Hymenothrix*, *Palafoxia* and *Schkuhria* taxa, showing group distances among the specimens
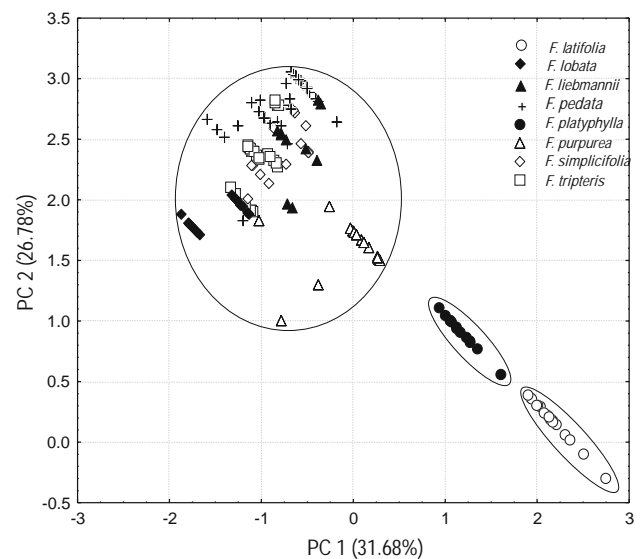
Fig. 3 *Scatterplot* of the first two components from principal component analysis (PCA) based on 26 characters of *Florestina* taxa, showing three separate groups

analysis recovered a highly resolved phylogeny (Figs. 6, 7). On the other hand, the aligned *rpl32*-trnL sequence encompassed a total aligned length of 920 and *trnC*-*pet*N was 759 sites. A phylogeny of the concatenated chloroplast sequences based on the total alignment of 1608 bp recovered a mostly unresolved phylogenetic tree (Fig. 8).

The findings of the partition homogeneity tests indicated the ETS and ITS data sets are congruent ($p = 0.14$) and reflect the same underlying phylogeny; thus they were analyzed together. Similarly, plastid *trnC*-*petN* and *rpl32*-*trnL* data sets ($p = 0.87$) are congruent with each other. However, for three combined matrices (nDNA-cpDNA, nDNA-morphology and cpDNA-morphology), the null hypothesis of homogeneity was rejected indicating that all three data sets are significantly heterogeneous ($p = 0.01$). These results confirm that the morphological, cpDNA and nDNA data are incongruent, and therefore combined analyses of these data sets are not justified. Therefore, the three data sets were analyzed separately.

Chromosome counts

Meiotic counts that were obtained represent first reports each for *F. lobata* ($n = 10$), *F. platyphylla* ($n = 12$) and *F. purpurea* ($n = 12$).

Fig. 4 Achene of **a**, **b** *Florestina latifolia* and **c** *F. platyphylla*, and pappus of **d** *Florestina latifolia* and **e** *F. platyphylla*. *Arrows* highlight the difference in features of the four shorter, ovate and obtuse scales, which alternating with four longer and lanceolate terminated by distinct awns scales

Reconstruction of basic chromosome numbers within clades

The results obtained in ChromEvol suggested that the best fitting model of chromosome evolution in *Florestina* was the hypothesis with gain and loss constant, no duplication. The rate parameters estimated were 2.91247 for loss, 2.29879e-010 for gain and the total inferred chromosome loss events were 12.4715. These results provide evidence that $n = 12$ is the most likely ancestral chromosome numbers in *Florestina* and suggest descending dysploidy events occurred along chromosome evolution in these species.

Discussion

Phylogenetic and statistical analyses of the morphological data

The resulting parsimony trees from morphological characters were very poorly resolved. The strict consensus tree was unresolved, showing the ingroup and the outgroup species in a polytomy with no synapomorphic characters supporting the monophyly of the genus *Florestina* (Fig. 1). However, two strongly supported clades were obtained, the first one formed by *F. latifolia* and *F. platyphylla,* and the second one constituted by *F. tripteris, F. liebmannii, F. pedata* and *F. simplicifolia*, but phylogenetic relationships in the second clade were also unresolved. Therefore, the parsimony analysis did not resolve the *Florestina*
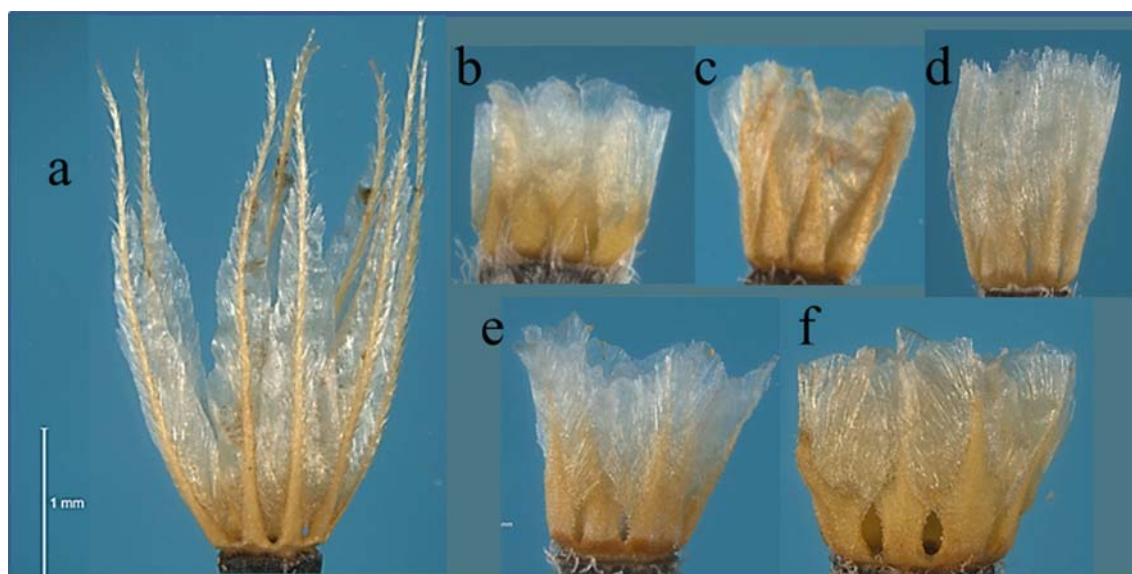
Fig. 5 Pappus of **a** *Florestina purpurea*, **b** *F. pedata*, **c** *F. liebmannii*, **d** *F. tripteris*, **e** *F. simplicifolia* and **f** *F. lobata*. Pappus of *Florestina purpurea* is formed by lanceolate scales with apex predominately acute, which are clearly different from the others taxa
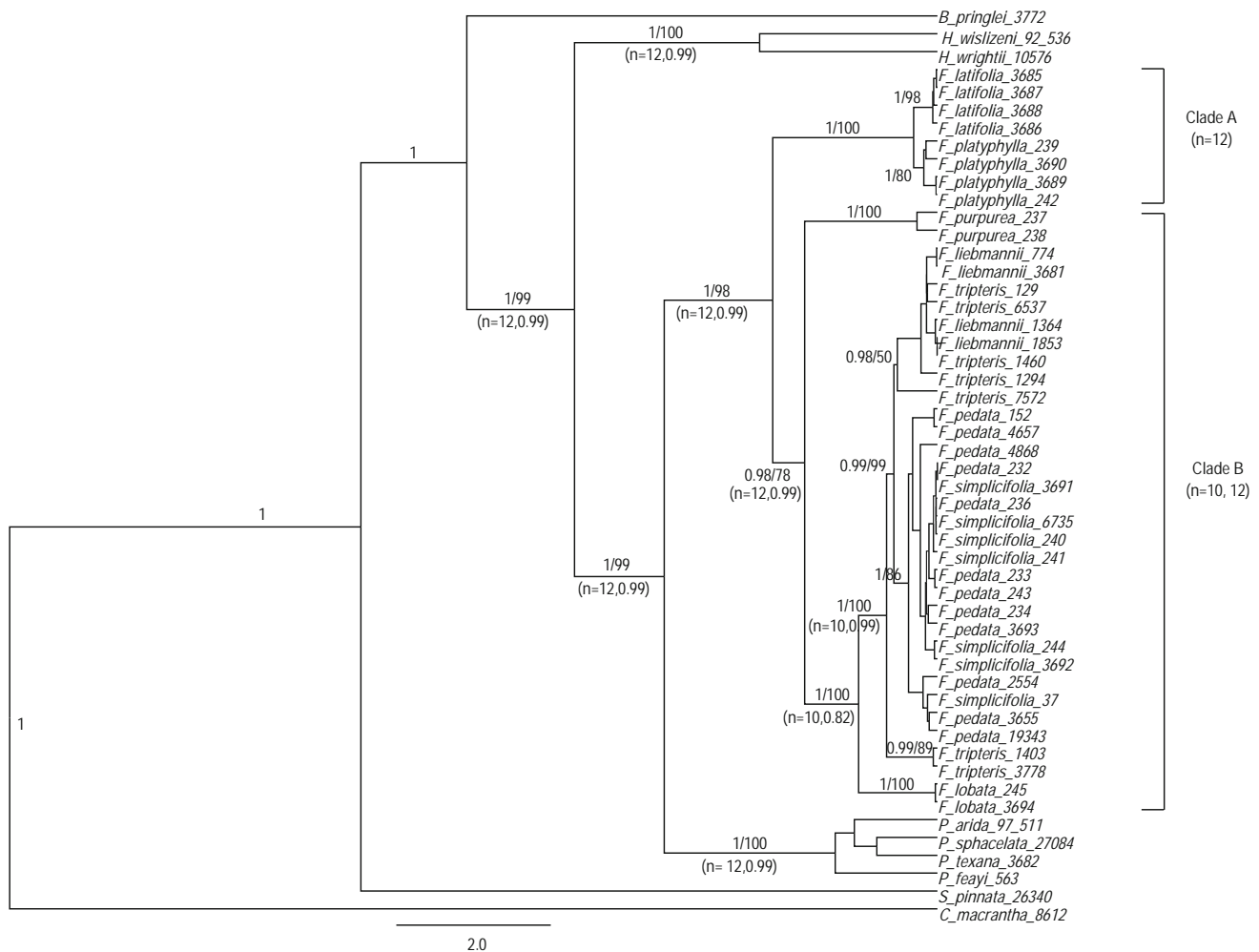
Fig. 6 Phylogenetic tree constructed from BI analysis of ETS and ITS sequences showing relationships of *Florestina* and its related genera *Bahia*, *Hymenothrix*, *Palafoxia*, *Schkuhria* and *Chaenactis*. *Numbers above* the branches indicate posterior probabilities/bootstrap values from BI and ML analyses, respectively. *Numbers below* the branches in *parentheses* indicate the estimated ancestral haploid chromosome numbers and their probabilities for clades

phylogeny and this might be due to the morphological characters included in our study are homoplastic or highly conserved.

PCA analysis showed that characters, such as the shape of the achene, number of ray florets, corolla type and apex of the stigmatic lines were the most important variables to separate *Bahia* and *Schkuhria* from the other genera (Fig. 2). However, the genera *Hymenothrix*, *Palafoxia* and *Florestina* formed a group, which revealed that they are morphologically similar (Fig. 2). The PCA results within *Florestina* revealed three groups along PC 1 (Fig. 3), the first two groups representing *F. latifolia* and *F. platyphylla*. These two taxa are externally very similar, and sometimes are very difficult to distinguish, but characters such as the lengths of both the achene and the pappus are useful in differentiating from each other (Fig. 4). *Florestina liebmannii*, *F. lobata*, *F. pedata*, *F. purpurea*, *F. simplicifolia*

and *F. tripteris*, which are morphologically very similar, formed the third group that revealed lack of useful taxonomic differences between them. However, PCA showed an apparent trend to separate *F. purpurea* from others according to the lengths of both the achene and the pappus (Fig. 5). Therefore, we conclude that the various morphological characters analyzed in the present study were not useful in recognizing taxonomic ranks within the third group; thus, we employed molecular markers to resolve their phylogenetic relationships.

Phylogenetic analyses of DNA

Given that in *Florestina* separate analyses of cpDNA (Fig. 8) and morphological data sets (Fig. 1) resulted in very poorly resolved trees, we concluded that nDNA provided the best estimate of phylogenetic relationships of

Fig. 7 Detail of Clade B from Fig. 6. *Numbers above* the branches indicate posterior probabilities/bootstrap values from BI and ML analyses, respectively. *Numbers below* the branches in *parentheses* indicate the estimated ancestral haploid chromosome numbers and their probabilities for clades
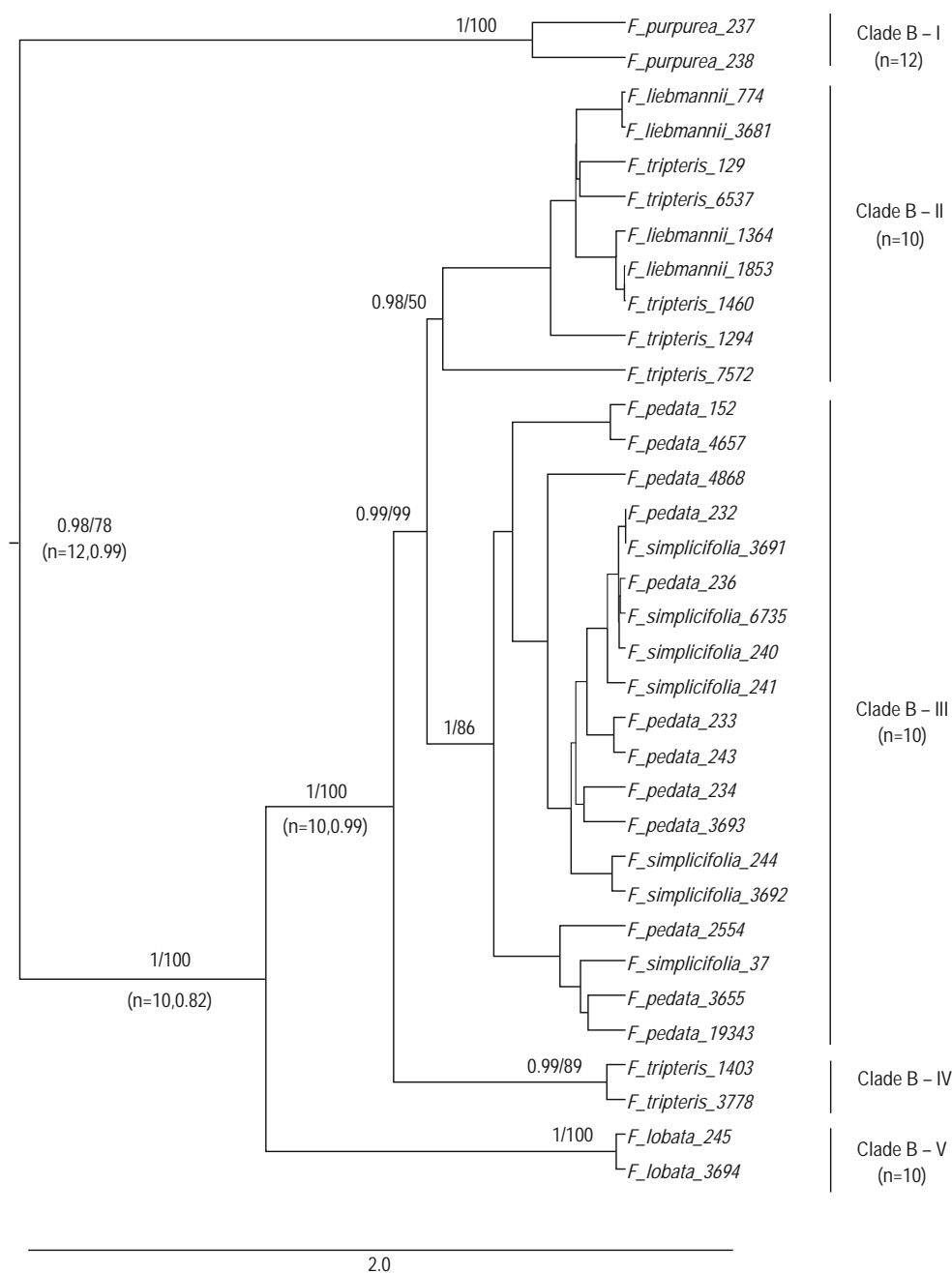
Florestina. ETS and ITS spacer-based phylogenetic analysis generated a highly resolved phylogeny (Figs. 6, 7), which strongly supported the monophyly of *Florestina*, and allowed us a number of well-supported conclusions and suggestions. In the former taxonomic treatment of the genus, Turner (1963) identified three main groups of taxa, but the relationships among these groups and among putative species in these lineages were unresolved. In contrast, our nuclear DNA-based results showed two main strongly supported clades and the relationships among species were mostly resolved. The first one, clade A, is

composed of *F. latifolia* and *F. platyphylla*, and clade B comprised the remaining species (Fig. 6). Turner (1963) treated the two taxa in clade A as different species, although he suggested that *F. platyphylla* could be a morphological variant of *F. latifolia*. Our results do not support this suggestion, since the tree recovered these two taxa as independent sister lineages in a strongly supported monophyletic group. *Florestina latifolia* and *F. platyphylla* are markedly different morphologically from the species included in clade B. For example, they display purplish corollas, simple leaves, and broad involucral bracts. In
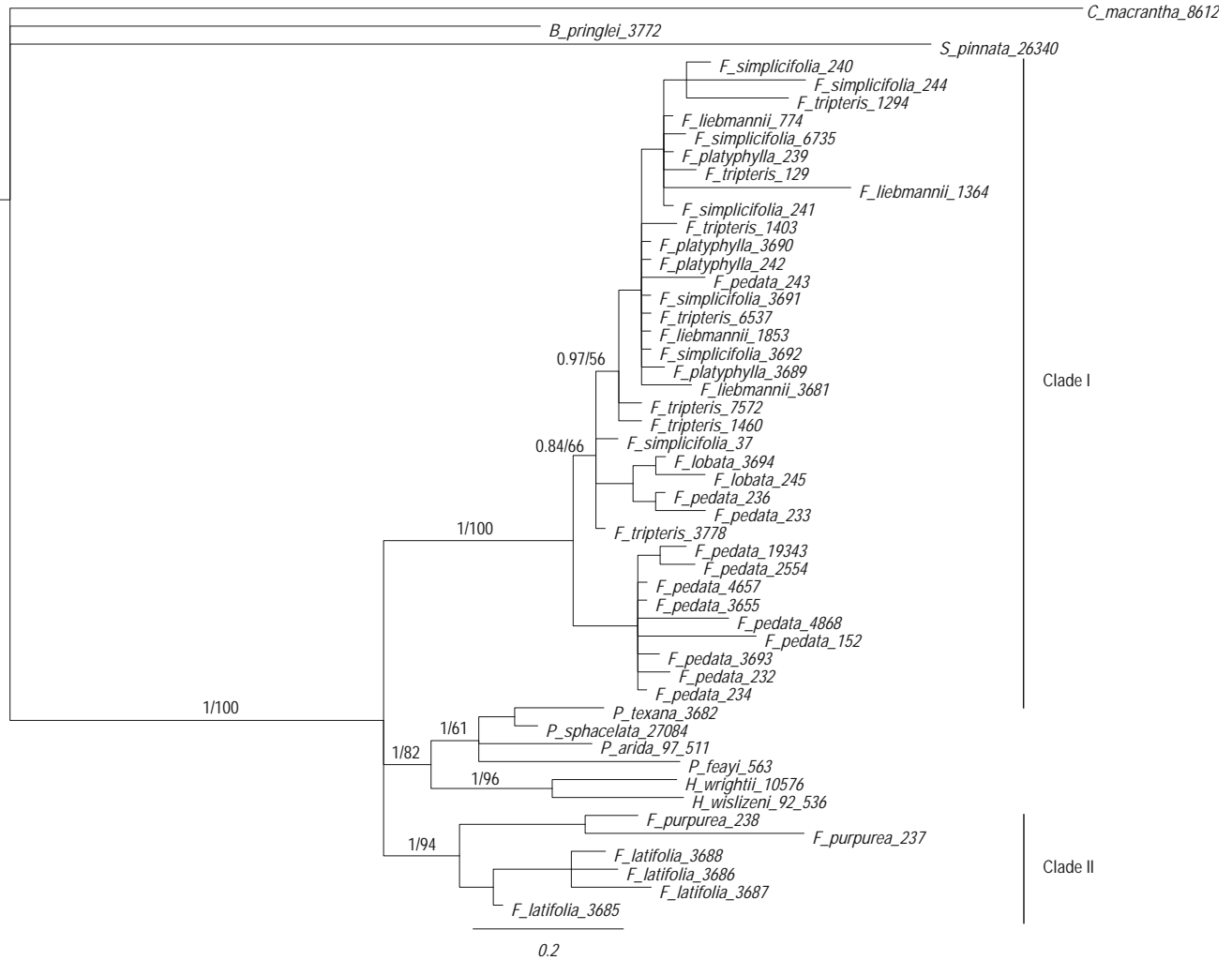
Fig. 8 Phylogenetic tree constructed from BI analysis of *rpl32-trn*L and *trn*C-*pet*N intergenic spacer sequences of *Florestina* and its related genera *Bahia*, *Hymenothrix*, *Palafoxia, Schkuhria* and *Chaenactis*. *Numbers above* the branches indicate posterior probabilities/bootstrap values from BI and ML analyses, respectively

addition, we confirm that these two taxa represent two independent species according to shape and size of the pappus scales (Figs. 3, 4), and they are parapatrically distributed. *Florestina platyphylla* is restricted to the central valleys in the state of Oaxaca, Mexico, and *F. latifolia* occurs from central Oaxaca to Nicaragua. Therefore, based on our phylogenetic analyses, supported by morphology and biogeography, we suggest that these two taxa should be treated as separate species.

Clade B was divided in five subclades (Fig. 7). The subclade B-I is constituted by *Florestina purpurea* and is sister to rest of clade B. This species was originally described as part of the genus *Hymenothrix*, and although Turner (1963) mentioned it as a "perplexing intermediate" between these two genera, results of our molecular analyses show it to clearly belong in *Florestina*. *F. purpurea* is morphologically well delimited by having biternately

dissected leaves, glabrous achenes with basal hairs and a pappus formed by lanceolate scales with their apex predominately acute. Furthermore, we observed leaflets with glandular trichomes, which were absent in the remaining species of clade B. The subclades B-II and B-III are most closely related within clade B. Subclade B-II includes *F. tripteris* and *F. liebmannii*, but neither species is recovered as monophyletic. According to Turner (1963), *F. liebmannii* is a weakly differentiated taxon occurring in sand dunes in coastal Veracruz, but different from *F. tripteris*, which is widely distributed in northern Mexico and southern Texas. However, our results indicate that these taxa belong to a single evolutionary lineage, but do not support recognizing them as separate species. Subclade B-III includes *F. pedata* and *F. simplicifolia*. *Florestina pedata* is a morphologically variable species that is widely distributed in central and southern Mexico, whereas *F.*

simplicifolia was described based only on a few specimens and is characterized by having simple leaves and located in the Tehuacán-Cuicatlán Valley in southern Mexico (Turner 1963). *Florestina simplicifolia* closely resembles *F. pedata*, and we recorded many specimens so morphologically similar that they may be allocated either as one species or the other. In fact, many plants with both simple and compound leaves grow intermixed with simple-leaved plants within populations of the putative *F. simplicifolia*. The findings of nDNA data allow us to hypothesize that *F. pedata* and *F. simplicifolia* actually form a single species, which shows wide morphological variation throughout its broad distributional range.

Sister to subclades B-II and B-III is subclade B-IV which includes samples of *F. tripteris* from eastern San Luis Potosí. Our analyses place *F. tripteris* in two separate, well-supported clades suggesting strongly that this taxon is polyphyletic. However, thus far subclade B-IV is only recognized by molecular characters, and additional studies are needed to test if this population should be considered a new species. Finally, subclade B-V is formed by samples of *F. lobata*, which was recovered as the sister taxon to subclades B-II to B-IV. This species is well distinguished by conspicuously lobed leaflets and glabrous involucral bracts and is locally restricted to the Balsas Basin in southern Mexico.

At the genus level, relationships of *Florestina* were clearly resolved on the ITS and ETS data-based tree (Fig. 6). Some genera, such as *Bahia*, *Hymenothrix*, *Palafoxia*, and *Schkuhria*, have been proposed to be closely related to *Florestina* or even to be congeneric with it (Hoffmann 1894; Shinners 1952; Turner 1963; Baldwin et al. 2002). According to Turner (1963), *Hymenothrix* is more closely related to *Florestina* than any other genus in Asteraceae; whereas *Palafoxia* is only distantly related. However, our molecular results clearly showed that *Florestina* and *Palafoxia* formed monophyletic sister groups, which is consistent with previous arguments by Shinners (1952) and Baldwin et al. (2002). Shinners (1952) argued that *Florestina* is closely related to *Palafoxia* and that they could not be separated in distinct genera, but our phylogenetic analyses showed that the members of *Florestina* and *Palafoxia* are clearly resolved based on nuclear DNA sequences with high levels of statistical support in the BI and ML analyses.

In contrast to the nuclear DNA-based phylogeny, the phylogenetic tree from chloroplast sequences neither resolved phylogenetic relationships in *Florestina* nor supported the monophyly of the genus (Fig. 8). However, two strongly supported clades were obtained, the first one (clade I) formed by *F. liebmannii*, *F. lobata*, *F. pedata*, *F. platyphylla*, *F. simplicifolia* and *F. tripteris*, but their phylogenetic relationships were unresolved. These taxa,

except *F. platyphylla*, are morphologically similar and closely related in the nuclear DNA-based phylogeny. The second clade (clade II) is comprised of *F. latifolia* and *F. purpurea*, these two species have a chromosome number of $n = 12$. These results suggest that cpDNA sequences are not sufficiently informative to resolve the phylogeny of *Florestina* and the phylogenetic relationships based on this marker may be misleading, probably due to hybridization/introgression (Rieseberg and Soltis 1991; Rieseberg and Carney 1998; Fehrer et al. 2007; Soltis and Soltis 2009). Hybridization and introgression have played an important role in plant speciation and evolution (Soltis and Soltis 2009). These processes occur frequently in desert plants, such as *Florestina*, and are favored by the peculiar habitat conditions that allow for recurrent contact of reproductively incompletely isolated species (Schneider et al. 2011). The findings of this study suggest that hybridization/introgression may be involved in the evolutionary history of *Florestina*. More detailed molecular and biogeographic studies are required to improve our insights into the role of these evolutionary processes in endemic taxa of arid and semiarid environments in Mexico.

According to the findings of the partition homogeneity tests, the morphological, cpDNA and nDNA data sets are incongruent with each other, and therefore combined analyses of these data sets are not justified for estimating phylogenetic relationships within *Florestina*. Theoretically, a combined analysis of different data sets could improve phylogenetic signal by increasing the number of characters (De Queiroz 1995), but could also increase the risk of mixing different phylogenetic signals. The different data sets might be incongruent and they may suggest alternative biological processes, such as hybridization or introgression (Wendel and Doyle 1998). Therefore, we consider that the results obtained by the combined analysis in *Florestina* might partially be overriding the true phylogenetic signal.

Chromosome evolution in *Florestina*

A high degree of congruence was found between chromosome number and the phylogenetic lineages in *Florestina* (Fig. 6). The results of the analysis of chromosome evolution support the hypothesis that the most likely ancestral chromosome number in *Florestina* is $n = 12$. Therefore, the chromosome number of $n = 10$ in taxa belonging to clade B might be interpreted as a reduction, as already suggested by Turner (1963). This chromosome number ($n = 10$) could have arisen through descending dysploidy, although the process remains hypothetical in the absence of in-depth studies in the genus. Dysploidy is considered as a very important evolutionary mechanism in plants and it is particularly common in many genera of Asteraceae (Guerra 2008; Galbany-Casals et al.

2009). Some authors have hypothesized that descending dysploidy is associated with a tendency to shorten the life cycle as an adaptation to extreme or xeric habitats [e.g. Garcia-Jacas et al. 1996 in Centaureinae (Asteraceae); Watanabe et al. 1999 in *Pogonolepis*, *Sondottia* and *Trichantodium* (Asteraceae); Vilatersana et al. 2000 in *Carduncellus*, *Carthamus* and *Phonus* (Asteraceae); Torrell et al. 2001 in *Artemisia*; Selvi and Bigazzi 2002 in *Nonea* (Boraginaceae); Garnatje et al. 2004 in the *Xeranthemum* (Asteraceae); Galbany-Casals et al. 2009 in *Helichrysum* (Asteraceae)]. In *Florestina*, species with $n = 10$ seem to occur in more xeric habitats; however, additional data are needed to assess whether the low chromosome numbers found in this genus are associated with an adaptation to more xeric habitats.

In summary, the phylogenetic analyses based on nuclear DNA (ETS and ITS) provided strong support for the monophyly of the genus *Florestina* and support *Palafoxia* as its closest relative. Based on comparison of our molecular hypotheses with morphological observations, the genus *Florestina* consists of six species: *F. pedata*, *F. tripteris*, *F. lobata*, *F. purpurea*, *F. latifolia*, and *F. platyphylla*. Two additional taxa, *F. simplicifolia* and *F. liebmannii*, are included within *F. pedata* and *F. tripteris*, respectively (Table 1). Both *F. pedata* and *F. tripteris* are widespread taxa that show ample morphological variation throughout their respective geographic distributional ranges.

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CAPÍTULO II

HISTORIA BIOGEOGRÁFICA DE *FLORESTINA* (ASTERACEAE, BAHIEAE) EN MÉXICO

Preparado para enviar como:

Biogeographic history of *Florestina* (Asteraceae, Bahieae) in Mexico's dry environments: testing hypotheses with biogeographic stochastic mapping

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ABSTRACT

Aim To infer t he biogeographic history o f t he ge nus *Florestina* in Mexico an d to demonstrate the ef fectiveness o f p robabilistic biogeographical methods i n s mall-data situations by qu antifying s tatistical s upport f or bi ogeography models, an cestral r ange estimates, and event counts.

Location Dry environments of Mexico

Methods We used a dated DNA phylogeny of *Florestina* estimated from nuclear ribosomal ITS a nd E TS regions. The s oftware BioGeoBEARS was us ed t o c ompare models, infer ancestral r ange p robabilities, an d es timate the n umber o f d ispersal, vicariance, an d sympatry events. BioGeoBEARS also implements stochastic mapping, a standard technique for DNA and morphological c haracters, here modified to enable conditional simulation of biogeographical histories under any chosen biogeographical model.

Results Our es timated d ates suggested that divergence b etween *Palafoxia* and *Florestina* occurred during the P liocene, approximately 3.54 Ma (HPD 2. 165–4.228 Ma); whereas diversification within *Florestina* occurred m ainly i n the Pleistocene. T he b iogeographical analyses indicated that there were substantial d ifferences in model performance, favouring a DEC model where dispersal probability is multiplied by distance^x.

Main conclusions Our f indings suggest t hat *Florestina* originated from a w idespread ancestor that p robably r anged from s outhern United S tates to s outhern M exico. T he divergence o f *Florestina* and *Palafoxia* was p robably d ue t o the development of t he Transvolcanic B elt. The m ost probable bi ogeographic m odel s howed a r elatively low number o f d ispersal e vents b etween a ll ar eas, s uggesting t hat s pecies e volved from o ne ancestral w idespread t axon dur ing the Pleistocene, leading t o the isolation o f p opulations

resulting in the diversification of *Florestina*. Phylogenetic and biogeographical analyses in *Florestina* highlight the influence of Pleistocene climate change in shaping biodiversity in Mexico's dry environments.

INTRODUCTION

Mexico is recognized as a biodiversity hotspot due to an extremely rich flora and fauna in terms both of overall species diversity and endemism (Mittermeier *et al*., 2011). This high species d iversity has been a ttributed t o geological and g eographical heterogeneity caused by both magmatic and tectonic activity, which have produced a wide diversity of climates and vegetation types. Furthermore, the complex overlap of Neotropical and Nearctic biotas has increased b oth diversity a nd endemism (Halffter, 1987; M orrone, 2015). Relatively recent major geological changes, such as the origin and development of the Transvolcanic Belt from the Miocene to Pleistocene, have increased a trend towards aridity by drastically reducing moisture and favoring the evolution of dry-forests and xerophytic communities in Mexico (Ferrusquía-Villafranca & González-Guzmán, 2005). Furthermore, the subsequent climate changes that occurred during the Pleistocene affected deeply the dry environments promoting the d iversification o f lineages of pl ants and a nimals with a r elatively high proportion o f e ndemic t axa (Hubbard, 1973; Ferrusquía-Villafranca & González-Guzmán, 2005). Among p lant gr oups of M exico, A steraceae e xhibits a notably high d iversity (Llorente-Bousquets & Ocegueda, 2008) , but the p atterns a nd c auses have yet t o be

carefully explored. The genus *Florestina* consists of annual herbs occurring mostly in arid and semiarid regions of Mexico, thus providing an interesting case for examination of the diversification of Asteraceae in Mexican dry environments. Recently, Soto-Trejo *et al.* (2015) developed a phylogeny based on DNA sequences from the ITS and ETS spacers of nuclear ribosomal DNA. Their study provided strong support for the monophyly of the genus and supported *Palafoxia* as its closest relative; and circumscribed the genus to six species: *F. latifolia* (DC.) Rydb., *F. lobata* B.L.Turner, *F. pedata* (Cav.) Cass., *F. purpurea* (Brandegee) Rydb., *F. platyphylla* (B.L. Rob. & Greenm.) B.L. Rob. & Greenm., and *F. tripteris* DC. Additionally, another potential taxon designated *F. sp.* may be a new species, but it is up to now only recognized by its distinctiveness in molecular characters and is still being evaluated. Based on the overall distribution of *Florestina* and its sister taxa, *Palafoxia* and *Hymenothrix* (Fig. 1), it might be expected that the genus originated in northern Mexico and subsequently expanded its range by movement along the eastern parts of the region. Results of the phylogenetic analyses, however, showed that the first diverging lineage of *Florestina* included two species endemic to southern Mexico, *F. latifolia* and *F. platyphylla* (Soto-Trejo *et al.*, 2015). Thus the area of origin of the genus, and its subsequent biogeographical history are still open questions.

The purpose of this study is to carry out biogeographical analyses of the genus *Florestina* using a dated phylogeny based on molecular data. Biogeographical analyses were conducted using the R package BioGeoBEARS (Matzke, 2015), which enables maximum likelihood (ML) inference using a variety of biogeographical models. Models can be statistically compared based on the likelihood that they confer on the geographic ranges observed at the tips of the phylogeny, and the relative importance of different biogeographic processes (e.g. vicariance vs. dispersal) under different models that can be

measured. BioGeoBEARS also implements biogeographical stochastic mapping, which is a formally appropriate method for estimating the number of events that have occurred on a phylogeny (see methods). We seek to show that these methods are useful even (and perhaps especially) on small datasets, like *Florestina*, where limited data means that uncertainty is likely to be high and needs to be quantitatively assessed.

The main objectives of this study were: 1) to estimate divergence times for the origin and diversification of *Florestina*, 2) to statistically compare models of geographic range evolution to infer the biogeographical history of the genus, 3) to evaluate hypotheses concerning biogeographical origin and evolutionary history of *Florestina* , 4) to analyze the likelihood of the historical processes (e.g. vicariance vs. dispersal) that have contributed to biogeographical and evolutionary history of *Florestina*, and (5) to assess how frequently shifts between major vegetation types have occurred.

METHODS

Divergence times. We used a DNA phylogeny produced for *Florestina*, which was constructed using sequences from ITS and ETS regions (Soto-Trejo *et al.*, 2015). Divergence dating was conducted using BEAST v2.1.2 (Drummond & Bouckaert, 2014). As *Florestina* lacks any fossil record, we were forced to use secondary calibrations derived from the time-calibrated supertree of Asteraceae (Torices, 2010, Fig. S1) to constrain the age of the root node (13.05 Ma), the *Bahia*/*Hymenothrix*/*Palafoxia*/*Florestina* node (5.8 Ma), the *Hymenothrix*/*Palafoxia*/*Florestina* node (4.35 Ma) and the *Palafoxia*/*Florestina* node (2.175 Ma). Each node date prior was given a normal distribution with a standard deviation of 1 Ma. We used a relaxed molecular clock with uncorrelated log-normal variation in branch rates, and the tree prior was birth-death process. The GTR + G model

was used for the concatenated sequence matrix of two partitions (ETS and ITS). The final analysis consisted of two independent MCMC analyses; each chain was run for 50 000 000 generations (burn-in 25%) with parameters sampled every 5000 steps. The independent runs converged on the same results, and were combined using LogCombiner v2.1.2 (Drummond & Bouckaert, 2014). Tracer v1.5.0 (Rambaut & Drummond, 2009) was used to confirm adequate mixing and likelihood stationarity of the MCMC chain. Finally, the maximum clade credibility tree were generated using Treeannotator v2.1.2 (Drummond & Bouckaert, 2014), and was visualized in FigTree v1.3.1 (Rambaut, 2009).

Areas. Seven discrete areas were defined and modified using natural eco-geographical boundaries from the biogeographic provinces map of the Nacional Commission of Biodiversity of Mexico (CONABIO, www.conabio.gob.mx) and biogeographic regionalisation by Morrone (2014). The regions were: North Plateau (A) (including S USA), South Plateau (B) (including Sierra Madre Oriental and Gulf of Mexico), South Pacific Lowlands (C), Balsas Basin (D), Transvolcanic Belt (E), Sierra Madre Sur (F), and Baja California and California (G). Presence-absence of each species in each area was coded according to collection localities from herbarium specimens of the National Herbarium of Mexico (MEXU). Figure 2 shows the geographical distribution of the *Florestina* species on the map of biogeographic provinces.

Biogeographical model selection. We conducted maximum likelihood analyses under 12 different biogeographical models. The first three are based on models that have received widespread use in the historical biogeography literature. First, the Dispersal-Extinction Cladogenesis (DEC) model (Ree & Smith, 2008), which models dispersal as an anagenetic range-expansion process with rate parameter d, and "extinction" as an anagenetic range-contraction process with rate parameter e. DEC models geographic range change during

cladogenesis by assigning equal per-event weights to sympatry, subset sympatry, and vicariance. The second model is DIVALIKE, which is a likelihood interpretation of the DIVA model of Ronquist 1997; it has the same anagenetic processes as DEC, but disallows subset sympatry and allows vicariance in which both descendants are widespread. The third model is BAYAREALIKE, a likelihood interpretation of the model of the BayArea program proposed by Landis *et al.* (2013). In BAYAREALIKE, no change to geographic range occurs during cladogenesis; instead, the ancestral range is copied to both descendants, even if the ancestral range covers several areas (widespread sympatry). A graphical depiction of the processes assumed by each model is available in Fig. 1 of Matzke (2013). An additional three models were created by adding a third free parameter, j, that represents the relative per-event weight of founder-event speciation ("jump dispersal") during cladogenesis, creating DEC+J, DIVALIKE+J, and BAYAREALIKE+J models. Founder-event speciation was found to be important in island clades (Matzke, 2014), but its importance in continental systems such as *Florestina* needs to be tested.

Finally, each of these six models was modified by adding the free parameter x, creating DEC+x, DEC+x+J, etc. In +x models, the base dispersal rate (for d) or relative weight (for j) is multiplied by distance^x. Here, distance was the minimum linear distance between areas, measured in kilometers (km) using an online calculator of the great circle distance (http://www.freemaptools.com/how-far-is-it-between.htm). In +x models, if $x=0$, distance has no effect on dispersal probability, and the model reduces to one of the six simpler models. If $x<0$, then dispersal probability declines as distance increases. In the case where areas are touching, we set the minimum distance between areas to be 1 km, such that the dispersal probability between adjacent areas is set by the other parameters, regardless of the value of x.

Due to the small size of our study clade, there is a significant chance that the dataset will lack power to reliably distinguish between some models. Therefore, the fit of each model to the data was compared with the Akaike Information Criterion corrected for small sample size (AICc), as recommended by Burnham & Anderson (2002). AICc was used to calculate the relative likelihood and model weight of each model. The likelihood ratio test (LRT) was also used to compare pairs of models in cases where models were nested (e.g. DEC+x+J reduces to DEC+J when $x=0$, and equals DEC when $x=0$, $j=0$).

Estimation of ancestral ranges and biogeographical event histories. Each inference run in BioGeoBEARS yields the ML estimates of each free parameter, and also calculates the probability of each possible ancestral state at each node (each "state" is a particular geographic range). These are ancestral state estimates under the global ML model (Felsenstein, 2004), and BioGeoBEARS produces charts showing either the single most-probable range at each node, or pie charts showing the probability of all ranges. These plots may be inspected to get the probable history of specific biogeographical events that have occurred, but it is important to keep in mind that ML ancestral range estimates represent averages over all histories possible under the model and the ML parameter estimates. An improved method is stochastic mapping, a standard technique for DNA and morphological characters (Nielsen, 2002; Huelsenbeck *et al.*, 2003; Ree, 2005; Bollback, 2006; Revell, 2011) which has recently been added to BioGeoBEARS for biogeographical models (tutorial and validation examples are available at http://phylo.wikidot.com/biogeographical-stochastic-mapping-example-script). Each stochastic map represents a simulated realization of a possible exact history, conditional on the dated tree, the model, the ML parameter estimates, and the observed geographic range data. By generating a large number of stochastic maps counting the biogeographical events and their directionality, a valid

estimate is o btained o f t he mean co unt o f eac h t ype o f e vent (e.g. range e xpansion dispersal, jump dispersal and vicariance), as well as the standard de viation in each e vent count. We r an 50 biogeographical stochastic maps u nder t he best-fitting bi ogeographical model; means and standard deviations of events across the 50 histories were calculated.

Ancestral vegetation type estimation. We also used BioGeoBEARS to evaluate ancestral vegetation type of *Florestina*. We defined three vegetation types: temperate forests, desert areas, and tropical dry forests. Each species was coded by whether or not it occupied each vegetation type. Here, the six basic biogeographical models were used for inference; in this context, the "geographic range" is really a "vegetation types occupied" character, and not the areas defined above, which can be modified by anagenetic or cladogenetic events.

RESULTS

Divergence time. Divergence t ime es timates from co ncatenated I TS and ETS r egions revealed that an initial divergence of *Palafoxia* and *Florestina* occurred during the Pliocene approximately 3.54 Ma (HPD 2.165–4.228 Ma) and the diversification within *Florestina* occurred from the late P liocene to th e Pleistocene (Fig. 3). Divergence o f cl ade A (*F. latifolia* and *F. platyphylla*) and clade B (includes the r emaining species) occurred 2.132 Ma (HPD 1.5–3.46 Ma). Within clade B, the oldest divergence was the split between *F. purpurea* and the rest of the species, estimated at 1.72 Ma (HPD 1.09–2.87 Ma); divergence of *F. lobata* and the clade formed by *F. tripteris* and *F. pedata* was estimated at 1.02 Ma (HPD 0.49–1.49 Ma); and the most recent divergence was the split between *F. tripteris* and *F. pedata* that was estimated at 0.56 Ma (HPD 0.2–0.67 Ma).

Biogeographical model selection. The lo g-likelihoods (LnL) o f t he d ata u nder the 1 2 models are presented in Table 1. Despite the small size of the dataset, there were substantial

differences in model performance: LnLs ranged from -47.51 (BAYAREALIKE) up to -30.97 (DEC+x). While differences in the cladogenesis assumptions tended to have only moderate impact on model performance (changing LnL by a few units at most), adding the x parameter, representing decay in dispersal probability with distance had a massive effect, typically increasing LnL by 10+ units. This represents an improvement of about $e^{10} \approx 22,000$ times in data probability. Overall, the six +x models garnered 99.99% of the model weight, with one model, DEC+x, gathering 88.8% of the model weight by itself. Most of the rest of the model weight, 9.67%, accrued to DEC+x+J.

As would be expected for such a strong result for +x models, the ML estimate of x was strongly negative (x=-4.8 under DEC+x), although this was reduced substantially under +J models (e.g. x=-0.9 under DEC+x+J). Other parameter estimates are shown in Table 1. The base rate of range-expansion (d) was much higher in the +x models, indicating that the dispersal rate between adjacent or nearby areas is actually high, even though the dispersal rate between distant regions is low. In the non-x models, d estimates are about 4.5 times lower, which makes sense as these models have to fit one constant dispersal rate between all regions, regardless of distance. The founder-event speciation weight parameter j, when present, is often substantial (j can range from 0 to 3, where 3 means that all cladogenesis events are jump dispersals, and the other three cladogenesis processes have 0 weight). However, the fact that estimates of d usually do not change very much when j is added, and neither do the log-likelihoods, indicate that j is not a particularly important explanatory variable in this dataset. The parameter e, representing the rate of range-loss, is typically estimated to be ≈0. This behavior is typical in DEC and DIVALIKE-type models, where it is difficult to detect extinction/local extirpation (Ree & Smith, 2008; Matzke, 2014). The exception is the BAYAREALIKE models, which, lacking vicariance and other processes

that r educe r ange s ize at c ladogenesis, h ave a s ubstantial e estimate. H owever, BAYAREALIKE-type models are poor fits to our dataset: all four put together only gather 0.20% of the total model weight.

Ancestral range estimates. Ancestral r ange es timates u nder t he o ptimum model (DEC+x) are presented in Fig. 4. The left panel shows the single-most probable range at each node, and t he r ight p anel shows p ie c harts g iving t he r elative p robability o f e ach o f t he 1 28 possible r anges at each n ode (without c onstraints o n the maximum r ange s ize, 7 ar eas means t hat t here ar e 2^7 possible co mbinations o f p resence/absence in t hese ar eas). As is expected for a small dataset with many widespread taxa, uncertainty is very high at many of the deeper nodes.

Biogeographic stochastic mapping. Plots of 5 0 r ealizations o f biogeographic history stochastically mapped u nder the DEC+x model. Each biogeographic stochastic map has a certain number o f d ispersal (range e xpansion), v icariance, s ubset s ympatry, a nd narrow sympatry events. Variability in these event counts across the 50 stochastic maps is shown in Fig. 5. It can be seen t hat narrow s ympatry is a quite r are ev ent (0-2 ev ents p er r ealized history), w hereas vicariance a nd s ubset s ympatry ar e more co mmon (4-6 e vents pe r realized history). R ange-expansion d ispersal is t he most c ommon e vent (about 1 1 e vents per realization).

The directionality of dispersal across stochastic maps is indicated in Fig. 6. It can be seen t hat d ispersal is o nly o bserved between ar eas that ar e ad jacent. T his is t he strongest form o f a d istance e ffect, b ut h as implications for the i nterpretation o f x estimates (see discussion).

Ancestral vegetation type estimation. The lo g-likelihood (LnL) o f t he d ata u nder t he 6 models f or estimation of a ncestral vegetation t ype are p resented in Table 2 . The L nLs

ranged from -0.23 (BAYAREALIKE) up to -19.45 (DEC+j). Our results indicate that the common ancestor of *Florestina* and *Palafoxia* occurred in desert habitats, whereas the tropical dry forest was the ancestral type of vegetation for *Florestina* species.

DISCUSSION

Biogeographic model selection and ancestral area estimation. The fact that the ancestral ranges estimates exhibit high uncertainty in the deeper nodes in the *Florestina* phylogeny is not unexpected. This is a small clade, several of the species are widespread, and sister species tend to have different ranges. In this situation, many of the ancestors will be widespread, and the nature of DEC-type models is that the number of possible cladogenetic range-inheritance scenarios increases as the size of the ancestral range increases. For example, under DEC, if the ancestor inhabits two areas (range AB), there are six possible cladogenesis scenarios, namely 2 vicariance (A, B and B, A) and 4 subset sympatry (A, AB; B, AB; AB, A; and AB, B); for 6 areas, there are 24 scenarios, and for 7 areas, 28 scenarios. In the *Florestina* analysis, any ancestral node could have a range from 1 to 7 areas. The resulting uncertainty is shown in Fig. 4.

However, despite this uncertainty, the probabilistic framework we have employed using BioGeoBEARS allows determine with confidence the strong effect of distance on dispersal probability between adjacent regions (see Fig. 6). It should be noted that adjacent-area-only dispersal can be modeled in other ways in BioGeoBEARS, for example with a manual dispersal constraints matrix that fixes a dispersal probability of 0 between non-adjacent regions. However, because of the complexity of the living and ancestral ranges in the case of *Florestina*, such a strategy was not an obvious one to take *a priori*. In contrast, proposing and testing a model where dispersal depended on distance allowed us to discover

the importance of adjacency in this clade. Furthermore, the manual dispersal constraints approach is a quite inflexible one: if our data had indicated even just a few dispersal events between non-adjacent regions, a model fixing the dispersal to 0 for non-adjacent areas would have yielded a low likelihood and been considered a bad model, even though there was adjacency information to discover. The +x models are a more flexible approach and may have widespread utility as a result.

Biogeographical stochastic mapping proved to be very useful for at least two reasons. First, visualization of the variability in biogeographic histories that could possibly explain the observed ranges provides appraisal of uncertainty, preventing the common problem of over-interpreting plots of "most probable range at each node". Naïve interpretation of such most-probable-range plots is hazardous when uncertainty is high, as even the "most probable" range at a node might have a total probability of 5% or less, compared to the summed probability of all of the other possible ranges. Stochastic mapping also helps to emphasize that in probabilistic biogeography modeling, *estimation* of ancestral ranges, and many ancestral ranges and histories may be reasonably probable given a particular dataset and model. This concept contrasts strongly with the ubiquitous "ancestral area reconstruction" terminology that is employed in historical biogeography. The reconstruction terminology derives from parsimony analyses, and tends to focus attention on one possible history, excluding some others that might have similar probability.

Stochastic mapping was also highly useful in estimating the number and directionality of dispersal events in the *Florestina* clade. Researchers interested in measuring dispersal in the history of a clade will often inspect ancestral range estimation plots and count dispersal events by eye (e.g. de Bruyn *et al.*, 2014), but this strategy tends to reinforce the above-mentioned problems of assuming a single possible history, and

furthermore it is particularly difficult to apply in a case like *Florestina* where uncertainty is high and no "simple" history presents itself in the summary ancestral range graphics.

Divergence time and ancestral area estimation of Florestina. Even though the ancestral ranges estimates showed a high uncertainty in the deeper nodes in the *Florestina* phylogeny, our calibrated phylogeny suggests that the ancestors of *Florestina* and *Palafoxia* diverged around 3.54 Ma (HPD 2.165–4.228 Ma) (Fig. 3). During this epoch, the development of Transvolcanic Belt was a major geologic and climatic event that contributed greater geographic heterogeneity and increasing aridity over most of the Mexican territory (Ferrusquía-Villafranca & González-Guzmán, 2005). Therefore, the results are consistent with the hypothesis that the Transvolcanic Belt formed a biogeographic barrier, which contributed to split of the ancestors of *Florestina* and *Palafoxia*. Probably, the most recent common ancestor of these two genera had a widespread range of distribution from the South United States and North Mexico to South and Central Mexico. After the separation, the *Palafoxia* lineage possibly occupied the southern United States and northern Mexico, whereas the *Florestina* lineage was probably confined to southern and central Mexico. Biogeographic studies indicate that the Transvolcanic Belt has been an important barrier influencing the diversification of several lineages (e.g. Sosa *et al.*, 2009; Bryson *et al.*, 2011, 2012; Ruiz-Sánchez *et al.*, 2012; Ruiz-Sánchez & Specht, 2013; Gándara & Sosa, 2014; Gándara *et al.*, 2014). Furthermore, we could hypothesize that the common ancestor of *Florestina* and *Palafoxia* was a taxon of desert floras from boreal dry lands, as suggested by Axelrod (1975, 1979) for the origin of the flora that colonized the North American Deserts, not from Neotropical regions as suggested by Turner & Morris (1976) for *Palafoxia*. This hypothesis is supported by our

estimation of the ancestral vegetation type, which indicated that the ancestors of *Florestina* and *Palafoxia* occurred in desert habitats.

Divergence time estimates revealed that the diversification within *Florestina* occurred from the late Pliocene to the Pleistocene (Fig. 3) suggesting that multiple events have occurred in a relatively short time period. Divergence of clade A and B occurred around 2.132 Ma (HPD 1.5–3.46 Ma), this split is significant in involving a change in base chromosome number. The two species in clade A have chromosome number $n = 12$, whereas three taxa, *F. lobata*, *F. tripteris* and *F. pedata*, belonging to clade B have $n = 10$, which could have arisen through descending dysploidy (Soto-Trejo et al. 2015). Within clade A, *F. platyphylla* and *F. latifolia* diverged very recently within the last 310,000 years in southern Mexico. *Florestina platyphylla* is endemic to central valleys in the state of Oaxaca, Mexico and is restricted to higher altitudes from 1200-2000 m, whereas *F. latifolia* is found from southern Oaxaca in the Isthmus of Tehuantepec to Nicaragua on lower altitudes from 100-1200 m (Fig. 2). Probably, the common ancestor of *F. platyphylla* and *F. latifolia* was a more widespread taxon in the southern Mexican lowlands. Additionally, we could hypothesize that *F. latifolia* reached its current distribution in Nicaragua lowlands via dispersal, which is consistent with the hypothesis that the Isthmus of Tehuantepec has acted as a corridor for the species from the Mexican lowlands to Mesoamerican lowlands at different times (Flores-Villela & Martínez-Salazar, 2009). Moreover, the diversification in clade B occurred during the Middle and Late Pleistocene and its most recent common ancestor probably occupied a widespread range of distribution in South and Central Mexico. The oldest divergence in the clade B was the split between *F. purpurea* and the remainder of the species, which occurred around 1.72 Ma (HPD 1.09–2.87 Ma). *Florestina purpurea* is restricted to the Tehuacán-Cuicatlán Valley in the Sierra Madre Sur where it

occurs mainly i n tropical dry f orests. Dávila *et al*. (2002) and Méndez *et al*. (2004) recognized t he Tehuacán-Cuicatlán V alley as a r egion w ith a r ich flora and fauna, and a particularly high endemism.

Florestina lobata is a species locally r estricted t o the B alsas B asin in s outhern Mexico, which diverged from subclade formed by *F. sp*., *F. tripteris* and *F. pedata* within 1.02 Ma (HPD 0.49–1.49 Ma). Regarding *F. sp*., it diverged r ecently within t he last 660,000 years (HPD 0.3–0.91 Ma) from the *F. tripteris* and *F. pedata* subclade. *Florestina sp*. is morphologically similar to *F. tripteris* and i t is restricted t o a s mall r egion near Ciudad M aíz in e astern S an Luis P otosí, but additional studies ar e needed t o test i f t his population should be considered a new species. Finally, the divergence between *Florestina tripteris* and *F. pedata* occurred recently 0.56 Ma (HPD 0.2–0.67 Ma). *Florestina pedata* is a morphologically variable species that is widespread in Central and South Mexico (Fig. 2). *Florestina tripteris* inhabits de serts o f southern Texas a nd northern Mexico and probably reached its current distribution via dispersal from the Central Mexico to the North Plateau.

Our d ivergence t ime es timates indicate t hat the diversification w ithin *Florestina* occurred m ainly in the Pleistocene (Fig. 3). The c limatic fluctuations o f t he P leistocene allowed t he e volution o f floristic e lements within r efugia, pr oducing numerous a llopatric and p arapatric p resent-day d istributions (Flores-Villela & M artínez-Salazar, 2009). T he current d istributions o f *F. latifolia*, *F. platyphylla*, *F. purpurea* and *F. lobata* (Fig. 2) suggest that these species e volved from one ancestral widespread taxon during P leistocene and that subsequent isolation o f populations resulted in the differentiation. This hypothesis is supported by our findings of the number of dispersal events is relatively low between all areas (Fig. 6), and it i s particularly low between the Balsas B asin, S ierra M adre S ur a nd

South Pacific Lowlands areas, where these four species are located. These regions are recognized as with high endemism had been considered as a refugium for plants and animals (e.g. Bryson *et al.*, 2011, 2012; Gámez, *et al.*, 2014; Gándara & Sosa, 2014). Furthermore, in Asteraceae the pappus is often involved in dispersal, and a very common type is to have a pappus of many capillary bristles which may aid in wind dispersal. In *Florestina*, however, the pappus consists of a relatively few scales, sometimes with awned tips. There is some variability in pappus size and prominence of awns, but the species with the most prominent awns, *F. purpurea*, has a relatively restricted distribution (Fig. 2). In contrast, the pappus of the two most widespread species, *F. tripteris* and *F. pedata*, are among the smallest. Therefore, this study suggests that the pappus does not appear to be involved in long distance dispersal in *Florestina*.

Additionally, our estimation of the type of ancestral vegetation indicates that the ancestor of *Florestina* probably inhabited tropical dry forests, which is one of the most widespread vegetation types of Mexico and is characterized by high endemism (Rzedowski, 1978). According to Becerra (2005), the Mexican tropical dry forest originated at least 20 Ma, long before the estimated divergence time of *Florestina* and *Palafoxia* (approximately 3.54 Ma, HPD 2.165–4.228 Ma), thus dry forests were already established in south and central Mexico, where the majority of *Florestina* species occurs and are endemics. The high endemism in tropical dry forests could be attributed to the climatic changes of the Pleistocene and probably functioned as refugium for a biota that earlier occupied larger and more continuous areas (Flores-Villela & Martínez-Salazar, 2009).

A final general point is that the analyses of smaller datasets tend to estimate the ancestral areas to be very uncertain, as in *Florestina*. Future studies analyzing a dataset that

comprises a larger clade including many of *Florestina*'s relatives, would yield more power in ancestral area estimation. In order to further substantiate that vegetation appears to be an important factor in the evolution of *Florestina*, we need better paleoclimatic estimates of past vegetation in the areas where the species occur. This would help us determine whether the habitat for these species is conserved and the implications this might have on the extinction risk of these species in the face of global climate change. Furthermore, studies including more endemic dry-land taxa with their paleo-histories might provide better understanding into the patterns and causes of diversity in arid and semiarid regions of Mexico. Finally, we must be kept in mind that for a given taxonomic group there may be several potential histories with varied probabilities. In this paper, we infer the most likely explanation of diversification history in *Florestina*, hoping to provide an insight into the diversification of Mexican Asteraceae in dry environments.

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BIOSKETCHES

Fabiola S oto-Trejo, a P hD ca ndidate at t he U NAM (Campus I ztacala), is interested in understanding distributional patterns in an evolutionary context for Mexican Asteraceae.

Author c ontributions: F.S., E .E.S. and P.D. conceived the i deas; F .S. collected t he d ata; F.S., K.M. and N.J.M. analyzed the data; and all authors shared in the writing.

FIGURE LEGENDS

Figure 1. Map o f t he ge ographical d istribution o f *Florestina* and its r elated g enera *Hymenothrix* and *Palafoxia*.

Figure 2. Geographical distribution of the *Florestina* species on the map of biogeographic provinces.

Figure3. Chronogram of the maximum clade credibility tree estimated from ETS and ITS sequences using BEAST of *Florestina* and its closest relatives. B., *Bahia*; C, *Chaenactis*; H., *Hymenothrix*; P., *Palafoxia*; and S., *Schkuhria*. Bars on the nodes represent 95% highest probability density (HPD) for the a ge o f t hat no de. Numbers a bove t he br anches indicate mean ages. A partial timescale is shown at the bottom, with units in millions of years.

Figure 4. Estimates o f a ncestral r anges for *Florestina* under t he M L D EC+x model. d=0.3211; e=0; j=0; x=-4.766; LnL=-31.11. Left: plot of the single most-probable range at each node. R ight: pie charts showing the probability o f all possible ancestral states at each node, showing considerable uncertainty in the exact geographic range at deeper nodes. The colors of w idespread r anges ar e mixtures o f t he co lors u sed for t he s ingle ar eas: for example, the range AB is light blue, as its constituent areas are A (dark blue) and B (cyan). Range co des: North P lateau (A) (i ncluding S U SA), South Plateau (B) (including S ierra Madre O riental and G ulf o f Mexico), S outh P acific Lowlands (C), B alsas Basin (D), Transvolcanic Belt (E), Sierra Madre Sur (F), and Baja California and California (G).

Figure 5. Distribution o f event counts across 50 biogeographic stochastic maps generated under the ML DEC+x model

Figure 6. Map showing the means and 95% confidence intervals for each possible dispersal event for t he w hole t ree, observed acr oss t he 50 biogeographic stochastic maps u nder t he DEC+x mo del. Arrows indicate the d irectionality o f d ispersal acr oss stochastic maps. Fo r this dataset and model, dispersal is perfectly correlated with adjacency, in that no dispersals happen between non-adjacent areas.

Figure 1
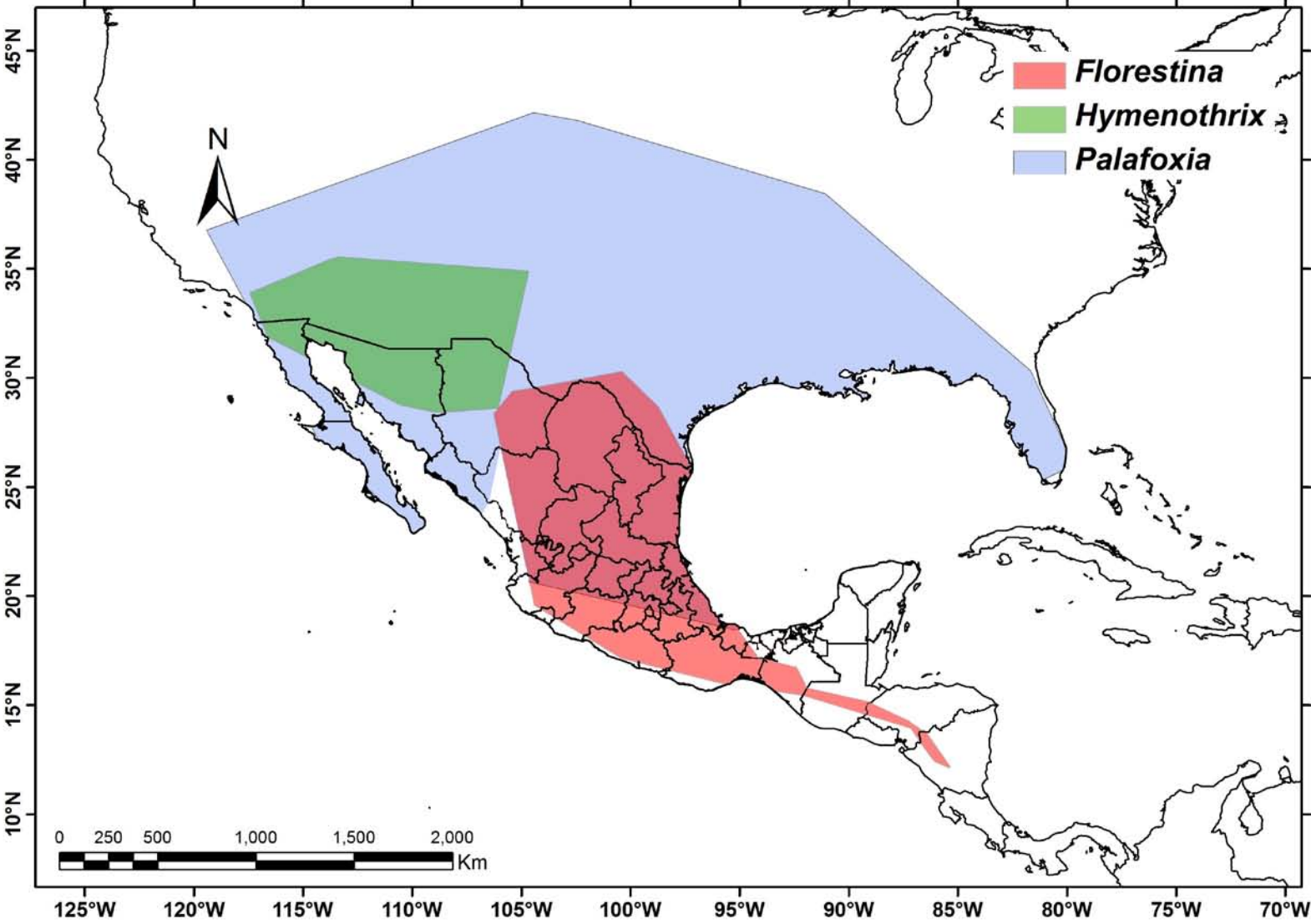
Figure 2
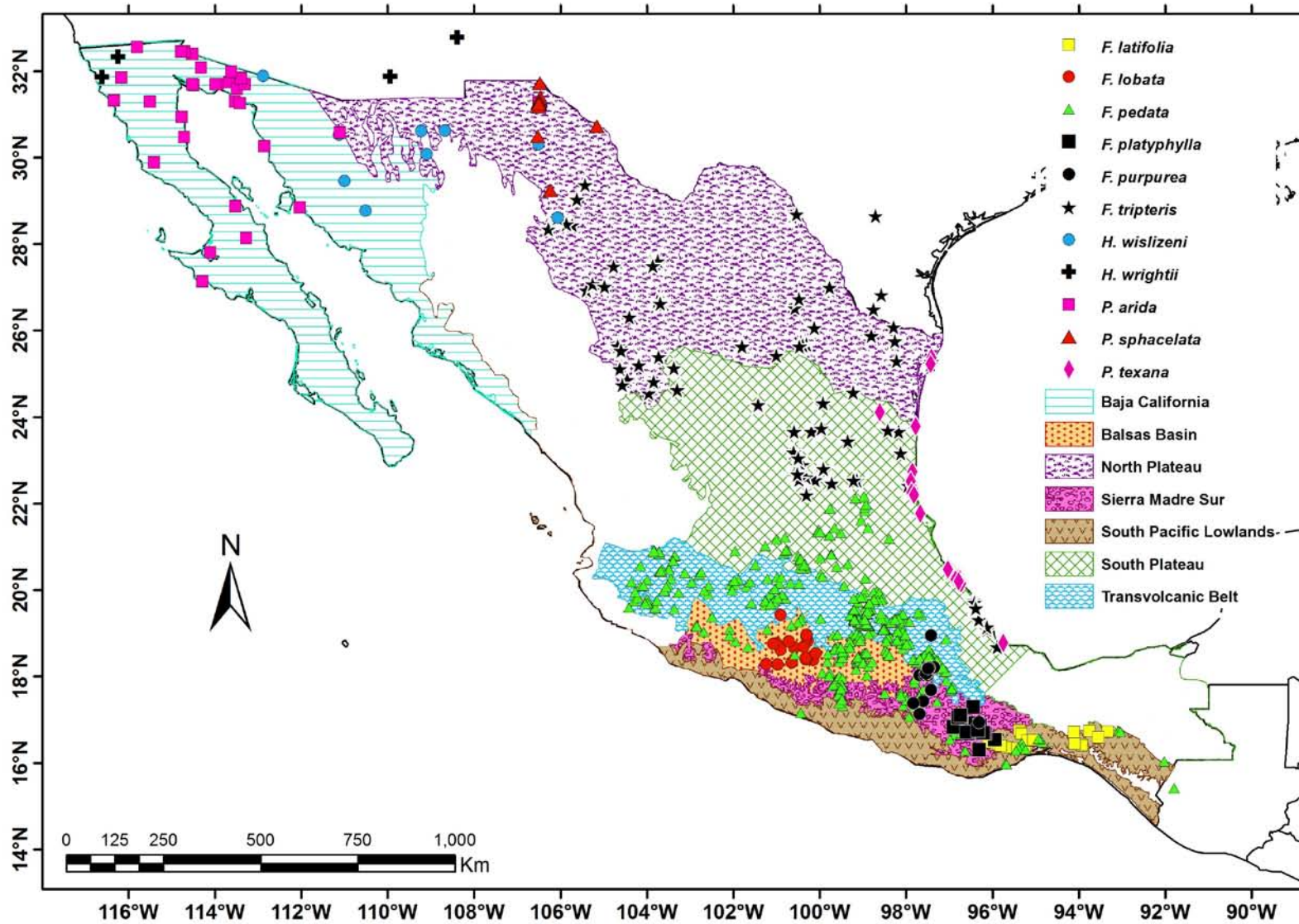
Figure 3

Figure 4

Figure 5

Event counts in each of 50 BSMs
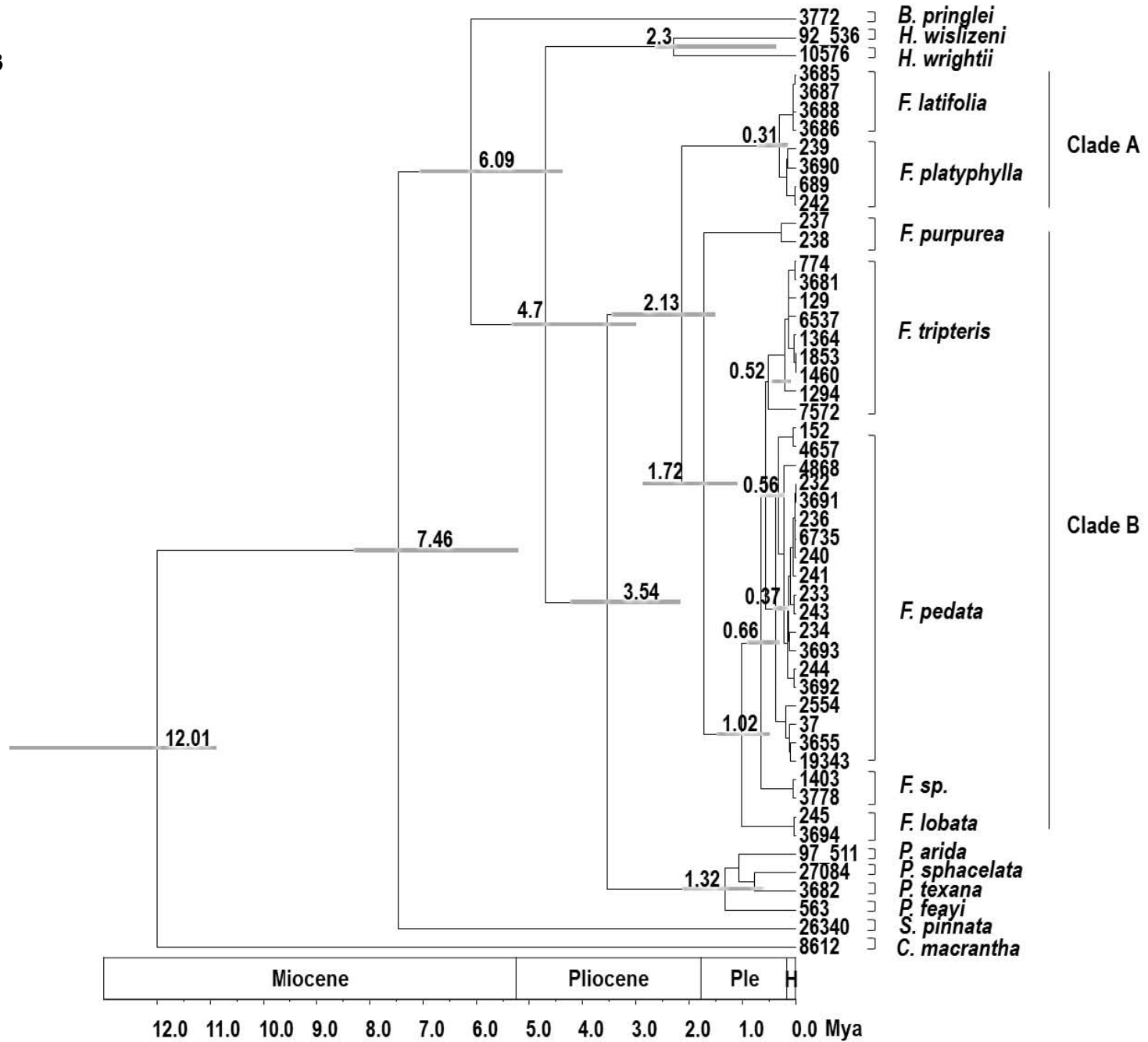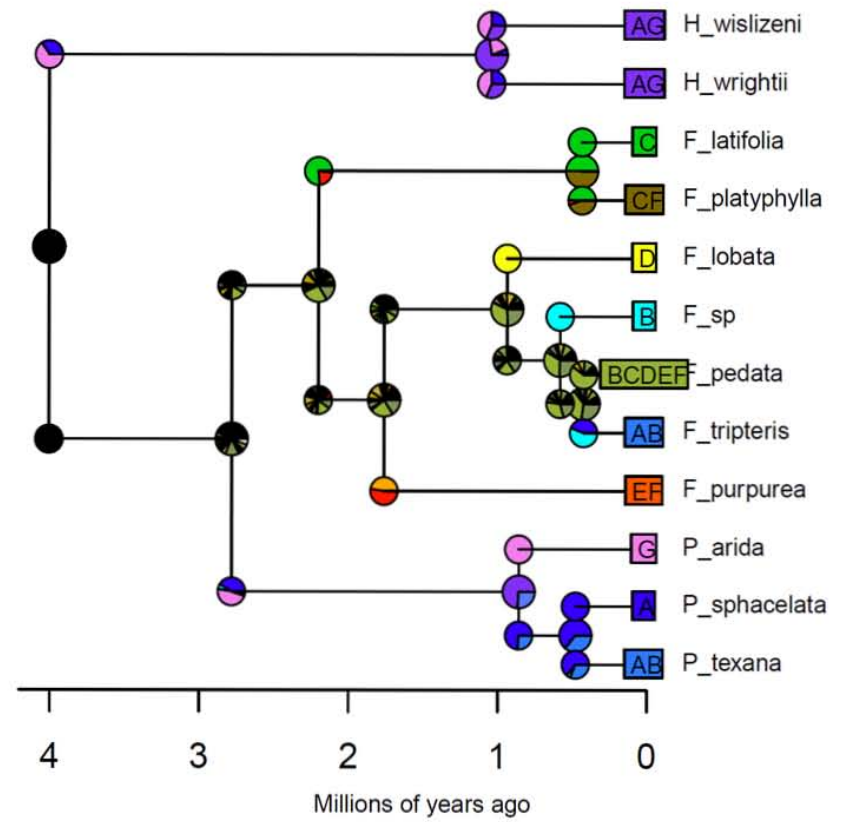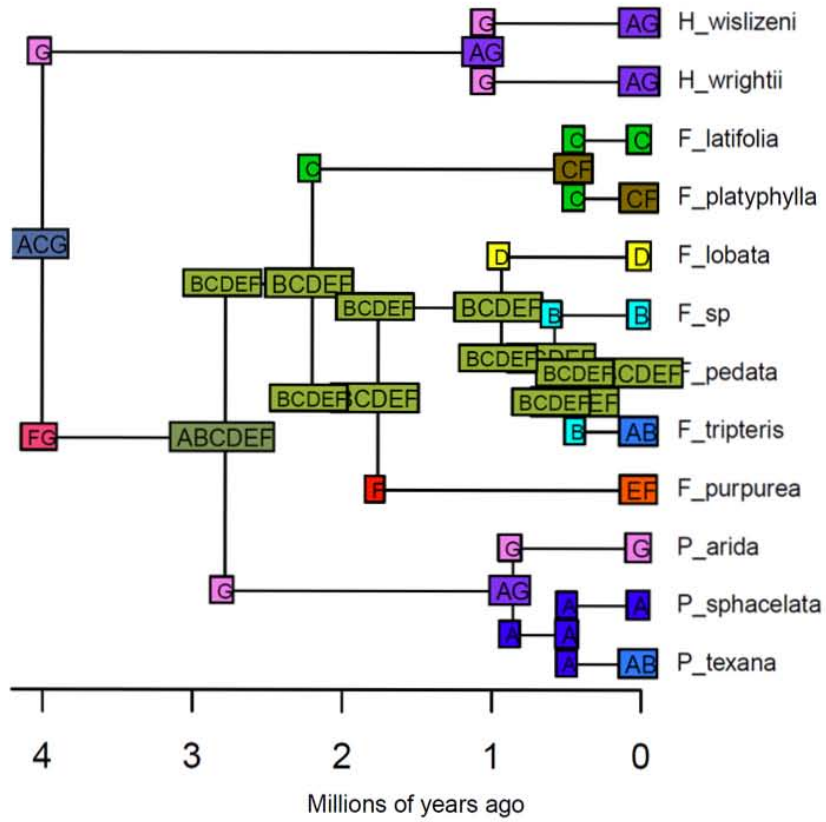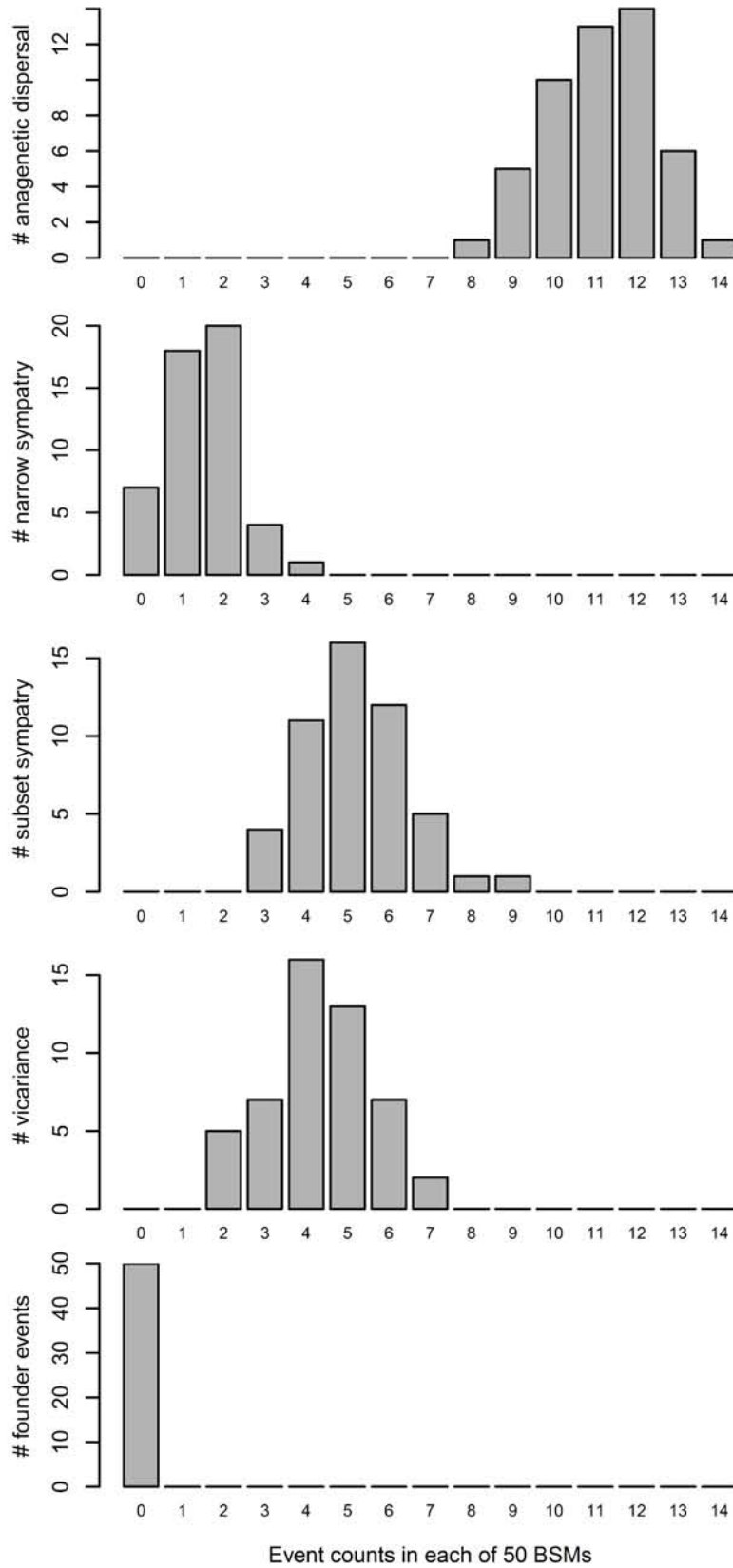
Figure 6
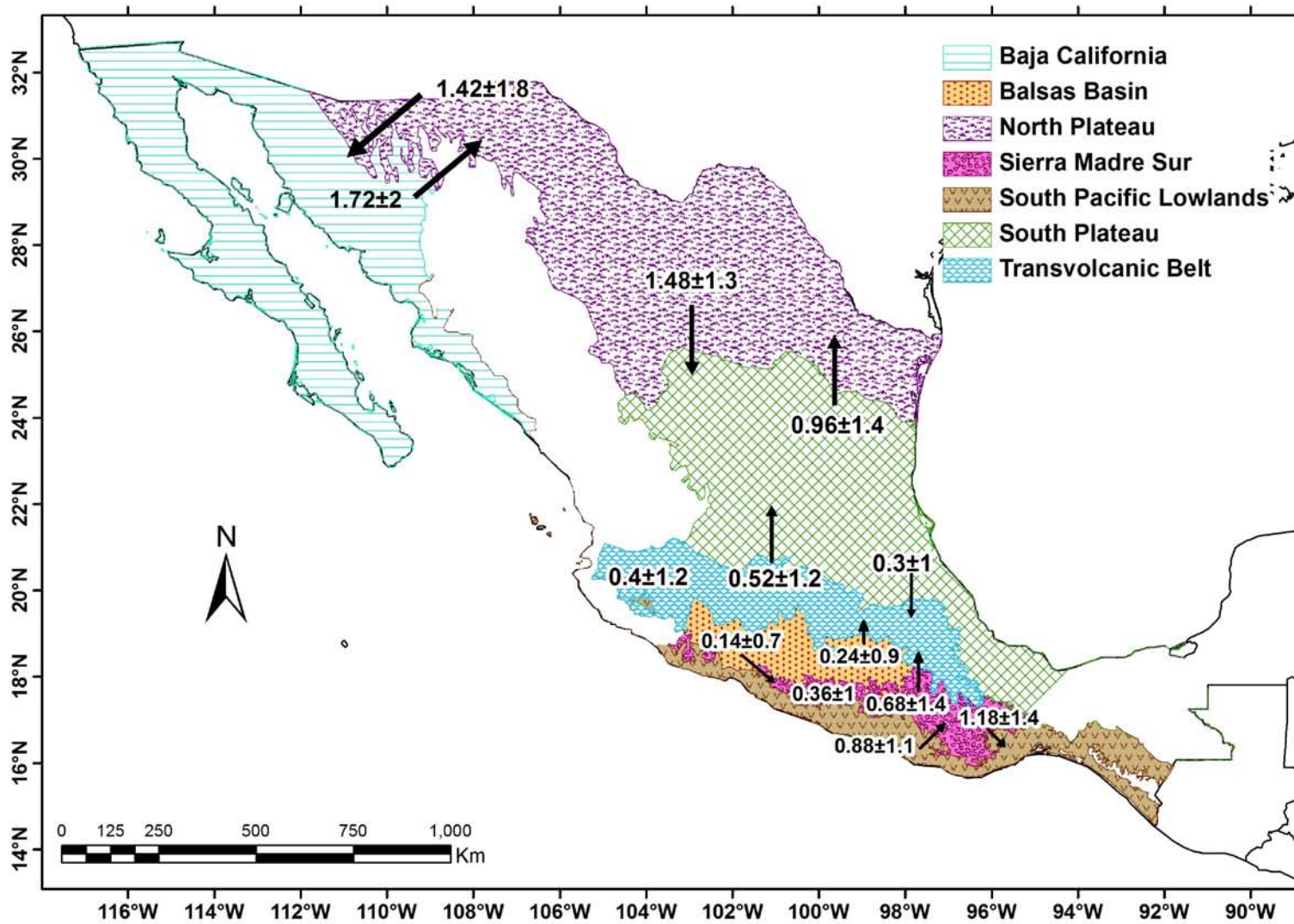
Table 1. Maximum log-likelihood of *Florestina* range data under each model, and resulting AICc and model weights. d = dispersal, e = extinction, j = jump dispersal, *x* = distance parameter. K = number of parameters, n = number of data, AICc = Akaike Information Criterion corrected for small sample size, RL = relative likelihood.

Model	*D*	*e*	*J*	*x*	LnL	K	n	AICc	delta AICc	RL	model weight
DEC	0.070	0.000	0.000	0.000	-42.68	2	12	90.70	19.47	5.9E-05	0.01%
DEC+J	0.068	0.000	0.032	0.000	-42.47	3	12	93.94	22.71	0.0000	0.00%
DIVALIKE	0.097	0.000	0.000	0.000	-46.07	2	12	97.48	26.25	2.0E-06	0.00%
DIVALIKE+J	0.097	0.000	0.000	0.000	-46.07	3	12	101.14	29.92	3.2E-07	0.00%
BAYAREALIKE	0.127	0.509	0.000	0.000	-47.51	2	12	100.35	29.13	4.7E-07	0.00%
BAYAREALIKE+J	0.057	0.132	0.138	0.000	-46.15	3	12	101.30	30.07	2.9E-07	0.00%
DEC+x	0.321	0.000	0.000	-4.767	-31.11	3	12	71.23	0.00	1.0000	88.80%
DEC+x+J	0.308	0.000	0.145	-0.903	-30.97	4	12	75.66	4.43	0.1089	9.67%
DIVALIKE+x	0.415	0.000	0.000	-4.808	-35.50	3	12	80.01	8.78	0.0124	1.10%
DIVALIKE+x+J	0.368	0.000	0.515	-0.746	-34.76	4	12	83.23	12.00	0.0025	0.22%
BAYAREALIKE+x	0.653	0.862	0.000	-1.618	-38.45	3	12	85.90	14.68	6.5E-04	0.06%
BAYAREALIKE+x+J	0.308	0.147	0.732	-0.735	-35.20	4	12	84.12	12.90	0.0016	0.14%

Table 2. Maximum log-likelihood of *Florestina* vegetation type data under each model, and resulting AICc. *d* = dispersal, *e* = extinction, *j* = jump dispersal, AICc = Akaike Information Criterion corrected for small sample size, K = number of parameters, n = number of data,

Model	*D*	*E*	*J*	LnL	K	n	AICc
DEC	0.1911	1.00E-12	0.0000	-19.474	2	12	42.95
DEC+J	0.1874	1.00E-12	0.0148	-19.452	3	12	44.90
DIVALIKE	0.2351	1.00E-12	0.0000	-20.022	2	12	44.04
DIVALIKE+J	0.2351	1.00E-12	1E-05	-20.022	3	12	46.04
BAYAREALIKE	0.2313	2.97E-01	0.0000	-23.347	2	12	50.69
BAYAREALIKE+J	0.2313	2.97E-01	1E-05	-23.347	3	12	52.69

CAPÍTULO III

REVISIÓN TAXONÓMICA DEL GÉNERO *FLORESTINA* (ASTERACEAE, BAHIEAE)

Taxonomic Revision of *Florestina* (Asteraceae, Bahieae)

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ABSTRACT

Florestina (Asteraceae, B ahieae) includes s ix annual he rbaceous species n ative t o the southern U nited S tates an d M exico. The taxonomy, t he phylogenetic r elationships, the chromosome e volution as well as the biogeographic history of *Florestina* are reviewed. A dichotomous ke y is pr ovided for t he s pecies o f t he ge nus. Additionally, l ectotype was designated here for *Florestina pedata*.

Keywords Asteraceae**,** *Florestina*, Mexico.

INTRODUCTION

Florestina Cass. (Asteraceae, B ahieae) comprises a s mall group o f annual herbs occurring from the southern United S tates t hrough M exico, and into Central America a long Guatemala, Honduras and Nicaragua. The species grow in arid and semiarid environments such a s xerophytic s crub and seasonal d eciduous forests. The genus i s characterized b y having uniseriate phyllaries, zygomorphic co rollas an d cu spidate ap pendages o f t he s tyle branches. The achene and flower morphology of *Florestina* species are illustrated in Fig. 1. All s pecies ar e morphologically s imilar an d frequently vegetative an d r eproductive characters o verlap a nd make it d ifficult t o d efine t he b oundaries o f eac h s pecies (Turner, 1963; Soto-Trejo et al., 2015).

Taxonomic history of *Florestina*. The ear liest taxonomic t reatment o f *Florestina* was performed by Rydberg (1914), who circumscribed six s pecies in the g enus: *F. latifolia* (DC.) Ry db., *F. liebmannii* Sch.Bip. e x G reenm., *F. pedata* (Cav.) C ass., *F. platyphylla*

(B.L. Rob. & Greenm.) B.L. Rob. & Greenm, *F. purpurea* (Brandegee) Rydb., and *F. tripteris* DC. Later, Turner (1963) added two newly-described species: *F. simplicifolia* B.L.Turner and *F. lobata* B.L.Turner. On the other hand, *Florestina* has been considered to be related to several genera by different authors, including *Schkuhria* Roth (Hoffmann 1894), *Hymenothrix* A.Gray (Turner 1962, 1963), and *Palafoxia* Lag. (Shinners 1952; Baldwin *et al.* 2002).

Phylogenetic relationships of *Florestina*. Phylogenetic relationships of *Florestina* were first examined by Turner (1963). According to the Turner´s morphology-based study, three main groups were suggested in the genus *Florestina*. Five species, *F. simplicifolia*, *F. liebmannii*, *F. tripteris*, *F. pedata*, and *F. lobata*, formed a group of morphologically very similar species, although their phylogenetic relationships were unresolved. Another group is formed by *F. latifolia* and *F. platyphylla*, which are morphologically similar from each other. Finally, *F. purpurea* is separated from the rest of the species. Therefore, the phylogenetic relationships among species were mostly unclear and unresolved.

Recently, the phylogenetic relationships of *Florestina* were examined by Soto-Trejo et al. (2015). This study presented results of a parsimony analysis of a morphological dataset, which did not resolve the *Florestina* phylogeny and failed to find synapomorphic characters supporting the monophyly of the genus. In addition, a principal component analysis was only useful in recognizing as distinct *F. latifolia* and *F. platyphylla,* but not for the rest of the species. Therefore, the *Florestina* phylogeny based on morphological characters complicated both the recognition of taxa and the unambiguous reconstruction of their phylogenetic relationships. In contrast, the ETS and ITS spacer-based phylogenetic analyses recovered a highly resolved phylogeny, which indicated that *Florestina* is a

monophyletic gr oup a nd s upported *Palafoxia* as its c losest r elative. Two main s trongly supported c lades a nd t he r elationships a mong s pecies w ere mostly r esolved (Fig. 2). T he first o ne is c omposed o f *F. latifolia* and *F. platyphylla*, these are m arkedly different morphologically from the rest of the species and they are closely related. The second clade comprised the remainder of species. In this clade, *F. pedata* and *F. simplicifolia*, formed a subclade, but neither species is recovered as monophyletic, and do not support recognizing them as s eparate s pecies. Similarly, *Florestina tripteris* and *F. liebmannii* formed a subclade, t hese t axa ar e morphologically very similar a nd t hey are not s upported as a different species. Finally, a subclade is formed by *Florestina lobata*, which was recovered as the sister taxon to *F. purpurea*. These results suggested that the genus is formed by six species: *F. latifolia*, *F. platyphylla*, *F. purpurea*, *F. lobata* B.L Turner, *F. pedata*, and *F. tripteris*. The two additional taxa, *F. simplicifolia* and *F. liebmannii,* were included within *F. pedata* and *F. tripteris*, respectively.

Chromosome evolution of *Florestina*. Chromosome n umbers f or all *Florestina* species were pr eviously r eported (Table 1). An analysis of c hromosome e volution s upported the hypothesis that the most likely ancestral chromosome number in *Florestina* is $n = 12$ (Soto-Trejo et al., 2015). Therefore, the ch romosome n umber o f $n = 10$ in the genus might be interpreted a s a r eduction, a nd t he de scending d ysploidy c ould have pr oduced t his chromosome number. Dysploidy is an important evolutionary mechanism in plants and it is particularly co mmon in A steraceae (Guerra 2008; Galbany-Casals et al. 2009) and it have been associated to a tendency to shorten the life cycle as an adaptation to extreme or xeric habitats (Garcia-Jacas et al. 1996; Watanabe *et al.* 1999; Vilatersana *et al.* 2000; Torrell *et al*. 2001 ; Selvi a nd B igazzi, 2002; Garnatje et a l. 2004; Galbany-Casals e t a l. 2009).

Florestina pedata and *F. tripteris* with *n* = 10 seem to occur in more xeric habitats, such as xerophytic scrub like vegetation.

Biogeographic history of *Florestina*. The biogeographic history of *Florestina* was studied by Soto-Trejo et al. (in prep.). Divergence time estimates from the ETS and ITS spacer-based phylogeny revealed that the divergence of *Palafoxia* and *Florestina* occurred during the Pliocene, around 3.54 Ma (HPD 2.165–4.228 Ma). The Transvolcanic Belt formed a biogeographic barrier, which influenced the split of the ancestors of *Florestina* and *Palafoxia*. Therefore, *Florestina* originated from a widespread ancestor that was probably distributed from southern United States and northern Mexico to southern Mexico. Probably, the common ancestor of *Florestina* and *Palafoxia* was a taxon from boreal dry lands, as suggested by Axelrod (1975, 1979) for the origin of the flora that colonized the North American Deserts, but not from Neotropical regions as suggested by Turner & Morris (1976) for *Palafoxia*. Finally, the diversification within *Florestina* occurred mainly in the Pleistocene and it was driven by Pleistocene climate changes.

Other studies in *Florestina*. Domínguez *et al.* (1988) analyzed the chemical constituents of *Florestina tripteris,* and detected eupatolide, eupatoriopicrin and a derivative corresponding to 11, 13-dihydro compound. Furthermore four substitution products of borneol and a trihydroxymanoyloxide were present. Comparison of these chemical compounds of *F. tripteris* with other members of tribe Bahieae suggested that it is phytochemically similar to *Chaenactis*, *Palafoxia* and *Schkuhria*.

Germination responses of *Florestina pedata* to substrate type, rainfall, and temperature was evaluated by Rivas-Arancibia et al. (2006). *Florestina pedata* germinated preferably in temperatures lower than 20°C and rainfall over 80 mm. The combination of

temperature an d p recipitation d etermines their g ermination p ercentages and g ermination speed. The s eed g ermination o f *Florestina pedata* is co mplicated when rainfall is lower than 40 mm.

Thus, t he purpose o f t his s tudy is t o p resent a s ummary o f t he t axonomy o f *Florestina*, and resolve some taxonomic issues for the genus.

MATERIALS AND METHODS

Herbarium s pecimens from t he N ational H erbarium of M exico (MEXU) o f t he National University o f México (UNAM) were s tudied. Additionally, n ew m aterial co llected i n central-southeastern Mexico in 2012-2014 was also included in the analysis. The taxonomic conclusions p resented are based on morphological a nd molecular s tudies c onducted by Soto-Trejo *et al.* (2015). Specimens w ith latitude an d longitude d ata o n the l abels w ere mapped.

TAXONOMIC TREATMENT

Florestina Cass. (1817: 11)

Type species: *Florestina pedata* (Cav.) Cass.

Annual herbs, 10–130 cm tall. Stems erect, simple or scarcely br anched, nearly glabrous to densely p ubescent, hairs straight, strigose and/or glandular. Leaves simple or c ompound, when c ompound 3 -5–foliate o r biternately d issected, lower ones opposite, the upper ones alternate, petiolate, blades o vate to l inear, pu bescent, hairs straight o r strigose, w ith o r without glandular hairs. Inflorescences terminal, dichotomous or corymbose, 5 t o numerous h eads. Heads homogamous, pedunculate. Involucres turbinate. Phyllaries uniseriate, 8–13, green o r p urplish, scarious margins a nd o ften s uffused w ith purple,

glabrescent to densely pubescent, with or without glandular hairs. Rays absent. Disk florets 6–40, hermaphrodite, zygomorphic, white or pink to purplish, tubular, 5–lobed; tube and throat well defined, nearly glabrous to densely pubescent, with or without glandular hairs. Anthers 5, sacs rounded at the base, apical appendages ovate to obtuse. Style branches flattened, stigmatic lines terminated by a penicillate, cuspidate appendage, otherwise glabrous. Achenes obpyramidal, 4–angled, the faces smooth or distinctly 3–ribbed, nearly glabrous to densely pubescent; pappus 8–10 scales, scales ovate–lanceolate, apex acute, rounded or truncate. Chromosome numbers $n = 10, 12$.

KEY TO THE SPECIES OF *FLORESTINA*

1. Leaf blades ovate to cordate; phyllaries with glandular trichomes; disk florets purplish; pappus of 8 heteromorphic scales (the 4 longer scales ovate–lanceolate with apex awned, the 4 shorter scales ovate with rounded apex)…………………………………….…..2

2. Disk florets 6.0–7.0 mm long, pappus 4.0–6.5 mm long.................…………..…..*F. latifolia*

2. Disk florets 2.5–3.0 mm long, pappus 1.5–4.0 mm long..………..…………..*F. platyphylla*

1. Leaves or leaflets lanceolate to linear; phyllaries without glandular trichomes; disk florets yellowish white; pappus 8 homomorphic scales …………………………………….3

3. Leaflets with some glandular trichomes; achenes mostly glabrous but with hairs at the base; pappus of 8 lanceolate scales, 2.5–4.5 mm, apex predominately acute..……………………………………………...……………………...*F. purpurea*

3. Leaflets without glandular trichomes; achenes pubescent; pappus 8–10 ovate to obovate scales, 0.5–2.0 mm, apex rounded or truncate……………………………………….…..4

4. Stems with short glandular trichomes, 0.25–1.0 mm long ....…......5

5. Leaflets conspicuously and irregularly lobed; phyllaries glabrous……................*F. lobata*

5. Leaflets entire; phyllaries pubescent…………………….…………………...*F. tripteris*

 4. Stems with long glandular trichomes, 1.5–3.0 mm long…………...……..*F. pedata*

Florestina latifolia (DC.) Rydb. (1914: 58). *Palafoxia latifolia* DC. (1836: 125). *Polypteris latifolia* (DC.) Hemsl. (1887: 59). Type: MEXICO. Oaxaca: Tehuantepec, April 1834, *G. Andrieux 286,* (holotype: G, photograph of holotype G, DC, MO; isotype: K!).

Annual herbs, 30–80 cm. Stems erect, scarcely branched, densely pubescent with glandular hairs. Leaves simple, with the pubescent petioles 0.5–5.0 cm long, the lower ones opposite, the upper ones alternate, ovate, lamina finely pubescent on both faces with glandular hairs, serrate, 1.5–6.0 cm wide, 2.5–8.0 cm long, the apex acute, the base obtuse to truncate. Inflorescence a dichotomous cyme with 10–80 heads, peduncles 1.0–6.0 cm, slender, pubescent with glandular hairs. Heads turbinate, homogamous, 9.0–12.5 high wide. Phyllaries uniseriate 7–10, ovate–lanceolate, 5.0–9.0 mm long, 2.5–6.0 mm wide, purplish, obtuse or rounded tip, pubescent with short, stiff, spreading hairs interspersed with glandular trichomes. Rays absent**.** Disk florets 20–40, zygomorphic, hermaphrodite, 6.0–7.0 mm long, purple, glandular–pubescent peduncles, 0.5–5.0 mm long; tube 1.5–2.0 mm long; throat cylindric, (3–) 4–5 mm long; lobes 0.5–2.5 mm long. Anthers 5, 1.5–3.0 mm long, sacs rounded at the base, apical appendages obtuse. Style branches flattened, about 2.5 mm long, stigmatic lines well defined, apices short, penicillate or with glabrous

cuspidate appendage. Achenes 4.0–6.5 mm long, obpyramidal, 4–sided, each side 3–ribbed, densely short pubescent with white hairs to nearly glabrous (often so in the same head); pappus of 8 scales, the longer scales lanceolate, awn 4.0–6.5 mm long, alternating with 4 shorter, 1.5–2.0 mm long, truncate, obtuse or awnless scales (the pappus scales often all alike). Chromosome number $n = 12$ (Turner 1963; Turner and Flyr 1966; Powell et al. 1975)

Distribution, habitat and phenology: *Florestina latifolia* grows in the forests and roadsides in dry forests. It grows from southern Mexico in the Oaxaca and Chiapas states and Central America in Honduras and Nicaragua, at elevations from 100 to 1200 meters (Fig. 4). Also, this species is expected in Guatemala, but there is not any record. Specimens of *F. latifolia* have been collected from flower in late July to September and in fruit from September to early November.

Taxonomic notes. *Florestina latifolia* is closely related to *F. platyphylla*, which form a strongly supported monophyletic group (Soto-Trejo *et al.*, 2015). These two species are markedly different morphologically from all of the remaining taxa. They display purplish corollas, simple leaves, and broad involucral bracts, but can be recognized by characters of reproductive structures (Fig. 1).

Selected Specimens Examined — HONDURAS. **El Paraíso**: Yuscarán, El rodeo, 500 m, 5 October 1994, J.L. Linares 1681 (MEXU!). **Francisco Morazán:** Las Canoas River E of 3 km Tegucigalpa, 1020 m, 30 October 1996, J.L. Linares 3863 (MEXU!). MEXICO. **Oaxaca**: 7 km of Lachiviza, 205 m, 16°45'23"N, 95°21'39"W, 27 August 1994, J.I. Calzada 19303 (MEXU!); 43 mi NW of Tehuantepec along the hwy, 900 m, 16°22'11"N,

95°38'47"W, 6 November 1965, A.J. Cronquist 10501 (MEXU!); 54 mi N W of Tehuantepec along the Pan-American hwy, 930 m, 16°25'2"N, 95°49'29"W, 13 October 1962, A.J. Cronquist 9660 (MEXU!); 5.9 km to Buenos Aires on Hwy Oaxaca-Tehuantepec, 215 m, 16° 20' 18.1"N, 95° 25' 8.1"W, 2 September 2012, U. Guzmán 3685 (MEXU!); Km 177.5 on Hwy Oaxaca-Tehuantepec, 726 m, 16°24'24.8"N, 95°47'37.8"W, 2 September 2012, U. Guzmán 3686 (MEXU!); 10 km de Coyul on Hwy Oaxaca-Tehuantepec, 1250 m, 16°32'39.5"N, 95°56'13.9"W, 2 September 2012, U. Guzmán 3687 (MEXU!); 3.2 km of San Carlos Yautepec, 830 m, 16° 32'28.1"N, 95°2'45.5"W, 2 September 2012, U. Guzmán 3688 (MEXU!); 77 km NW of Tehuantepec on Hwy 195, 500 m, 16°24'52"N, 95°48'11"W, 20 October 1978, B.E. Leuenberger 2536 (MEXU!); Ixtepec, 16°32'42"N, 95° 4'31"W, 24 July 1932, E. Matuda 28531 (MEXU!); Tehuantepec, 16°19'0"N, 95°25'0"W, 5 July 1938, E. Matuda 1545 (MEXU!); 37 km NNE of San P. Tapanatepec, 1200 m, 16°43'0"N, 94°6'0"W, 7 August 1985, S. Maya 2017 (MEXU!); 5 km NW of Santa María Laxizonace, 950 m, 16°26'32"N, 95°50'48"W, 6 August 1989, J. Reyes 1902 (MEXU!); 7 km S of Las Margaritas, 900 m, 25 September 1988, A. Saynes 1311 (MEXU!); 21 km N of Laollaga, 460 m, 16°41'0"N, 95°19'28"W, 23 August 1984, R. Torres 5858 (MEXU!); 21 km N of Laollaga, 460 m, 16°41'0"N, 95°19'28"W, 23 August 1984, R. Torres 5858 (MEXU!); 0.5 km W of Buenos Aires, 250 m, 16°20'10"N, 95°28'53"W, 12 September 1985, R. Torres 7295 (MEXU!); 4 km NE of El Limón and 20 km al de Tehuantepec, 195 m, 16°20'8"N, 95°25'22"W, 23 October 1998, J.L. Villaseñor 1493 (MEXU!); 2 mi NW of Río Hondo, 660 m, 16°25'0"N, 95°50'0"W, 5 September 1976, G.L. Webster 21008 (MEXU!); 12 km SW of El Camarón on the way from Hwy 190 to Yautepec, 16°28'53"N, 96°5'17"W, 1 October 1994, T. Yahara 145 (MEXU!); 10 km SW of El Camarón, 880 m, 1 November 1998, T. Yahara 1375 (MEXU!). **Chiapas**: 5 km

W of Rizo de Oro, 900 m, 16°50'0"N, 92°27'0"W, 26 August 1974, D.E. Breedlove 36684 (MEXU!); 5 km N of Cintalapa, 800 m, 16°44'41"N, 93°45'1"W, 23 August 1974, D.E. Breedlove 36581 (MEXU!); 580 m, 16°36'0"N, 93°33'0"W, 1 November 1991, O. Farrera 18 (MEXU!); 10 mi NE of Las Cruces along route 190, 16°43'42"N, 93°19'1"W, 22 July 1960, R.M. King 3442 (MEXU!). NICARAGUA. **Estelí:** Km 163 on Hwy 1, 920 m 13°13'0"N, 86°23'0"W, 5 August 1977, W.D. Stevens 3001 (MEXU!).

Florestina lobata B.L.Turner (1963: 35). Type: MEXICO. Estado de México: Temascaltepec, Ixtapan, 900 m, 18° 50' 24" N, 99° 40' 12" W, 5 August 1932, *G. B. Hinton 1261* (holotype GH!; isotypes, F!, K!, MO!, NY!).

Annual herbs, 30–120 cm. Stems erect, simple or sparsely branched, nearly glabrescent to pubescent with short appressed white hairs, with glandular trichomes 0.5-1.0. Leaves trifoliate, 3-6 cm long, lower ones opposite, the upper ones alternate, pubescent with sparse appressed white hairs, petioles 1-2 cm long; leaflets ovate, 2.0-6.0 cm long, 0.4-2.0 cm wide, irregularly lobed. Inflorescence a subcorymbose cyme with 5 to numerous heads. Heads subturbinate, homogamous, 5.5-7.0 mm high, 4.0-7.0 mm wide, peduncles 3.0-10.0 mm long. Phyllaries uniseriate, 10-12, 4.0-6.0 mm long, 1.5-2.5 mm wide, green, with white membranous margins, apex obtuse or acute, glabrous or almost glabrous. Rays absent. Disk florets 18-20, white, 2.0-3.0 mm long, zygomorphic; tube glandular-pubescent, 1.0 mm long; throat flaring, 1.0 mm long; lobes 1.0-1.50 mm long. Anther 5, sacs rounded at the base, apical appendages ovate to obtuse. Style branches flattened, 1 mm long; stigma lobes penicillate, with cuspidate appendage, otherwise glabrous. Achenes 2.5-4.0 mm long, obpyramidal, 4-sided, densely to sparsely pubescent with appressed hairs;

pappus of 8-10 scales, 1.0-2.0 mm long, apex rounded to truncate. Chromosome number *n* = 10 (Soto-Trejo *et al.* 2015; Fig. 3-B).

Distribution, habitat and phenology: *Florestina lobata* is restricted to the Balsas Basin in the states of México, Guerrero and Michoacán in Mexico (Fig. 5). This species grows in seasonal deciduous forests and along roadsides at elevations from 200 to 1100 meters. *F. lobata* have been collected flowering and fruiting from July to October.

Taxonomic notes. *Florestina lobata* is closely related to *F. pedata* and *F. tripteris* (Fig. 2), but can be recognized by conspicuously lobed leaflets and glabrous involucral bracts.

Selected Specimens Examined — MEXICO: **Estado de México**: El Zapote on Hwy to Acamuchitlán, 1050 m, 18°49'28"N, 100°16'23"W, 17 August 1979, E. Guízar 561 (MEXU!); Luvianos, 300 m W de Cajón de Agua, 1118 m, 18°57'48"N, 100°17'43"W, 4 September 2012, U. Guzmán 3694 (MEXU!); 810 m W of Caja de Agua on way to Cerro Culebra, 1090 m, 18°57'44"N, 100°17'48"W, 19 August 2005, V. Juárez 742 (MEXU!); Palmar Chico in Amatepec, 750 m, 18°41'40"N, 100°22'6"W, 24 August 1954, E. Matuda 31308 (MEXU!); San A. Tlatlaya, 250 m, 18°23'46"N, 100°18'34"W, 19 July 1954, E. Matuda 31217 (MEXU!); 6.6 km N of Peña del Organo, 440 m, 18°26'0"N, 100°19'0"W, 29 July 1996, H. Vibrans 5914 (MEXU!); Road from Sultepec to S Sierra Goleta, 800 m, 18°32'0"N, 100°5'0"W, 28 July 1996, H. Vibrans 5889 (MEXU!). **Guerrero**: 8.5 km N of Placeres de l Oro, 18° 16'35"N, 100° 57'57"W, 26 August 1999, J. Calónico 15695 (MEXU!); Alborejo, 18°40'7"N, 100°34'50"W, 10 August 1981, A. Díaz 230 (MEXU!); Hwy Zitácuaro-Huetamo, 18° 48'50"N, 100°42'29"W, 7 September 1981, A. Díaz 340 (MEXU!); El Petatillo, 17°54'4"N, 101°49'59"W, 13 August 1993, E. Guízar 2849

(MEXU!); 3 km E of Guayameo, 800 m, 18°17'13"N, 101°14'17"W, 14 July 1982, E.M. Martínez 1368 (MEXU!); 14 k m N o f L a U nión o n H wy t o C oahuayutla, 130 m, 18°5'0"N, 101°42'0"W, 24 October 1983, J.C. Soto 6032 (MEXU!); 7 km NE of Ciudad Altamirano on Hwy to Iguala, 250 m, 18°19'47"N, 100°38'16"W, 12 September 1985, J.C. Soto 10426 (MEXU!). **Michoacán**: Road to Tierras Blancas-Bastán near to Rancho Viejo, 630 m, 18°45'50"N, 100°58'59"W, 6 October 1981, J.C. Soto 3205 (MEXU!); Barrio de las Colonias, Huetamo, 400 m, 18°37'27"N, 100°53'54"W, 3 September 1982, J.C. Soto 4282 (MEXU!); 18 k m S W o f T iquicheo, La Crucita, 18 km al SO de Tiquicheo, 640 m, 18°45'13"N, 101°4'28"W, 15 July 2003, J.C. Soto 15112 (MEXU!); Along the road from Tzitzio to El Limón, 0.5 km S of the turnoff to Tafetán, 950 m, 19°25'46"N, 100°54'0"W, 8 August 2004, V.W. Steinmann 4519 (MEXU!).

Florestina pedata (Cav.) Cass. (1820: 155) . *Stevia pedata* Cav. (1797: 33) . *Ageratum pedatum* Ortega (1797: 38) . *Hymenopappus pedatus* (Cav.) Cav. ex Lag. (1816: 28) . *Hymenopappus pedatus* (Cav.) Kunth (1820: 261). *Achyropappus pedatus* (Cav.) Less. (1832: 239) . *Palafoxia pedata* (Cav.) Shinners (1949: 25). Type: MEXICO. Plants cultivated in the Botanical Garden at Madrid from seeds obtained in Mexico, July 1797, *Luis Née s.n.* (lectotype (here designated): MA 476379 (lectotype MA!)

Schkuhria viscosissima Standl. & Steyerm. (1840: 318). *Florestina viscosissima* (Standl. & Steyerm.) Heiser (1845: 278). Type: GUATEMALA. El Quiché: near Sacapulas,1040-1240 m, 12-14 January 1939, *P. C. Standley 62513* (Holotype F).

Schkuhria glomerata B.L. Rob. & S eaton (1893: 109). T ype: M EXICO. Estado de México: Rio H ondo, 5 S eptember 1891, C . G . *Pringle 5006* (Holotype G H!; isotype F, GH!, O, NY, UC, US).

Florestina simplicifolia B.L.Turner (1963: 39). Type: MEXICO. Oaxaca: Cuicatlán, 600 m, 2 December 1895, *C. Conzatti 141*(holotype GH!, isotype GH!).

Annual herbs, 10–60 c m. Stems erect, simple o r s parsely branched, d ensely p ubescent t o nearly glabrescent with spreading, pubescence decreasing in density towards the apex, with glandular t richomes, 1 .5–3.0 mm l ong. Leaves simple and c ompound, the lower o nes simple t o 3 –foliate a nd opposite, t he uppe r o nes 3 –foliate an d/or 5 –foliate an d alternate, new growth o n secondary stems shows s imple l eaves throughout, pubescent w ith scattered appressed hairs, p etioles 1 .0–2.0 c m long, leaflets lanceolate t o o blanceolate, 2.0–5.0 c m long, 0.2–1.0 cm w ide, acute at the ap ex, en tire o r r arely lobed. Inflorescence subcorymbose cyme, 5 to numerous heads. Heads subturbinate, 5.5–8.0 mm long, 3.0–5.0 mm wide. Phyllaries 8–12, green or purplish, 3.5–6.0 mm long, 1.0–3.0 mm wide, ovate to lanceolate, with w hite a nd/or r ed–tinged margins, a pex o btuse o r r ounded, s parsely pubescent t o ne arly g labrescent. Rays absent. Disk florets 6–18, hermaphrodite, 2.0–4.0 mm long, w hite, z ygomorphic; tube 0. 75–1.0 mm long, g labrous t o s parsely pubescent, with g landular trichomes; th roat s ometimes p urplish; lobes 0 .5–2.0 m m l ong. Anthers 5, sacs rounded at the base, apical appendages o vate to obtuse. Style branches flattened, 1.5–2.0 mm long, w ith w ell–defined s tigmatic lines, abruptly t erminated b y a p enicillate an d cuspidate appendage, otherwise glabrous. Achenes 2.5–4.5 mm long, obpyramidal, 4–sided, finely p ubescent w ith a scending hairs t o n early g labrous; pa ppus o f 8 –10 s cales, 0.5–2.0

mm long, apex rounded. Chromosome number $n = 10$ (Turner and Johnston 1961; Beaman and Turner 1962; Turner 1963; Keil et al. 1988; Sundberg *et.al.* 1986 as *F. simplicifolia*).

Distribution, habitat and phenology: *Florestina pedata* is known from northeastern, central and southern Mexico to Guatemala (Fig. 6). It grows in seasonal deciduous forests, xerophytic scrub, oak forest and along roadsides at elevations from 100 to 2800 meters. *F. pedata* have been collected flowering and fruiting from June to November.

Lectotypification. During this taxonomic study of *Florestina*, we discovered that *F. pedata* has never been typified. The basionym, *Stevia pedata*, was described by Antonio José Cavanilles in Icones et Descriptiones Plantarum based on plants grown in the Real Jardín Botánico from Mexico. Three sheets of *Stevia pedata* in the Cavanilles herbarium has label information annotated by Cavanilles indicating that the plant was cultivated in the Real Jardín Botánico. We designated the original sheet MA 476379 specimen as lectotype for *Florestina pedata*. This specimen is marked as Icon. Tab. 356, and is annotated the name Née as collector. This choice is based on the specimen, which is a complete plant with leaves and inflorescences; further the specimen has information about collector, collection date and locality.

Taxonomic notes. *Florestina pedata* is a morphologically variable species that is widely distributed in central and southern Mexico, whereas *F. simplicifolia* was described based only on a few specimens located in the Tehuacán-Cuicatlán Valley in southern Mexico. According to Turner (1963), *Florestina simplicifolia* is characterized by having simple leaves and the uppermost portion of the stem and inflorescence densely short glandular-pubescent. This taxon is closely resembles *Florestina pedata*, and we recorded many specimens so morphologically similar that they may be allocated either as one species or

the other. In fact, many plants with both simple and compound leaves grow intermixed with simple-leaved plants within populations of the putative *F. simplicifolia*. Regarding presence and density of glandular trichomes, we do not observed a clear difference between *Florestina pedata* and *F. simplicifolia*. Actually, we observed specimens without glandular trichomes on branches of the inflorescences of *F. simplicifolia* (e,g. H. Vibrens 7852, C. Rodríguez 1681, F. González 1310, F. Chiang F-2200, R. Rosas 68). Glandular trichomes play a defensive role against herbivores in plants (Levin, 1973) and intraspecific variation for trichome type and density has been reported in many species (Ehrendorfer, 1953; Heiser, 1961; Hardin, 1964; Schaeftlein, 1968; Levin & Levy, 1971; Levin, 1973). This variation has often been correlated to environmental components, such as temperature, herbivore abundance, solar radiation and soil water content (Levin, 1973; Buta *et al.*, 1993; Pérez-Estrada *et al.*, 2000; Fordyce, & Agrawal, 2001; Wagner *et al.*, 2004; Hare & Smith, 2005; Jaime *et al.*, 2013). Therefore, the presence and density of glandular trichomes in stems and inflorescences of *Florestina* species could be correlated environmental factors, such as aridity and herbivore pressure (Levin, 1973; Ehleringer, 1984; Pérez-Estrada *et al.*, 2000). According to our morphology-based observations and the nuclear DNA-based phylogeny by Soto-Trejo *et al.* (2015), we propose that *Florestina pedata* and *F. simplicifolia* actually form a single species, which shows wide morphological variation throughout its broad distributional range.

Selected Specimens Examined — MEXICO: **Distrito Federal**: Reserva Ecológica Pedregal de San Angel, 2300 m, 19°18'48"N, 99°10'21"W, 2 September 2007, L.A. Céspedes 186 (MEXU!); Reserva Ecológica del Pedregal de San Angel, 2309 m, 19°19'5"N, 99°10'52"W, 19 September 2008, L.A. Céspedes 420 (MEXU!); Cerro Teutli,

19°13'23"N, 99 °1'48"W, 20 S eptember 1974, D .J. F lores 79 (MEXU!); S an L uis Tlaxialtemalco-Serranía, 19° 15'29"N, 99° 2'10"W, 15 O ctober 1992, V . N ava 24 (MEXU!); Xochimilco, Valley of Mexico, 19°16'30"N, 99°8'20"W, 3 October 1910, C.R. Orcutt 4251 (MEXU!); Reserva Pedregal de San Angel, 2310 m, 19°19'6"N, 99°11'37"W, 4 July 2006, F . S oto-Trejo 99 (MEXU!); J ardín Botánico U NAM, 2310 m, 19°18'59"N, 99°11'30"W, August 2011, F . S oto-Trejo 232 (MEXU!). **Estado de México**: C hapingo, 19°30'0"N, 98° 53'18"W, 31 A ugust 1976, F . B olaños 38 (MEXU!); S an A. O metusco, 19°45'34"N, 98°34'17"W, 30 August 1979, F.J. Espinosa 712 (MEXU!); near of Huitzila, 19°48'42"N, 98° 57'32"W, 1 O ctober 1979, F .J. E spinosa 777 (MEXU!); 6 k m S E o f Texcoco, 2300 m, 19°30'58"N, 98°49'59"W, 9 S eptember 1977, J. García 424 (MEXU!); 2.8 mi just SE of Amecameca, 19°2'12"N, 98°46'53"W, 31 December 1971, M. Leake 95 (MEXU!); C hapingo, 2240 m, 19°30'1"N, 98°53'18"W, 22 S eptember 1979, R . R amírez 28 (MEXU!); E l T ejocote, 2270 m, 19° 26'28"N, 98°54'15"W, 20 S eptember 2000, H . Vibrans 6673 (MEXU!); Ojo de Agua, 235 m, 18°40'40"N, 101°39'13"W, 30 July 2003, S. Rangel 913 (MEXU!); 9 k m al N de Nocupétaro, carr. a Villa Madero, 865 m, 19°4'49"N, 101°12'31"W, 28 September 1982, J.C. Soto 4626 (MEXU!); 19 km SW of Buenavista on Hwy Apatzingán-Tepalcatepec, 19°1'45"N, 102°40'31"W, 24 August 1980, J.C. Soto 2468 (MEXU!); 4 k m S o f T epalcatepec o n r oad t o C oalcoman d e M atamoros, 1 000 m , 19°9'43"N, 102°50'12"W, 22 September 1984, D.M. Spooner 2554 (MEXU!); Along Mex 51, 6 km N of La Tiringucha, 20 k m N of Huetamo, 625 m, 18°48'20"N, 101°56'46"W, 4 October 2003, V.W. Steinmann 3631 (MEXU!). **Guanajuato**: NO of San J. del Carmen on Cerro del Conejo, 1750 m, 20°17'8"N, 100°53'4"W, 23 September 1993, J. Castañeda 152 (MEXU!); S an M iguel Allende o n H wy t o P resa de l O braje, 1850 m, 20 °55'9"N, 100°46'40"W, 9 August 1979, J . K ishler 722 (MEXU!); 1 k m N o f G aytán, 2000 m,

20°9'15"N, 100°40'5"W, 8 October 1987, A. Rubio 683 (MEXU!); Near to Salvatierra, 1800 m, 20°17'0"N, 100°59'0"W, 15 June 1985, J. Rzedowski 38585 (MEXU!); 15 km N of Xichú in Río Alamo, 1500 m, 21°24'1"N, 100°1'8"W, 8 September 1989, E. Ventura 7247 (MEXU!). **Guerrero**: Km 119 Hwy Ixtapa-Grutas, 1200 m, 18°39'38.6"N, 99°30'27.8"W, 4 September 2012, U. Guzmán 3693 (MEXU!); 8 km N of Comala, 1335 m, 18 August 2007, O. Hinojosa 158 (MEXU!); Huitziltepec, 1315 m, 17°47'52"N, 99°29'19"W, 25 October 1994, J. Jiménez 992 (MEXU!); 2 km W of Milpillas on road to Xochipala, 850 m, 17°47'29"N, 99°35'27"W, 16 January 1983, E.M. Martínez 4868 (MEXU!); Km 62 Hwy México-Acapulco on Cañón del Zopilote, 910 m, 17°56'10"N, 99°36'10"W, 4 July 1980, D. Sánchez (MEXU!); 69 km S of Iguala on road to Filo de Caballo, 740 m, 17°47'53"N, 99°33'52"W, 14 August 1985, J.C. Soto 9802 (MEXU!). **Hidalgo**: 8 mi W of Tula on Hwy 126, 2100 m, 20°5'50"N, 99°25'43"W, 27 August 1960, W.L. Ellison 79 (MEXU!); 3 km N of Puente Tasquillo, 2100 m, 20°35'29"N, 99°20'19"W, 31 August 1990, R. Fernández 4657 (MEXU!); Zempoala, 19°54'54"N, 98°40'11"W, 2 September 1981, L. Moreno 40 (MEXU!). **Jalisco**: Fields along route 15, 40 mi SE of Guadalajara, 20°15'17"N, 103°26'6"W, 7 August 1960, R.M. King 3655 (MEXU!); Near Km 647, 5 road-mi SW of Santa Cruz de Las Flores, 1550 m, 22°20'14"N, 103°52'19"W, 24 August 1957, R. McVaugh 16301 (MEXU!); 1.3 mi NNE of Santa Cruz, 1440 m, 20°29'39"N, 103°29'12"W, 2 September 1973, T.F. Stuessy 3071 (MEXU!); 6.6 mi E of Los Pocitos along the hwy from Guadalajara to Ameca, 20°34'28"N, 103°51'17"W, 29 September 1984, S.D. Sundberg 2932 (MEXU!); Km 7 way to C. de Buenos Aires, 1640 m, 19°55'45"N, 103°15'32"W, 22 August 1978, L.M. Villarreal 10544 (MEXU!). **Morelos**: Cuernavaca, 1620 m, 18°56'2"N, 99°15'10"W, 12 April 1963, D.B. G. 67 (MEXU!); 2 km N of Santa Catarina, 1625 m, 18°59'9"N, 99°8'24"W, 26 July 1979,

A. Díaz 14 (MEXU!); 2 km NW of Yautepec on Hwy Yautepec-Tepoztlán, 1210 m, 18°49'44"N, 99°3'9"W, 5 October 1986, G. Flores 117 (MEXU!); 7 km S of Alpuyeca on Hwy Cuernavaca-Acapulco, 993 m, 18°39'44"N, 99°17'43"W, 23 September 2005, H. Vibrans 7796 (MEXU!); Km 5 Hwy Puente de Ixtla-Taxco, 1468 m, 18°33'58"N, 99°27'55"W, 20 October 2010, J.L. Villaseñor 1812 (MEXU!). **Oaxaca**: 5 km of San Sebastían Tecomaxtlahuca on Hwy to Coicoyan de Las Flores, 1710 m, 17°19'30"N, 98°3'28"W, 6 October 1994, J.I. Calzada 19399 (MEXU!); Road from Santiago Astata to San P. Huamelula, 120 m, 15°58'0"N, 95°40'0"W, 30 August 1994, J.I. Calzada 19343 (MEXU!); 37 miles SE of Oaxaca, SW slopes, 1650 m, 16°45'48"N, 96°19'54"W, 13 October 1962, A.J. Cronquist 9659 (MEXU!); 5 km SE of Cuicatlán, 760 m, 17°46'0"N, 96°55'0"W, 27 August 1980, F. González 1520 (MEXU!); 5.7 km NE of Santiago Chazumba, 1971 m, 18°12'46.4"N, 97°27'56.4"W, 3 September 2012, U. Guzmán 3692 (MEXU!); 11 km NW of Tehuantepec on Cerro Coyote, 16°21'0"N, 95°19'0"W, 30 August 1988, C. Martínez 1783 (MEXU!); 9 km S of San Agustín Atenango on Hwy to Juxtlahuaca, 17°33'0"N, 98°1'0"W, 3 November 1976, J. Rzedowski 34514 (MEXU!); Along Hwy toward Huajuapan de León, 17°51'11.3"N, 97°47'53.1"W, 30 September 2011, F. Soto-Trejo 234 (MEXU!); Santa María Ayú, 1765 m, 17°56'2"N, 97°48'17.2"W, 30 September 2011, F. Soto-Trejo 235 (MEXU!); Ahuehuetitlán de González, 1734 m, 17°56'19.2"N, 97°47'24"W, 30 September 2011, F. Soto-Trejo 236 (MEXU!); Oaxaca City, 1600 m, 17°5'47"N, 96°43'53"W, 4 October 2013, F. Soto-Trejo 243 (MEXU!); 3 km S de Teotitlán, 18°6'28.5"N, 97°4'8.5"W, 3 October 2013, F. Soto-Trejo 240 (MEXU!); San José El Chilar, 930 m, 17°42'52.8"N, 96°56'26.4"W, 3 October 2013, F. Soto-Trejo 241 (MEXU!); 7 km NE of Santiago Chazumba, 1991 m, 18°12'57"N, 97°38'46"W, 30 June 2004, O. Téllez 16400 (MEXU!); 3 km S of Teotitlán, 965 m,

18°6'25"N, 97° 4'6"W, 2 October 2005, H. Vibrans 7852 (MEXU!); **Puebla**: Meseta de San Lorenzo, 4 km SW of Tehuacán, 1800 m, 18°28'0"N, 97°26'0"W, 4 August 1985, O. R. Dorado 2764 (MEXU!); 4 km NE of Santo Tomás Otlaltepec, 1610 m, 18°19'2"N, 97°45'24"W, 23 August 1980, F. González 1310 (MEXU!); 5 km E of Santa Catarina Tehuixtla,1820 m, 18°22'0"N, 97°42'26"W, 23 August 1980, F. González 1345 (MEXU!); Meseta San Lorenzo, 1825 m, 18°26'58.1"N, 97°27'46.4"W, 3 September 2012, U. Guzmán 3691 (MEXU!); 1 km NW of San Lorenzo, W of Tehuacán, 1750 m, 18°28'0"N, 97°26'0"W, 16 October 1971, W.L. Graham 1507 (MEXU!); NE of San R., 1010 m, 18°12'11"N, 97°8'20"W, 14 October 1998, R. Luna 21 (MEXU!); 10 km Hwy Tehuacán-Teotitlán, 1500 m, 18°23'44"N, 97°20'46"W, 3 April 1976, C. Rodríguez 1681 (MEXU!); 2 km of Meseta San Lorenzo, 1777 m, 18°27'56"N, 97°27'32"W, 26 October 2005, I. Rosas 68 (MEXU!); 7 km of Meseta San Lorenzo, 1895 m, 18°26'16"N, 97°28'17"W, 26 October 2005, I. Rosas 149 (MEXU!); 2 km NW of San A. Nanahuatipan, 850 m, 18°6'0"N, 97°7'0"W, 30 July 1985, A. Salinas 2663 (MEXU!); Santa María Ixitlán, 1769 m, 18°0'43.9"N, 97°45'51"W, 30 September 2011, F. Soto-Trejo 233 (MEXU!); NE of San Rafael, Coxcatlán, 1010 m, 18°12'11"N, 97°8'20"W, 4 October 2013, F. Soto-Trejo 244 (MEXU!); 6.1 km of Hwy Tehuacán-Puebla, 1927 m, 18°26'36"N, 97°27'42"W, 8 September 2005, O. Téllez 19477 (MEXU!); 4 km W of Santa Ana Teloxtoc, 1820 m, 18°22'42"N, 97°34'40"W, 22 September 1984, P. Tenorio 7271 (MEXU!); Los Tepetates, N of Caltepec, 1930 m, 18°11'0"N, 97°28'0"W, 28 July 1984, P. Tenorio 6735 (MEXU!); 2 km NW OF San Juan Raya, 1800 m, 18°24'0"N, 97°48'0"W, 5 November 1991, A. Valiente 195 (MEXU!); 2.5 km S of San Martín, 1741 m, 18°15'26"N, 97°32'34"W, 25 October 2005, F. Victorino 37 (MEXU!); 5 mi N of Tehuacán, 1650 m, 18°32'0"N, 97°24'0"W, 11 August 1976, G.L. Webster 20826 (MEXU!). **Veracruz:** Chalma 3 km NE

of Huejutla on road to Platón Sánchez, 250 m, 21°11'0"N, 98°23'0"W, 22 June 1980, M. Nee 18438 (MEXU!).

Florestina platyphylla (B.L.Rob. & Greenm.) B.L.Rob. & Greenm. (1896: 49). *Schkuhria platyphylla* B.L.Rob. & Greenm. (1895: 156). Type: MEXlCO. Oaxaca: Monte Alban, 1676 m, 17° 03' 00" N, 96° 46' 48" W, 8 October 1894, *C. G. Pringle 4975* (holotypes GH; isotypes: BR!, E, JE!, K!, M!, MO!, NY, PH, UC, US,).

Annual herbs, 30–80 c m tall. Stems erect, scarcely branched, d ensely pubescent w ith glandular hairs. Leaves simple with the pubescent petioles 0.5–5.0 cm long, the lower ones opposite, t he u pper o nes a lternate, o vate, lamina finely pubescent o n bo th faces w ith glandular hairs, serrate, 2.5–8.0 cm lo ng, 1.5–6.0 cm wide, the apex acute, the ba se obtuse to truncate. Inflorescence a dichotomous c yme with 1 0–80 he ads, peduncles 1.0–6.0 cm, slender, p ubescent w ith glandular hairs. Heads turbinate, h omogamous, 8.0–12.0 high. Phyllaries uniseriate, 7–10, ovate–lanceolate, 5.0–8.0 mm long, 2.5–6.0 mm wide, purplish, obtuse or rounded t ip, pu bescent w ith short, stiff, spreading hairs interspersed w ith glandular t richomes. Rays absent. Disk florets 20–40, zygomorphic, hermaphrodite, 2.5–3.0 mm l ong, purple, g landular–pubescent pe duncles 0.5–5.0 mm l ong; tube 1.5–2.0 mm long; throat cylindric, 3.0–5.0 mm l ong; lobes 0.5–2.5 mm l ong. Anthers 5, 1.5– 3.0 mm long, s acs r ounded at t he base, apical ap pendages obtuse. Style branches f lattened, about 2.5 m m long, stigmatic lines w ell defined, apices short, p enicillate or gl abrous cuspidate appendage. Achenes 3.5–5.5 mm lo ng, o bpyramidal, 4–sided, each s ide 3–ribbed, de nsely short pubescent with white hairs to nearly glabrous (often so in the same head); pappus of 8

scales, the 4 longer scales lanceolate or ovate, 1.5–4.0 mm long, apex acute or obtuse, alternating with 4 shorter ovate, 1.0–1.5 mm long, apex rounded or obtuse (the pappus scales often all alike). Chromosome number $n = 12$ (Soto-Trejo *et al.* 2015; Fig. 3-A)

Distribution, habitat and phenology: *Florestina platyphylla* is restricted to central valleys of the state of Oaxaca and in southern Puebla, Mexico. It grows in forests and roadsides in seasonal deciduous forests at elevations from 1200 to 2000 meters (Fig. 4). *F. platyphylla* have been collected in flower from late July to September and in fruit from September to early November.

Taxonomic notes. Turner (1963) suggested that *Florestina platyphylla* could be a morphological variant of *F. latifolia*. However, Soto-Trejo et al. (2015) showed that these two taxa are independent sister lineages that form a monophyletic group. These two taxa are externally very similar, and sometimes are very difficult to distinguish, but characters such as the lengths of the corolla, achene and the pappus are useful in differentiating from each other (Fig.1). Therefore, we confirm that these two taxa represent two independent species.

Selected Specimens Examined — MEXICO: **Oaxaca**: 5 km NW of Las Margaritas, 1050 m, 16°41'42"N, 96°12'30"W, 17 July 1987, R. Aguilar 255 (MEXU!); Km 74 on Hwy 190 from Oaxaca to Tehuantepec, 1200 m, 16° 43'0"N, 96° 18'0"W, 1 August 1978, T.S. Cochrane 8634 (MEXU!); Canteras de Ixcolel, 1550 m, 17°2'17"N, 96°43'52"W, 24 October 1932, C. Conzatti 4004 (MEXU!); El Crestón, 1600 m, 17°4'41"N, 96°43'36"W, 12 November 1932, C. Conzatti 4828 (MEXU!); 10 km NW of Totolapan along the Pan-American hwy, 1200 m, 16°40'43"N, 96°9'59"W, 2 July 1974, A.J. Cronquist 11145

(MEXU!); Km 98 Hwy Oaxaca-Tehuantepec, 1148 m, 16°41'20.7"N, 96°12'34"W, 2 September 2012, U. Guzmán 3689 (MEXU!); Km 67 Hwy Oaxaca-Tehuantepec, 1535 m, 16°45'2.5"N, 96°20'0"W, 2 September 2012, U. Guzmán 3690 (MEXU!); Cerro Colorado, 1730 m, 16°40'47"N, 96°33'24"W, 18 August 2005, M.R. Hernández 92 (MEXU!); San J. Taviche, 16°42'40"N, 96°35'35"W, 15 July 2005, M.R. Hernández 63 (MEXU!); 48 mi NW of El Camarón along Hwy 190, 700 m, 16°45'0"N, 96°20'0"W, 4 September 1975, G. Holstein 20297 (MEXU!); Edge, San Juan Mixtepec, 2000 m, 16°18'6"N, 96°18'1"W, 12 November 1996, E.S. Hunn 491 (MEXU!); 7.3 mi NW of Totolapan on Mex Rte 190, 15.9 mi Se of turnoff to Mitla, 1410 m, 16°47'7"N, 96°19'58"W, 13 September 1981, D.J. Keil 15532 (MEXU!); Vicinity of San Bernardo Mixtepec, 1560 m, 16°49'27"N, 96°53'53"W, 25 June 1984, G.I. Manzanero 1046 (MEXU!); 1 km S Portillo Nejapa on Hwy Tehuantepec-Oaxaca, 1240 m, 16°32'21"N, 95°56'0"W, 17 August 1989, M. Martínez 373 (MEXU!); N of Totolapan on Hwy 190 S of Oaxaca, 1600 m, 16°45'0"N, 96°25'0"W, 18 July 1991, M.H. Mayfield 959 (MEXU!); Vicinity of Mitla, valley of Río Grande de Mitla, 1600 m, 16°55'0"N, 96°24'0"W, 4 October 1972, E. Messer 148 (MEXU!); Oaxaca city, 1600 m, 17°5'47"N, 96°43'53"W, 4 October 2013, F. Soto-Trejo 242 (MEXU!); Hierve el Agua, 1640 m, 16°51'55"N, 96°16'40"W, 23 October 2003, E. Torres 2328 (MEXU!).
Puebla: Caltepec, 1902 m, 18°10'48.1"N, 97°28'54.5"W, 2 October 2013, F. Soto-Trejo 239 (MEXU!).

Florestina purpurea (Brandegee) Rydb. (1914: 57). *Hymenothrix purpurea* Brandegee (1909: 392). Type: MEXICO. Puebla: San Luis Tultitlanapa, August 1908, 18° 10' 48" N,

97° 27' 00" W, *C. A. Purpus 3119* (holotype: UC; isotypes: BM!, E!, F, GH!, MO!, NY, US).

Annual herbs, 10–50 cm tall. Stems erect, unbranched or branched, with short appressed hairs interspersed with glandular trichomes. Leaves biternately dissected with the pubescent or glandular petioles; lower ones opposite, the upper ones alternate; the lamina linear to linear–lanceolate, 10–30 mm long, 1–3 mm wide, densely pubescent with glandular trichomes. Inflorescence a subcorymbose cyme with 15 to numerous heads, peduncles pubescent with glandular hairs. Heads turbinate, homogamous, 6.5–10.5 mm high, 6–10 mm wide. Rays absent. Phyllaries uniseriate, 8–10, linear–lanceolate 4.0–7.0 mm long, 1.5–2.5 mm wide, purple, pubescent to nearly glabrous, membranous margins, obtuse to rounded apex. Rays absent. Disk florets 20–28, zygomorphic, hermaphrodite, 3.0–4.0 mm long, white, peduncles 12.0–30.0 mm long, pubescent with glandular hairs; tube glabrous, 1.5 mm long. Anthers 5, sacs rounded at the base, apical appendages ovate to obtuse. Style branches flattened (about 1.3 mm long), stigmatic lines purplish, apices terminated by a penicillate caudate appendage. Achenes 3.5–5.0 mm long, obpyramidal, 4–sided, glabrous above with pubescent at the base with stiff ascending hairs; pappus 8, ovate–lanceolate scales, 2.5–4.5 mm long, apex predominately acute, formed by the midnerve, 0.1–0.2 mm long. Chromosome number $n = 12$ (Soto-Trejo *et al.* 2015).

Distribution, habitat and phenology: *Florestina purpurea* is located in a restricted area of the states of Oaxaca and Puebla, Mexico. It grows in seasonal deciduous forests, xerophytic scrub and oak forest, at elevations from 1200 to 2300 meters (Fig. 5). *F. purpurea* have been collected in flower and fruit from September to November.

Taxonomic notes. *Florestina purpurea* was originally described as part of the genus *Hymenothrix* and Turner (1963) mentioned it as a ''perplexing intermediate'' between these two genera. Soto-Trejo *et al.* (2015) showed that this species clearly belong in *Florestina* and *Hymenothrix* is not the most closely related genus. *Florestina purpurea* is morphologically well delimited by having biternately dissected leaves, glabrous achenes with basal hairs and a pappus formed by lanceolate scales with their apex predominately acute (Fig. 1).

Selected Specimens Examined — MEXICO: **Oaxaca**: 7.3 km NNW of Santiago Tejupan on Hwy to Coixtlahuaca, 2010 m, 17°41'21"N, 97°25'12"W, 28 October 1996, J.L. Panero 6788 (MEXU!); 15 km NW of San Juan Mixtepec, 1900 m, 17°23'0"N, 97°50'0"W, 4 October 1988, J. Reyes 775 (MEXU!); 7.3 km of Santiago Tejupan, Hwy toward Coixtlahuaca, 2010 m, 17°41'23"N, 97°25'13"W, 30 September 2011, F. Soto-Trejo 237 (MEXU!); 4 mi S of Tlaxiaco, near Km 64 of Hwy 125, 17°8'0"N, 97°41'0"W, 7 October 1984, S.D. Sundberg 3048 (MEXU!); Cerro Chicamole, N of Guadalupe Membrillos, 2300 m, 18°2'35"N, 97°33'38"W, 22 November 1991, P. Tenorio 18140 (MEXU!); Membrillos, 2310 m, 18°1'0"N, 97°33'0"W, 10 September 2001, P. Tenorio 20865 (MEXU!); Guadalupe Cuautepec on Hwy Tehuacán-Huajuapan, 2159 m, 18°2'0"N, 97°41'0"W, 2 October 2001, P. Tenorio 21258 (MEXU!); E of San Miguel Aztatla, 2044 m, 18°57'0"N, 97°25'0"W, 6 November 2001, P. Tenorio 21568 (MEXU!); Corral del Cerro, 1700 m, 16°56'25"N, 96°18'25"W, 12 September 1978, F. Ventura 15512 (MEXU!); **Puebla**: Caltepec, 1958 m, 18°10'53"N, 97°28'58.1"W, 2 October 2013, F. Soto-Trejo 238 (MEXU!); El Tecomite, W of San Simón, 2080 m, 18°2'0"N, 97°30'0"W, 3 October 1984, P. Tenorio 7552 (MEXU!); Barranca Seca, S of Cruz Chica on road to San Simón,

18°4'0"N, 97°31'0"W, 2 October 1984, P. Tenorio 7544 (MEXU!); Portezuelo de Santa Lucía, Coatepec, 1280 m, 18°11'0"N, 97°29'0"W, 19 October 1984, P. Tenorio 7481 (MEXU!); Cerro El Gavilán, SE of Caltepec, 1880 m, 18°4'0"N, 97°32'0"W, 20 October 1983, P. Tenorio 4779 (MEXU!); 1 km NW of Los Reyes Metzontla, 1800 m, 18°13'0"N, 97°29'0"W, 15 November 1991, A. Valiente 432 (MEXU!); 3 km W of San Luis Atolotitlán, 18°11'0"N, 97°27'0"W, 10 November 1983, J.L. Villaseñor 598 (MEXU!); Cerro El Gavilán, 4 km SE of Caltepec on road to San Luis Atoolitlán, m, 18°4'0"N, 97°32'0"W, 12 November 1983, J.L. Villaseñor 621 (MEXU!).

Florestina tripteris DC. (1836: 655). *Palafoxia tripteris* (DC.) Shinners (1949: 24). Type: United States. Texas: Laredo, August 1829, *Berlandier 2077* (holotype GDC, isotypes F!, GH!, K!, MO!).

Palafoxia tripteris (DC.) Shinners *var. brevis* Shinners (1952: 94). Type: UNITED STATES. Texas. Crockett County. Texas Range Station, about 22 1/2 miles north of Ozona; 1.9 miles N by W of headquarterns (east of highway). 10 October 1942, *V. L. Gory 40710* (Holotype BRIT!)

Florestina liebmannii Sch. Bip. ex Greenm. (1907: 272). *Palafoxia liebmannii* (Sch. Bip. ex Greenm.) Shinners (1949: 25). Type: MEXICO. Veracruz: Boca del Rio, just south of the city of Veracruz, July 1841, *Liebmann 71* (holotype C!, K!; isotype NY!)

Annual herbs, 10–60 cm. Stems erect, simple or sparsely branched, nearly glabrescent to pubescent with short appressed white hairs, with glandular trichomes 0.25–0.4 mm long, increasing in density towards the branches of the inflorescence. Leaves simple and

compound, lower ones simple a nd o pposite, t he uppe r o nes t rifoliate a nd a lternate, pubescent with scattered appressed white hairs, petioles 1–3 cm long; leaflets ovate, 0.4–2.0 cm wide, 2–6 cm long, entire, apex acute. Inflorescence a subcorymbose cyme with 5 to numerous heads. Heads turbinate, homogamous, 5.0–8.0 mm long, 4.0–7.0 mm wide, peduncles 5.0–25.0 mm long. Phyllaries uniseriate, 8–14, green, 4.0–6.0 mm long, 1.5–2.5 mm wide, with white membranous margins (very rarely somewhat reddish), apex obtuse or rounded, pubescent with appressed hairs to nearly glabrous. Rays absent. Disk florets 10–25 white, 4.0–5.0 mm long, zygomorphic; tube glandular–pubescent, 1.0–1.5 mm long, the throat flaring, 2.5–3.0 mm long (including the lobes), the lobes 0.5–2.0 mm long. Anthers 5, sacs rounded at the base, apical appendages ovate to obtuse. Style branches flattened, 1.5–2.0 mm long; stigma lobes penicillate with cuspidate appendage, otherwise glabrous. Achenes 2.5–4.5 mm long, obpyramidal, 4–sided, densely to sparsely pubescent with appressed hairs; pappus of 8–10 scales, 0.5–2.0 mm long, apex rounded to truncate. Chromosome number $n = 10$ (Turner and Johnston 1961 as *F. liebmannii*; Turner 1963; Keil and Stuessy 1975; Keil and Stuessy 1977 as *F. liebmannii*; Powell et al. 1975; Watanabe *et al.* 2007, $2n = 20$).

Distribution, habitat and phenology: *Florestina tripteris* grows in xerophytic scrub, along roadsides and disturbed areas from southern and south-central Texas, northern and eastern Mexico, at elevations from sea level to 2000 meters (Fig. 6). *F. tripteris* have been collected in flower and fruit from July to November. This species was accidentally introduced to Australia from the USA, and it has become an exotic weed in Tambo and Barcaldine (McKenzie et al., 2014). *Florestina tripteris* has the ability to survive dry conditions due to the fact that its seeds are easily spread and they germinate very fast after

rain. Recently, a chemical screening study was conducted by McKenzie *et al.* (2014) to identify methods for effective herbicide control of this invasive species in Australia.

Taxonomic notes. According to Turner (1963), *Florestina liebmannii* is a weakly differentiated taxon, but different from *F. tripteris*, that is characterized by having leaves coarsely pubescent, the hairs with conspicuous swollen basal cells and inflorescence without glandular trichomes. Similarly to *Florestina pedata* and *F. simplicifolia*, intraspecific variation for the type and density of trichomes were observed in stems, leaves and inflorescences of both *F. tripteris* and *F. liebmannii*. We observed leaf trichomes with conspicuous swollen basal cells in both *Florestina tripteris* and *F. liebmannii*. Also, we observed specimens with glandular trichomes on branches of the inflorescences of putative *Florestina liebmannii* (e,g. P. Zamora 156, M.C. Johnston 4791, J. Dorantes 774, B.L. Turner 15368 and P. Moreno 1445). Therefore, the use of trichomes as a taxonomic character in *Florestina* is complicated by their wide intraspecific variation. In accordance with our morphology-based observations and the nuclear DNA-based phylogeny by Soto-Trejo *et al.* (2015), we propose that *Florestina tripteris* and *F. liebmannii* actually belong to a single species, which shows wide morphological variation throughout its broad distributional range.

Selected Specimens Examined — MEXICO: **Chihuahua**: 9 km NW of Las Pampas ranch, on the road to Camargo, 1450 m, 27° 30'0"N, 104° 46'0"W, 24 August 1972, F. Chiang 8821 (MEXU!); 40 km SE of Chihuahua, 1000 m, 28°21'32"N, 106° 16'38"W, 2 October 1970, A.J. Cronquist 10753 (MEXU!); 26 km S of ciudad on Hwy to Torreón, 1480 m, 28°28'16"N, 105°50'58"W, 4 October 1978, J. García 754 (MEXU!); 26.6 mi NE of Aldama, along Hwy 16, 1320 m, 29° 3'0"N, 105° 37'0"W, 15 September 1972, J.S.

Henrickson 7572 (MEXU!); On Hwy 45, 22 mi WSW of junction of Hwys 45 and 49, 1420 m, 27°5'0"N, 105°15'0"W, 23 August 1988, G.L. Nesom 6537 (MEXU!); 23.8 mi S of edge of Chihuahua on Hwy 45, 28°27'24"N, 105°43'23"W, 7 August 1984, D. Randolph 189 (MEXU!); 3 km N of Rancho El Vergel on road to Penjamo and Sacramento, 1550 m, 29°23'0"N, 105°25'30"W, 24 October 1972, T.L. Wendt 9875 (MEXU!). **Coahuila**: N end of Bolson de los Lipanes, 27°37'24"N, 103°44'23"W, 12 September 1940, I.M. Johnston 1228 (MEXU!). **Durango**: 60 mi NE of Durango, 14 miles SW of Yerbesina, 1950 m, 24°33'31"N, 103°57'2"W, 26 September 1962, A.J. Cronquist 9520 (MEXU!); 63 miles N of Durango on Hwy 45, 1710 m, 24°45'5"N, 104°33'35"W, 3 September 1960, W.L. Ellison 129 (MEXU!); 10 de Abril on way Rodeo-Nazas, 1300 m, 25°12'30"N, 104°11'0"W, 9 July 2000, S. González 6248 (MEXU!); Ceballos, Mohovano de Lilas, 1095 m, 26°40'27"N, 103°41'9"W, 9 September 1994, G.B. Hinton 24759 (MEXU!); 3.6 miles SW of Cuencame on route 40, 1850 m, 24°50'0"N, 103°50'0"W, 25 August 1991, J.A. Soule 2817 (MEXU!); 6 km NW of Las Higueras, 1470 m, 25°7'28"N, 104°37'17"W, 5 September 1983, P. Tenorio 4246 (MEXU!). **Nuevo León**: El Obispado, Monterrey City, 660 m, 25°41'24"N, 100°17'58"W, 3 November 1978, J. García 840 (MEXU!); Doctor Arroyo, 1750 m, 23°40'20"N, 100°10'49"W, 1 August 1990, G.B. Hinton 20 619 (MEXU!); Km 49 on Hwy Monterrey-Laredo, 600 m, 26°4'34"N, 100°7'0"W, 3 June 1994, G.B. Hinton 24269 (MEXU!); 30 km ENE of Doctor Arroyo, 2.5 km ENE of San A. de la Peña Nevada, W base of Cerro Peña Nevada, 1980 m, 23°45'1"N, 99°57'21"W, 3 August 1981, G.L. Nesom 4337 (MEXU!); Cañon de Bustamante, 7.2 km W of Bustamante, 550 m, 26°32'0"N, 100°34'0"W, 16 October 1992, A. Prather 1294 (MEXU!). **San Luis Potosí**: Km 81 Hwy Federal 57 San Luis Potosí-Matehuala, 1410 m, 22°41'4"N, 100°29'13"W, 23 September 1977, J. García 472 (MEXU!); 4.3 km N of Las Palomas,

1014 m, 22°29'31.6"N, 99°48'51.6"W, 13 November 2012, U. Guzmán 3778 (MEXU!); 7 km SE of Buena Vista on road to Cerros Blancos, 1300 m, 22°33'18"N, 100°6'4"W, 22 September 1996, C. Gómez 1323 (MEXU!); El Huizache on Hwy 57, 22°41'4"N, 100°29'13"W, 31 July 1983, J.S. Marroquín 3960 (MEXU!); 2 km E of El Huizache on road to Ciudad del Maíz, 1300 m, 22°54'33"N, 100°22'57"W, 22 July 1983, J.L. Villaseñor 473 (MEXU!). **Tamaulipas**: El Mezquital, 61 km E on Hwy Victoria-Matamoros, 25°14'0"N, 97°26'0"W, 3 October 1984, D. Baro 492 (MEXU!); 32 km SW of Tula, 1900 m, 22°48'49"N, 99°54'13"W, 9 August 1972, F. González 4374 (MEXU!); 48 mi from Reynosa on the San Fernando road, 25°18'42"N, 98°12'39"W, 19 October 1959, M.C. Johnston 4377 (MEXU!);15 mi from Tampico on the Mante highway, 22°25'28"N, 97°56'51"W, 27 September 1959, M.C. Johnston 4079 (MEXU!); 6 mi S of the jct of Mex 70 and Mex 180 along Mex 180, 23°40'22"N, 98°10'3"W, 23 April 1987, A.W. Lievens 2466 (MEXU!). **Veracruz**: Estación Biológica La Mancha, 0 m, 19°35'24"N, 96°22'52"W, 9 October 1991, G. Castillo 6873 (MEXU!); Laguna Verde (Punta Limón), 19°43'12"N, 96°24'17"W, 19 June 1972, J. Dorantes 774 (MEXU!); Playa de Riachuelos in Tecolutla, 10 m, 20°25'3"N, 96°57'43"W, 15 July 1977, J.J. Fay 922 (MEXU!); 1 km N of Las Casitas, 19 m, 20°15'53.8"N, 96°48'30.5"W, 31 August 2012, U. Guzmán 3681 (MEXU!); 1 mi N of Las Casitas, 2 m, 20°15'19"N, 96°48'3"W, 20 November 1959, M.C. Johnston 4804 (MEXU!); 0.5 mi W of Antigua on the road to Cardel, 6 m, 19°19'23"N, 96°19'53"W, 19 November 1959, M.C. Johnston 4791 (MEXU!); Road from Punta Limón to Cerro Monte de Oro, 25 m, 18°41'42"N, 95°53'0"W, 20 June 1972, A. Lot 1853 (MEXU!); Estación Morro de La Mancha, 0 m, 19°35'24"N, 96°22'52"W, 31 October 1978, P. Moreno 1478 (MEXU!); Morro de La Mancha, 0 m, 19°35'24"N, 96°22'52"W, 4 August 1978, P. Moreno 1445 (MEXU!); Estación Morro de La Mancha, 0 m, 19°35'24"N,

96°22'52"W, 31 October 1978, P. Moreno 1461 (MEXU!); Beach front at Palma Sola, 0 m, 19°46'11"N, 96°25'26"W, 7 October 1983, B.L. Turner 15368 (MEXU!); Mocambo, 10 m, 19°9'0"N, 96°7'0"W, 23 October 1975, F. Ventura 12018 (MEXU!); Playa Palma Sola, 0 m, 29 October 1998, T. Yahara 1364 (MEXU!); Hwy Cardel-Veracruz, 200 m, 19°19'7"N, 96°18'41"W, 15 October 1986, P. Zamora 156 (MEXU!). **Zacatecas**: 35 km al S de C. del Oro, Noria de Guadalupe, 24°17'43"N, 101°25'14"W, 15 July 1975, F. González 8096 (MEXU!). UNITED STATES OF AMERICA. **Texas: S** U.S. Navy's Escondido Ranch, 107 m**,** 28°40'0"N, 98°42'13.6"W, 27 October 2006, W.R. Carr 25273 (MEXU!).

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Figures

Fig. 1 Achene and flower morphology of A) *Florestina latifolia* B) *F. platyphylla*, C) *F. purpurea*, D) *F. lobata*, E) *F. tripteris*, and F) *F. pedata.* Scale bar 1 mm.

Fig. 2 Phylogenetic tree constructed from Bayes Inference analysis of ETS and ITS sequences showing relationships of *Florestina* (modified from Soto-Trejo et al., 2015). Numbers above the branches indicate posterior probabilities values. A partial timescale is shown at the bottom, with units in millions of years.

Fig. 3 Pollen mother cells of *Florestina platyphylla n* = 12 (A) and *F. lobata n* = 10 (B) at meiotic metaphase. Scale bar 10 μm.

Fig. 4 Distribution of *Florestina platyphylla* and *F. latifolia.*

Fig. 5 Distribution of *Florestina purpurea* and *F. lobata.*

Fig. 6 Distribution of *Florestina tripteris* and *F. pedata.*
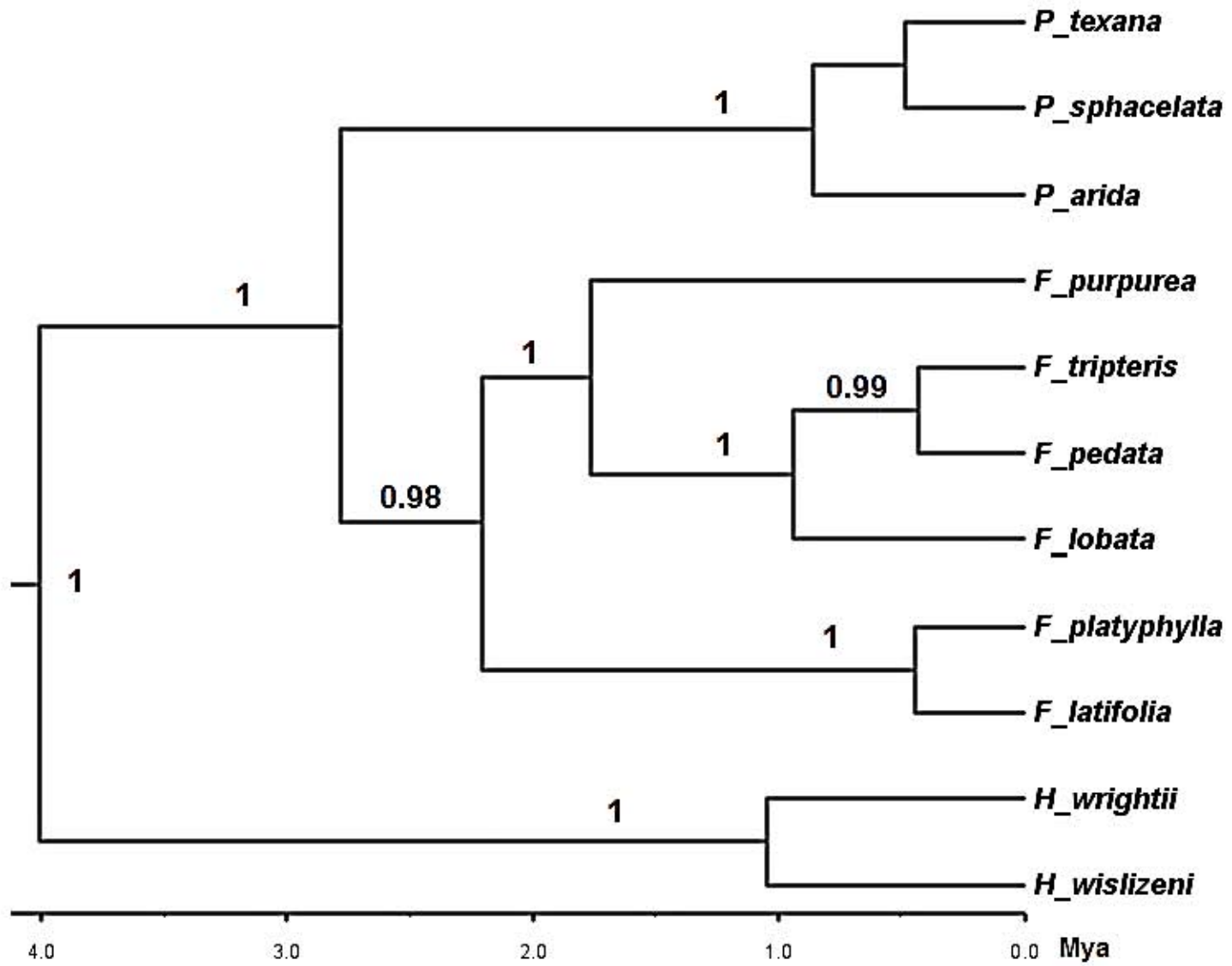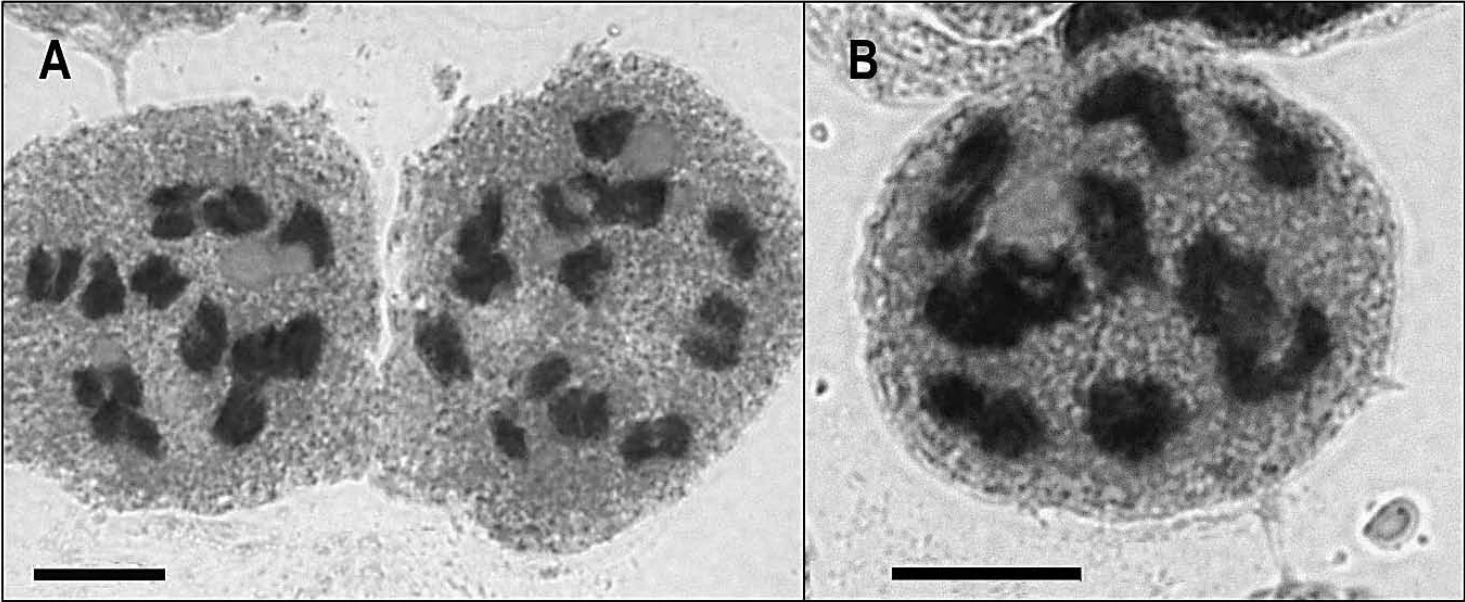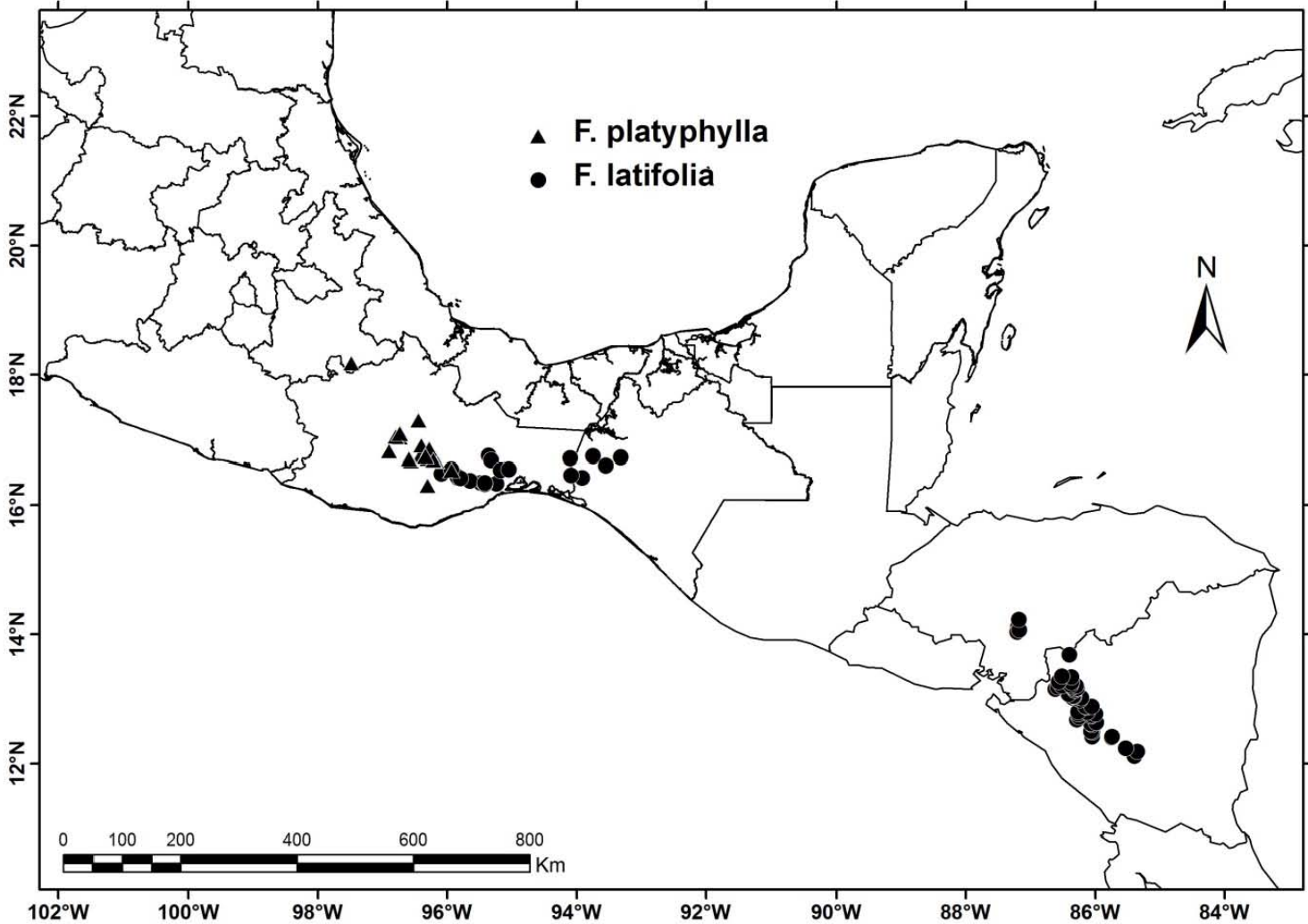
Fig. 1

Fig.2

Fig.3

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Fig. 5
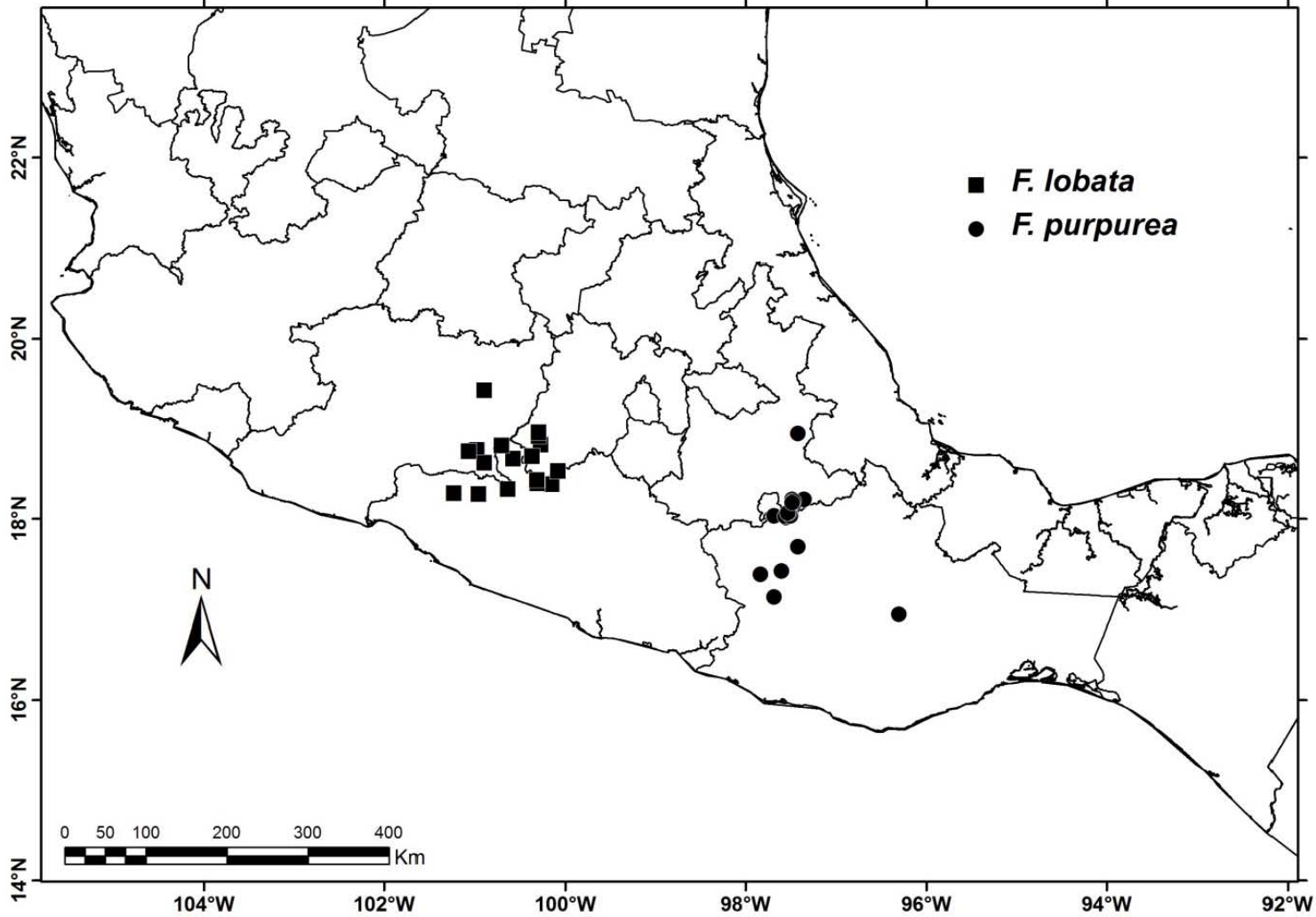
Fig. 6
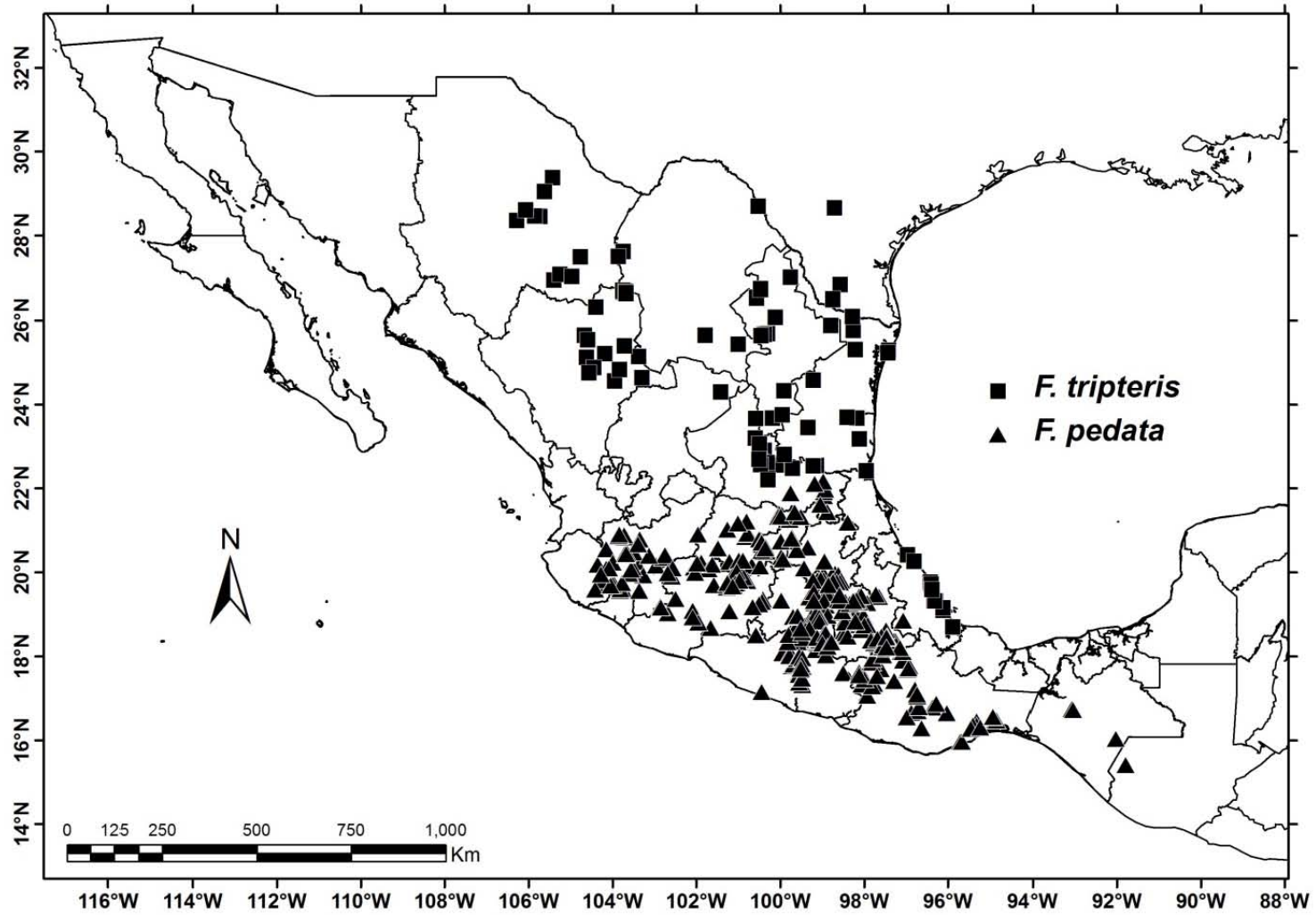
Table 1. Chromosome numbers for *Florestina* species.

Species	*n*	References
Florestina latifolia	12	Turner 1963; Turner and Flyr 1966; Powell et al. 1975
F. lobata	10	Soto-Trejo et al. 2015
F. pedata	10	Turner a nd Johnston 1961; Beaman and T urner 1962; Turner 1963; Keil et al.1988; Sundberg *et.al*. 1986 as *F. simplicifolia*
F. platyphylla	12	Soto-Trejo et al. 2015
F. purpurea	12	Soto-Trejo et al. 2015
F. tripteris	10	Turner a nd J ohnston 1961 as *F. liebmannii*; Turner 1963 ; Keil and S tuessy 1975 ; Keil and S tuessy 1977 a s *F. liebmannii*; Powell et al. 1975; Watanabe *et al*. 2007, $2n = 20$

CONCLUSIONES GENERALES

Generalmente, los es tudios filogenéticos q ue i ncorporan datos m orfológicos y moleculares en u n análisis combinado, proporcionan mejores e stimaciones de la filogenia que a quellos que a nalizan los d iferentes c onjuntos de da tos po r s eparado (De Q ueiroz 1995). Sin embargo, en el estudio filogenético de *Florestina*, los resultados de la prueba de homogeneidad de p articiones mostró que los c onjuntos d e d atos m orfológicos, de ADN nuclear y de ADN d e c loroplasto s on incongruentes e ntre s í. P or l o t anto, un análisis combinado de es tos c onjuntos d e d atos n o es tán justificados p ara es timar las relaciones filogenéticas del género. De hecho, un análisis combinado en *Florestina* podría incrementar el r iesgo d e m ezclar d iferentes señales f ilogenéticas, l o cual podría o bscurecer p rocesos biológicos alternativos, tales como hibridación o introgresión (Wendel y Doyle 1998).

Los datos morfológicos s on la fuente primaria e n e l estudio d e la gran mayoría d e los grupos de organismos. Sin embargo, en el caso de taxones morfológicamente similares, como *Florestina*, pocos caracteres morfológicos se emplean en estudios t axonómicos. Los resultados de l a nálisis d e pa rsimonia de los caracteres m orfológicos n o contribuyeron a resolver la filogenia de *Florestina* y no mostraron caracteres sinapomórficos que apoyen la monofilia de l gé nero. Además, el análisis d e c omponentes p rincipales s ólo fue útil e n e l reconocimiento de *F. latifolia* y *F. platyphylla*, pero no para el resto de las especies. Por lo tanto, la morfología no contribuyó a e sclarecer las r elaciones filogenéticas e n *Florestina* y no p ermitió el reconocimiento d e cada u na d e las especies. Por s u p arte, los d atos moleculares si fueron útiles para esclarecer las relaciones filogenéticas en *Florestina*.

En general, en los estudios filogenéticos moleculares se utilizan d iferentes genes (o regiones g enéticas), los cu ales p ueden p resentar diferentes t asas d e s ustitución y p ueden ayudar a resolver diferentes n iveles j erárquicos d e una f ilogenia (Fisher-Reid y Wi ens,

2011; Gibson y Baker, 2012). Los espaciadores ETS e ITS son comúnmente utilizados para realizar inferencias filogenéticas a nivel genérico e infragenérico, debido principalmente a sus elevadas tasas de sustitución (Álvarez y Wendel, 2003). Los análisis filogenéticos basados en los espaciadores ETS e ITS recuperaron una filogenia altamente resuelta, la cual indica que *Florestina* es un grupo monofilético y apoya que *Palafoxia* es el género más cercanamente relacionado. Los resultados muestran dos clados principales, en los cuales las relaciones filogenéticas entre las especies fueron resueltas en su mayoría. El primer clado está formado por *F. latifolia* y *F. platyphylla*, mientras que el segundo clado comprende el resto de las especies. En el segundo clado, cuatro taxones morfológicamente muy similares, *F. liebmannii*, *F. pedata*, *F. simplicifolia*, y *F. tripteris*, están muy cercanamente relacionados y los datos moleculares sugieren que sólo dos especies deben ser reconocidas: una que comprende a *F. pedata* y *F. simplicifolia*, la cual muestra amplia variación morfológica a través de su área de distribución; y la otra que comprende a *F. liebmannii* y *F. tripteris*. Por otra parte, *Florestina lobata* y *F. purpurea* son especies claramente distintas del resto y fuertemente apoyadas como grupos monofiléticos. Adicionalmente, otro taxón potencial designado como *Florestina sp.* podría ser una nueva especie, pero hasta ahora sólo ha sido reconocida por caracteres moleculares y aún está siendo evaluada.

Por otro lado, el ADN de cloroplasto ha sido particularmente útil en estudios filogeográficos y de estructura genética poblacional, debido a su herencia uniparental, a las altas tasas de sustitución y a que no recombinan (Avise, 2000). En contraste con la filogenia basada en ADN nuclear, los análisis filogenéticos de las secuencias de ADN de cloroplasto (*rpl32-trn*L y *trn*C-*pet*N) en *Florestina* no contribuyen a resolver las relaciones filogenéticas ni apoyan la monofilia del género. Esta incongruencia entre ambas filogenias

podría d eberse a p rocesos d e hibridación e introgresión (Rieseberg y S oltis, 1991; Rieseberg y Carney,1998; Soltis y Soltis, 2009). La hibridación e introgresión han jugado un papel importante en la especiación y evolución de plantas (Soltis y Soltis, 2009). Estos procesos o curren frecuentemente en p lantas d e h ábitats ár idos y s emiáridos, c omo las especies d e *Florestina*, y s on f avorecidos por condiciones que permiten e l co ntacto recurrente d e las es pecies a isladas r eproductivamente d e forma incompleta (Schneider et al., 2011).

Las d iferencias e ntre los r esultados d e los es tudios filogenéticos b asados e n d atos morfológicos y moleculares pueden deberse a varias causas. Las diferencias en el diseño y en el análisis de los datos son las más comúnmente referidas en la literatura, por ejemplo: i) diferencias en la obtención de los datos (p. ej. en el muestreo taxonómico, en el muestro insuficiente d e car acteres); ii) diferencias en los m étodos d e a nálisis (p. e j. máxima parsimonia v s má xima v erosimilitud); iii) el uso de diferentes opciones en los métodos de análisis (p. e j. o ptimización, e nraizamiento, método d e al ineamiento d e s ecuencias). Sin embargo, cuando los datos moleculares producen una filogenia robusta que contrasta con una filogenia poco resuelta obtenida a p artir d e la mo rfología, como en e l caso d e *Florestina*, la s incongruencias pueden deberse principalmente a diferencias en la tasas de evolución morfológica y molecular (Wiens y Hollingsworth, 2000; Poisot et al., 2011). Por lo tanto, la divergencia molecular no está necesariamente asociada a cambios morfológicos importantes y v isceversa. Por ejemplo, estudios moleculares han mostrado la existencia de especies "crípticas", las c uales so n morfológicamente in distinguibles, p ero presentan u na amplia divergencia genética (Witt et al., 2006; Bickford et al., 2007).

El número de cromosomas es una herramienta útil en el estudio de la evolución y sistemática de plantas, que puede complementar la información obtenida a partir de datos morfológicos y moleculares (Guerra, 2008). Recientemente, se desarrolló un método para inferir la evolución del número de cromosomas por medio de modelos probabilísticos (Mayrose et al., 2010). Este análisis permite probar hipótesis acerca de los patrones de evolución cromosómica a lo largo de una filogenia. El análisis de la evolución cromosómica en *Florestina* apoyó la hipótesis que n = 12 es el número ancestral de cromosomas más probable para el género. Por lo tanto, el número de cromosomas de n = 10 en el género podría ser interpretado como una reducción producida a través de disploidía descendente. La disploidía es un importante mecanismo evolutivo de las plantas y es particularmente común en Asteraceae (Guerra, 2008; Galbany-Casals et al., 2009), el cual se ha asociado con una tendencia a acortar el ciclo de la vida como una adaptación a hábitats extremos o xerófilos como parece ser el caso de *Florestina pedata* y *F. tripteris* (p.e. García-Jacas et al., 1996; Watanabe et al., 1999; Vilatersana et al., 2000; Torrell et al., 2001; Selvi y Bigazzi, 2002; Garnatje et al., 2004; Galbany-Casals et al., 2009).

Los estudios filogenéticos moleculares y el desarrollo de modelos probabilísticos en biogeografía histórica han generado cambios importantes respecto a sus métodos y sus supuestos básicos. Recientemente, se han desarrollo distintos modelos probabilísticos que permiten la inferencia biogeográfica a partir de enfoques de Máxima Verosimilitud e Inferencia Bayesiana. En particular, BioGeoBEARS es un herramienta que permite comparar estadísticamente una variedad de modelos biogeográficos con base en la probabilidad que le confieren a las áreas geográficas observadas en la filogenia y la importancia relativa de los diferentes procesos biogeográficos (p.e. vicarianza vs. dispersión) (Matzke, 2015). Esta herramienta también implementa el método de mapeo

estocástico, el cual es apropiado para estimar el número de eventos que han ocurrido en una filogenia y es útil para analizar pequeños conjuntos de datos. Tal es el caso de *Florestina*, donde la incertidumbre es alta y requiere ser evaluada cuantitativamente. Los resultados de los análisis biogeográficos usando BioGeoBEARS sugieren que: 1) *Florestina* se originó a partir de un ancestro ampliamente distribuido desde el sur de Estados Unidos hasta el sur de México; 2) la divergencia de *Florestina* y *Palafoxia* fue probablemente influenciada por el desarrollo del Eje Neovolcánico Transversal; 3) el modelo biogeográfico más probable mostró un número relativamente bajo de eventos de dispersión entre todas las áreas; y 4) la diversificación de *Florestina* fue probablemente resultado de los cambios climáticos ocurridos durante el Pleistoceno. Finalmente, los análisis filogenéticos y biogeográficos en *Florestina* destacan la influencia de los cambios climáticos durante Pleistoceno en la conformación de la biodiversidad en ambientes secos de México.

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