Research article

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Phylogeny of *Lysiloma* (Fabaceae), a genus restricted to Megamexico with outliers in the West Indies and Florida

Filogenia de *Lysiloma* (Fabaceae), un género restringido a Megaméxico con especies atípicas en las Antillas y Florida

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Abstract:

Background and Aims: *Lysiloma* is a Neotropical genus in the Fabaceae family that comprises eight species, six of which are widely distributed in Mexico, and two more that occur in the Antilles and Florida. *Lysiloma* is frequent in Megamexico's dry forests. A previous phylogenetic study included three species of *Lysiloma* and *Hesperalbizia occidentalis*. Both genera are closely related, but their divergence has weak support. Our objectives were to test the monophyly of the genus, evaluate the sister relationships within the genus, and estimate the divergence times.

Methods: A phylogenetic analysis based on morphological characters, molecular markers (ETS, *mat*K, and *trn*K), as well as a combined analysis (morphology + molecules) was performed. The data matrices were analyzed both individually and concatenated (total evidence approach) with Bayesian inference and Maximum Parsimony. In addition, molecular divergence times were estimated from the ETS dataset with a Bayesian uncorrelated lognormal relaxed clock.

Key results: The morphological analysis supports the monophyly of *Lysiloma* with *Hesperalbizia* as sister group. However, the individual and the combined molecular analyses do not provide resolution to clarify the relationships between *Hesperalbizia occidentalis*, *Lysiloma sabicu*, and core *Lysiloma*. The total evidence analysis (including morphology) supports the monophyly of *Lysiloma*, yet with low support. According to our molecular clock model, the clade *Lysiloma+Hesperalbizia* diverged from other members of the tribe Acacieae+Ingeae about 32 million years ago, and the diversification of the core of *Lysiloma* occurred during the Miocene.

Conclusions: *Lysiloma+Hesperalbizia* is an early divergent clade of tribes Acacieae+Ingeae. There are enough morphological differences to recognize both linages. Morphological characters informally used for taxonomic delimitation seem to have evolved homoplasiously. The clade *Lysiloma* and *Hesperalbizia* separated from other members of the tribe Acacieae+Ingeae in the Oligocene, but the diversification of the core of the genus coincides with the expansion of the dry forest at the beginning of the Miocene.

Key words: Acacieae, *Hesperalbizia*, Ingeae, Leguminosae, molecular clock, Neotropics.

Resumen:

Antecedentes y Objetivos: *Lysiloma* es un género neotropical de la familia Fabaceae que comprende ocho especies, seis de las cuales se distribuyen ampliamente en México y dos más que ocurren en las Antillas y La Florida. *Lysiloma* es frecuente en los bosques secos de Megaméxico. Un estudio filogenético previo incluyó tres especies de *Lysiloma* y *Hesperalbizia occidentalis*. Ambos géneros están estrechamente relacionados, pero su divergencia tiene un apoyo débil. Nuestros objetivos fueron probar la monofilia del género, evaluar las relaciones de grupo hermano dentro del género y estimar los tiempos de divergencia.

Métodos: Se realizó un análisis filogenético basado en caracteres morfológicos, marcadores moleculares (ETS, *mat*K y *trn*K), así como un análisis combinado (morfología + moléculas). Las matrices de datos se analizaron tanto individualmente como concatenadas (enfoque de evidencia total) con inferencia Bayesiana y máxima parsimonia. Además, los tiempos de divergencia molecular se estimaron a partir del conjunto de datos ETS con un modelo de reloj bayesiano relajado lognormal no correlacionado.

Resultados clave: El análisis morfológico respalda la monofilia del *Lysiloma* con *Hesperalbizia* como grupo hermano. Sin embargo, los análisis moleculares individuales y combinado no proporcionan resolución para aclarar las relaciones entre *Hesperalbizia occidentalis*, *Lysiloma sabicu* y el núcleo de *Lysiloma*. El análisis de evidencia total (incluida la morfología) respalda la monofilia de *Lysiloma*, pero con un bajo soporte. Según nuestro modelo de reloj molecular, el clado *Lysiloma+Hesperalbizia* se separó de otros miembros de la tribu Acacieae+Ingeae hace unos 32 millones de años y la diversificación del núcleo del *Lysiloma* se produjo a lo largo del Mioceno.

Conclusiones: *Lysiloma+Hesperalbizia* es un clado de divergencia temprana de las tribus Acacieae+Ingeae. Existen suficientes diferencias morfológicas para reconocer ambos linajes. Los caracteres morfológicos utilizados informalmente para la delimitación taxonómica parecen haber evolucionado de manera homoplásica. El clado de *Lysiloma* y *Hesperalbizia* se separó de otros miembros de la tribu Acacieae+Ingeae en el Oligoceno, pero la diversificación del núcleo del género coincidió con la expansión del bosque seco a principios del Mioceno.

Palabras clave: Acacieae, *Hesperalbizia*, Ingeae, Leguminosae, Neotrópico, reloj molecular.

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Introduction

Lysiloma Benth. (Bentham, 1844) is one of 36 genera within the tribe Ingeae (Brown, 2008) in the extended subfamily Caesalpinioideae (LPWG, 2017). Two recent phylogenetic analyses (Brown et al., 2008; Iganci et al., 2015) suggest that *Lysiloma* and the monotypic *Hesperalbizia* Barneby & J.W. Grimes are sister taxa. As currently understood, it is composed of eight tree species with a primarily Neotropical distribution, ranging from Arizona and Florida through Mexico, Central America, and the Antilles (Thompson, 1980; Gale and Pennington, 2004; Andrade and Sousa, 2012) (Table 1).

Lysiloma acapulcense (Kunth) Benth. and *L. divaricatum* (Jacq.) J.F. Macbr. are the species with the widest distribution, ranging from northern Mexico to Central America (Nicaragua and Costa Rica). Both taxa include a long list of synonyms and broad morphological variation. These taxa urgently require a deeper future reassessment. The rest of the species have more restricted distributions (Table 1). The general distribution of the genus coincides in general terms with Megamexico 3, a biogeographic area that includes the entire Mexican territory, but also the southern part of the United States of America (the northern portions of the Chihuahuan and Sonoran deserts in California, Arizona, New Mexico, and Texas) in the north, and Central American to Nicaragua north of the lakes, in the south (Rzedowski, 1991). Two exceptions are *L. latisiliquum* (L.) Benth., and *L. sabicu* Benth., which are found in Florida, the Bahamas, and the West Indies (Thompson, 1980). In general, *Lysiloma* species occupy a wide variety of dry forest types, being dominant or codominant elements in many of them (Rascón-Ayala et al. 2018; Ancona et al., 2019; Ortiz-Ávila et al., 2020).

The generic name of this group of plants derives from the Greek *Lysis* (to lose) and *loma* (edge), referring to the shedding of the legume edge at fruit maturity that allows for the dispersion of the seeds. This type of dehiscence has been referred to as craspedial (Thompson, 1980; Gale and Pennington, 2004). Two distinctive species of the genus lack this feature, *Lysiloma sabicu* and *L. latisiliquum* (Fig. 1E, F), where the valves remain together at maturity. An additional, distinctive feature of the genus is the membranous, foliaceous stipule (Fig. 1C) (Bentham, 1875), a character state that is shared by *Hesperalbizia*. Thompson (1980) proposed an informal classification system with two subgenera based upon fruit dehiscence characters: *Lysiloma* subg. *Lysiloma* Thompson (*nom. nud.*), with indehiscent or late dehiscent legumes and *Lysiloma* subg. *Lysivalva* Thompson (*nom.*

Table 1: General distribution of the genus *Lysiloma* Benth. We included *L. divaricatum* (Jacq.) Macbr. and *L. microphyllum* Benth. as two species following Thompson's manuscript (1980), but Gale and Pennington (2004) considered them coespecific. Our results support Gale and Pennington's point of view.

Species (according to Thompson, 1980)	Distribution and elevation in m above sea level
Lysiloma acapulcense (Kunth) Benth.	Mexico, Central America to El Salvador, and Honduras. 600-2000
Lysiloma auritum (Schltdl.) Benth.	Southwest Mexico to Central America except Belize. 100-2400
Lysiloma candidum Brandegee	Mexico (Baja California Norte, Baja California Sur, and Sonora). 0-400
Lysiloma divaricatum (Jacq.) Macbr.	Mexico (Chiapas, Oaxaca, and Veracruz), Central America to Costa Rica. 20-1700
Lysiloma latisiquum (L.) Benth.	USA (Florida), southeast Mexico, Belize, Guatemala, and the Bahamas, Cuba, and Hispaniola. 0-400
Lysiloma microphyllum Benth.	Mexico (Baja California, Chihuahua, Colima, México, Morelos, Nayarit, Oaxaca, Querétaro, San Luis Potosí, Sonora, and Tamaulipas). 0-1600
Lysiloma sabicu Benth.	The Bahamas, Cuba, and Hispaniola. 50-900
Lysiloma tergeminum Benth.	Mexico (Colima, Guerrero, México, Michoacán, Morelos, Nayarit, and Puebla). 0-2000
Lysiloma watsonii Rose	USA (Arizona) and Mexico (Chihuahua, and Sinaloa). 300-1300

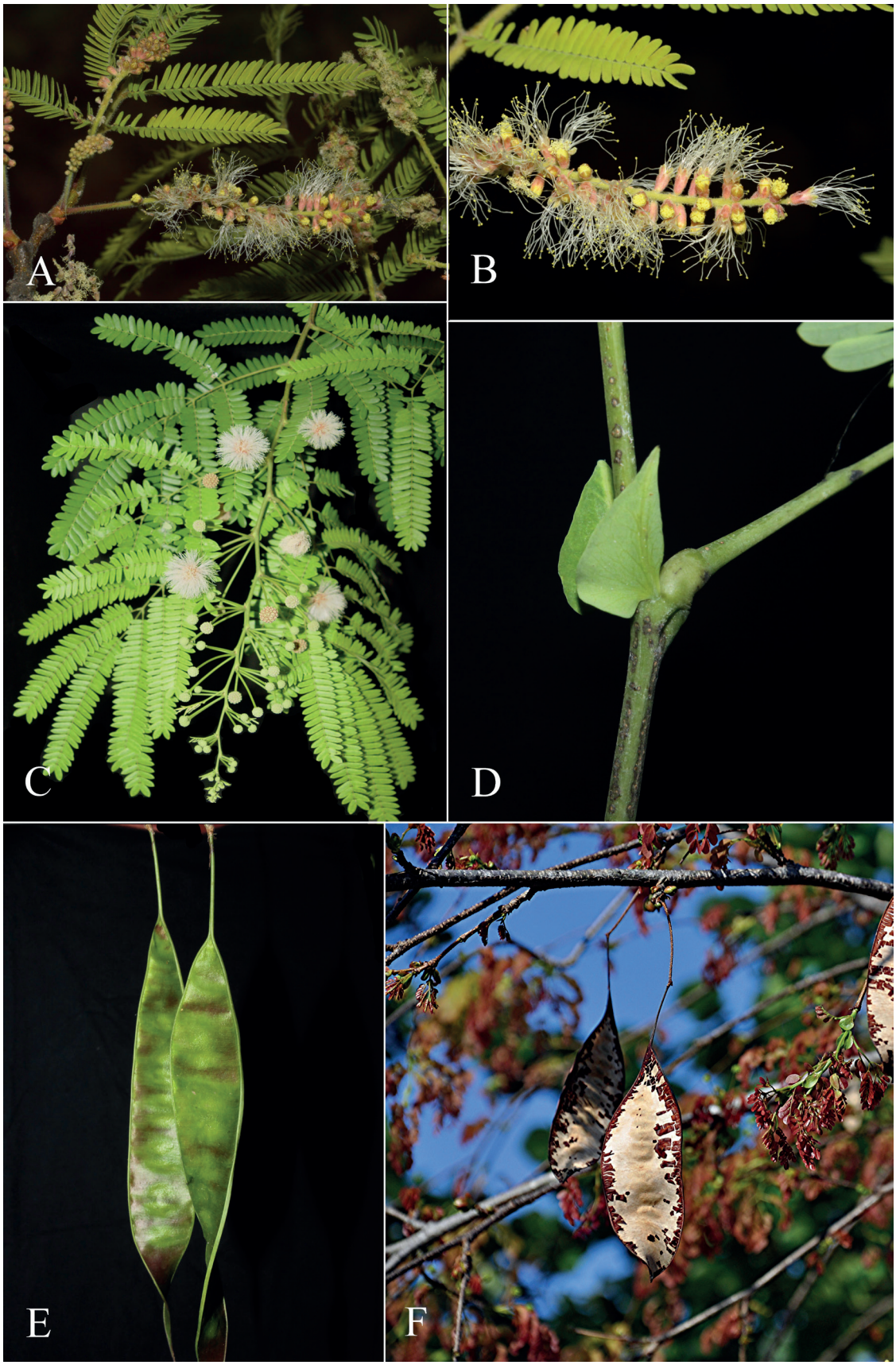

Figure 1: General morphology of *Lysiloma* Benth. A. leaves, and inflorescences of *Lysiloma acapulcense* (Kunth) Benth.; B. inflorescence of *L. acapulcense*; C. leave and stipule of *Lysiloma latisiliquum* (L.) Benth.; D. inflorescence of *Lysiloma latisiliquum*; E. young and old fruits of *L. latisiliquum*; F. fruit of *Lysiloma sabicu* Benth. Pictures A, B: Claudia Ramírez; C, D, E: Rodrigo Duno de Stefano; F: Susan Ford Collins.

nud.), where the legume is dehiscent, and the valves break apart at the margins. Subgenus *Lysiloma* is composed of two sections defined by inflorescence types (capitulum *vs*. raceme) and flower pedicels (sessile *vs*. pedicellate): *Lysiloma* subg. *Lysivalva* sect. *Capitata* Thompson (*nom. nud.*), and sect. *Racemosa* Thompson (*nom. nud.*) (Fig. 1A, B, D).

As is the case of most members of tribe Ingeae, *Lysiloma* pollen grains are arranged in polyads of 16 (28-32 in *L. divaricatum* (Jacq.) J.F. Macbr.) (Guinet and Grimes, 1997). In general, polyads are 46-79 µm diameter, isodiametric where the eight radial pollen grains are longer than wide, whereas the internal tetrad is composed of square pollen grains. The external pollen grains are dissymmetric in thickness; whereas the tectum is perforated with 20-100/µm^2 ornamentation is uniform throughout, fossulate, or polygonal with more or less rounded areoles (Sorsa, 1969; Guinet and Grimes, 1997). *Hesperalbizia occidentalis* (Brandegee) Barneby & J.W. Grimes is characterized by similar yet larger, symmetrical polyads. Both *Lysiloma* and *Hesperalbizia* feature 2n=26 chromosomes (Thompson, 1980; Rico Arce, 1992).

Lysiloma is known from a Tertiary (Olygocene) fossil record, *L. mixtecana* Magallón-Puebla & Cevallos-Ferriz (Magallón-Puebla and Cevallos-Ferriz, 1993). This fossil was recovered in a sedimentary layer in Puebla, along with records of other members of tribe Ingeae: *Pithecellobium grimesii* Calvillo-Canadell & Cevallos-Ferriz and *Pithecellobium barneby* Calvillo-Canadell & Cevallos-Ferriz (Calvillo-Canadell and Cevallos-Ferriz, 2005).

Assessing the monophyly of *Lysiloma* is particularly relevant since two related genera studied from a molecular perspective were non-monophyletic: *Abarema* Pittier (Iganci et al., 2015), and *Zygia* P. Brown (Ferm et al., 2019). Aiming at testing the monophyly and sister-group relationships of *Lysiloma*, we performed phylogenetic analyses combining morphological and molecular datasets. We employed morphological characters, plastid (*trn*K and *mat*K), and nuclear (ETS) DNA sequence data.

These analyses were carried out with parsimony for the morphological characters and parsimony and Bayesian inference for the molecular and total evidence approach. This study will allow us to test three hypotheses: 1) *Lysiloma* is monophyletic as currently circumscribed; 2) *Lysiloma* and *Hesperalbizia* are closely related (Brown et al., 2008; Iganci et al., 2015), and 3) the infrageneric relationships between the species as proposed by Thompson (1980). Regarding infrageneric relationships, the informal classification proposal of Thompson (1980) was used as a null hypothesis. Moreover, we were interested in estimating the chronology of the diversification under the uncorrelated lognormal relaxed molecular clock approach. To do so, we adopt the same assumption of Becerra (2005) regarding *Bursera* Jacq. ex L. (Burseraceae) and the expansion of the dry forest. In other words, the history and evolution of *Bursera* in Mexico mirrors the history of the dry forest in Mexico. Thus, the very forces that drove the range expansions and contractions of the dry forests also shaped the diversification of *Bursera.* Because *Lysiloma* is highly adapted to the ecological conditions of the dry forest, we test whether the diversification of this genus is related to the expansion of the dry forest in the Miocene (20-5 Mya (million years ago)), when arid environments expanded across the world.

Material and Methods

Taxon sampling

The ingroup includes the nine *Lysiloma* species recognized by Thompson (1980) and *Hesperalbizia occidentalis* whereas the outgroup is composed of basal members of the Mimosoid clade, including *Acaciella angustissima* (Mill.) Britton & Rose, *Calliandra eriophylla* Benth., *C. haematocephala* Hassk., *Faidherbia albida* (Delile) A. Chev., *Mariosousa dolichostachya* (S.F. Blake) Seigler & Ebinger, *Senegalia parviceps* (Speg.) Seigler & Ebinger, *Vachellia farnesiana* (L.) Wight & Arn., *Zapoteca formosa* (Kunth) H.M. Hern., and *Zapoteca tetragona* (Willd.) H.M. Hern. (Table 2). An attempt was made to include two or three accessions per species to sample their geographical and ecological range. However, some species feature wide distributions (e.g. *Lysiloma acapulcense*) and it is possible that an even broader sampling may be required to represent the morphological and distributional range of these particular species.

DNA extraction, amplifications, and sequencing

For the molecular analyses, fresh leaflet tissue collected in the field, in the Regional Botanical Garden Roger Orellana of the Centro de Investigación Científica de Yucatán,

Table 2: Summary of the botanical material used for molecular analysis of the genus *Lysiloma* Benth., including accessions extracted from GenBank. In bold GenBank accession numbers of sequences generated in the present study.

Species	**ETS**	***mat*K**	***trn*K**
Acaciella angustissima (Mill.) Britton & Rose	EF638082.1	HM020733.1	*E. López 1128* (CICY) **MN755794**
Calliandra eriophylla Benth.	*E. López 1099* (CICY) **MN755770**	EU025883.1	*E. López 1099* (CICY) **MN755797**
Calliandra haematocephala Hassk.	*Bot. Garden Fairchild* 2007 0163A **MN755769**	MH749029.1	*R. Duno 2425* (CICY) **MN755796**
Faidherbia albida (Delile) A. Chev.	EF638163.1	JF270778.1	AF274129
Hesperalbizia occidentalis (Brandegee) Barneby & J.W. Grimes	*R. García Sosa 71* (MO) **MN755772**	*R. García Sosa 71* (MO) **MN755817**	*R. García Sosa 71* (MO) **MN755799**
Hesperalbizia occidentalis (Brandegee) Barneby & J.W. Grimes	*Santana and Cervantes 868* (ZEA) **MN755773** **MN755774**	*A. Sánchez and A. Nava 399* (ZEA) **MN755818** **MN755819**	*C. E. Hughes 1543* (MEXU) **MN755800** **MN755801**
Lysiloma acapulcense (Kunth) Benth.	*J. Calónico Soto 7192* (FCME) **MN755778** **MN755779**	*O. Alcántara and M. Paniagua 5831* (FCME) **MN755822**	*O. Alcántara and M. Paniagua 5831* (FCME) **MN755803**
Lysiloma acapulcense (Kunth) Benth.	-	*E. López s.n* (CICY) **MN755823**	*E. López s.n* (CICY) **MN755804**
Lysiloma auritum (Schltdl.) Benth.	*Bot. Garden Fairchild 62265* **MN755780** **MN755781**	*Bot. Garden Fairchild 62265* **MN755824**	*Bot. Garden Fairchild 62265* **MN755805**
Lysiloma auritum (Schltdl.) Benth.	-	JQ587745.1	-
Lysiloma candidum Brandegee	-	KX302335.1	-
Lysiloma divaricatum (Jacq.) J.F. Macbr.	*M. Ayala et al. 918* (FCME) **MN755783** **MN755784**	*M. Ayala et al. 918* (FCME) **MN755826**	*M. Ayala et al. 918* (FCME) **MN755807**
Lysiloma divaricatum (Jacq.) J.F. Macbr.	-	-	AF523088.1
Lysiloma latisiliquum (L.) Benth.	*P. Simá 2287* (CICY) **MN755785** **MN755786** **MN755787**	*P. Simá 2287* (CICY) **MN755827** **MN755828**	*P. Simá 2287* (CICY) **MN755808**
Lysiloma latisiliquum (L.) Benth.	-	-	*S. Villanueva s.n.* (CICY) **MN755809** **MN755810**
Lysiloma microphyllum Benth.	*J. Calónico Soto 9422* (FCME) **MN755788** **MN755789**	*I. Rosas et al. 2284* (MO) **MN755829**	*I. Rosas et al. 2284* (MO) **MN755811**
Lysiloma microphyllum Benth.	-	*M. Ordóñez 2* (CHIP) **MN755830**	-

Table 2: Continuation.

Lysiloma microphyllum Benth.	*E. López 1134* (CICY) **MN755790**	*E. López 1134* (CICY) **MN755831**	*E. López 1134* (CICY) **MN755812** **MN755813**
Lysiloma sabicu Benth.	*Bot. Garden Fairdchild* 2012-039 **MN755775**	*Bot. Garden Fairdchild* 2012-039 **MN755820** **MN755821**	*Bot. Garden Fairdchild* 2012-039 **MN755802**
Lysiloma sabicu Benth.	*J. R. Abbott 24059* (MO) **MN755776** **MN755777**		-
Lysiloma tergeminum Benth.	*S. Valencia 4058* (FCME) **MN755791** **MN755792**	-	*S. Valencia 4058* (FCME) **MN755814**
Lysiloma tergeminum Benth.	-	-	EU812062.1
Lysiloma tergeminum Benth.	*J. Calónico Soto 84* (FCME) **MN755793**	*J. Calónico Soto 84* (FCME) **MN755832** **MN755833** **MN755834**	*J. Calónico Soto 84* (FCME) **MN755815**
Lysiloma watsonii Rose	*Regional Bot. Garden Roger Orellana* (RD-001) **MN755782**	*Regional Bot. Garden Roger Orellana* (RD-001) **MN755825**	*Regional Bot. Garden Roger Orellana (*RD-001*)* **MN755806**
Mariosousa dolichostachya (S.F. Blake) Seigler & Ebinger	EF638084.1	EU812056.1	AF523190.1
Senegalia parviceps (Speg.) Seigler & Ebinger	*L. E. Quispe 63* (MO) **MN755767** **MN755768**	*L. E. Quispe 63* (MO) **MN755816**	*L. E. Quispe 63* (MO) **MN755795**
Vachellia farnesiana (L.) Wight & Arn.	EF638128.1	FJ711552.1	AY574103.1
Zapoteca formosa (Kunth) H.M. Hern.	*J. Peñaranda 216* (MO) **MN755771**	AY125854.1	*R. Duno s.n*. (CICY) **MN755798**
Zapoteca tetragona (Willd.) H.M. Hern.	EF638133.1	JQ587912.1	AF523097

A. C., and herbarium material were employed. Herbarium specimens used in these analyses came from CICY, FCME, MA, MEXU, MO, UCOL, and ZEA (acronyms as in Thiers, 2020 continuously updated). Permission was requested from herbarium curators to use leaflet material for DNA extraction. Sixty-five new sequences were generated (17 ETS: MN755767-MN755793, 19 *trn*K: MN755794-MN755815, and 14 *mat*K: MN755816-MN755834). Additional sequences were downloaded from GenBank (GenBank, 2020), particularly from datasets created by Miller and Bayer (2001), Miller et al. (2003), Brown et al. (2008), and Heil et al. (2009). For GenBank accessions see Supplementary Material (Table 2, 3).

The *mat*K gene has been among the most useful loci for resolving plant phylogenetic relationships at different evolutionary timescales (Hilu et al., 2008). It has been used to assess and monitor biodiversity and, via community phylogenetics, to investigate ecological and evolutionary processes that may be responsible for the community structure of particular forests (DNA barcoding) (Heckenhauer et al. 2017). The *trn*K intron sequences also provide similar levels of phylogenetic information as *mat*K. Combining the *trn*K with *mat*K increases overall bootstrap support (Hilu et al., 2008). The external transcribed spacer (ETS) of 18S-26S nuclear ribosomal DNA has been used intensely in phylogenetic studies of the tribes Acacieae and Ingeae, generally

Table 3: Additional accessions for the large ETS analysis.

Taxon	GenBank's accession number
Abarema jupunba (Willd.) Britton & Killip	EF638109, EF638110
Abarema piresii Barneby & J.W. Grimes	KF921624
Acacia chartacea Maslin	DQ029304, DQ029305
Acacia dempsteri F. Muell.	DQ029300
Acacia karina Maslin & Buscumb	KC796100
Acacia pyrifolia DC.	DQ029293
Acacia ryaniana Maslin	DQ029303
Acacia semicircinalis Maiden & Blakely	KC283889
Acacia strongylophylla F. Muell.	DQ029299
Acacia victoriae Benth.	DQ029310, DQ029311
Acaciella angustissima (Mill.) Britton & Rose	EF638082.1
Albizia adinocephala (Donn. Sm.) Britton & Rose ex Record	EF638144
Albizia polycephala (Benth.) Killip,	KF921625
Archidendron ellipticum (Blume) I.C. Nielsen	EF638153
Archidendron hendersonii I.C. Nielsen	HM800427
Archidendron kanisii R.S. Cowan	EF638098
Archidendron lucyi F. Muell.	HM800428
Archidendron whitei I.C. Nielsen	EF638099
Blanchetiodendron blanchetii (Benth.) Barneby & J.W. Grimes	KF921626
Cojoba arborea (L.) Britton & Rose	EF638108, EF638095
Cojoba undulatomarginata L. Rico	EF638096
Ebenopsis confinis (Standl.) Britton & Rose	KF921650, EF638100
Ebenopsis ebano (Berland.) Barneby & J.W. Grimes	EF638101, EF638102, KF921651
Enterolobium contortisiliquum (Vell.) Morong	EF638151
Enterolobium gummiferum (Mart.) J.F. Macbr.	KF921652
Enterolobium timbouva Benth.	KF921654
Faidherbia albida (Delile) A. Chev.	EF638163.1
Falcataria moluccana (Miq.) Barneby & J.W. Grimes	HM800429, HM800430
Havardia mexicana (Rose) Britton & Rose	KF921655
Havardia pallens (Benth.) Britton & Rose	EF638146, EF638147, KF921656
Hydrochorea corymbosa (Rich.) Barneby & J.W. Grimes	KF921657
Inga thibaudiana DC.	KF921659
Leucochloron bolivianum C.E. Hughes & Atahuachi	KF921660
Macrosamanea pubiramea (Steud.) Barneby & J.W. Grimes	KF921665
Mariosousa dolichostachya (S.F. Blake) Seigler & Ebinger	EF638084.1
Pararchidendron pruinosum (Benth.) I.C. Nielsen	EF638129
Paraserianthes lophantha (Willd.) I.C. Nielsen	KU727929, KU727943, HM800432
Paraserianthes toona (Bailey) I.C. Nielsen	EF638106, EF638107
Pithecellobium diversifolium Benth.	KF921666
Pithecellobium dulce (Roxb.) Benth.	EF638142, EF638143
Samanea saman (Jacq.) Merr.	KF921668
Samanea tubulosa (Benth.) Barneby & J.W. Grimes	EF638135
Sphinga acatlensis (Benth.) Barneby & J.W. Grimes	KF921669, EF638145

Table 3: Continuation.

Thailentadopsis nitida (Vahl) G.P. Lewis & Schrire	KF921670
Vachellia farnesiana (L.) Wight & Arn.	EF638128.1
Wallaceodendron celebicum Koord.	EF638097
Zapoteca tetragona (Willd.) H.M. Hern.	EF638133.1
Zygia racemosa (Ducke) Barneby & J.W. Grimes	KF921671

together with ITS (Brown et al., 2008; Murphy et al., 2010). The ETS has also a high rate of sequence evolution by nucleotide substitution (Baldwin and Markos, 1998).

Total DNA from leaflets (fresh or from herbarium material) was obtained with the DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, California) following the provider's specifications. To assess concentration and relative quality of DNA, 3 µl of the final volume plus 2 µl loading buffer were run for 30 minutes at 6 volt/cm in a 1% agarose gel prepared with TBE 0.5X. The resulting gel was revealed by immersion for 20-30 minutes in a 0.1 µg/ml ethidium bromide solution and later observed in a DigiDoc-It Imaging System (v. 6.7.1; UVP, Inc., Cambridge, UK) transilluminator. Furthermore, DNA purity and concentration were quantified with a NanoDrop 2000c (Thermo Scientific™, Waltham, USA). Then, DNA samples were standardized at 10 ng μl^{-1}. Amplifications were performed in an Applied Biosytems Veriti 96 Well Thermal Cycler (Applied Biosystems, Foster City, USA). Volumes of reagents and conditions for the amplifications were as follows:

ETS: 30 µl of mix containing 3 µl 10X Buffer, 2.5 µl $MgCl_2$, 0.6 µl (~10 ng) primer, 4 µl Q solution, 1 µl 1.25 mM l^{-1} dNTP, 0.2 µl (1 U) TAQ polymerase, 2 µl (~10 ng) DNA, then completed to volume (approx. 16.1 µl) with ultra-pure water. PCR's were conducted under the following protocol: 94 °C for 3 min + 30 cycles (94 °C for 1 min + 60.5 °C for 1 min + 72 °C for 2 min) + 72 °C for 7 min. Primers were 18S-IGS and 26S-IGS (Baldwin and Markos, 1998).

***trn*K**: 20 µl containing 2.0 µl 10X Buffer, 0.8 µl of $MgCl_2$, 1 µl (~ 10 ng) of primers, 0.8 µl $MgCl_2$, 1 µl (~ 10 ng) primers, 4 µl Q solution, 1.5 µl 1.25 mM l^{-1} of dNTP, 0.2 µl (1 U) TAQ polymerase, 3 µl (~10 ng) DNA, then completed to volume (approx. 6.5 µl) with ultra-pure water. PCR's were conducted under the following protocol: 94 °C for 3 min + 30 cycles of (94 °C for 1 min + 55 °C for 1 min + 72 °C for 2 min) + 72 °C for 7 min. Primers were *trn*K 3914 and Ac 283R (Johnson and Soltis, 1994).

***mat*K**: We used 20 µl reaction mix composed of 2.0 µl Buffer 10X, 0.8 µl $MgCl_2$, 1 µl (~ 10 ng) primers, 4 µl Q-solution, 1.5 µl 1.25 mM l^{-1} dNTP, 0.2 µl (1 U) TAQ polymerase, 3 µl (~10 ng) DNA, then completed to volume (0.5 µl) with ultra-pure water. PCR reactions were conducted under the following protocol: 94 °C for 3 min + 30 cycles (94 °C for 1 min + 55 °C for 1 min + 72 °C for 2 min) + 72 °C for 7 min. Primers were Ac 12F and Ac 1290R (Miller and Bayer, 2001).

PCR products were sent for sequencing to Macrogen Korea. Assemblage and edition of the sequencing products were carried out in BioEdit v. 7.0.9 (Hall, 1999). The data were partitioned into three blocks according to the following gene regions: ETS, *trn*K, and *mat*K. Each of the three partitions was aligned independently using MAFFT (Katoh et al., 2002; 2017) in the online server (https://mafft.cbrc.jp/alignment/server/). Alignments for each partition were generated using the default settings (gap opening penalty=1.53 and offset value=0.00). Finally, visual inspection and refinements were performed to optimize homology of the alignment.

Morphological characters

We assembled a morphological matrix with information compiled from the studies by Thompson (1980) and Gale and Pennington (2004) (Table 4). These characters and their states were assessed for structure, homology assumptions, and coding. To do so, we studied ~300 exsiccata studied at or else borrowed from the following herbaria: CICY, CIQRO, ENCB, FCME, GH, GUADA, MEXU, MO, UADY, UAMIZ, UCAM, UCOL, and US (acronyms as in Thiers, 2020, continuously updated). Thirty-five characters (both binary and multistate) were coded (Table 4). Of these, 26 were discrete, four were discrete numerical and, five were nu-

Table 4: Morphological characters and character states for the phylogenetic analysis of the genus *Lysiloma* Benth. based on Thompson (1980). Some characters are based on previous references, which are indicated in each case. The morphological matrix is included below the table.

[01] Habit: 0 =tree, 1 =shrub, 2 =liana

[02] Stipule: 0 =foliaceous or subfoliaceous, 1 =espiniform

[03] Stipule, shape: 0 =ovate to widely ovate, 1 =linear (does not apply for the spiniform stipules)

[04] Stipules develop: 0 =absent, 1 =present

[05] Paraphyllidia: 0 =absent, 1 =present (Rico et al., 2008)

[06] Number of pinnae: 0 =1-2 pairs, 1 =3 or more pairs

[07] Number of leaflets per pinna: 0 =3-20, 1 =21 or more

[08] Mid vein: 0 =central, 1 =off center

[09] Size of leaflet: 0 =microphyll (<15 mm long), 1 =macrophyll (>20 mm long)

[10] Leaflet, shape: 0 =narrowly oblong, 1 =elliptic to widely elliptic

[11] Inflorescence, type: 0 =capitulum, 1 =raceme, 2 =fascicle

[12] Flower: 0 =sessile, 1 =pedicellate

[13] Number of flowers per head: 0 =few (<29), 1 =intermediate (30-35), 2 =many (>40)

[14] Bracteole, shape: 0 =spatulate, 1 =oblanceolate-linear, 2 =triangular-rhombic, 3 =cuneate

[15] Corolla, length: 0 =short (<5 mm long), 1 =long (>6.5-11 mm long)

[16] Stamens, connation: 0 =free, 1=joined into a short tube

[17] Stamens, number: 0 =few (<29) 1 =intermediate, (30-35), 2 =many (>40)

[18] Stamens, length (mm): 0 =short (<11 mm long), 1 =intermediate (15-20 mm long), 2 =large (>25 mm long)

[19] Fruit twisted in young state: 0 =absent, 1 =present

[20] Pod consistency: 0 =membranaceous, 1 =cartilaginous, 2 =chartaceous to crustaceous, 3 =coriaceous

[21] Pod, permanence: 0 =remain only one year, 1 =remains two or more year

[22] Pod, lateral sutures: 0 =always joined to the valves 1 =separating

[23] Craspedial pod dehiscence: 0 =tardily, 1 =early (does not apply to species without lateral sutures).

[24] Stipe of the pod, length: 0 =short (0.1- 1.5 cm), 1 =long (>2 cm).

[25] Pod, base: 0 =attenuate, 1 =obtuse 2 =truncate,

[26] Pod, shape of the apex: 0 =acute to narrowly acute 1 =obtuse o rounded-truncate to emarginated.

[27] Seeds leaving deep marks on the surface of the pod valves with ups and downs: 0 =absent, 1 =present

[28] Pod, exfoliation: 0 =absent, 1 =present

[29] Pod, venation: 0 =reticulate, 1 =parallel

[30] Funicule, shape: 0 =elongated and straight, 1 =sigmoid, 2 =almost absent, short

Table 4: Continuation.

[31] Seed, shape: 0 =ovate to oblong, 1 =circular to quadrangular

[32] Seed, color: 0 =yellow to light brown, 1 =dark brown to black

[33] Pleurogram, relative size regarding the surface of the seed; 0 =less than 33, 1 =40-50%, 2 =more than 70%

[34] Seed areolate: 0 =absent, 1 =present (Rico et al., 2008)

[35] Cotyledon: 0 =not auriculate, 1 =auriculate

MATRIX

(Polymorphism is indicated by *, and $, no apply is indicated by n).

Vachellia farnesiana 111n01*100000n0n0n10301nn0000120000

Acaciella angustissima $011131*100012300200201n00000021110

Acaciella villosa $011131*1000123002002012011000021110

Hesperalbizia occidentalis 00000110111000211220301n000100011 11

Mariosousa dolichostachya 111n011110011n1n0n10101n00000 1n0100

Senegalia parviceps 211n001110000n0n0n10201n00000110010

Lysiloma acapulcense $000001110011110101121010*011001110

Lysiloma auritum 000001111001111011112101000110011110

Lysiloma candidum 00000n000010000011112101000110001 10

Lysiloma divaricatum 000000110000000010112101000110001 10

Lysiloma latisiliquum 000000*10000001010$1210010010000110

Lysiloma microphyllum 0000001100000000101121010001100011 0

Lysiloma sabicu 000001*001101001100101001111100011 0

Lysiloma tergeminum 000001001120000010012101011110 0*110

Lysiloma watsonii 100001111001101011112101000110011110

merically continuous. In all cases, continuous characters which featured clear discontinuities in between were coded as distinct character states (for example character 9, leaflets size: microphyll (<15 mm long) or macrophyll (>20 mm long), character 15, corolla length: short (<5 mm long), or long (6.5-11 mm long), character 18, stamens length: short (<11 mm long), intermediate (15-20 mm long), and large (>25 mm long).

Phylogenetic analyses

Morphological analysis

We prepared a matrix composed by morphological data only (Table 4) that was analyzed with a Maximum Parsimony Analysis done with NONA v. 2.0 (Goloboff, 1993) through the Winclada v. 1.00.08 (Nixon, 2002) shell. Non-parsimony

informative coding characters were deactivated. Informative characters were considered unordered and given the same weight (Fitch Parsimony). To identify maximally parsimonious topologies, we performed a ratchet algorithm analysis with 5000 iterations, 10 trees held at each iteration, whereas 10% of the characters were sampled in each iteration (mult* 10000, ho/10; max*). Clade support was assessed with 1000 iterations of bootstrap. The topologies retrieved are shown here only for morphological characters, but our complete set of results is available from the corresponding author upon request.

Molecular analyses

There has been much debate over the merits of different algorithms for phylogenetic inference (Rindal and Brower, 2010). However, parametric Bayesian methods have become very popular in molecular phylogenetics due to the availability of user-friendly software implementing sophisticated models of evolution (Nascimento et al., 2017). These methods have the advantage of including nucleotide evolutionary models and a solid statistical framework. In the present study, we use maximum parsimony as an exploratory analysis, obtaining topologies (not shown) that are highly congruent with those resulting from Bayesian analyses.

We assembled several DNA matrices, one composed only of rDNA-ETS sequences that included 112 taxa and account for most of the major clades in tribes Ingeae and Acacieae that have been identified in recent analyses (e.g. Brown et al., 2008, 2011, Iganci et al., 2015, Ferm, 2018). The analysis was carried out with MrBayes version v. 3.2.7 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and Tracer v. 1.6 (Rambaut et al., 2014).

This analysis was designed to test the monophyly of *Lysiloma* as well as the position of the genus within Ingeae + Acacieae. The matrix is available from the corresponding author upon request. Except for the sequences of *Lysiloma*, *Zapoteca* H.M. Hern., and some members of the *Pithecellobium* alliance, most of the sequences used were retrieved from GenBank (GenBank, 2020; Table 2). The individual matrices were integrated with our data and completed with GenBank sequences to match the composition of the morphological matrix.

Combined analyses

We concatenated the three molecular alignments and morphological data in a matrix and analyzed it under the Bayesian Inference paradigm using MrBayes v. 3.2.7 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and Tracer v. 1.7.1 (Rambaut et al., 2014).

Each partition was treated as independent and associated with its own model. Our analyses were performed with the default parameters of the software, except for the number of generations, which were five million. Two independent threads were run. Convergence was assessed with both MrBayes v. 3.2.7 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and Tracer v. 1.7.1 (Rambaut et al., 2014). Posterior Probabilities (PP) of <0.95 were considered weakly supported, whereas PP of 0.95-1.0 were deemed as strongly supported. Missing data were coded as "?" in the concatenated matrix. To assess the best evolutionary model for all the molecular matrices, we used jModelTest v. 2.1.7 (Guindon and Gascuel, 2003; Darriba et al., 2012). In each case, three substitution schemes were used; the search criterion was a maximum likelihood tree estimated with the "best" option of the software. The selected model was suggested by the Akaike Information Criterion (AIC). For the larger ETS matrix, the best model was GTR + G, as well as for the smaller matrices (*mat*K, *trn*K and ETS).

Fossil calibration and diversification times

A Bayesian analysis to estimate divergence times was conducted using the ETS matrix under the uncorrelated log-normal relaxed molecular clock approach implemented in the program BEAST v. 1.10 (Suchard et al., 2018). The main reason to use this marker is the high number of accessions available in GenBank (Brown et al., 2008; Murphy et al., 2010; Ferm. 2018).

In each BEAST 1-10 run, we used pure-birth (Yule) tree prior, and a Monte Carlo Markov chain (MCMC) of 25,000,000 generations, sampling every 1000 generations, with parameters sampled every 1000 generations. For this matrix, the nucleotide substitution model was GTR + I + G using the AIC criterion with jModelTest v. 2.1.6 (Posada, 2008).

Tracer v. 1.7.1 (Rambaut et al., 2014) was used to assess effective sample sizes (ESS >200) for all estimated

parameters. We used TreeAnnotator v. 1.8.2 (part of the BEAST package) to discard 10% of the saved trees as burn-in and to combine trees. Maximum clade credibility trees with mean node heights were visualized using FigTree v. 1.4.2 (Rambaud, 2014). We report highest posterior densities intervals, the interval containing 95% of the sampled values.

Three fossils were used to calibrate the divergence dating analysis. The first one was *Lysiloma mixtecana* (Magallón-Puebla and Cevallos-Ferriz, 1993) assigned to the crown group of *Lysiloma* at 28.4 million years (myr). The second point was based on pollen assigned to *Calliandra* Benth. (Caccavari and Barreda, 2000), with 16 myr and referred to the core group of *Calliandra*, whereas the third one was the tree root with 45 Ma, a date taken from the fossil pollen of the most recent common ancestor assigned to Ingeae and Acacieae (Simon et al., 2009). All fossils were defined as minimum age constraints and implemented in the dating analysis as a lognormal statistical distribution. We choose lognormal distribution because it is appropriate for calibrations derived from fossils. A log normal density distribution to calibration points allows for uncertainty associated with a fossil representing a minimum age where the clade in question could have evolved earlier but not later than the age of the fossil. The maximum clade credibility tree (MCC) was visualized using FigTree v.1.4.2 (Rambaud, 2014) and the means and 95% higher posterior densities (HPD) were obtained.

We carried out a second estimate of divergence times analysis intended to evaluate the origin of the genus without any temporal constraint. For this reason, we did not include the fossil of *Lysiloma mixtecana*, but we used the other two fossils plus *Acaciapollenites myriosporites* (Cookson) Mildenhall (23 Ma) (Macphail and Hill, 2001), which was assigned to the *Acacia* s.s. clade.

For this analysis we had to implement a normal prior with a stdev=2 for the older date (Ingeae and Acacieae 45 Ma) and the other fossils were assigned with a gamma prior and stdev=2 distribution, because these distributions allowed us to obtain the best results for the effective sample size (ESS). These parameters have also been implemented in other studies (Gustafsson et al., 2010; Chomicki et al., 2015; Pérez-Escobar et al., 2017).

Results

Morphology

The parsimony analysis based on morphological characters yielded eight most parsimonious trees (L=63, CI=69, RI=80). Figure 2 shows the strict consensus tree, where three nodes have no support and collapse. *Vachellia farnesiana,* upon which the cladograms are rooted, is followed by a poorly supported grade that includes *Senegalia parviceps*, *Mariosousa dolichostachya*, *Acaciella* spp. plus a clade containing *Lysiloma* and *Hesperalbizia*. The clade including *Acaciella* Britton & Rose as a sister group of *Lysiloma* and *Hesperalbizia* has moderate bootstrap support (77%) and is held together by four synapomorphies. *Acaciella* has a strong bootstrap support (97%) and is supported by two synapomorphies. The clade *Hesperalbizia* and *Lysiloma* (clade A) also features a high bootstrap support (91%) and is supported by four synapomorphies. *Hesperalbizia* is supported by one synapomorphy. On the other hand, *Lysiloma* (clade B) is monophyletic and has a low bootstrap support (68%); this clade features five morphological synapomorphies. Internally, *Lysiloma* is poorly resolved; there are three terminal taxa; *L. auritum* (Schltdl.) Benth., *L. acapulcense* and *L. watsonii* Rose and clade C. The last clade lacks bootstrap support, but it is held together by four reversions. It is composed of *L. latisiliquum* and two clades. The first, including *L. divaricatum* + *L. microphyllum* Benth. with a low bootstrap support (53%), and the second composed of three species with moderate bootstrap supported (77%): *L. candidum* Brandegee as a sister group of *L. tergeminum* Benth., and *L. sabicu*.

Clade C, which is composed by *L. watsonii* and the clade *L. acapulcense* and *L. auritum* is strongly supported by the bootstrap (94%). Clades D (*L. candidum* plus the clade *L. sabicu* + *L. tergeminum*), E (*L. sabicu* + *L. tergeminum*), and F (*L. divaricatum* + *L. microphyllum*) lacked significant bootstrap support (<50%).

Molecular data

Figure 3 shows the result of the Bayesian analysis (rDNA-ETS). In this analysis, some of the genera segregated from *Acacia* Mill. such as *Acaciella*, *Mariosousa* Seigler & Ebinger, *Senegalia* Raf., and *Vachellia* Wight & Arn. are poorly represented. However, this large analysis evaluates the relationship of the genus *Lysiloma* in a more general context (see Brown et al.,

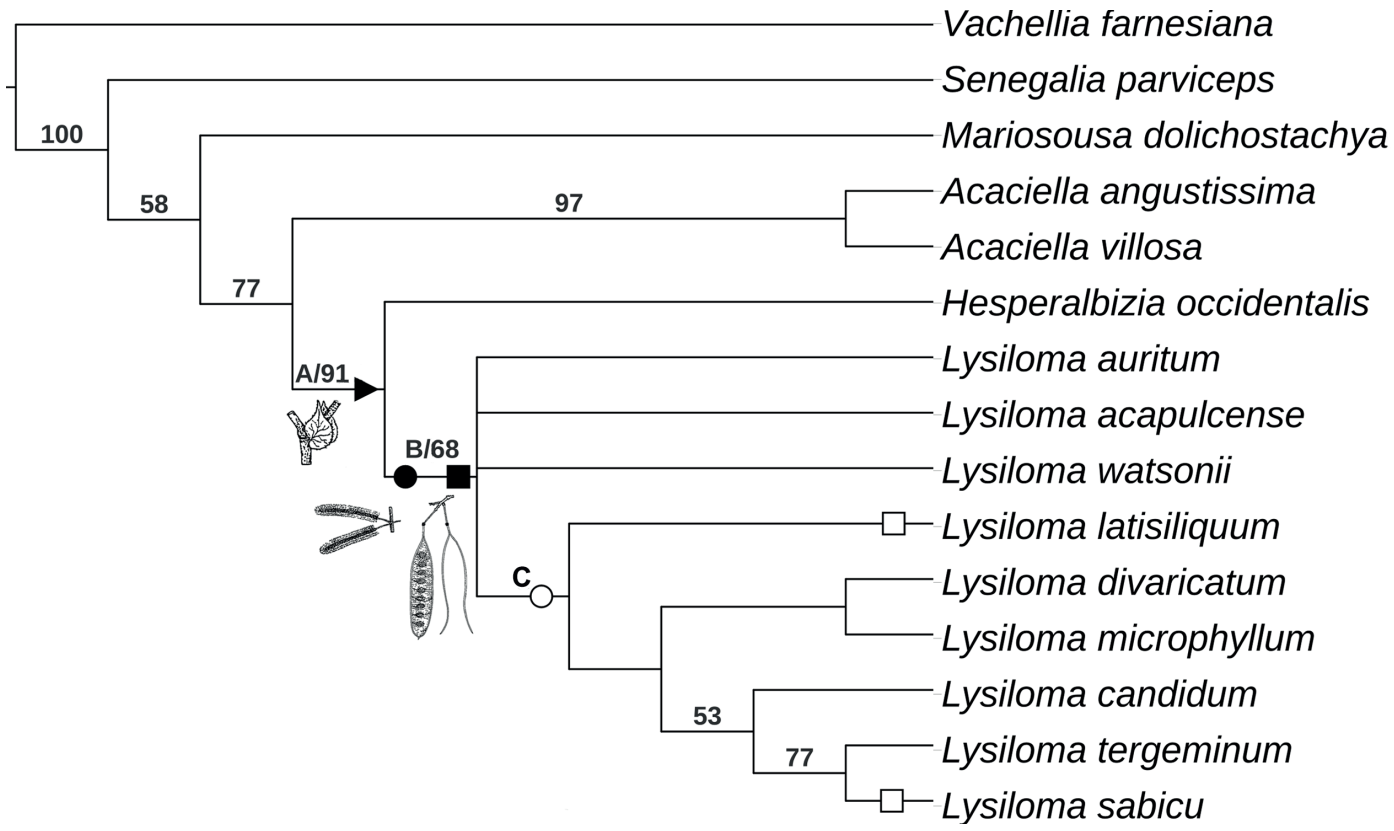

Figure 2: Results of parsimony analysis of the genus *Lysiloma* Benth. based on morphology (value below the node, bootstrap support, simbols: A, clade *Hesperalbizia* and *Lysiloma*; B, clade *Lysiloma*; C, species with inflorescence racemose; black triangle (▶), synapomorphy: stipule developed, black circle (●) and black square (■): synapomorphies for inflorescence racemose and pod craspedial; white circle (○): inflorescence capitate (reversion), and white square (□): pod indehiscent (reversion).

2008, for a complete list of the genera of the Acacieae+Ingeae tribes). *Acaciella*, *Senegalia*, *Mariosousa* comprise a basal grade. The clade A (PP=1.0) is formed for two clade: *Calliandra* is the sister group *Zapoteca* and both are the sister group of Ingeae, including *Acacia* (B Clade, PP=1.0). This clade has two subclades: the clade *Viguieranthus* sensu Rodriguez de Souza et al. (2016) including *Faidherbia* A. Chev., *Sanjappa* É.R. Souza & M.V. Krishnaraj, *Thailentadopsis* Kosterm., and *Viguieranthus* Villiers (PP=0.95) and clade C. This clade included *Cojoba* Britton & Rose as sister group of *Lysiloma* and Hesperalbizia, and clade D (PP=0.99). This last clade includes four subclades whose relationships are unresolved, namely the New World Ingeae p.p. clade, which is strongly supported (PP=1.0), the poorly supported (PP=0.76) Old World Ingeae clade, including *Acacia*, and lastly, the *Pithecellobium* clade (part of the New World Ingeae), which is strongly supported (PP=1.0).

Combined molecular and total evidence analyses

From all regions analyzed, we found ETS to be the most informative: 26 taxa, 482 characters, from which 44.73% were informative, followed by *trn*K, 21 taxa, 999 characters, 5.4% informative, and by *mat*K, 18 taxa, 767 characters, 3.65% informative. The results of the combined molecular analysis and those of the total evidence analysis are very similar; thus, we only show the second in Figure 4. At the base of the tree is a polytomy conformed by *Vachellia farnesiana* (root), *Acaciella,* and then it follows a clade with all the other taxa (Clade A). This polytomy is the sister group of a grade with *Senegalia parviceps*, *Mariosousa dolischotachya*, and clade B. This last clade included four subclades without resolution among them, namely *Calliandra* spp., *Faidherbia, Zapoteca* spp., and finally a clade with *Lysiloma* and *Hesperalbizia* (Clade C). In the combined molecular analysis as well as each individual analysis (ETS, *mat*K, *trn*K), there is no resolution for *Hesperalbizia*

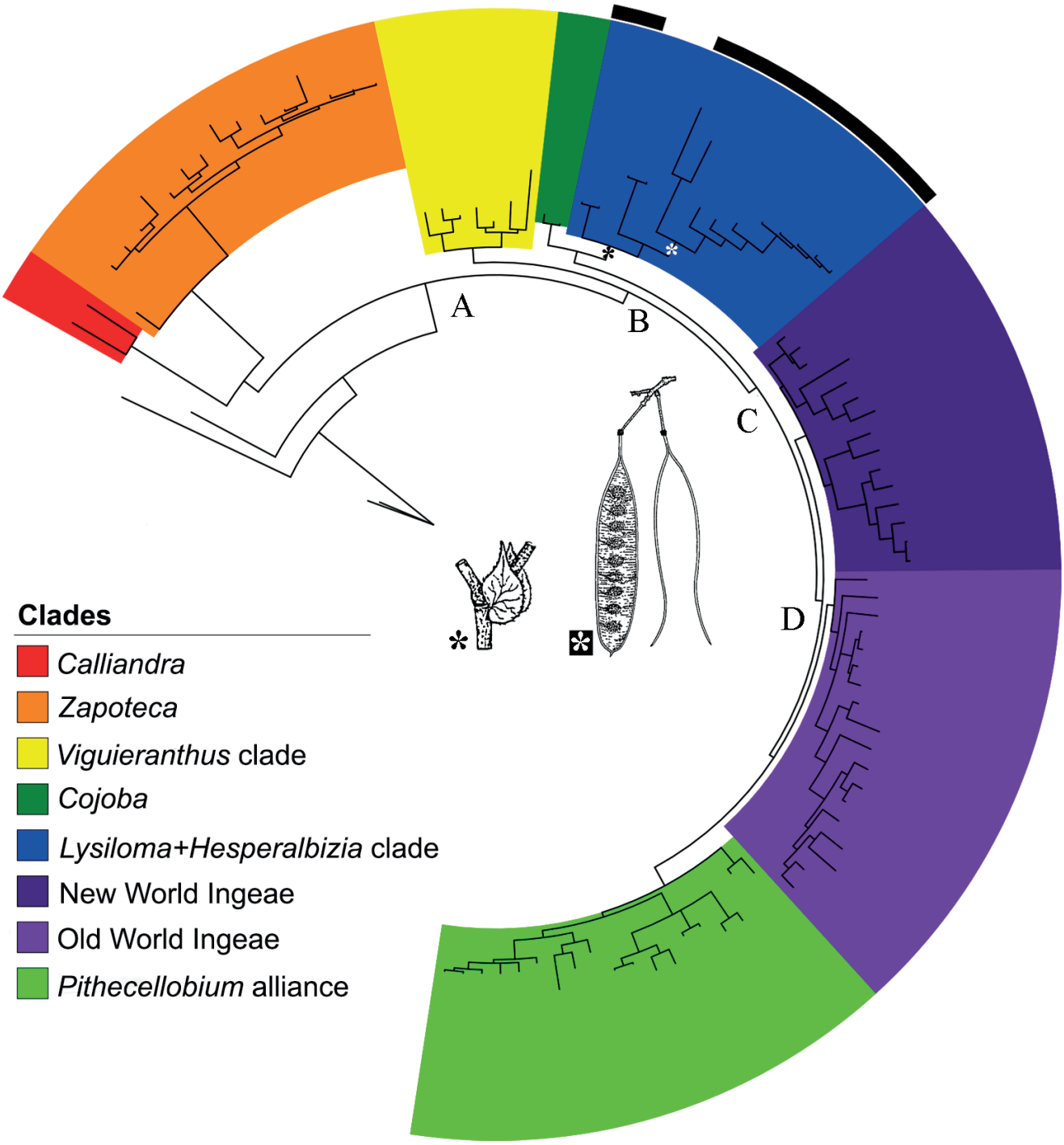

Figure 3: Results of Bayesian analysis of the genus *Lysiloma* Benth. based on a large molecular matrix of DNA-ETS (Clade A: core Ingeae, including *Acacia* Mill. clade B: clade A- clade *Calliandra*, and *Zapoteca*; clade C: *Cojoba*, sister group of *Lysiloma* and *Hesperalbizia*, and Ingeae p.p. including *Acacia*, Clade D: New World Ingeae p.p., Old World Ingeae and *Acacia*, and *Pithecellobium* alliance. Important synapomorphies: stipule developed for *Lysiloma* + *Hesperalbizia*, and pod craspedial for *Lysiloma*.

occidentalis, *Lysiloma sabicu* and the core of *Lysiloma*. However, in the total evidence analysis, *Hesperalbizia* was recovered as sister group of *Lysiloma* with low support.

Diversification of the genus *Lysiloma*

The exclusion of the *Lysiloma* fossil from the molecular clock analyses does not result in relevant differences in chronology. Hence, the results are not contingent upon the

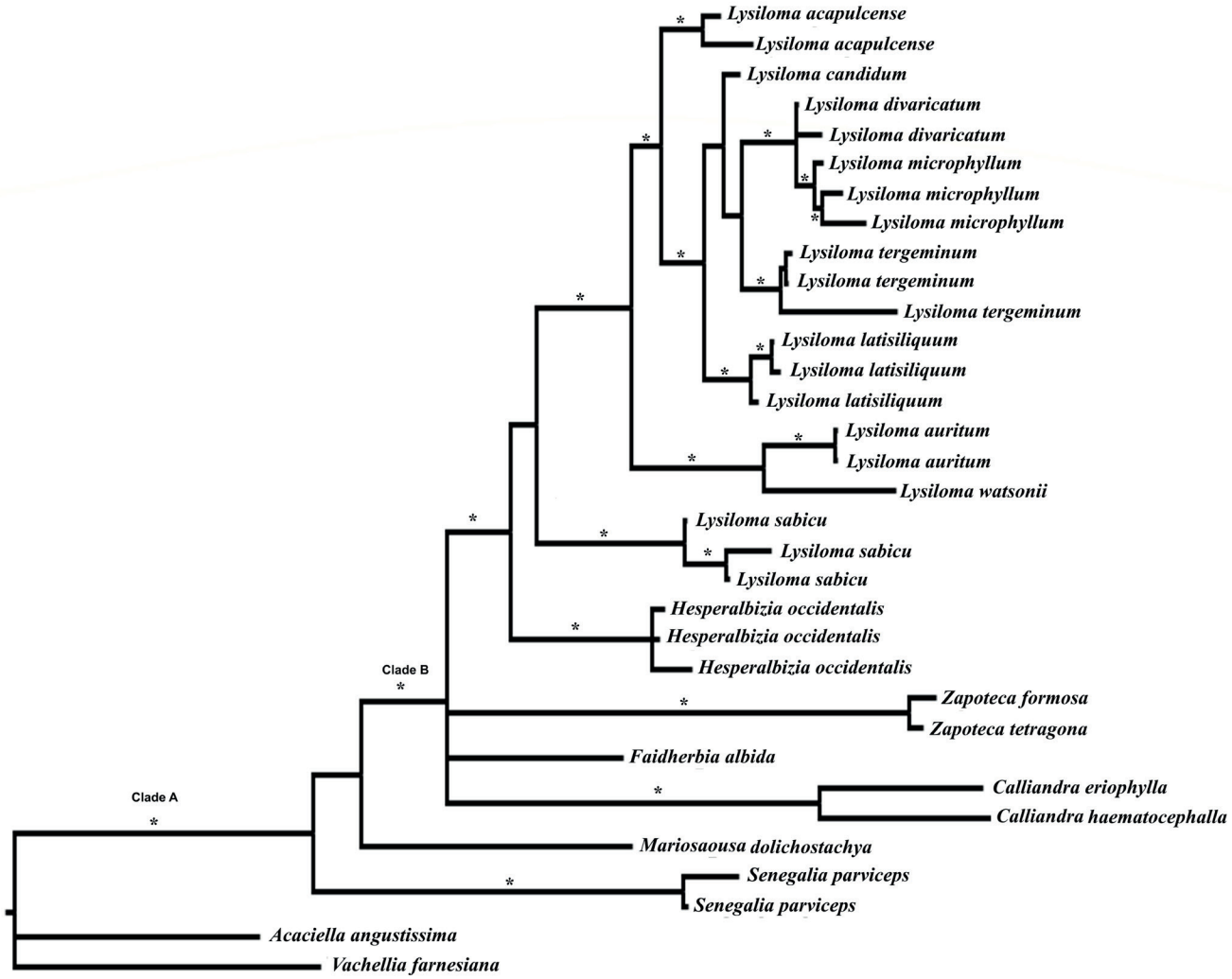

Figure 4: Results of total Bayesian analysis the genus *Lysiloma* Benth. (morphology, ETS, *mat*K and *trn*K). * PP >95 %.

restriction imposed by the inclusion of this fossil and associated date.

Figure 5 shows the result molecular clock analyses. The divergence of *Lysiloma* and *Hesperalbizia* (L1) was estimated at about 28.22 myr (95% HPD: 19.5-36.25) in the beginning of the Oligocene. The second node (L2) corresponds to the divergence of *Lysiloma* and was estimated at about 29.86 myr (95% HPD: 28.64-39.13). Node L3, the divergence of the *Lysiloma* core, was estimated at about 22.65 myr (95% HPD: 15.78-28.33) in the beginning of the Miocene.

Discussion

Phylogeny reconstruction is a crucial aim of contemporary systematics. The success of phylogenetic inference can be measured in terms of resolution, support, and accuracy (Wortley et al., 2005). Many studies suggest that increasing sequence data is a better way to improve support, resolution, and accuracy of the phylogenetic trees (Rokas and Carroll, 2005) at any level; the ordinal level (Li et al., 2019), family level (Stull et al., 2015), generic level (Cardoso et al., 2015; Stull et al., 2015), and at the species level (Nicholls et al., 2015). We consider that additional DNA regions must be explored to test and improve the resolution of topology, particularly regarding *Lysiloma* and *Hesperalbizia*. The morphological analysis (maximum parsimony) confirms the monophyly of *Lysiloma* and its close relationship with *Hesperalbizia*, in concordance with two previous molecular analyses, which include at least one accession of each genus (Brown et al., 2008; Iganci et al., 2015). However, the

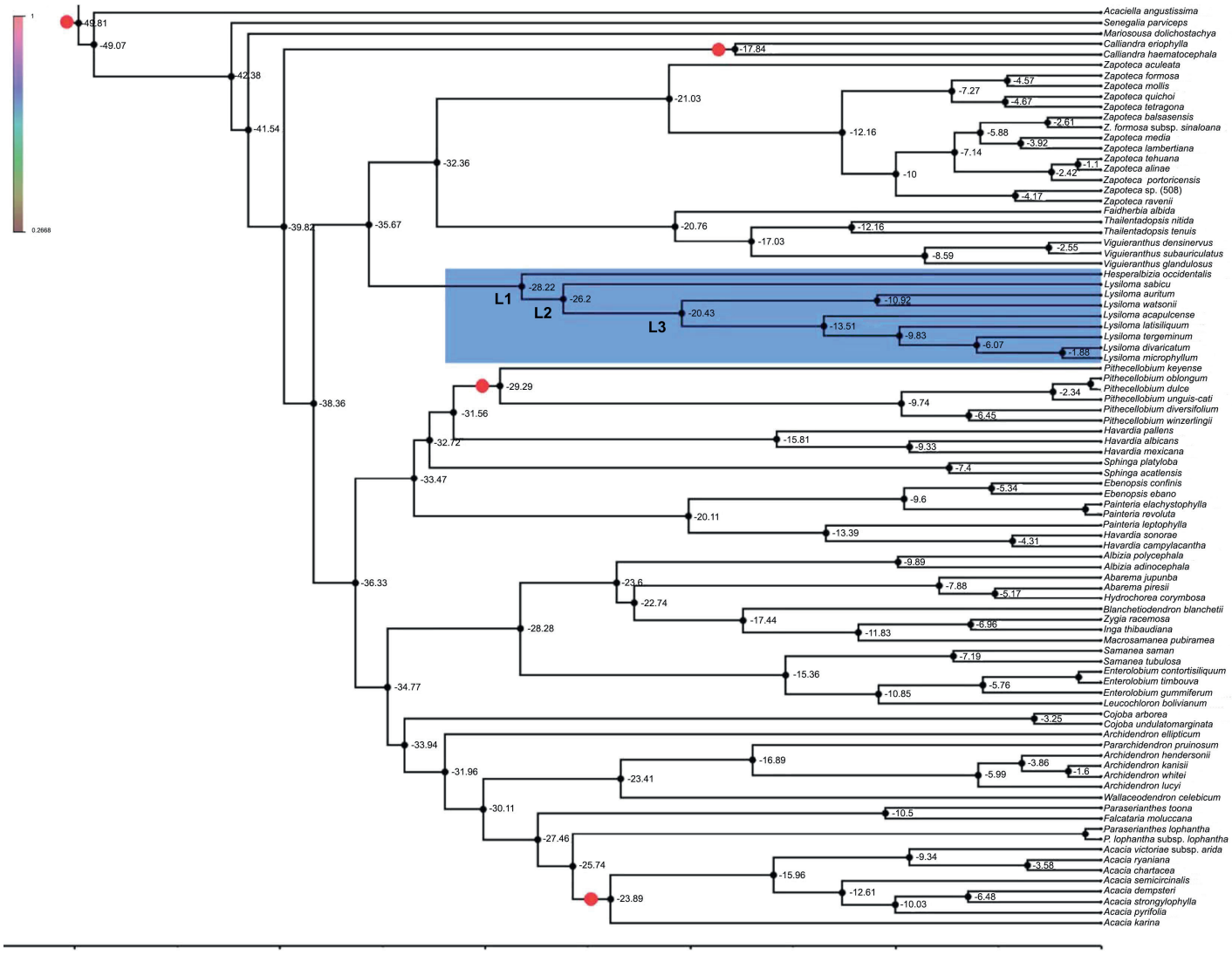

Figure 5: Chronogram of *Lysiloma*, and *Hesperalbizia* and other related taxa from the tribe Acacieae and Ingeae (Caesalpinioideae) based on ETS data. Divergence times are shown using the computer program BEAST v. 1.10. The calibration nodes 1 (28.4 myr), and 2 (16 myr), are marked by dots based on the fossil records. The root of the tree was set to no more than 45 myr.

current morphological analysis does not allow to recover a complete picture of the relationships of *Lysiloma* and *Hesperalbizia* with other members of tribes Acacieae and Ingeae, because only a few taxa were included.

Morphological analysis

In a previous morphological analysis that included many members of the tribe and in which 75 characters were used (Grimes, 1995), a different picture of the relationships of *Lysiloma* and *Hesperalbizia* was retrieved. Grimes (1995) found that *Lysiloma* is most closely related to the *Pithecellobium* alliance, along with *Faidherbia*, *Senegalia*, and *Vachellia*. However, *Hesperalbizia* is more related to *Samanea* (Benth.) Merr., and *Pseudosamanea* Harms. The main explanation for this result is the unorthodox way of coding some characters by the abovementioned author (e.g. characters 51 to 58 are derived from the fruit and represent one or two, probably strongly correlated characters).

According to our morphological analysis, *Lysiloma* has five synapomorphies (Fig. 2, characters 16, 18, 20, 21, and 29). The fact that some of these characters states are synapomorphies may be related to the sampling strategy. Hence, if more taxa of the tribe were included, some would most likely become homoplasic. For example, the character "fruits remaining united to the mother plant for long

time" is found in *Albizia* Durazz. (not included in the current analysis). In addition, the character "prominent foliaceous stipules" a synapomorphy for *Hesperalbizia+Lysiloma* is present in *Albizia chinensis* (Osbeck) Merr.

Molecular and total evidence analyses

In a more general context, three previous phylogenetic analyses have been published with abundant accessions of ITS, and/or ETS (Brown et al., 2008; Rodriguez de Souza et al., 2013; Iganci et al., 2015). In concordance with those, our large (rDNA-ETS, Fig. 2) analysis supports a basal position for the segregates of *Acacia*, namely *Acaciella*, *Mariosousa*, *Senegalia*, and *Vachellia* as well as for the *Lysiloma+Hesperalbizia* clade. It also supports the monophyly of the *Pithecellobium* alliance and the clade *Viguieranthus* as a sister group of *Zapoteca* (Rodriguez de Souza et al., 2016). None of these earlier phylogenetic analyses support the relationship of *Lysiloma* and *Hesperalbizia* found by Grimes (1995), nor support the relationship of *Hesperalbizia* with *Albizia* (Rico Arce et al., 2008). In the current analysis some clades are strongly supported (e.g. *Zapoteca*, *Cojoba*, and the *Pithecellobium* alliance (PP >98%)), but not the *Lysiloma+Hesperalbizia* clade. The basal clade of the current tree (Fig. 2) is a mixture of elements restricted to the New World (*Acaciella*, *Mariosousa*, and *Zapoteca*), others from the Old Word (*Faidherbia*), as well as elements from both regions (*Calliandra+Afrocalliandra* É.R. Souza & L.P. Queiroz, and *Senegalia*). There are three clades with no resolution which reveal geographic coherence: The New World Ingeae (e.g. *Abarema*, *Inga* Mill., *Samanea*, *Enterolobium* Mart., etc.), the Old World Ingeae (e.g. *Archidendron* F. Muell., *Paraserianthes* I.C. Nielsen, *Wallaceodendron* Koord., etc.), plus *Acacia s.s.*, and the *Pithecellobium* alliance from the New World.

Regarding the internal relationship of the genus, *Lysiloma latisiliquum* and *L. sabicu*, the two taxa bearing fruits with persistent lateral sutures are not retrieved as a clade; instead, the two species are nested in widely diverging clades, pointing to an independent evolution of this fruit type. Our results suggest that the distribution of *L. sabicu* in the Antilles can be explained by an old vicariance process but in the case of *L. latisiliquum*, it requires long-distance dispersal (from mainland Mexico to south Florida, Bahamas, Turks and Caicos Islands, Cuba and Haiti). A fact that still has no explanation is why *L. sabicu* has been less successful in its expansion in the Antilles compared to *L. latisiliquum*, considering the long occupation time of the lineage in the area.

Lysiloma taxa with capitate inflorescences and those with racemose inflorescences constitute polyphyletic assemblages. In summary, we found no support for Thompson's infrageneric informal classification (1980). This was already proposed by Barneby and Grimes (1996), who did not find any reason to maintain such a classification and proposed that character states observed in *L. latisiliquum* and *L. sabicu* probably evolved independently. The evolution of fruit dehiscence in *Lysiloma* is of ecological interest, because indehiscent fruits, which are buoyant and probably an apomorphic condition, have most likely allowed the genus to invade the West Indies.

In our analysis, we did not find a clear biogeographical pattern with the three species distributed in the Nearctic region (the southwestern coast of USA, and Mexico): *L. candidum* Brandegee, *L. microphyllum* (partially) and *L. watsonii,* because they are not closely related, suggesting the genus invaded the Nearctic region thrice. However, a structurally and geographically coherent clade recovered in both the morphological and total evidence analyses was found to occur along the Pacific coast of Mexico: *L. candidum*, *L. tergeminum*, and *L. divaricatum*. One of the morphological features of this clade are the leaves with few pinnae. Therefore, diversification in *Lysiloma* seems to have followed an isolation by allopatry pattern, whereby sister taxa are currently allopatric and sympatric taxa are not closely related. Thus, *Lysiloma latisiliquum* and *L. sabicu*, which are sympatric in the West Indies, belong to different clades in the genus, whereas the sister pair of *L. watsonii* and *L. auritum* occur one each on one of the opposing drainages of Mexico, the former on the Pacific slope, the latter on the Gulf slope.

Lysiloma sabicu is the most distinct taxon within the genus. This taxon shares with *Hesperalbizia* leaves with few pinnae with a few large leaflets, which can imply a morphological transition. It is possible that additional evidence will eventually increase the support for a sister group relationship of *Hesperalbizia* and *Lysiloma*. In the total evidence

analysis (Bayesian), *Hesperalbizia* is the sister group of *Lysiloma,* but the relationship is poorly supported (PP <0.95%).

The advantages of including *Hesperalbizia* in the genus *Lysiloma* are similar to those of creating a monotypic genus for *Lysiloma sabicu*. Any decision here, regarding the generic boundaries of *Lysiloma* and *Hesperalbizia,* should be well thought-out and meet the mandatory rule of monophyly, as well as the secondary criteria proposed by Backlund and Bremer (1998): support, diagnosability, maximum informativity, and stability. There are morphological differences in the most distinctive character of *Lysiloma* as compared to *Hesperalbizia*; for example, the fruit in *Lysiloma* exfoliates, eventually revealing the pale brown endocarp (not in *Hesperalbizia*). Another difference is related to flower number: fewer with longer petals in *Hesperalbizia*. Seeds are also different (lens-like, pale brown, and areolate in *Hesperalbizia*), as opposed to ovate, oblong to elliptic and dark brown to black in *Lysiloma*. Thus, here we argue that until more data become available, it is best to retain *Lysiloma* in its present circumscription.

Despite *Lysiloma* being a small genus, it shows a complex evolutionary structure. The phylogenetic molecular analysis points at the homoplasic evolution of many morphological characters, which strongly suggest that different lineages were modeled by similar ecological pressures. Other examples of a similar pattern are the lizards of the genus *Anolis* Daudin, where in the case of Caribbean species, the evidence strongly supports the hypothesis of repeated and independent development of similarly shaped body on each island (Losos, 2001). In the case of flowering plants, the genus *Manihot* Mill. is another good example of convergent evolution (in this case, growth forms) (Cervantes-Alcayde et al., 2015). Both examples are of speciose lineages with 400 and 100 species, respectively. Because of this phenomenon (repeated evolutionary convergence), the classifications based only on morphology may be misleading, being not natural (monophyletic), but polyphyletic or paraphyletic. Moreover, although these two examples may suggest that repeated evolutionary convergence is characteristic of diverse lineages, our data of *Lysiloma* show that even small genera with more restricted distributions can also show this pattern. A similar pattern of evolution could be found in other members of the tribe Ingeae, involving some Old World *Albizia* species and New World *Albizia* species associated with flooded forests, all the species of the genus *Hydrochorea* Barneby & J.W. Grimes, and the two species of *Balizia* Barneby & J.W. Grimes section *Balizia*. All these lineages are subject to environmental pressures (seasonally flooded environments) and present indehiscent, lomentiform fruit.

In addition, there is a long-standing argument on the position of *Lysiloma microphyllum* relative to *L. divaricatum*, to whose synonymy it has been relegated. Thompson (1980) recognized both as distinct, albeit morphologically very similar. However, Barneby and Grimes (1996) and Gale and Pennington (2004) were unable to identify consistent characters to separate them. Upon evaluating morphological features in herbarium specimens, we did not find differences in character states between both taxonomic concepts. Furthermore, molecular data do not support both taxa as separate entities, as their ETS, *mat*K, and *trn*K sequences are almost identical. Neither is there a geographical discontinuity in their distributional ranges (Thompson, 1980). Thus, here we conclude that *L. divaricatum* and *L. microphyllum* should be treated as the same species (see Gale and Pennington, 2004; Andrade and Sousa, 2012).

Lysiloma acapulcense requires special consideration. As presently circumscribed, this taxon includes a long list of synonyms, reflecting the fact that it is an extremely polymorphic and widely distributed species. A review of the fruit of the types allows us to recognize at least two additional morphotypes: one characterized by very long and thin fruits (*Lysiloma jorullense* Britton & Rose), and a second morph bearing fruits with a broad base (*Lysiloma platycarpa* Britton & Rose). Thus, this species requires a taxonomic re-evaluation.

Diversification of the genus *Lysiloma*

The divergence between *Lysiloma* and *Hesperalbizia* (Fig. 5, L1) was estimated at 28.2 myr at the beginning of the Oligocene (Fig. 5, Table 4). This date coincides with a drastic and abrupt decrease in temperature and humidity at the beginning of the Oligocene (Galeotti et al., 2016). The second node (L2) corresponds to the divergence of the *Lysiloma* estimated at 26 myr, coinciding with two important facts, a low global temperature and humidity as well as a proxim-

ity between the Caribbean Arc and the American continent through the Yucatan peninsula during the Oligocene (Pindel and Kennan, 2009). An important lineage divergence occurred in the upper Miocene, whereas additional minor ones happened below this node (N3) all along the Miocene, which agree with Becerra's (2005) findings regarding the evolution and divergence of *Bursera* (Burseraceae) in the dry forests.

We can assume that the history of *Lysiloma* seems to be tightly related to the last 30 to 5 myr corresponding with the formation of the main orographic systems in Mexico, such as the Sierra Madre Occidental and later the Neovolcanic belt and the dry forest (Gómez-Tuena et al., 2007). The last uplift of the Sierra Madre Occidental occurred between 34 and 15 myr, whereas the Neovolcanic axis was formed in several stages along a west-east progression that started in the west (Sosa et al., 2018). The uplift of these two orographic systems was presumably responsible for the climatic conditions necessary (high temperature and low humidity) for the development and maintenance of the dry forests (Sosa et al., 2018).

Conclusion

Lysiloma and *Hesperalbizia* are sister genera, albeit with low clade support, with *Lysiloma* often resolved as paraphyletic with respect to *Hesperalbizia*. More data are necessary to confirm the generic status of *Lysiloma*. This sister clade is early branching within the clade comprising the rest of the tribe Ingeae. Indeed, we estimate the stem age of this sister clade to average about 32 Ma. In addition, our analysis shows that, although *Lysiloma* is a small genus, it shows a complex evolutionary structure that may be modeled in different lineages of the genus by the same ecological pressures, suggesting that the phenomena of convergence and/or parallel evolution occur regardless of species richness. One consequence of this phenomenon (repeated evolutionary convergence) is that classifications based on morphology are not necessarily natural (monophyletic), but instead, a conglomerate of polyphyletic or paraphyletic taxa. This applies not only to morphology, but also to patterns of geographic distribution: unrelated species can occupy the same distribution.

Regarding the divergence time, the history of the genus *Lysiloma* and *Hesperalbizia* begins in the Oligocene and diversifies into the beginning of the Miocene. This time frame coincides with low global temperatures, a proximity between the Caribbean Arc and the American continent through the Yucatan peninsula, and the uplift of the Sierra Madre Occidental and later of the Neovolcanic axis. Presumably, these conditions were responsible for the climatic conditions associated with the development and maintenance of the dry forest in Mexico (Sosa et al., 2018), the habitat in which *Lysiloma* diversified.

Author contributions

RD, JL, and CT conceived and designed the study. Laboratory work and the acquisition of other data (sequences) were carried out by RD, JL and LC. The analyzes were performed by CT, IT, JR and RD. Finally, RD, CT, GC and CL performed the interpretation. All authors wrote and contributed to the discussion, review, and approval of the final manuscript.

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