PHYLOGENETIC RELATIONSHIPS OF THE MEXICAN TUSSILAGINOID GENERA (ASTERACEAE: SENECIONEAE)

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ABSTRACT

The Mexican tussilaginoid genera (Asteraceae: Senecioneae) consists of 13 genera distributed from the USA to Panama, with most species occurring in montane regions from Central Mexico to Guatemala. Presently, 142 species are recognized in this clade, with many considered to be endemic to threatened pine-oak or cloud forest ecosystems. Endemism is abundant among the clades identified, with over half of the species restricted to relatively small geographic areas. Moreover, most members of the Mexican tussilaginoid inhabit montane regions at or above 1500 m in Mexico and Guatemala and are thus under immense pressure from human land use. We conducted a phylogenetic investigation of the Mexican tussilaginoid genera and outgroup taxa, using nuclear ribosomal DNA sequences of 74 species from 17 different genera. We also compared competing hypotheses regarding the monophyly of three genera that were supported as monophyletic in previous investigations using topology-based hypothesis testing. The results of our analyses support thirteen clades within the Mexican tussilaginoid genera. Topology-based hypothesis testing indicated that the genera *Pittocaulon, Psacaliopsis,* and *Roldana* are not monophyletic. The genus *Telanthophora,* by contrast, was found monophyletic, but nested within *Roldana.*

Key Words: Guatemala, hypothesis testing, Mexico, Senecioneae

RESUMEN

Los géneros tussilaginoides mexicanos (Asteraceae: Senecioneae) son 13 géneros distribuidos desde Estados Unidos a Panamá, y la mayoría de las especies ocurren en las regiones montañosas desde el centro de México hasta Guatemala. Actualmente, se reconocen 142 especies muchas de éstas consideradas endémicas de ecosistemas amenazados como los bosques de pino-encino o nefosilvas. Los endemismos son abundantes en el clado, con más de la mitad de las especies restringidas a áreas geográficas relativamente pequeñas. Además, la mayoría de los miembros del grupo están presentes en las regiones montañosas a 1500 m de elevación o más en México y Guatemala, donde existe una inmensa presión por las prácticas humanas de uso de la tierra. Se incluyeron sesenta y cuatro especies de 17 géneros dentro del grupo de estudio y los grupos externos en un análisis filogenético combinado de dos regiones de la repetición ribosómica nuclear, los espaciadores transcritos internos y externos. Los resultados del análisis combinado de nrDNA respaldaron fuertemente trece clados dentro los géneros tussilaginoides mexicanos. Sin embargo, la topología y la prueba de hipótesis utilizando modelos de restricción indicaron que los géneros *Pittocaulon*, *Psacaliopsis* y *Roldana* no eran monofiléticos. *Telanthophora* es monofilético, pero este género estaba anidado dentro de *Roldana*.

INTRODUCTION

The Mexican tussilaginoid genera (Asteraceae: Senecioneae) consists of 13 genera (Table 1) and 142 species distributed from the United States to Panama, with centers of diversity in central Mexico and southern Mexico-Guatemala (Barkley 1985a, b; Barkley et al. 1996). This diverse group consists of small trees, shrubs, herbs and epiphytes, most of which occur at elevations above 1500 m in cloud and/or pine-oak forests. Endemism in this group is high, with approximately 60% percent of species occurring in Guatemala only, in Guatemala and the adjacent Mexican state of Chiapas, or in two or fewer states of Mexico. The species share the following characters: stigmatic surfaces united across at least the upper third of the inner face of the style branches; cylindrical anther collars; principal phyllaries with midrib thickened at the base; chromosome

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Table 1. Overview of the genera in Mexican tussilaginoid genera and the genus and species numbers included in this investigation.

Genera in Mexican tussilaginoids	Number of species per genus	Number of species sampled
Arnoglossum	9	2
Barkelyanthus	1	1
Digitacalia	5	2
Nelsonianthus	2	1
Pippenalia	1	1
Pittocaulon	5	4
Psacaliopsis	5	5
Psacalium	46	4
Robinsonecio	2	1
Roldana	55	35
Telanthophora	9	7
Villasenoria	1	1
Yermo	1	1

numbers *n* = ca. 30. The Mexican tussilaginoid and other endemic Asteraceae that inhabit threatened montane ecosystems have been promoted for conservation efforts (Villaseñor et al. 1998).

The Senecioneae is the largest tribe of Asteraceae with respect to species numbers, comprising approximately 3000 species in 150 genera (Nordenstam 2003, 2007). The 'Mexican tussilaginoid' genera are comprised of members of the subtribe Tussilagininae that are most diverse in the montane regions of Mexico and Guatemala, and this group of plants is part of the 'tussilaginoid' clade of the Senecioneae (Barkley & Funston 1996). The Mexican tussilaginoid either have radiate ('senecionoid' genera) or discoid capitula ('cacalioid' genera). Barkley et al. (1996) referred to the 'Mexican tussilaginoid' genera as the 'tussilaginoid genera of Mexico and Central America', as the species are distributed also in areas outside Mexico. The genera *Arnoglossum* Raf. and *Yermo* Dorn were excluded from Barkley's treatment due to their exclusive occurrence in the USA.

Several taxonomic treatments of the Mexican tussilaginoid genera (Barkley 1985a, b; Jeffrey 1992; Barkley et al. 1996) or sections within this group (Rydberg 1924a, b; Pippen 1964, 1968; Robinson & Brettell 1973a, b, 1974; Robinson 1974; Barkley & Janovec 1996; Clark 1996; Funston 2008) were previously published. However, their generic and species delimitations remain largely unresolved (e.g. compare most recent *Roldana* treatments by Funston 2008 and Turner 2005). Only three studies have so far analyzed DNA sequence data of representatives of the Mexican tussilaginoid genera in a phylogenetic context (Bain & Golden 2000; Pelser et al. 2007; Pelser et al. 2010). Although these investigations supported the monophyly of the Mexican tussilaginoid genera, they did not clarify inter- and intrageneric relationships of the Mexican tussilaginoids. Thus, phylogenetic relationships of the entire group have yet to be investigated with DNA sequence data using a taxon sampling that is sufficient to clarify both inter- and intrageneric relationships. Pelser (2010) illustrated that incongruence between nuclear and plastid phylogenies was common in the Senecioneae, including among the study genera.

In the present investigation, we conduct a phylogenetic analysis of the inter- and intrageneric relationships of the Mexican tussilaginoid genera based on DNA sequence data of two nuclear ribosomal markers. The goals of this study are to (1) reevaluate the phylogenetic relationships among these genera using novel sequence data and a dense taxon sampling, and (2) evaluate the monophyly of the genera *Pittocaulon* H. Rob. & Brettell, *Psacaliopsis* H. Rob. & Brettell, *Roldana* La Llave, and *Telanthophora* H. Rob. & Brettell.

MATERIALS AND METHODS

Taxon sampling

A total of 262 accessions, representing 74 species in 17 genera, were collected and assembled for this investigation. Our sampling hereby comprises accessions from each genus of the Mexican tussilaginoid genera and covers the entire geographic distribution and morphological variation among the genera. The plant material

was taken from herbarium specimens, silica-dried and living plant material. Live plants were grown at the Brackenridge Field Laboratory of The University of Texas at Austin. Silica-dried specimens were collected during field trips to Mexico and Guatemala between 2009 and 2013. Herbarium specimens were deposited at the University of San Carlos of Guatemala (BIGU), the National Autonomous University of Mexico (UNAM) and the University of Texas (TEX). To supplement our taxon sampling, we included 39 DNA sequences obtained from GenBank to our sequence data set. Table 1 summarizes the genera and the number of species analysed in this investigation; Appendix 1 provides a list of all analysed accessions, corresponding herbarium vouchers and GenBank/ENA accession numbers.

Selection of DNA markers

We selected two nuclear ribosomal markers for phylogenetic analysis of the Mexican tussilaginoid genera: the internal transcribed spacer (ITS) and the external transcribed spacer (ETS). The utility of both markers for interspecific phylogenetic studies in Asteraceae has been demonstrated by numerous investigations (e.g., Baldwin 1992; Baldwin & Markos 1998; Linder et al. 2000; Lee et al. 2003; Roberts & Urbatsch 2003; Sanz et al. 2008). Combining these two markers typically yields more resolved phylogenies than either one individually (Baldwin & Markos 1998; Clevenger & Panero 2000; Markos & Baldwin 2001; Roberts 2002; Roberts & Urbatsch 2003). We conducted phylogenetic analyses of these two nuclear DNA markers separately and after concatenation.

The ITS was sequenced for 144 accessions, representing 72 species, with 26 sequences from previous investigations (Pelser et al. 2007, 2010); the ETS was sequenced for 119 accessions, representing 67 species, with 18 sequences from previous investigations (Pelser et al. 2007, 2010); the combined ITS/ETS data set contained 100 accessions, representing 67 species. Each marker-specific data set included DNA sequences of outgroup taxa, with nine outgroup accessions in the ITS, five outgroup accessions in the ETS data set, and five outgroup accessions in the combined data set. In total, 221 novel sequences were included in the study. Species of the genera *Aequatorium* B. Nord. and *Gynoxys* Cass. from South America were used as outgroup taxa for the Tussilagininae, as they are members of the sister group of the Mexican tussilaginoid genera (Pelser et al. 2007). The species *Rugelia nudicaulis* Shuttlew. ex Chapm., a more distantly related Tussilagininae genus from eastern USA, and *Senecio vulgaris* L., a non-tussilaginoid member of in the Senecioninae, were also included as outgroups.

DNA extraction, amplification and sequencing

Genomic DNA was isolated from herbarium vouchers, silica-dried and living plant material using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). DNA markers were amplified through polymerase chain reaction (PCR) in 25 µL volumes containing 14.6 µL of ddH_2O, 7.5 µL of FailSafe reaction buffer (Epicentre Biotechnologies, Madison, Wisconsin), 0.25 µL of forward and reverse primers (at a concentration of 20 µM each), 0.4 µL of *Taq* polymerase and 2 µL of DNA template. The ETS was amplified using the primers ETS2 (Bayer et al. 2002) and 18S-ETS (Baldwin & Markos 1998); the ITS was amplified using the primers ITS1A (Sharpe et al. 2000) and ITS4 (White et al. 1990). Reaction conditions included an initial denaturation step at 94°C for 1 min 30 sec, followed by 30 cycles of 94°C for 30 sec, 53°C for 30 sec and 72°C for 1 min 30 sec, with a final extension step of 72°C for 30 min.

Different nuclear ribosomal alleles were isolated via cloning using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, California). Plasmid inserts were amplified under the same conditions as the nuclear ribosomal markers, except for the use of pUC-18 primers and an increased amount of FailSafe reaction buffer. PCR conditions were kept unchanged, except for an increase in the denaturation and annealing time to 45 sec each, an increase in the annealing temperature to 58°C and an increase in the cycle number to 35. Unused primer and residual nucleotides were removed from the amplicons through the application of Exonuclease I (New England Biolabs, Inc., Ipswich, Massachusetts) and Shrimp Alkaline Phosphatase (Promega Corp., Madison, Wisconsin). Sanger sequencing was carried out at the Institute for Cellular and Molecular Biology Core Facilities at The University of Texas at Austin.

For each species, between two to five clones were sequenced per accession. For ITS, any clone that

differed by more than one nucleotide in the 5.8S region was excluded from our datasets as a paralogous copy. To avoid the combination of clones from different accessions in our combined data sets, at least two clones for both ETS and ITS were sequenced from those accessions that were successfully amplified in each marker. Final DNA sequences were submitted to GenBank/ENA with the help of custom Python scripts (https://github.com/michaelgruenstaeudl/annonex2embl).

Sequence alignment, model selection and data concatenation

DNA sequences were edited in Geneious Pro 4.0.4 (Drummond et al. 2006) and aligned using ClustalX v.1.8 (Thompson et al , 1997), followed by a manual adjustment in Mesquite (Maddison & Maddison 2011) using the rules of Kelchner (2000). Upon alignment, the ITS data set had a length of 827 nucleotides; 418 (50%) were invariant, 304 (37%) were variable and parsimony-informative and 105 (13%) were variable but uninformative. The aligned ETS data set had a length of 469 nucleotides; 205 (44%) were invariant, 179 (38%) were variable and parsimony-informative and 85 (18%) were variable but uninformative. Table 2 summarizes sequence characteristics for the combined ITS/ETS data set and the separate ITS and ETS data sets.

Best-fitting models of nucleotide substitution were selected using the Akaike information criterion (AIC; Akaike 1974) in Modeltest v.3.06 (Posada & Crandall 1998). The GTR+G model was determined to be the best-fitting model for both the separate ITS and ETS data sets as well as the combined data set. Phylogenetic congruence between the ETS and ITS data sets and their suitability for combination into a single data set was evaluated and confirmed via the incongruence length difference test (ILD; Farris et al. 1994) in PAUP v. 4.0b10 (Swofford 2002). The concatenated ITS/ETS data set contained the common set of taxa of the individual data sets and had a length of 1296 nucleotides; 261 (21%) were invariant, 501 (38%) were variable and parsimony-informative, and 534 (41%) were variable but uninformative.

Phylogenetic reconstruction

Phylogenetic reconstructions were performed via maximum likelihood (ML) and Bayesian inference (BI). Reconstructions under ML were performed with RAxML v.7.2 (Stamatakis 2008) using the GTR+G model of nucleotide substitution with unlinked data partitions. Node support for phylogenetic inferences under ML was calculated using 100 bootstrap (BS) replicates. Reconstructions under BI were performed with MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003) using two Markov Chain Monte Carlo (MCMC) runs, each with four parallel chains, for a total five million generations and saving every 10,000th generation. Independent sampling of generations and the convergence of Markov chains were confirmed in Tracer v.1.4 (Rambaut & Drummond 2007). The initial 50% of all MCMC trees were discarded as burn-in. Post burn-in trees were summarized as maximum clade credibility (MCC) trees and visualized via FigTree v.1.4.2 (Rambaut 2008). Node support for phylogenetic inferences under BI is given as posterior probability (PP) values.

Evaluation of competing hypotheses

We evaluated the hypothesis of monophyly of three genera (i.e., *Pittocaulon, Psacaliopsis,* and *Roldana)*, which were considered monophyletic in previous investigations (Robinson & Brettell 1974; Turner 2005; Funston 2008), under our new sequence data using topology-based hypothesis testing. In this type of hypothesis testing, the likelihood of the optimal tree topology is compared to the likelihood of a tree topology inferred under a topological constraint, with both inferences based on our new sequence data. Specifically, competing hypotheses were evaluated via marginal likelihood estimation using stepping-stone sampling of harmonic means in MrBayes. The stepping-stone sampling was based on 50 steps, with 19,500 generations (39 samples) within each step. A log difference above 10 was considered strong evidence in favor of the model with a better model fit (i.e., the optimal BI tree; Kass & Raftery 1995; Xie et al. 2011).

RESULTS

Phylogenetic reconstruction

Thirteen strongly supported (PP > .97) clades in the Mexican tussilagnoid genera were recovered under BI of the ITS data set (Fig. 1), whereas eleven strongly supported clades were recovered under BI of the ETS data set

TABLE 2. Overview of the DNA markers under study, the number of accessions per marker and the number of total and of phylogenetically informative characters. Columns 4 (ITS) and 5 (ETS) refer to the marker-specific data set partitions of the combined data set. Abbreviations used: acc. = accessions

Data set	ITS	ETS	Combined	ITS (101 acc.)	ETS (101 acc.)
# of accessions (# of species)	144 (72)	119 (69)	100 (67)	100 (67)	100 (67)
Total characters	827	469	1296	827	469
# of informative characters	304	179	501	285	178
% of informative characters	37%	38%	38%	34%	38%

(Fig. 2). Both data sets support the monophyly of the Mexican tussilagnoid genera and its sister relationship to the representative South American gynoxoid taxa, although more taxa from the gynoxoid clade would be required to confirm this relationship, as demonstrated in Pelser et. al 2007, 2010. The MCC tree based on ITS was better resolved, and most clades received stronger support than in the MCC tree based on ETS. The ILD test indicated that the ITS and ETS data sets were not significantly incongruent ($P = 0.19$) and were thus combined. Thirteen clades were recovered using BI of the combined data set (Fig. 3), with clade support higher than in the individual data sets. In addition, ITS and the combined ITS/ETS data supported 13 different clades, often with strong BS and PP support. The genera *Roldana*, *Pittocaulon* and *Psacaliopsis* were hereby identified as not monophyletic by the optimal ML and BI trees.

Evaluation of competing hypotheses

The results of our topology-based hypothesis testing indicated that none of the three evaluated genera of the Mexican tussilagnoid clade (i.e., *Roldana*, *Pittocaulon* and *Psacaliopsis*) were likely monophyletic (Table 3). The optimal tree topology inferred under BI, which found *Roldana* s.s. (log difference = 17.46), *Pittocaulon* s.s. (log difference = 13.41) and *Psacaliopsis* (log difference = 108.37) as polyphyletic, displayed a significantly better fit to the data than the constraint topology, in which these genera were constrained to be monophyletic. An overview of the results of our topological hypothesis testing is given in Table 3.

DISCUSSION

Phylogenetic relationships of the Mexican tussilaginoid were evaluated using two nuclear ribosomal DNA markers and a dense taxon sampling. Our analyses resolved several relationships among the Mexican tussilaginoid that have so far been unresolved (Pelser et al. 2007, 2010). The most profound result of this study is that the genus *Roldana* is not monophyletic in its current circumscription, even though it has been considered a distinct, morphologically well-circumscribed genus (Robinson & Brettell 1974; Turner 2005; Funston 2008).

Previous molecular phylogenetic investigations

Several molecular phylogenetic analyses that include representatives of the Mexican tussilaginoid were previously published. Bain & Golden (2000) included four Mexican tussilaginoid species (*Barkleyanthus salicifolius*, *Robinsonecio gerberifolius, Pittocaulon praecox* and *Psacalium peltatum*) in a molecular phylogenetic analysis of *Packera*. They demonstrated moderate support for a clade formed by these four genera. Pelser et al. (2007) inferred the phylogenetic relationships of the Mexican Tussilaginoid clade using nuclear ribosomal DNA sequences from 13 species in eight genera. Their study was the first to demonstrate strong support for the monophyly of the Mexican tussilaginoid clade as well as for the South American Tussilagininae 'gynoxoid' clade, which was recovered as sister to the former. Pelser et al. (2010) later expanded their work, sequencing the external transcribed spacer (ETS), the internal transcribed spacer (ITS) and five plastid loci from 13 accessions that represented 13 different genera of Mexican tussilaginoid. This investigation confirmed the monophyly of the group and its sister relationship to the gynoxoid clade. Although major clades in the Senecioneae that were recovered through DNA sequence data of nuclear ribosomal markers were also supported by plastid DNA sequence data, several taxa and clades were found incongruent when comparing these data sets (Pelser et al. 2010).

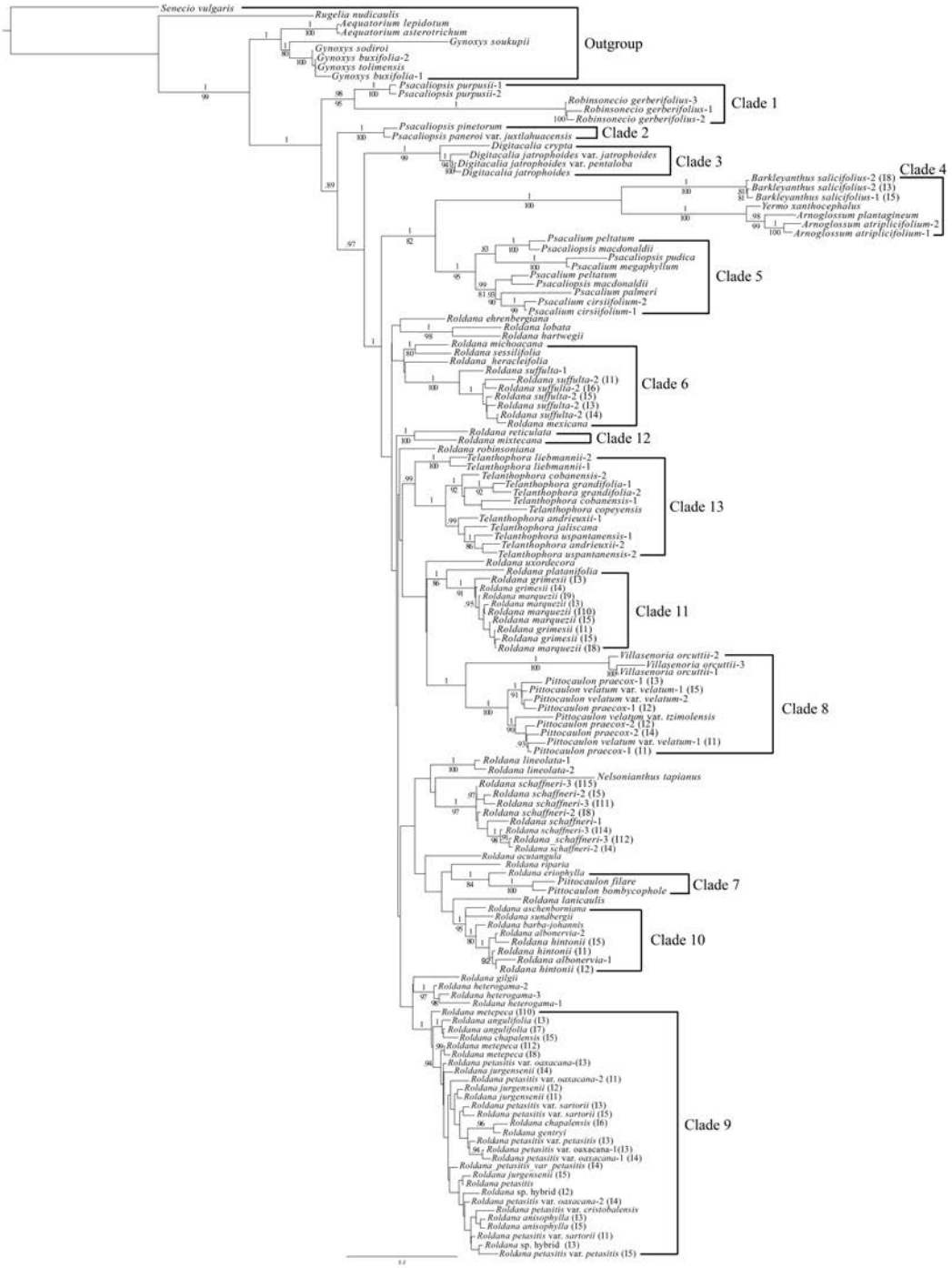

Fig. 1. MCC tree of the posterior tree distribution inferred under BI of the ITS sequence data. Numbers above branches indicate PP values, numbers below branches indicate BS of the corresponding ML tree inference.

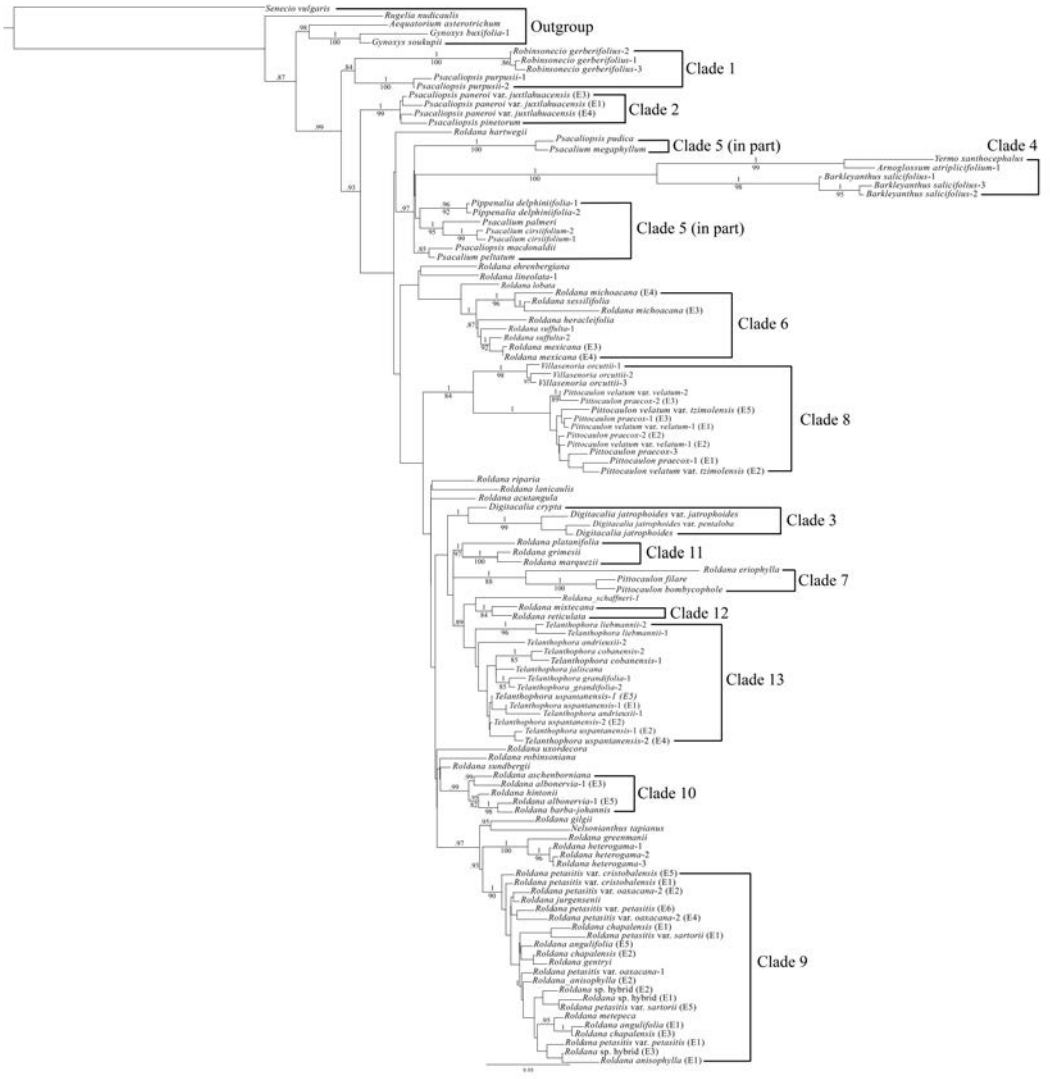

Fig. 2. MCC tree of the posterior tree distribution inferred under BI of the ETS sequence data. Numbers above and below branches are as in Figure 1.

Table 3. Overview and results of our topology-based hypothesis testing regarding the monophyly of three genera that were considered monophyletic in previous investigations. Marginal likelihoods are given in natural log units. Log differences above 10 are considered strong evidence for the alternative model.

Genus	Marginal likelihood of best tree (not monophyletic)	Marginal likelihood of constrained tree (monophyletic)	Difference (log units)	Significance (>10)
Pittocaulon	-13246.18	-13232.77	13.41	*
Psacaliopsis	-13321.85	-13213.48	108.37	*
Roldana	-13207.82	-13225.28	17.46	*

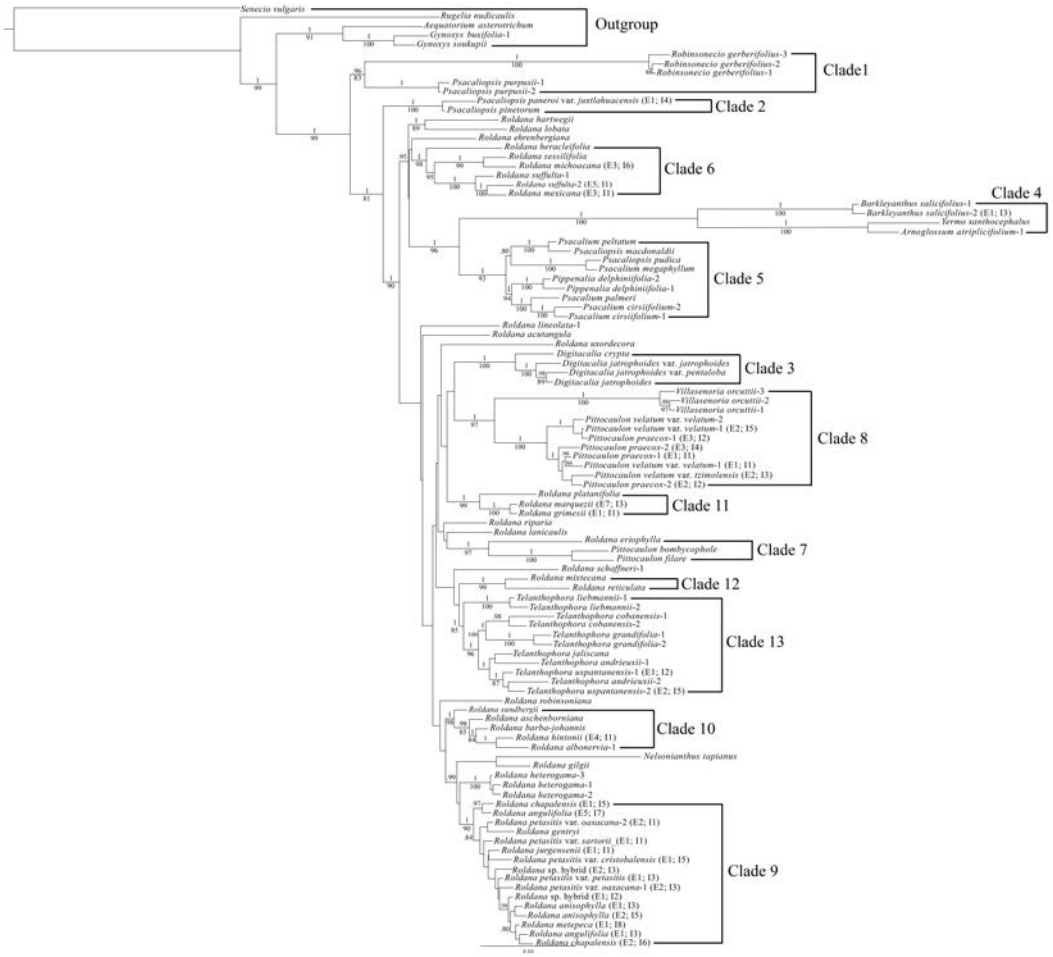

Fig. 3. MCC tree of the posterior tree distribution inferred under BI of the combined ITS/ETS sequence data. Numbers above and below branches are as in Figure 1.

Relationships with clades

Species-rich genera (i.e., *Psacalium* Cass. and *Roldana*) have undergone adaptive radiation in montane Mexico and Guatemala, and the age of the origin of *Psacalium* is estimated to ca. 2.84–3.49 mya (Pelser et al. 2010). The monophyly of the Mexican tussilaginoid group, its sister relationship to the 'gynoxoid' group from South America, and the existence of thirteen major clades are strongly supported through our phylogenetic tree inference (Figs. 1–3). Descriptions of the thirteen supported clades as recovered in the combined ITS/ETS analysis (Fig. 3), with information regarding morphological synapomorphies, geographical distribution, taxonomic implications and purported number of species are provided below.

Clade 1.—This clade comprises the species *Psacaliopsis purpusii and Robinsonecio gerberifolius*, which was sister to the remainder of the Mexican tussilaginoid clade in a previous study using ITS (Pelser et al. 2010), and the ITS data supported *Robinsonecio* as sister the rest of Mexican tussilaginoid genera (Pelser et al. 2007). However, the 2007 study did not include *Pscaliopsis* in the analyses. In the present study, all analyses support clade 1 as sister to the remaining Mexican tussilaginoid genera. The taxa in this clade are found at high elevations in Mexico and Guatemala from 2,600–4,200 m. This clade consists of subalpine and alpine herbs with

radiate yellow capitula, rosulate leaves with denticulate margins and woody rhizomes. Clade 1 is strongly supported as sister to the rest of the Mexican tussilaginoid clade. *Psacaliopsis purpusii* has petiolate, peltate leaves that are orbicular and secondarily lobed. *Robinsonecio* T.M. Barkely & Janovec has subsessile leaves that are ovate to spathulate. Barkley & Janovec (1996) originally described two species of *Robinsonecio* and proposed its relationship to other tussilaginoid genera.

Psacaliopsis purpusii occurs in pine-oak forests at 2,200–2,600 m and has only been reported from Oaxaca and Puebla. *Robinsonecio porphyresthes* was not included in the study but this species is morphologically and anatomically similar to *R. gerberifolius* (Barkley & Janovec 1996; Pruski 2012). Both species of *Robinsonecio* occur in alpine habitats and can occur above 4,000 m; *R. gerberifolius* occurs in central Mexico (Distrito Federal, Mexico, Puebla, Tlaxcala, and Veracruz) and in Huehuetenango, Guatemala. *Robinsonecio porphyresthes* is endemic to alpine habitats in Tamaulipas, Mexico.

Clade 2.—The two species in this clade are likely conspecific. Phenotypic plasticity is evident among herbarium specimens of *Psacaliopsis pinetorum* and *P. paneroi*. The centrally-peltate leaves have lobes that range from rounded to acute and undulate to shallowly lobed (up to ¼ way to center of the blade). Similarly, depending on the age of the plants collected and the amount of the material collected for each specimen, the number of capitula ranges from 5 to over 80 and levels of pubescence on the abaxial leaf surfaces is highly variable. Morphologically, *P. pinetorum* is similar to *P. purpusii* from Clade 1 but the leaves of *P. pinetorum* are not secondarily lobed as in *P. purpusii*. Leaves of *P. pinetorum* are shallowly lobed with sinus depths to ¼ towards the center of the blade vs. sinus depths ½ to ¾ towards the center of the blade in *P. purpusii*, the capitula number 5–80+ in *P. pinetorum* vs. 5–18 in *P. purpusii,* the phyllaries are in two subequal series vs. one series in *P. purpusii* and the corolla tube 5–6 mm long in *P. pinetorum* vs. ca. 3.5 mm long in *P. purpusii*. *Psacaliopsis paneroi* var. *juxtlahuacensis* is based on a single population from Oaxaca, Mexico and the characters that define this variety are found in many herbarium specimens that have been determined as the alternate variety, *P. paneroi* var. *paneroi*. When describing *Psacaliopsis* (*Senecio* L.) *paneroi*, Turner (1989) did not look at specimens of *Senecio pinetorum*. It is obvious that these two species are the same entity and *S. pinetorum* is the older name. *Psacaliopsis paneroi* var. *paneroi* became a taxonomic synonym of *Psacaliopsis pinetorum* var. *pinetorum*. Therefore, *P. paneroi* var. *paneroi* needs to be transferred to *Psacaliopsis pinetorum* var. *pinetorum* and the other variety *P. paneroi* var. *juxtlahuacensis* needs to be transferred to *P. pinetorum* var. *juxtlahuacensis*. This species occurs in pine-oak forests of central to southern Mexico, and it is also reported from El Salvador and Honduras. Although it has not been collected in Guatemala, it is expected to reside there as well.

Clade 3.—This clade includes the *Digitacalia* species examined and Pippen (1968) first proposed *Digitacalia* Pippen as a small genus of perennial herbs with leafy stems, discoid capitula with the corollas deeply cleft and exothecial thickenings on the transverse walls. *Digitacalia heteroidea* was transferred to *Roldana* based on exothecial thickenings on the vertical walls in *R. heteroidea* compared to exothecial thickenings on the transverse walls in the other species of *Digitacalia* and larger capitula in a more lax capitulescence in *R. heteroidea* as compared to smaller capitula and a dense capitulescence in *Digitacalia* (Robinson & Brettell 1974). Presently, *R. heteroidea* remains in *Roldana* (Turner 2005; Funston 2008). In a previous study Turner (1990a) included five species of *Digiticalia* that occur in montane central and southern Mexico but only two of the five species were included in the present study. Species delimitations are vague and based on variable morphological characters and few herbarium specimens.

Clade 4.—This group contains an assemblages of three morphologically diverse genera. Pelser et al. (2007, 2010) demonstrated through molecular evidence that these three genera form a well-supported clade, and that *Barkleyanthus* diverged from the other two genera 3.7–4.8 million years ago (Pelser et al. 2010). *Arnoglossum* is strongly supported as the sister taxon to *Yermo* and these two genera are strongly supported as a sister group to *Barkleyanthus*. The entire clade is well-supported as the sister group to Clade 5. *Arnoglossum* consists of nine species of herbs with discoid capitula that occur in the central and southeastern USA. This genus was originally treated as *Cacalia* in a North American treatment (Pippen 1978). *Barkleyanthus salicifolius* is a shrub or small tree with radiate capitula that is widespread from southern Arizona to Honduras at

1000–3000 m. *Yermo xanthocephalus* is a small, suffruticose herb with radiate capitula endemic to Fremont, County, Wyoming at 2000 m. *Arnoglossum* and *Yermo* are the only two genera in the Mexican tussilaginoid group that are restricted to the USA.

Clade 5.—This clade includes four species of *Pscalium,* two species of *Pscaliopsis* and the single species of *Pippenalia* McVaugh. *Pippenalia delphiniifolia* was originally treated as a species in *Odontotrichum* Zucc. (Rydberg 1924b) based on its non-peltate, pinnatisect leaf blades. This species is the only member of Clade 5 with radiate capitula and it is the only species with this feature in the Mexican tussilaginoid group that lacks a pappus. *Psacalium* was originally characterized by Rydberg (1924a), and later revised by Pippen (1968) and more recently by Robinson (1973b). Rydberg (1924a, b) recognized the peltate leaved *Psacalium* as distinct from the non-peltate or subpeltate leaved *Odonotrichum*. In this and previous studies, *Odontotrichum* is included within *Psacalium* (Pippen 1968; Robinson 1973b). *Psacaliopsis macdonaldii* and *P. pudica* share vegetative characters (basal peltate leaves with rounded lobes and hirsute pubescence of the leaf bases) and floral characters (discoid capitula) with *Psacalium*. The nrDNA data also support *Pippenalia* as being closely related to *Psacalium cirsiifolium and P. palmeri,* and both of these species were previously placed in *Odontotrichum* along with *Pippenalia delphinifolia* (Rydberg 1924b), and *P. cirsiifolium* was the type species for the former *Odontotrichum*. *Pippenalia delphinifolia* occurs at elevations of 2400–3000 m in pine-oak forests. *Psacaliopsis macdonaldii* is endemic to Oaxaca, Mexico, and *Psacaliopsis pudica* is endemic to the Sierra Cuchumatanes of Guatemala and has only been reported from the department of Huehuetenango and Quiche. *Psacalium,* in the broadest sense with the inclusion of *Odontotrichum* (Robinson & Brettell 1973b) occurs in montane forests and meadows from southern Arizona to Guatemala. Only one species, *P. decompositum,* occurs in the USA. Two species, *P. guatemalense* and *P. pinetorum*, are endemic to Guatemala. The five species of *Pscaliopsis* are present in the three different clades in the phylogeny and warrant further investigations into their placement among the Mexican tussilaginoid genera.

Clade 6.—This group includes five species that are placed in *Roldana* but were previously considered in the genus *Pericalia* Cass. In 1827, Cassini first proposed the genus *Pericalia,* although it was not validly published until 1924 (Rydberg 1924a). Pippen (1964, 1968) treated *Pericalia* as a distinct entity with its discoid capitula with underground tubercles attached to the base of the stem and it included the following species: *P. mexicana, P. michaocana, P. sessifolia* (type species), and *P. suffulta*. *Pericalia* has been placed within because these taxa share morphological features of the leaves and flowers, although all three treatments recognize *Pericalia* as a morphologically distinct group of several *Roldana* species due to the presence of discoid capitula *Roldana* (Robinson & Brettell 1974; Turner 2005; Funston 2008). In the present study *Pericalia* (sensu Pippen 1968) is monophyletic with the inclusion of *Roldana heracleifolia,* a species with radiate capitula. This species shares characters with *R. mexicana* and *R. suffulta* such as a single-stemmed herbaceous growth habit to 1–3 m tall, funnelform corollas ca. 8 mm long and pubescent cypselae. Moreover, Turner (2005) suggested that *R. sessifolia* and *R. michocana* may be confused due to certain phenotypical constraints in particular geographic regions (i.e., forms with petiolate leaves), and this supports the present phylogenetic placement of these two species as each other's closest relatives. *Roldana mexicana* has been treated as a variety of *R. suffulta* (Gibson 1969) and this also supports the current placement of these two species within the '*Pericalia*' clade. *Roldana mexicana* and *R. suffulta* have glabrous cypselae and occur sympatrically in four central Mexican states. *Roldana heteroidea* and *R. subpeltata* should be included in a future phylogenetic analysis to confirm the monophyly of this group. There is relatively high morphological variation among *Roldana* (*Pericalia*) species, which can confound field identification of members of this clade (Turner 2005). More sampling across a wider geographic distribution should be included in future studies. Expanded investigations may lead to the resurrection of *Pericalia* as a genus. With the inclusion of the radiate *R. heracleifolia*, the *Pericalia* group consists of seven species distributed in cloud forests and pine-oak forests of central Mexico, and five species of this group were included in the present study.

Clade 7.—*Pittocaulon* has been circumscribed to contain five species with succulent stems, chambered piths and seasonally deciduous leaves (Robinson & Brettell 1973a; Clark 1996). This clade contains two

species in *Pittocaulon* that have pubescent leaves and 13–20 involucral bracts, although a third species, *P. hintonii,* is purported to be related to these species. *Roldana eriophylla* is strongly supported as a member of this clade, and further inspection of voucher specimens at TEX, MEXU and US made it apparent that the material resembled other species in *Pittocaulon* with its succulent stems, chambered piths and deciduous leaves. However, *R. eriophylla* only has eight involucral bracts, a feature not shared with the other two species in Clade 7. The three species formerly placed in *Pittocaulon; P. bombycophole, P. filare* and *P. hintonii*, occur in rocky, deciduous scrublands. *Roldana eriophylla* occurs in pine-oak forests. Two of the species, *P. filare* and *P. hintonii*, are narrow endemics. With the inclusion of *R. eriophylla,* this group is purported to contain four species that occur from central to southern Mexico at elevations between 500–1,700 m.

Clade 8.—This clade consists of two morphologically and geographically distinct groups. The *Pittocaulon praecox/velatum* complex includes succulents with chambered-piths and simple leaves that are deciduous prior to anthesis and are associated with volcanic soils. *Villasenoria orcuttii* is a small tree to four meters in height with a solid pith and pinnately compound leaves. The phylogenies based on ITS/ETS data confirm the previously demonstrated well-supported relationship between *P. praecox* and *Villasenoria orcuttii* (Pelser et al. 2010). Although Pelser et al.'s (2007) data suggested that *P. bombycophole* and *P. praecox* are not closely related, support values for the branches in the combined analysis were weak. *Pittocaulon praecox* and *P. velatum* occur sympatrically in central Mexico, and they differ only in a lack of pubescence just beneath the stem apices in the former (Robinson & Brettell 1973a). *Pittocaulon praecox* occurs from central Mexico to Oaxaca and *P. velatum* and its two varieties occur from central Mexico to Guatemala. These species are most common in deciduous forests or scrublands between 1,000–2,500 m. *Villasenoria* B.L. Clark occurs in Oaxaca and Veracruz and is the only genus in the Mexican tussilagnoid genera restricted to elevations below 1,100 meters in Mexico. (Clark 1996, 1999).

Clade 9.—This group includes seven species and four varieties of mostly small shrubs or suffruticose herbs found in montane regions from northern Mexico (Tamaulipas) to Central America, excluding Belize. Funston (2008) and Turner (2005) disagreed in their species delimitations within *Roldana*. In particular, Funston (2008) treated several members of this complex as varieties. Turner (2005) recognized the varieties as species without a clear explanation. For the above reasons, Funston (2008) is followed with respect to the taxonomy and species delimitations. *Roldana petasitis* var. *cristobalensis* is based solely on the presence of eradiate capitula. Populations of *Roldana anisophylla* have been collected with radiate or eradiate capitula. Although non-peltate leaves are most common among species in Clade 9, *Roldana chapalensis* has peltate leaves, *R. angulifolia* has individuals with peltate or non-peltate leaves, and *R. petasitis* var. *oaxacana* has non-peltate and peltate leaves on the same plant. Based on observations made in a greenhouse, species within this complex are obligate outcrossers but F1 hybrids between *Roldana petasitis* var. *oaxacana* and *R. p.* var. *petasitis* can be readily made and a result of this cross was used in the analyses.

Clade 10.—This clade (Fig. 3) consists of five species, four of which have ovate to cordate leaves with callus denticulate margins and densely pubescent adaxial leaf surfaces. *Roldana hintonii* has elliptic to obovate leaves with arachnoid pubescence on the veins of the adaxial leaf surfaces. *Roldana aschenborniana* is a widespread species that exhibits morphological variation throughout it range from northern Mexico to Guatemala. Two of the four specimens that Turner used in describing *R. sundbergii* were suggested to be hybrids by Gibson (1969). *Roldana albonervia, R. aschenborniana* and *R. barba-johannis* are widespread from northeastern Mexico to Honduras in pine-oak forest and cloud forest habitats most common at 2000–3200 m. *Roldana hintonii* is endemic to the state of Mexico and is only reported from the vicinity of Temascáltepec in pine-oak and fir forests at 2100–3000 m. *Roldana sundbergii* is endemic to Nuevo Leon from 800–2100 m in tropical deciduous and pine-oak forests.

Clade 11.—The three representative species in this complex have 9–13 phyllaries and palmatifid leaves, common to many species of *Roldana*. *Roldana platanifolia* is a small herb from woody rhizomes with mostly basal leaves. *Roldana grimesii* and *R. marquezii* are small shrubs with cauline leaves distributed on the upper half of the stems. *Roldana platanifolia* occurs in pine-oak and fir forests of central Mexico from 2700–4100 m.

Roldana grimesii and *R. marquezii* occur in central Mexico in pine-oak forests from 1500–2700 m. The number of species in this group is unknown, although it is likely that there are only a few. This number depends on whether one accepts Funston's classification of a broad *R. grimesii* or Turner's concept of three separate species based on dubious distinctions.

Clade 12.—The two species in this clade are similar with respect to their leaf morphology and radiate capitula that are arranged in corymbiform cymes, features that are uncommon in *Roldana*. *Roldana mixtecana* has more stems than *R. reticulata* (3–5 vs. 1), the leaf blade margins are entire vs. serrate, and *R. mixtecana* is the only species in the Mexican tussilaginoid genera with phyllaries in five graduated, dimorphic series, the outer three series rimmed with deep purple. The phyllaries are stramineous, a characteristic not found in other species of *Roldana* (Panero & Villaseñor 1996). Panero & Villaseñor (1996) suggested a relationship of *R. mixtecana* to *R. michoacana* and *R. sessifolia* based on the shared small, angular leaves and the short and several-stemmed herbaceous habitat. These species differ from *R. mixtecana* in the presence of discoid capitula with white corollas. Funston (2008) suggested a relationship between *R. mixtecana* and *R. hederifolia,* although the latter species has discoid capitula and uniseriate phyllaries. *Roldana mixtecana* has been reported from two districts in Oaxaca in pine-oak forests from ca. 2000 meters. *Roldana reticulata* is widespread at 2800–3600 m in pine-oak forests in central Mexico.

Clade 13.—*Telanthophora* was originally proposed by Robinson & Brettell in 1974, and at that time it included 14 species of suffruticose herbs, shrubs or small trees with solid piths and leaves restricted to just below the capitulescence. Clark (1996) revised the genus and her treatment included nine species. Moreover, she indicated a phyletic relationship of *Telanthophora* with *Pittocaulon* and *Villasenoria* based on morphological characters, although these relationships are not supported in the present study. In recent nrDNA phylogenies of the Senecioneae, the relationship of *Telanthophora* to the remainder of the Mexican tussilaginoid genera is weakly supported based on ITS (Pelser et al. 2007) and ITS/ETS (Pelser et al. 2010) data sets. In our trees (Figs. 1–3), *Telanthophora* is monophyletic, however, it is nested within species of *Roldana.* The small shrub with serrated leaves that is endemic to Oaxaca, *T. liebmannii,* is sister to the rest of Clade 13. The subclade containing *T. andrieuxii, T. jaliscana* and *T. uspantanensis* contains species that are very similar with respect to their glabrous leaves and many aspects of the floral morphology. *Telanthophora andrieuxii* differs from the other two species in having eight involucral bracts as opposed to five to six. The subclade containing *T. cobanensis* and *T. grandifolia* consists of the two most widespread species in the genus, each of which is represented by two varieties. More collections of this genus need to be included in order to understand its evolutionary relationships better, especially the under-collected endemic species *Telanthophora bartlettii* (Belize) and *T. sublaciniata* (Guatemala). *Telanthophora bartlettii* is restricted to below 1100 meters in the Maya Mountains of western Belize. Moreover, more accessions from southern Central America should also be included in any future phylogenetic analysis. The nine species of *Telanthophora* occur in montane habitats and are common between 1200 and 2700 meters from northern Mexico to Panama.

Species of uncertain placement.—With respect to *Roldana,* it is evident based on morphological variation and nrDNA data that this genus needs a thorough phylogenetic survey that would involve extensive fieldwork, herbaria visits and molecular work using nuclear and sufficient plastid data at both the species and population level in order to further elucidate the relationships among this polyphyletic genus. The type species, *Roldana lobata,* is ambiguously placed within the Mexican tussilaginoid group, and it is related to the widespread species *R. hartwegii*. Although the combined analysis supported a clade including *Roldana hartwegii* and *R. lobata,* these two species are not discussed as a clade because of the lack of support for the relationship of *R. ehrebergiana* to *R. lobata* and *R. hartwegii* in the combined analyses, and their ambiguous placement in the separate ITS and ETS analyses. After further molecular analyses are conducted that include plastid data, only those species that formed a monophyletic group that included *Roldana lobata* would be able to be considered *Roldana* s.s. Another unresolved species of *Roldana*, *R. schaffneri,* morphologically resembles *Telanthophora* in having branches that terminate in inflorescences and pinnately-veined leaves. Specimens of *R. shaffneri* have been incorrectly identified as *Telanthophora grandifolia* based on material observed at BIGU,

MEXU, TEX, and US. *Roldana schaffneri* is highly variable with respect to its morphology and it is widely distributed from central Mexico to Nicaragua. *Nelsonianthus* is not a well-supported taxon in this study but further investigations of this epiphytic taxon are needed not only to understand its phylogenetic placement, but also this under-collected genus' range as well.

Biogeographic implications.—A relatively recent origin (9.5–15 mya) has been suggested for the Mexican tussilaginoid group (Pelser et al. 2010), which would place its origin in a period when Asteraceae showed an explosive radiation throughout Mexico and Central America (Raven & Axelrod 1974). Among the three species that occur near the Arizona–Mexico border, *Barkleyanthus salicifolius, Psacalium decompositum, Roldana hartwegii* and clade 4 (consisting of *Arnoglossum*, *Barkleyanthus* and *Yermo*) are the only taxa that occur in the USA. The most recent common ancestor between *Arnoglossum* (Midwest and the Southeast USA) and *Yermo* (Fremont County, Wyoming) is estimated to have occurred 1.17–1.43 mya, suggesting a recent origin of the clade into the USA (Pelser et al. 2010). The genus *Roldana* is widespread and displays a high diversity in the mountains of central and southern Mexico. Two species (*R. heterogama* and *R. scandens*) occur as far south as Costa Rica and *R. heterogama* occurs in northern Panama. With respect to *Telanthophora,* both the ITS and combined ITS/ETS data sets support *T. liebmannii* as sister to the rest of the genus. The highlands of Guatemala share most of their species composition with southern Mexico. However, a few taxa at the species-level are endemic to Guatemala (i.e., *Psacaliopsis pudica, Psacalium guatemalense, P. pinetorum, Roldana riparia, Telanthophora sublaciniata*). Two of the previously mentioned four species are described based on a single population. One species, *Telanthophora bartlettii,* is endemic to western Belize.

Conservation implications.—Montane forests of Mexico and Central America are under intense negative pressures from human land use and climate change. In Mexico, endemic montane Asteraceae, including many taxa within the Mexican tussilaginoid group have been used to illustrate regions of high biodiversity (Villaseñor et al. 2006, 2007) and endemism (Villaseñor et al. 1998; González-Zamora et al. 2007). The Mexican tussilaginoid group comprises an ecologically and morphologically diverse group of plants that are common in threatened montane ecosystems. Once the phylogenetic relationships of the Mexican tussilaginoids are better understood threatened species may be used as tools to assess plant community richness in these ecosystems of Mexico and Central America.

CONCLUSION

The primary findings of this study are that (1) the genera *Pittocaulon, Psacaliopsis,* and *Roldana* are not monophyletic, (2) the genus *Telanthophora* is monophyletic, but nested within *Roldana,* and (3) the Mexican tussilagnoid genera form a clade but require additional markers from both the plastid and nuclear genomes to attempt to clearly resolve a phylogeny. A combined ITS and ETS nrDNA data set supports the monophyly of the Mexican tussilaginoid clade, and a sister relationship to the 'gynoxoid' group of South America is resolved and well-supported. Our results also support the establishment of 13 well-supported clades within the North and Central American Mexican tussilaginoid group. Previous studies using nuclear and plastid data sets strongly support the relationships that are supported in the current study (i.e. Clades 1, 4, 5, 8; Pelser et al. 2007, 2010). However, taxon sampling in those previous studies was minimal compared to the present study and therefore no other conclusions can be inferred when broadly comparing the phylogenies. Although this study provides a phylogenetic analysis to elucidate relationships of the Mexican tussilaginoid group, field biologists are still left to cope with difficult taxonomic keys and an incomplete knowledge of morphological variation, phenotypic plasticity and actual range distributions for these species. Increasing the number of populations of species complexes, adding more species of *Roldana* would provide a better understanding of the evolution and origin of montane endemism within this genus. As this study only included four of the forty-six described species in *Psacalium,* a thorough sampling of this diverse montane genus would elucidate evolutionary relationships. A more comprehensive study that includes more taxa and analyzes a plastid data set that included several non-coding regions is required to improve the understanding of this diverse montane plant group.

APPENDIX 1

List of all analyzed accessions, corresponding herbarium vouchers and GenBank/ENA accession numbers. Asterisks indicate sequences obtained from GenBank that were originally published in Pelser et al. (2002*, 2007**, 2010***) and Bayer et al. (2002****). Herbarium abbreviations follow *Index Herbariorum* (Thiers, 2017). Cloned sequences are listed with marker abbreviation (E = ETS; I = ITS) and clone number, followed by GenBank/ENA accession numbers. Accessions in bold were used in the combined analyses (Fig. 3) and the reduced separate analyses (Figs. 1–2). An "*x*" represents missing data for either ETS or ITS. The information is listed in the following order and format: Species, country, state or department, herbarium, collector and collection number, (ETS accession number; ITS accession number).

Aequatorium *asterotrichum* B. Nord., Ecuador, Napo, S, *Asplund 18263*, (GU818111*; GU818489**), *Aequatorium lepidotum* B. Nord., Ecuador, Carchi, MO, *Palacios & Tipaz 10538*, (*x;* EF538148**). ***Arnoglossum*** *atriplicifolium*-1 (L.) H. Rob., USA, Ohio, MU, *M.A. Vincent 3925*, (GU818115***; EF538154**). *Arnoglossum atriplicifolium*-2 (L.) H. Rob., USA, Tennessee, TEX, *V.E. McNeilus 97-897*, (*x;* KR010705). *Arnoglossum plantagineum* Raf., USA, Iowa, MU, *M.A. Vincent 5576*, (*x*; EF538155**). ***Barkleyanthus*** *salicifolius*-1 (Kunth) H. Rob & Brettell, Cultivated from Guatemala, Quetzaltenango, TEX, *T. Sultan Quedensley s.n.* (E3=KR010709; I5=KR010885). *Barkleyanthus salicifolius*-2 (Kunth) H. Rob & Brettell, Mexico, Chiapas, TEX, *C. Santíz R. 585*, (E1=KR010710; I3=KR010884, I8=KR010883). *Barkleyanthus salicifolius*-3 (Kunth) H. Rob & Brettell, Mexico, B, *P. Genelle & G. Fleming 861*, (GU818120***; *x*). ***Digitacalia*** *crypta* B.L. Turner, Mexico, Guerrero, TEX, *J.L. Panero 6186* (E1=KR010711; I2=KR010878). *Digitacalia jatrophoides* (Kunth) Pippen, Mexico, Oaxaca, TEX, *Panero 2330 & Salinas*, (GU818152***; GU818545***). *Digitacalia jatrophoides* var. *jatrophoides* (Kunth) Pippen, Mexico, Michoacan, TEX, *J.M. Estebado 2111* (E3=KR010712; I2=KR010926). *Digitacalia jatrophoides* var. *pentaloba* B.L. Turner, Mexico, Puebla, TEX, *B.L. Turner 80A-4c* (E5=KR010713; I10=KR010872). ***Gynoxys*** *buxifolia*-1 (Kunth) Cass., Ecuador, Napo/Cotapaxi border, TEX, *J.L. Luteyn 13431* (E4=KR010714; I1=KR010911). *Gynoxys buxifolia*-2 (Kunth) Cass., Ecuador, S, (*x;* EF538218*). *Gynoxys sodiroi* Hieron., Ecuador, Carchi, F, *F. Hekker & W.H.A. Hekking 10 285*, (*x;* EF538219**). *Gynoxys soukupii* Cuatrec., Peru, L, *Hutchison & Wright 5352*, (GU818174***; AF459963*). *Gynoxys tolimensis* Cuatrec., Ecuador, Carchi, B, *B. Øllgaard & H. Balslev 8418*, (*x;* EF538220**). ***Nelsonianthus*** *tapianus* (B.L. Turner) C. Jeffrey, Mexico, Chiapas, MO, *I. Pérez & L. Kendizabal 457*, (GU818211***; GU817580***). ***Pippenalia*** *delphinifolia*-1 (Rydb.) McVaugh, Mexico, MO, D.E. *Breedlove 59032 & F. Almeda*, (GU818230***; GU818627***). *Pippenalia delphinifolia*-2 (Rydb.) McVaugh, Mexico, Durango, TEX, *T.S. Quedensley 10133* (E3=KR010715; I6=KR010877). ***Pittocaulon*** *bombycophole* (Bullock) H. Rob. & Brettell, Mexico, Guerrero, MEXU, *J.L. Villaseñor s.n.* (E4=KR010716; I1=KR010707). *Pittocaulon filare* (McVaugh) H. Rob. & Brettell, Mexico, Zacatecas, TEX, *T.S. Quedensley 10142* (E4=KR010717; I7=KR010876). *Pittocaulon praecox*-1 (Cav.) H. Rob. & Brettell, Mexico, Jalisco, US, *H.H. Iltis et al. 31157*, (E1=KR010718, E3=KR010719; I2=KR010811, I2=KR010866, I3=KR010865). *Pittocaulon praecox*-2 (Cav.) H. Rob. & Brettell, Cultivated from Mexico, Distrito Federal, TEX, *T. Sultan Quedensley s.n.* (E2=KR010720, E3=KR010721; I2=KR010924, I4=KR010814). *Pittocaulon praecox*-3 (Cav.) H. Rob. & Brettell, Cultivated from Mexico, MJG, *R. Greissl s.n.*, (GU818231***; *x*). *Pittocaulon velatum* var. *tzimolensis* (T.M. Barkley) B.L. Clark, Mexico, Oaxaca, MEXU, *C. Gallardo H. et al. 1477*, (E2=KR010722, E5=KR010723; I3=KR010921). *Pittocaulon velatum* var. *velatum*-1 (Greenm.) H. Rob. & Brettell, Mexico, Jalisco, TEX, *G. Flores M. et al 1819*, (E1=KR010724, E2=KR010725; I1=KR010810, I5=KR010875). *Pittocaulon velatum* var. *velatum*-2 (Greenm.) H. Rob. & Brettell, Mexico, Jalisco, MEXU, *G. Flores M. et al 1819*, (E4=KR010726; I8=KR010919). ***Psacaliopsis*** *macdonaldii* (B.L. Turner) C. Jeffrey, Mexico, Oaxaca, TEX, *G.B. Hinton et al. 26794*, (E4=KR010728; I1=KR010864). *Psacaliopsis paneroi* var. *juxtlahuacensis* Panero & Villaseñor, Mexico, Oaxaca, TEX, *J.I.Calzada 19663*, (E1=KR010731, E3=KR010732, E4=KR010733; I4=KR010863). *Psacaliopsis pinetorum* (Hemsl.) Funston & Villaseñor, Mexico, Guerrero, TEX, *T.S. Quedensley 10197*, (E1=KR010735; I2=KR010882). *Psacaliopsis pudica* (Standl. & Steyerm.) H. Rob. & Brettell, Guatemala, Huehuetenango, TEX, *M. Véliz 7222*, (E5=KR010736; I1=LT992449). *Psacaliopsis purpusii*-1 (Greenm. ex Brandegee) H. Rob. & Brettell, Mexico, Oaxaca, TEX, J.L. *Panero 2607 with Davila & Tenorio*, (GU818235***; GU818629***). *Psacaliopsis purpusii*-2 (Greenm. ex Brandegee) H. Rob. & Brettell, Mexico, Oaxaca, TEX, J.L. *Panero 2607 with Davila & Tenorio*, (E3=KR010737; I5=KR010861). ***Psacalium*** *cirsiifolium*-1 (Zucc.) H. Rob. & Brettell, Mexico, WIS, *R.R. Kowal 3053*, (GU818236***; EF538270**). *Psacalium cirsiifolium*-2 (Zucc.) H. Rob. & Brettell, Mexico, Guerrero, MEXU, *J.L. Villaseñor s.n.*, (E4=KR010727; I2=KR010907). *Psacalium megaphyllum* (B.L. Rob. & Greenm.) Rydb., Mexico, Guerrero, MEXU, *J.L. Villaseñor s.n.*, (E3=KR010729; I1=KR010914). *Psacalium palmeri* (Greene) H. Rob. & Brettell, Mexico, Jalisco, TEX, *J. Villa C. & H. Chávez L. 835*, (E1=KR010730; I1=KR010909). *Psacalium peltatum* (Kunth) Cass., Mexico, Oaxaca, TEX, *T.S. Quedensley 7076*, (E5=KR010734; I1=KR010862). ***Robinsonecio*** *gerberifolius*-1 (Sch. Bip. ex Hemsl.) T.M. Barkley & Janovec, Mexico, Tlaxcala, TEX, *R. Acosta P. 2449*, (E2=LT992451; I1=KR010809). *Robinsonecio gerberifolius*-2 (Sch. Bip. ex Hemsl.) T.M. Barkley & Janovec, Mexico, Mexico, MEXU, *J. Garcia P. 171*, (E2=KR010738; I4=KR010808). *Robinsonecio gerberifolius*-3 (Sch. Bip. ex Hemsl.) T.M. Barkley & Janovec, Mexico, Mexico, MO, *J. Garcia P. 171*, (GU818239***; GU818630***). ***Roldana*** *acutangula* (Bertol.) Funston, Guatemala, Quetzaltenango, TEX, *T.S. Quedensley 1817*, (E3=KR010739; I8=KR010920). *Roldana albonervia*-1 (Greenm.) H. Rob. & Brettell, Mexico, Jalisco, US, *M. A. Wetter et al. 2043*, (E3=KR010740, E5=KR010741; I9=KR010813). *Roldana albonervia*-2 (Greenm.) H. Rob. & Brettell, Mexico, L, *J. Garcia P. 969*, (*x;* EF538291**). *Roldana angulifolia* (DC.) H. Rob. & Brettell, Mexico, Michoacan, MEXU, *B. Farfán Heredia 334*, (E1=KR010742, E5=KR010743; I3=KR010860, I7=KR010859). *Roldana anisophylla* (Klatt) Funston, Mexico, Oaxaca, TEX, *T.S. Quedensley 7082*, (E1=KR010744, E2=KR010745; I3=KR010858, I5=KR010857). *Roldana aschenborniana* (S. Schauer) H. Rob. & Brettell, cultivated from Mexico, TEX, *T. Sultan Quedensley s.n.*, (E2=LT992452; I5=KR010899). *Roldana barba-johannis* (DC.) H. Rob. & Brettell, Mexico, Oaxaca, TEX, *T.S. Quedensley 7039*, (E5=KR010746; I1=KR010874). *Roldana chapalensis* (S. Watson) H. Rob. & Brettell, cultivated from Mexico, TEX, *T. Sultan Quedensley s.n.*, (E1=KR010749, E2=KR010747, E3=KR010748; I5=KR010856, I6=KR010855). *Roldana ehrenbergiana* (Klatt) H. Rob. & Brettell, Mexico, Puebla, MEXU, *P. Tenorio L.et al. 8927*, (E6=KR010750; I4=KR010826). *Roldana eriophylla* (Greenm.) H. Rob. & Brettell, Mexico, Oaxaca, MEXU, *C. Gallardo H. 1487 et al.*, (E4=KR010751; I1=KR010854). *Roldana gentryi* H. Rob. & Brettell, cultivated from Mexico, TEX, *T. Sultan Quedensley s.n.*, (E6=KR010752; I3=KR010853). *Roldana gilgii* (Greenm.) H. Rob. & Brettell, Guatemala, Quetzaltenango, TEX, *T.S. Quedensley 1987*, (E1=KR010753; I4=KR010852). *Roldana greenmanii* H. Rob. & Brettell, Mexico, Chiapas, MEXU, *A. Espejo et al 963*, (E2=KR010754; *x*). *Roldana grimesii* (B.L. Turner) C. Jeffrey, Mexico, Hidalgo, MEXU, *I. Luna et al. 1863*, (E1=KR010755; I1=KR010825, I3=KR010851, I4=KR010852, I5=KR010824). *Roldana hartwegii* (Benth.) H. Rob. & Brettell, Mexico, Nayarit, MEXU, *G. Flores-Franco et al. 3190*, (E3=KR010756; I6=KR010850). *Roldana heracleifolia* (Hemsl.) H. Rob. & Brettell, Mexico, Zacatecas, MEXU, *J.J. Balleza C. 9822 con M. Adame G.*, (E2=KR010757; I9=KR010870). *Roldana heterogama*-1 (Benth.) H. Rob. & Brettell, Guatemala, Quetzaltenango, TEX, *T.S. Quedensley 5114*, (E1=KR010758; I2=KR010901). *Roldana heterogama*-2 (Benth.) H. Rob. & Brettell, cultivated from Guatemala, Quetzaltenango, TEX, *T.S. Quedensley s.n.*, (E5=KR010759; I6=KR010849). *Roldana heterogama*-3

(Benth.) H. Rob. & Brettell, cultivated from Mexico, Chiapas, TEX, *T.S. Quedensley s.n.,* (E2=LT992453; I4=KR010848). *Roldana hintonii* H. Rob. & Brettell, Mexico, Mexico, MEXU, *J.L. Villaseñor 1328 et al.,* (E4=KR010760; I1=KR010812, I2=KR010923, I5=KR010908). *Roldana jurgensenii* (Hemsl.) H. Rob. & Brettell, Guatemala, Quetzaltenango, TEX, *T.S. Quedenlsey 5188,* (E1=KR010764; I1=KR010847, I2=KR010846, I4=KR010845, I5=KR010844). *Roldana lanicaulis* (Greenm.) H. Rob. & Brettell, Mexico, Oaxaca, TEX, *T S. Quedensley 7070,* (E3=KR010765; I2=KR010843). *Roldana lineolata*-1 (DC.) H. Rob. & Brettell, Mexico, Oaxaca, TEX, *T.S. Quedensley 7073,* (E3=KR010766; I2=LT992450). *Roldana lineolata*-2 (DC.) H. Rob. & Brettell, Mexico, MU, *R. Bye 11751*, (*x;* EF538292*). *Roldana lobata* La Llave, Mexico, Mexico, MEXU, *J.L. Villaseñor R. 1517,* (E5=KR010767; I12=KR010918). *Roldana marquezii* (B.L. Turner) C. Jeffrey, Mexico, Puebla, MEXU, *J.L. Contreras J. 4896,* (E7=KR010768; I3=KR010842, I5=KR010841, I8=KR010897, I9=KR010925, I10=KR010916). *Roldana metepeca* (B.L. Turner) C. Jeffrey, Mexico, Puebla, MEXU, *J.L. Contreras J. 6763,* (E1=KR010769; I8=KR010840, I10=KR010839, I12=KR010915). *Roldana mexicana* (McVaugh) H. Rob. & Brettell, Mexico, Michoacan, MEXU, *S. Zamudio 10059,* (E3=KR010770, E4=KR010771; I1=KR010903). *Roldana michoacana* (B.L. Rob.) H. Rob. & Brettell, Mexico, Michoacan, MEXU, *J.L. Linares 4473,* (E3=KR010772, E4=KR010773; I6=KR010906). *Roldana mixtecana* J.L. Panero & Villaseñor, Mexico, Oaxaca, TEX, *J.I. Calzada 19488,* (E4=KR010774; I7=KR010881). *Roldana petasitis* var. *cristobalensis* (Greenm.) Funston, Mexico, Oaxaca, TEX, *T S. Quedenlsey 7040,* (E1=KR010775, E5=KR010776; I5=KR010838). *Roldana petasitis* var. *oaxacana*-1 (Hemsl.) Funston, cultivated from Guatemala, Quetzaltenango, TEX, *T. Sultan Quedensley s.n.,* (E2=KR010777; I3=KR010823, I4=KR010837). *Roldana petasitis* var. *oaxacana*-2 (Hemsl.) Funston, Mexico, Oaxaca, TEX, *T.S. Quedensley TQ 7049,* (E2=KR010778, E4=KR010779; I1=KR010905, I3=KR010904, I4=KR010913). *Roldana petasitis* var. *petasitis* (Sims) H. Rob. & Brettell, cultivated from Mexico, TEX, *T. Sultan Quedensley s.n.,* (E1=KR010780, E6=KR010781; I3=KR010822, I4=KR010836, I5=KR010869). *Roldana petasitis* var. *sartorii* (Sch. Bip. ex Hemsl.) Funston, Mexico, Oaxaca, MEXU, *J.J. Calzada 20853,* (E1=KR010782, E5=KR01078; I1=KR010835, I3=KR010834, I5=KR010880). *Roldana platanifolia* (Benth.) H. Rob. & Brettell, Mexico, Hidalgo, MEXU, *N.P. Rodriguez C. 7.,* (E2=KR010784; I4=KR010912). *Roldana reticulata* (DC.) H. Rob. & Brettell, Mexico, Mexico, MEXU, *G.C. Tenorio 1846.,* (E5=KR010785; I1=KR010873). *Roldana riparia* Quedensley, Véliz & L. Velásquez, Guatemala, Huehuetenango, TEX, *T.S. Quedensley 10188,* (E6=KR010786; I7=KR010868). *Roldana robinsoniana* (Greenm.) H. Rob. & Brettell, Mexico, Oaxaca, MEXU, *J.J. Calzada 19895,* (E2=KR010787; I8=KR010879). *Roldana schaffneri*-1 (Sch. Bip. ex Klatt) H. Rob. & Brettell, Mexico, Guerrero, MEXU, *N. Diego et al. 8543,* (E2=KR010788; I3=KR010893). *Roldana schaffneri*-2 (Sch. Bip. ex Klatt) H. Rob. & Brettell, Guatemala, Quetzaltenango, BIGU, *T S. Quedensley 1980,* (*x*; I4=KR010892, I5=KR010891, I8=KR010890). *Roldana schaffneri*-3 (Sch. Bip. ex Klatt) H. Rob. & Brettell, Guatemala, Quetzaltenango, TEX, *T.S. Quedensley 5120,* (*x*; I11=KR010889, I12=KR010888, I14=KR010894, I15=KR010887). *Roldana sessifolia* (Hook. & Arn.) H. Rob. & Brettell, Mexico, Michoacan, F, *E. Peréz & E. Garcia 1800,* (E1=KR010789; I4=KR010896). *Roldana* sp. hybrid, Mexico, Oaxaca, TEX, *T S. Quedensley 7077,* (E1=KR010761, E2= KR010762, E3= KR010763; I2=KR010917, I3=KR010898). *Roldana suffulta*-1 (Greenm.) H. Rob. & Brettell, Mexico, L, *Rzedowski 36569,* (GU818246**; GU818631**). *Roldana suffulta*-2 (Greenm.) H. Rob. & Brettell, Mexico, Nayarit, MEXU, *G. Flores Franco 4188 et al.,* (E5=KR010790; I1=KR010821, I3=KR010902, I4=KR010820, I5=KR010819, I6=KR010818). *Roldana sundbergii* (B.L. Turner) B.L. Turner, Mexico, Nuevo Leon, MEXU, *J.A. Villareal & M.A. Carranza V-4204,* (E3=KR010791; I5=KR010900). *Roldana uxordecora* Quedensley & Villaseñor, Mexico, Oaxaca, TEX, *T S. Quedensley 7050,* (E3=KR010792; I1=KR010833). ***Rugelia*** *nudicaulis* Shuttlew. ex Chapm., USA, Tennessee, TENN, *M.A. Feist, L.R. Phillippe, B.,Molano-Flores, D. Busemeyer & C. Carroll 714* (ETS), *L.R. Phillippe, J. Payne & P.B. Marcum 37061* (ITS), (GU818247***; GU818632***). ***Senecio*** *vulgaris* L., Canada, Alberta, CANB, *Bayer AB-95006* from (ETS), New Zealand, CHR, *S.J. Wagstaff 10.05 2002* (ITS), (AF319755****; EF538396**). ***Telanthophora*** *andrieuxii*-1 (DC.) H. Rob. & Brettell, Mexico, Oaxaca, TEX, *T S. Quedensley 7083,* (E3=KR010793; I3=KR010832). *Telanthophora andrieuxii*-2 (DC.) H. Rob. & Brettell, Mexico, Tamaulipas, TEX, *M.C. Johnston 7402,* (E2=KR010794; I1=KR010831). *Telanthophora cobanensis*-1 (J.M. Coult.) H. Rob. & Brettell, cultivated from Mexico, TEX, *T. Sultan Quedensley s.n.,* (E2=KR010795; I5=KR010830). *Telanthophora cobanensis*-2 (J.M. Coult.) H. Rob. & Brettell, Guatemala, Quetzaltenango, TEX, *T.S. Quedensley 2649,* (E2=KR010796; I6=KR010910). *Telanthophora copeyensis* (Greenm.) H. Rob. & Brettell, Costa Rica, San Jose, U, *M. Kapelle MK16,* (*x;* EF538404**). *Telanthophora grandifolia*-1 (Less.) H. Rob. & Brettell, cultivated, MJG, (GU818318***; EF538405**). *Telanthophora grandifolia*-2 (Less.) H. Rob. & Brettell, cultivated from Mexico, TEX, *T. Sultan Quedensley s.n.,* (E6=KR010797; I4=KR010829). *Telanthophora jalisciana* (Buchinger ex Klatt) H. Rob. & Brettell, Mexico, Guerrero, MEXU, *C. Catalan H. 2352,* (E1=KR010798; I1=KR010895). *Telanthophora liebmannii*-1 (Buchinger ex Klatt) H. Rob. & Brettell, Mexico, Oaxaca, TEX, *T.S. Quedensley 7084,* (E5=KR010799; I5=KR010828). *Telanthophora liebmannii*-2 (Buchinger ex Klatt) H. Rob. & Brettell, Mexico, Oaxaca, TEX, *L. Woodruff 197 with C. Todzia & A. Campos V.,* (E1=KR010800; I2=KR010827). *Telanthophora uspantanensis*-1 (J.M. Coult.) H. Rob. & Brettell, Mexico, Oaxaca, TEX, *T.S. Quedensley 7068,* (E1=KR010801, E2=KR010802, E5=KR010803; I2=KR010817). *Telanthophora uspantanensis*-2 (J.M. Coult.) H. Rob. & Brettell, Mexico, Oaxaca, TEX, *A. Campos V. 4389 con J.L. Panero,* (E2=KR010804, E4=KR010805; I5=KR010867). ***Villasenoria*** *orcuttii*-1 (Greenm.) B.L. Clark, Mexico, Oaxaca, TEX, *T S. Quedensley 7086,* (E5=KR010806; I3=KR010816). *Villasenoria orcuttii*-2 (Greenm.) B.L. Clark, Mexico, Veracruz, TEX, *E. Estrada M. 1003,* (E1=KR010807; I6=KR010815). *Villasenoria orcuttii*-3 (Greenm.) B.L. Clark, Mexico, XAL, *L. Robles 389,* (GU818324***; GU818726***). *Yermo xanthocephalus* Dorn, USA, Wyoming, MO, *L.C. Anderson 13691,* (GU818327***; GU818727***).

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