



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

Sistemática de *Deppea* (Hamelieae, Rubiaceae)

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

María Aguilar Morales

TUTORA PRINCIPAL DE TESIS:

DRA. HELGA OCHOTERENA BOOTH

INSTITUTO DE BIOLOGÍA, UNAM

COMITÉ TUTOR:

DRA. MARÍA HILDA FLORES OLVERA

INSTITUTO DE BIOLOGÍA, UNAM

DR. DANIEL PIÑERO DALMAU

INSTITUTO DE ECOLOGÍA, UNAM

TUTOR INVITADO: DR. THOMAS BORSCH

BOTANISCHER GARTEN UND BOTANISCHES MUSEUM BERLIN

CIUDAD UNIVERSITARIA, CD. MX.

2023



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COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

OFICIO CPCB/904/2022

ASUNTO: Oficio de Jurado

M. en C. Ivonne Ramírez Wence
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Presente

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **25 de abril de 2022** se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **AGUILAR MORALES MARÍA** con número de cuenta **304285097** con la tesis titulada **“SISTEMÁTICA DE DEPPEA (HAMELIEAE; RUBIACEAE)”**, realizada bajo la dirección de la **DRA. HELGA OCHOTERENA BOOTH**, quedando integrado de la siguiente manera:

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

ATENTAMENTE
“POR MI RAZA HABLARÁ EL ESPÍRITU”
Ciudad Universitaria, Cd. Mx., a 04 de octubre de 2022

COORDINADOR DEL PROGRAMA



DR. ADOLFO GERARDO NAVARRO SIGÜENZA



AGRADECIMIENTOS INSTITUCIONALES

Agradezco a la Universidad Nacional Autónoma de México y en particular al Posgrado en Ciencias Biológicas.

Agradezco al Consejo Nacional de Ciencia y Tecnología (CONACyT) por la beca otorgada (320242).

Agradezco a mi tutora principal: Dra. Helga Ochoterena y a los miembros de mi comité tutor: Dra. María Hilda Flores Olvera, Dr. Daniel Piñero Dalmau y al tutor invitado Dr. Thomas Borsch.

AGRADECIMIENTOS

A la UNAM y al Instituto de Biología

A mi tutor principal la Dra. Helga Ochoterena por permitirme desarrollar esta tesis e impulsar mi formación académica

A los miembros de mi comité tutor: Dra. Hilda Flores Olvera, Dr. Daniel Piñero y Dr. Thomas Borsch por sus aportaciones al desarrollo de este proyecto y sus comentarios semestre a semestre.

A la Dra. Teresa Terrazas y al Dr. Leonardo Alvarado por fungir como revisores de actividades académicas complementarias.

A los miembros de mi comité de candidatura: Dra. Teresa Terrazas, Dr. David Gernandt, Dra. Rosaura Grether, Dra. Victoria Sosa y Dra. Hilda Flores.

Al Dr. Daniel Piñero por abrirme las puertas de su laboratorio y financiar la secuenciación. A Azalea Guerra García por su apoyo en el trabajo de laboratorio molecular.

Al Dr. David Gernandt por el gran apoyo y tiempo otorgado para el procesamiento bioinformático de los datos y acceso a su servidor para el ensamblaje de secuencias. A José Rubén Montes por su ayuda en bioinformática.

A quienes me apoyaron en campo: M. en C. Carlos Gómez, Dra. Helga Ochoterena, Dra. Hilda Flores, Biol. Melissa Galvan y Biol. Biaanni Velasco, en particular a Héctor Gómez por su invaluable guía en el estado de Chiapas.

A los curadores y personal de los herbarios consultados: CHAP, CHIP, CIDIIR, ECOSUR, ENCB, FCME, HEM, HGO, MEXU, QRO, SERO, XAL.

Al herbario C (Copenhagen) y la Dra. Helga Ochoterena por la muestra de *Schenckia*.

A Clemente Verdugo, Héctor Gómez y Pedro de la cruz por las muestras facilitadas de *Eizia* y *Csapodya*.

A la M. en C. María Berenit Mendoza Garfias por todo el apoyo en el Microscopio Electrónico de Barrido del IBUNAM.

A la Biól. Susana Guzmán Gómez por su ayuda en la toma de imágenes del Microscopio estereoscópico en el Laboratorio de Microscopía y Fotografía de la Biodiversidad.

A Rocío González y al personal del del Posgrado en Ciencias Biológicas.

DEDICATORIA

Para Andrea

Para Tormenta

Para Alejandro

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RESUMEN

Hamelieae es una tribu de Rubiaceae caracterizada por las inflorescencias ebracteadas, a menudo flores amarillas o amarillentas y la presencia de rafidios. La circunscripción de la tribu aún es debatible ya que se reconocen de 10 a 16 géneros dependiendo del autor. Estas diferencias en diversidad se deben al complejo *Deppea*, el cual es un grupo de 10 géneros que fueron segregados de *Deppea* o están morfológicamente relacionados con este género: *Bellizinca*, *Csapodya*, *Deppea*, *Deppeopsis*, *Edithea*, *Omiltemia*, *Pinarophyllon*, *Plocaniophyllon*, *Pseudomiltemia*, y *Schenckia*.

El complejo *Deppea* se caracteriza por tener flores 4-meras, corolas en botón con estivación contorta, ovario bilocular, placentación axilar, numerosos óvulos, frutos capsulares con dehiscencia típicamente poricida. Dentro del complejo, la circunscripción del género *Deppea* esta en debate. Algunos autores sinonimizan *Bellizinca*, *Csdapodya*, *Deppeopsis*, *Edithea* y *Schenckia* con *Deppea*, mientras que otros autores reconocen estos seis géneros, confusión debida en parte al alto traslapamiento en los caracteres morfológicos empleados para definir los géneros y a la carencia de un análisis filogenético robusto. Para los géneros *Omiltemia*, *Pinarophyllon*, *Plocaniophyllon*, y *Pseudomiltemia*, miembros del complejo *Deppea*, existe consenso en su aceptación y reconocimiento morfológico.

El objetivo de esta tesis fue poner a prueba la monofilia del género *Deppea* con respecto a otros géneros del complejo *Deppea*, mediante análisis filogenéticos basados en datos morfológicos y moleculares. De igual forma, me propuse estudiar algunos caracteres morfológicos de potencial relevancia taxonómica (inflorescencia, morfología floral, fruto y semilla) en la delimitación genérica dentro del complejo *Deppea*. Para la morfología se exploraron caracteres microestructurales en la semilla empleando técnicas de microscopía electrónica y microscopía de luz, asimismo realicé la revisión de ejemplares de herbario y referencias bibliográficas para los caracteres macromorfológicos. En la parte molecular, descargué, aliné y analicé las secuencias disponibles de Genbank para la tribu, además de la extracción, secuenciación, alineamiento y análisis de muestras propias empleando secuencias nucleares de bajo número de copia (Angiospermas353).

Debido a que los caracteres microestructurales de la semilla han sido poco investigados en la tribu, analicé 16 caracteres en 37 especies de Hamelieae, correspondientes a 15 de los

posibles 16 géneros reconocidos dentro de la tribu. Los resultados muestran que la combinación de caracteres de la semilla como forma, color, compresión doriventral, posición del hilo y el microrelieve periclinal, ayuda al diagnóstico de algunos géneros y también de especies.

El análisis de secuencias de GenBank incluyó una matriz con los marcadores de cloroplasto (*trnL-F*) y núcleo (la región del ITS de ADN ribosomal) en combinación con una matriz de 24 caracteres morfológicos reproductivos (flor, fruto y semilla), donde se incluyen todos los 10 géneros y 17 especies del complejo *Deppea*. El resultado de este análisis muestra al género *Deppea* como parafilético, dentro de un clado que incluye a *Bellizinca*, *Csapodya*, *Edithea*, *Schenckia*. El resto de los géneros del complejo *Deppea* incluidos en el análisis se agrupan en un clado con poca resolución. En cuanto a los caracteres morfológicos, se corroboró que el color de la flor, la inserción de los estambres y la forma y dehiscencia del fruto, pueden ser útiles en el reconocimiento de algunos géneros.

En el análisis filogenético basado en sondas de RNA específicas para 353 genes nucleares (kit Angiospermas353 que aplica captura de secuencias) se incluyeron 10 géneros del complejo *Deppea* y 40 especies, en una matriz con 164 genes nucleares y más de 166,259 pares de bases. En este análisis se mapearon 31 caracteres morfológicos tanto vegetativos como reproductivos. Con base en esta última filogenia, *Omiltemia*, *Plocaniophyllon* (incluyendo *Deppea tenuiflora*) *Pseudomiltemia* y *Pinarophyllon* son reconocidos como géneros válidos, y se propone a *Bellizinca*, *Csapodya*, *Edithea* y *Schenckia* como sinónimos de *Deppea*. De igual forma se postulan dos nuevos géneros, *Pseudodeppea* y *Pseudodeppeopsis*, con base en caracteres morfológicos y genéticos. Con estos cambios la tribu Hamelieae queda conformada por 12 géneros: *Cosmocalyx*, *Deppea*, *Deppeopsis*, *Hamelia*, *Hoffmannia*, *Omiltemia*, *Pinarophyllon*, *Plocaniophyllon*, *Pseudodeppea*, *Pseudodeppeopsis*, *Pseudomiltemia* y *Syringantha*.

ABSTRACT

Hamelieae stands out as a Rubiaceae tribe characterized by ebracteate inflorescences, often yellow or yellowish flowers, and the presence of raphides. The circumscription of the tribe is still debatable because 10 to 16 genera are recognized depending on the author. These differences in diversity are mainly due to the *Deppea* complex, which is a group of 10 genera that were segregated from *Deppea* or are morphologically closely related to it: *Bellizinca*, *Csapodya*, *Deppea*, *Deppeopsis*, *Edithea*, *Omittemia*, *Pinarophyllon*, *Plocaniophyllon*, *Pseudomittemia*, and *Schenckia*.

The *Deppea* complex is characterized by having 4-merous flowers, button-shaped corollas with contorted aestivation, bilocular ovary, axillary placentation, numerous ovules, capsular fruits with typically poricidal dehiscence. Within the complex, the circumscription of *Deppea* is debatable; some authors synonymize *Bellizinca*, *Csapodya*, *Deppeopsis*, *Edithea* and *Schenckia* with *Deppea*, while others recognize these six genera as different. This confusion is due in part to the high overlap in the morphological characters used to define the genera and to the lack of a robust phylogenetic analysis. For the genera *Omittemia*, *Pinarophyllon*, *Plocaniophyllon* and *Pseudomittemia*, other members of the *Deppea* complex, there is consensus on their recognition and morphological recognition.

The objective of this thesis was to test the monophyly of *Deppea* genus with respect to the other genera of the *Deppea* complex by phylogenetic analyses based on morphological and molecular data. Also to study morphological characters of potential taxonomic relevance (inflorescence, floral morphology, fruit and seed) for generic delimitation. For morphology, microstructural characters in the seed were explored using electron microscopy and light microscopy, as well as a review of herbarium specimens and bibliographic references for macromorphological characters. For the molecular part, the sequences available from Genbank for the tribe were downloaded, aligned and analyzed, in addition to the extraction, sequencing, alignment and analysis of our own samples using low copy nuclear sequences (Angiosperms353 kit).

Because the microstructural characters of the seed have been little investigated in the tribe, we studied 16 seed characters for 37 species corresponding to 15 of the possible 16 genera recognized for the Hamelieae tribe. The results show that the combination of characters

such as shape, color, dorsiventral compression, hilum position and periclinal microrelief helps diagnose genera and species.

The GenBank sequence analysis included a matrix with chloroplast (*trnL-F*) and nuclear (ITS region) markers in combination with 24 reproductive morphological characters (flower, fruit, and seed), including all 10 genera and 17 species of the *Deppea* complex. The result of this analysis shows *Deppea* as paraphyletic with respect to *Bellizinca*, *Csapodya*, *Edithea* and *Schenckia*, while the rest of the genera of the *Deppea* complex are included in the analysis are grouped in a clade with poor resolution. Regarding the morphological characters, many of them can be useful in the recognition of genera such as the color of the flower, the insertion of the stamens and the shape and dehiscence of the fruit.

In the phylogenetic study based in RNA specific for 353 nuclear genes (Angiosperms353 kit of targeted sequencing) a total of 10 genera of the *Deppea* complex and 40 species were included in a matrix with 164 nuclear genes and more than 166,259 bp. Thirty one morphological characters were mapped in this analysis, both vegetative and reproductive. Based on the resulting topology the genera *Omitemia*, *Plocaniophyllon* (including *Deppea tenuiflora*) *Pseudomitemia* and *Pinarophyllon* should be accepted, also *Bellizinca*, *Csapodya*, *Edithea* and *Schenckia* should be synonymized with *Deppea*. Also, two new genera *Pseudodeppea* and *Pseudodeppeopsis* are postulated based on various morphological and molecular evidence; with these changes the circumscription of Hamelieae tribe includes 12 genera: *Cosmocalyx*, *Deppea*, *Deppeopsis*, *Hamelia*, *Hoffmannia*, *Omitemia*, *Pinarophyllon*, *Plocaniophyllon*, *Pseudodeppea*, *Pseudodeppeopsis*, *Pseudomitemia* and *Syringantha*.

I. INTRODUCCIÓN

A. La familia Rubiaceae y la tribu Hamelieae

Las Rubiaceae son la cuarta familia más diversa de plantas vasculares en el mundo con 500 a 700 géneros y aproximadamente 13200 especies (Standley, 1918; Robbrecht, 1988; Taylor, 2001; KEW, 2017). Son de distribución cosmopolita, con centro de diversidad en las regiones tropicales y subtropicales. Esta familia comprende árboles, arbustos, lianas o hierbas, generalmente terrestres, rara vez epífitas con hojas opuestas o verticiladas, con margen entero, estípulas, partes florales connatas y ovario ínfero (Robbrecht, 1988).

La clasificación a nivel de subfamilias, tribus y géneros ha sido controversial (Bremer *et al.*, 1995). La primera división de la familia en subfamilias la propuso Schumann (1891) con dos grupos principales basándose en el número de óvulos por lóculo: Cinchonoideae y Coffeoidae. Por su parte, Verdcourt (1958) reconoció tres subfamilias: Cinchonoideae, Rubioideae y Guettardoideae. Posteriormente, después de varias propuestas de escasa relevancia, Robbrecht (1988) estableció uno de los principales esquemas de clasificación para Rubiaceae que se basa en características morfológicas, donde se reconocen cuatro subfamilias: Antirrhoideae, Cinchonoideae, Ixoroideae y Rubioideae. Con la evidencia molecular, el esquema de clasificación reconoce dos o tres subfamilias: Cinchonoideae (que incluye Ixoroideae) y Rubioideae (Robbrecht & Manen, 2006) vs Cinchonoideae, Ixoroideae y Rubioideae (Bremer *et al.*, 1995, 1996).

La tribu Hamelieae, es un linaje neotropical que originalmente fue incluido en la subfamilia Rubioideae con base en la presencia de rafidios (Lorence & Dwyer, 1988); no obstante, los análisis filogenéticos más recientes la ubican dentro de Cinchonoideae (Bremer *et al.*, 1995, Andersson & Rova, 1999). La tribu Hamelieae se reconoce por las inflorescencias ebracteadas, a menudo flores amarillas, pared exterior de la testa granulada o tuberculada y la presencia de rafidios (Manns & Bremer, 2010; Martínez-Cabrera *et al.*, 2010). Esta tribu cuenta con 11 a 18 géneros, dependiendo del autor (Tabla 1).

Tabla 1. Géneros considerados en la tribu Hamelieae (Cinchonoideae) por diferentes autores

Género	Robbrecht (1988)	Borhidi (2006)	Manns & Bremer	Stranzing <i>et al.</i>
---------------	-----------------------------	---------------------------	-------------------------------	------------------------------------

1	<i>Bellizinca</i> Borhidi	--	Rondeletieae	(2010) = <i>Deppea</i>	(2014) Hamelieae
2	* <i>Bertiera</i> Aubl.	<i>Inc. sedis</i>	Hamelieae	--	--
3	<i>Cosmocalyx</i> Standl.	<i>Inc. sedis</i>	Ixoreae	Hamelieae	--
4	<i>Csapodya</i> Borhidi	--	Rondeletieae	= <i>Deppea</i>	Hamelieae
5	<i>Deppea</i> Schtdl. & Cham.	Hamelieae	Rondeletieae	Hamelieae	Hamelieae
6	<i>Deppeopsis</i> Borhidi & Stranzinger	--	= <i>Deppea</i>	--	Hamelieae
7	<i>Edithea</i> Standl.	Hamelieae	Rondeletieae	= <i>Deppea</i>	Hamelieae
8	<i>Eizia</i> Standl.	Rondeletieae	Rondeletieae	Hamelieae (?)	--
9	<i>Hamelia</i> Jacq.	Hamelieae	Hamelieae	Hamelieae	Hamelieae
10	<i>Hoffmannia</i> Sw.	Hamelieae	Hamelieae	Hamelieae	Hamelieae
11	<i>Omiltemia</i> Standl.	Hamelieae	Rondeletieae	Hamelieae	Hamelieae
12	<i>Patima</i> Aubl.	Isertieae	--	Hamelieae (?)	--
13	<i>Pinarophyllon</i> Brandege	Hamelieae	Rondeletieae	Hamelieae	Hamelieae
14	<i>Plocaniophyllon</i> Brandege	--	Mussendeae	Hamelieae	Hamelieae
15	<i>Pseudomiltemia</i> Borhidi	--	Rondeletieae	--	Hamelieae
16	* <i>Renistipula</i> Borhidi	--	Rondeletieae	--	Hamelieae
17	<i>Schenckia</i> K. Schum.	= <i>Deppea</i>	--	= <i>Deppea</i>	--
18	<i>Syringantha</i> Standl.	Cinchoninae	Cinchoneae	Hamelieae	Hamelieae

*Excluidos de Hamelieae por reciente evidencia molecular (Andreasen & Bremer, 2000, Torres-Montúfar, 2018)

La tribu Hamelieae posee una marcada diversidad en México con 14 géneros y de 95 a 101 especies, con alto grado de endemismo (Stranzinger *et al.*, 2014). De acuerdo con Manns *et al.* (2012) la tribu Hamelieae es un linaje neotropical de origen centroamericano, que ha adquirido su distribución actual por varias dispersiones hacia el Caribe y Sudamérica. En la Tabla 2 se presenta la distribución general y diversidad de los géneros incluidos en Hamelieae.

Tabla 2. Número de especies y distribución geográfica general de los géneros considerados en la tribu Hamelieae (Cinchonoideae) por diferentes autores					
Género	Lozano	Lozano	Borhidi	Kew	Distribución

		& Dwyer (1998)	& Taylor (2012)	(2012)	(2017)	
1	<i>Bellizinca</i>	--	--	1	--	Mx
2	<i>Cosmocalyx</i>	--	1	1	1	Mx, Ca
3	<i>Csapodya</i>	--	--	3	--	Mx
4	<i>Deppea</i>	25	26	24	39	Mx, Ca, Sa
5	<i>Deppeopsis</i>	--	--	5	--	Mx, Ca
6	<i>Edithea</i>	--	--	8	--	Mx
7	<i>Eizia</i>	--	1	1	1	Mx
8	<i>Hamelia</i>	--	16	16	16	Mx, An, Ca, Na, Sa
9	<i>Hoffmannia</i>	--	100	100-150	100	Mx, Ca, Sa
10	<i>Omiltemia</i>	--	--	2	2	Mx
11	<i>Patima</i>	--	--	--	2	Sa
12	<i>Pinarophyllon</i>	1	2	2	2	Mx, Ca
13	<i>Plocaniophyllon</i>	1	1	1	1	Mx, Ca
14	<i>Pseudomiltemia</i>	--	2	2	2	Mx
15	<i>Schenckia</i>	= <i>Deppea</i>	--	--	1	Sa
16	<i>Syringantha</i>	--	--	1	1	Mx

An: Antillas, Ca: Centroamérica, Mx: México, Na: Norteamérica, Sa: Sudamérica

B. El complejo *Deppea*

El género *Deppea* Schtdl. & Cham. es uno de los más conflictivos taxonómicamente dentro de la tribu Hamelieae. Ha sido ubicado en distintas tribus e incluso en otra subfamilia (Rubioidae: Hedyotideae; Cinchonoideae: Rondeletieae, Argostemmateae, Hillieae, Deppeae y Hamelieae) (Chamisso & Schlechtendal, 1830; Verdcourt, 1958; Bremekamp, 1966; Kirkbride, 1984; Robbrecht, 1988; Lorence & Dwyer, 1988). Actualmente existe consenso en que *Deppea* pertenece a la tribu Hamelieae según evidencia molecular y anatómica (Bremer *et al.*, 1995; Martínez-Cabrera *et al.*, 2007, 2009, 2010; Bremer & Eriksson, 2009; Manns & Bremer, 2010; Stranzinger *et al.*, 2014).

El género *Deppea* se caracteriza por tener flores 4-meras, principalmente amarillas, corolas en botón con estivación contorta, ovario bilocular, placentación axilar, numerosos óvulos, frutos capsulares con dehiscencia loculicida y numerosas semillas pequeñas con testa foveolada o reticulada (Lorence & Dwyer, 1988). Recientes estudios en el género *Deppea* han resultado en la segregación de varios géneros: *Bellizinca* Borhidi, *Csapodya* Borhidi, *Deppeopsis* Borhidi & Stranzinger, *Edithea* Standl. con base en características morfológicas del tipo de inflorescencias, tamaño de la corola y ornamentación de la testa de

las semillas (Borhidi *et al.*, 2004; Borhidi & Reyes-García, 2007; Borhidi & Stranzinger 2012a; Borhidi & Stranzinger 2012b).

Estos géneros no han sido aceptados de manera consensuada en tratamientos regionales de la familia o trabajos filogenéticos (Lorence & Dwyer, 1988; Lorence, 1999; Lorence & Taylor, 2012; Borhidi, 2006; Stranzinger *et al.*, 2014). De igual manera, existen géneros morfológicamente relacionados a *Deppea* por lo que algunas de sus especies han sido segregadas o consideradas sinónimos de *Deppea* (*Omiltemia*, *Plocaniophyllon* y *Pinarophyllon*). Para Lorence & Dwyer (1988) las especies que después fueron tratadas como *Bellizinca*, *Csapodya*, *Deppeopsis* y *Edithea* son parte de *Deppea*, mientras que la especie posteriormente incluida en *Pseudomiltemia* se consideraron como parte de *Omiltemia*.

Borhidi (2012) reconoce los ocho géneros (no incluye a *Schenckia*, pues su tratamiento se restringe a México). Al mismo tiempo, Lorence & Taylor (2012) consideran que *Bellizinca*, *Csapodya* y *Edithea* son sinónimos de *Deppea* y también reconocen a *Plocaniophyllon* y a *Pseudomiltemia* (no incluyen a los otros géneros, pues su tratamiento se restringe a Mesoamérica). La complicación para la circunscripción genérica se debe a que entre estos géneros se forma un mosaico de caracteres morfológicos con traslapamientos complejos. A continuación, se desglosa la historia taxonómica y diversidad de cada uno de los géneros considerados aquí como el complejo *Deppea*.

***Bellizinca* Borhidi**

Género monotípico mexicano, conocido de Oaxaca y Puebla (Borhidi, 2012). De acuerdo con Borhidi *et al.* (2004), el nombre del género *Bellizinca* está compuesto por el vocablo italiano “bello” (de belleza) en combinación con *Lysimachia* (Gentianaceae). La especie *Bellizinca scoti* (J.H. Kirkbr.) Borhidi fue transferida de *Deppea scoti* (J.H. Kirkbr.) Lorence, descrita originalmente como *Omiltemia scoti* J.H. Kirkbr. Más adelante, esta especie fue transferida al nuevo género *Bellizinca* debido a la inflorescencia péndula y con dos lóbulos del cáliz más grandes que los otros, así como por la corola tubular y los filamentos cilíndricos insertados en la base de la corola (Borhidi *et al.* 2004).

***Csapodya* Borhidi**

Género endémico de Chiapas, en México, que cuenta con tres especies (Borhidi, 2012). *Csapodya* es un género descrito por Borhidi *et al.* (2004), en honor a los botánicos húngaros Verae Csapody e István Csapody. Este género se reconoció por la pubescencia del estilo, la posición de los estambres insertos en la base, así como a la forma y textura de las semillas.

La especie tipo de este género es *Csapodya splendens* (Breedlove & Lorence) Borhidi, la cual fue descrita inicialmente como *Deppea splendens* Breedlove & Lorence. Las tres especies del género: *C. challengerii* Borhidi & Reyes-García, *C. sousae* Borhidi & Reyes-García y *C. splendens* se distribuyen en la Sierra Madre de Chiapas (Borhidi, 2004; Borhidi & Reyes-García, 2007).

En el estudio de Stranzinger *et al.* (2014) dos especies de *Csapodya* forman un clado que se anida en el clado sin resolución con *Deppea*, *Bellizınca* y *Edithea*, por lo que aún no es clara la relación filogenética entre dichos taxones y tampoco la pertinencia de su reconocimiento, sin embargo, el muestreo de caracteres en ese estudio no fue extenso (ITS y *trnL-F*) y el resultado carece de soporte. Por su parte, en el trabajo de Manns & Bremer (2010), *Csapodya splendens* (tratada como *Deppea splendens*) se recupera como hermana del resto de las especies de *Deppea* incluidas en el estudio, pero, ese trabajo presenta un escaso muestreo de especies.

Asimismo, existe controversia a nivel de especies; de acuerdo con Lorence & Taylor (2012) *C. challengerii* y *C. sousae* fueron colectadas aparentemente de la misma población y representan individuos con diferentes estadios de desarrollo de la inflorescencia y ligeras diferencias en la pubescencia, características que permiten suponer que se trata de variación de *Deppea splendens*, considerando así además que no es justificado el reconocimiento del género *Csdapodya* y reconociéndolo como sinónimo de *Deppea*.

Deppea

El género *Deppea* es particularmente interesante para México dado que en este país tiene su centro de diversidad (Borhidi, 2012; Lorence & Taylor, 2012). Este género fue descrito por

Chamisso & Schlechtendal (1830) en honor al naturalista Ferdinand Deppe, quien colectó el ejemplar con el que se describiría *Deppea* (Stresemann, 1954).

Inicialmente el género fue ubicado en la tribu Hedyotideae debido a la similitud de sus frutos con los de *Hedyotis* (Chamisso & Schlechtendal, 1830), de la subfamilia Rubioideae, donde tradicionalmente se le ubicó debido a la presencia de rafidios (Lorence & Dwyer, 1988). Ya desde temprano, la clasificación de *Deppea* en Hedyotideae causó controversia.

Con base en la presencia de más de un óvulo por lóculo, frutos capsulares secos, semillas no aladas y lóbulos de la corola contortos en botón, Hooker (1873) trasladó *Deppea* a la tribu Rondeletieae de la subfamilia Cinchonoideae (Lorence & Dwyer, 1988). Por su parte, Verdcourt (1958) discutió que los rafidios de *Deppea* son poco visibles y aunque presenta estivación contorta, la corola se divide en la base como en Argostemmataeae, mientras que el fruto capsular es como en Hedyotideae. Bremekamp (1966) incluyó tentativamente a *Deppea* en Hillieae por la presencia de estructuras en la pared de la testa, sin embargo, aclara que falta generar información para un mejor entendimiento.

Poco después Kirkbride (1984) propone la tribu Deppeae, incluyendo además a *Omittemia* y *Schenckia*, basado en la forma de vida (arbolitos o arbustos), presencia de rafidios, tricomas septados, estivación de la corola principalmente contorta y numerosos óvulos. Posteriormente Deppeae fue considerada como sinónimo de Hamelieae y consecuentemente *Deppea* se reubicó como parte de ésta (Bremer, 1987; Robbrecht, 1988). Los análisis filogenéticos con evidencia molecular, así como distintos trabajos anatómicos, apoyan la inclusión de *Deppea* en la tribu Hamelieae (Bremer *et al.*, 1995; Bremer & Eriksson, 2009; Martínez-Cabrera *et al.*, 2007, 2009, 2010; Manns & Bremer, 2010; Stranzinger *et al.*, 2014).

Para distinguir entre las especies de *Deppea* los principales caracteres utilizados en los tratamientos taxonómicos que incluyen al género son: el tipo y número de flores por inflorescencia (monocasiales, escorpioideas, dicasiales, umbeliformes o tirsiformes), la pubescencia, la forma y tamaño de las cápsulas y la disposición de las hojas (Lorence & Dwyer, 1988).

El género *Deppea* presenta su centro de diversidad en México. De acuerdo con KEW (2017), en México existen 39 especies, siendo Oaxaca (18 sp) y Chiapas (17 sp) los estados con mayor número de ellas (Tabla 3). De acuerdo con Borhidi (2012), 25 especies de *Deppea* se distribuyen en México, de las cuales 18 son endémicas (nueve para Oaxaca). Otra particularidad del género *Deppea* es la distribución disyunta de tan sólo una especie, ya que *D. blumenaviensis* se distribuye en Brasil y Argentina (Lorence & Dwyer, 1988). Es por ello que esta especie se clasificó en su propio género *Schenckia* (Borhidi *et al.*, 2004).

Tabla 3. Distribución del género *Deppea* y número de especies correspondientes a las diferentes estados de México o países en donde se distribuye

	México		Otros países	
1	Oaxaca	24	Guatemala	5
2	Chiapas	10	Costa Rica	2
3	Veracruz	6	El Salvador	2
4	Hidalgo	5	Honduras	1
5	Estado de México	4	Panamá	1
6	Guerrero	4	*Argentina	1
7	Puebla	4	*Brasil	1
8	Querétaro	2		
9	Michoacán	2		
10	Otros	1		

**Deppea blumenaviensis/Schenckia blumenaviensis*

En bases de datos (TROPICOS, Kew) se enlistan 54 nombres de especies en *Deppea*, de los cuales existe consenso en que nueve de ellos pertenecen a otros géneros, incluso de otras tribus (ej. *Deppea stenosepala*= *Arachnothryx jurgensenii* y *Deppea venezuelensis*= *Amphidasya venezuelensis*). Para el resto no existe consenso en el número de especies de *Deppea*: KEW (2017) reconoce 39 especies, Tropicos 37 y The Plant List 25. En la única revisión para el género se reconocen 25 especies (Lorence & Dwyer, 1988). Por su parte Boridhi (2012) reconoce 25 especies, muchas de las cuales no son las mismas que las del tratamiento de Lorence & Dwyer (1988) puesto que se han descrito nuevas especies y otras han sido segregadas.

***Deppeopsis* Borhidi & Stranzinger**

Borhidi & Stranzinger (2012b) describieron al género *Deppeopsis* con base en el hipanto y frutos cilíndricos. Este género cuenta con cinco especies y es casi endémico de México, aunque existen unas pocas localidades de colecta en Guatemala (Departamento de Izabal y Suchitepéquez). Asimismo, *Deppeopsis* presenta distribución disyunta en México, puesto que la especie tipo *D. hernandezii* (Lorence) Borhidi & Stranzinger es endémica de Hidalgo, mientras que las otras especies se ubican en la zona sur del país (Chiapas, Oaxaca y Veracruz).

Tres especies de *Deppeopsis* fueron incluidas en el estudio de Stranzinger *et al.* (2014) en el cual *D. foliolosa* y *D. tubaeana* se recuperaron como grupo hermano de *Plocaniophyllon* que forman parte de un clado sin resolución en el que se incluye *Pseudomiltemia filisepala*, un clado con especies de *Hoffmannia*, el clado *Omiltemia-Renistipula* y *D. hernandezii*.

***Edithea* Standl.**

El género *Edithea* fue descrito por Standley en 1933 en honor a Edith M. Vincent, bibliotecaria del Departamento de Botánica en el Field Museum of Natural History. Se caracteriza por la corola tubular-infundibuliforme, tubo ligeramente ensanchado, estambres insertos en la garganta y filamentos cortos (Standley, 1933). Es un género con ocho especies reconocidas endémicas de México, seis de ellas en Oaxaca y dos más en Guerrero (Borhidi, 2012).

La especie tipo *Edithea floribunda* Standl. fue transferida a *Deppea* y renombrada *Deppea schultzei* Lorence con base en morfología floral (Lorence & Dwyer, 1988). Posteriormente, Borhidi *et al.* (2004) resucitaron *Edithea* con evidencias micromorfológicas y anatómicas (no especificadas en su trabajo), además de transferir a dicho género seis especies de *Deppea*, mismas que anteriormente habían sido descritas por Borhidi & Velasco-Gutiérrez (2010) como la sección *Paradeppea* dentro del género *Deppea* (Borhidi, 2006; Borhidi & Stranzinger, 2012b).

De acuerdo con Lorence & Taylor (2012), *Edithea* debe ser tratado como sinónimo de *Deppea*, puesto que la evidencia molecular disponible (ITS y trnL-F) no apoya la separación de ambos géneros según los resultados no publicados de Galambos (2009); de

igual forma los resultados de Stranzinger *et al.* (2014) muestran que tres especies de *Edithea* forma un clado sin resolución anidado en el grupo formado por *Deppea*, *Bellizinca* y *Csapodya*.

***Omiltemia* Standl.**

El género *Omiltemia* fue descrito por Paul C. Standley en 1918 con base en la corola roja y las flores solitarias. El nombre hace referencia a la localidad de Omiltemia, Guerrero, donde *O. longipes* fue colectada. Posteriormente Morton (1968) transfiere como parte del género *Omiltemia* a la especie *Kohleria filisepala* (género perteneciente a la familia Gesneriaceae) y J. Kirkbride transfiere *Edithea floribunda* a *Omiltemia*, debido al parecido del tamaño de la corola y la forma del fruto. Asimismo, Kirkbride (1984) describe la nueva especie *Omiltemia scoti* J.H. Kirkbr. Sin embargo, de acuerdo con Borhidi (2004) las especies *O. scoti* y *O. floribunda* (Standl.) J.H. Kirkbr. no deben incluirse en *Omiltemia* debido a la presencia de “haces” en el fruto y a la dehiscencia apical loculicida, describiendo así el género *Bellizinca* y resucitando el género *Edithea*. Asimismo, Borhidi (2004) describió el género *Pseudomiltemia* a partir de *O. filisepala* con base en la forma de la corola, largo de los filamentos y la inserción de las anteras.

Recientemente se han descrito dos nuevas especies bajo el género de *Omiltemia*: *O. parvifolia* Borhidi & K. Velasco (Borhidi & Velasco-Gutiérrez, 2010) del estado de Oaxaca y *O. guerrerensis* Lozada-Pérez & J. Rojas (Lozada-Pérez & Rojas Gutiérrez, 2016) del estado de Guerrero. Con esto el género *Omiltemia* cuenta con tres especies endémicas a México.

En el estudio filogenético de Stranzinger *et al.* (2014) se incluye únicamente *Omiltemia longipes* Standl. la cual se recupera como hermana de *Renistipula* (género reconocido como sinónimo de *Arachnothryx* en Guettardeae, por lo que posiblemente representa un error en el etiquetado de su material). Tanto *Renistipula* como *Omiltemia longipes* están en un clado sin resolución formado por *Deppeopsis hernandezii*, *Pseudomiltemia filisepala*, el clado de especies de *Hoffmannia* y el clado *Plocaniophyllon-Deppeopsis*.

Pinarophyllon

Género que se distribuye en México y Guatemala, descrito por Brandegee (1914), cuenta con dos especies. Se caracteriza por el hábito herbáceo, hojas arrosadas y frutos capsulares loculicidas. *Pinarophyllon flavum* Brandegee es la especie tipo, conocida de Chiapas. La otra especie del género fue descrita originalmente como *Deppea lundelli* Dwyer, posteriormente considerada como sinónimo de *Pinarophyllon bullatum* Standl. (Lorence & Dwyer; 1988, Borhidi, 2012; Lorence & Taylor, 2012). En la filogenia de Stranzinger et al. (2014) solo se representa a *P. bullatum*, muestra que se recupera como hermana del clado formado por *Deppeopsis*, *Pseudomiltemia*, *Hoffmannia*, *Omiltemia*, *Plocaniophyllon* y *Renistipula*.

Plocaniophyllon

Género monotípico que se distribuye en México y Guatemala, descrito por Brandegee (1914). En la descripción resalta su parecido con *Hoffmannia* dado el fruto inmaduro, no obstante, se destaca que la venación lineolada es un carácter fundamental para reconocerlo. En la filogenia de Stranzinger et al. (2014) *Plocaniophyllon flavum* se recupera como hermana del clado formado por dos especies de *Deppeopsis*, grupo incluido en el clado sin resolución formado por *Pseudomiltemia filisepala*, el clado de especies de *Hoffmannia*, el clado *Omiltemia-Renistipula* y *Deppeopsis hernandezii*.

Pseudomiltemia Borhidi

Borhidi (2004) describió al género *Pseudomiltemia* basado en *Omiltemia filisepala*. De acuerdo con el autor, *Pseudomiltemia* se distingue de *Omiltemia* por la estivación contorta, forma de la corola, largo de los filamentos y la inserción de las anteras. Recientemente se describió *Pseudomiltemia davidsonii* Martínez-Camilo & Lorence, especie que encaja en la circunscripción de dicho género, sin embargo, los autores reconocen que la delimitación genérica no está bien definida ya que se basa solo en caracteres morfológicos y no se dispone de evidencia molecular suficiente (Martinez-Camilo et al., 2011). Por tanto, el género *Pseudomiltemia* cuenta con dos especies endémicas a México.

En el estudio de Stranzinger et al. (2014), *Pseudomiltemia filisepala* se incluye en el clado sin resolución formado por *Deppeopsis hernandezii*, el clado de especies de *Hoffmannia*, el clado *Omiltemia-Renistipula* y el clado *Plocaniophyllon-Deppeopsis*.

***Schenckia* K. Schum.**

El género monotípico *Schenckia* fue descrito por Karl M. Schumann en 1889 y dedicado a H. Schenck, quien la colectó cerca de la ciudad brasileña de Blumenau. La especie *S. blumenaviensis* K. Schum. fue transferida a *Deppea* por Lorence & Dwyer (1988), debido a la estivación de la corola, dehiscencia del fruto, estructura de la estípula y el tamaño y forma de la corola. Recientemente, se reconoce la presencia de esta especie también en Argentina (KEW, 2017; Tropicos, 217).

La evidencia molecular sugiere que *Schenckia* es sinónimo de *Deppea* (Manns & Bremer, 2010; Stranzinger *et al.*, 2014), con lo cual *Deppea* tendría una distribución disyunta de México a Panamá y en Brasil-Argentina. No obstante, Borhidi *et al.* (2004) reconocen a *Schenckia* como un género aceptado.

C. Relaciones filogenéticas dentro del complejo *Deppea*

En la literatura existen dos estudios recientes que han abordado a la tribu Hamelieae. Manns & Bremer (2010), en el contexto de una filogenia de la tribu Cinchonoideae, empleando cinco marcadores moleculares (*atpB-rbcL*, *ndhF*, *rbcL*, *rps16*, *trnL-F*) incluyen pocos representantes de Hamelieae (10 géneros y 12 especies) y el estudio Stranzinger *et al.* (2014) enfocados en la tribu Hamelieae (13 géneros y 34 especies) con marcadores moleculares (región del ITS del nrDNA y *trnL-F*), en ambos trabajos *Deppea* no se recupera monofilético.

En resumen, para la tribu Hamelieae el árbol de consenso de mayor probabilidad posterior reportado por Stranzinger *et al.* (2014) (Figura 1) está evidentemente menos resuelto que el presentado por Manns & Bremer (2010) (Figura 2). Los resultados de Stranzinger *et al.* (2014) muestran tres linajes principales sin resolución en Hamelieae: 1) *Syringantha-Hamelia* (PP 0.99, BS 98%), 2) *Bellizinca-Csdapodya-Deppea-Edithea* (PP 1.00, BS 97 %) y, 3) *Deppeopsis-Hoffmannia-Omiltemia-Pinarophyllon-Plocaniophyllon-Pseudomiltemia-Renistipula* (PP 0.96, BS 78%). El complejo *Deppea* posee representantes en los clados 2 y 3. Con base en el análisis de Stranzinger *et al.* (2014), ningún género del complejo *Deppea* se recupera como monofilético. El clado 2 está formado por muchos de los géneros del complejo *Deppea*. El género *Deppea* resulta parafilético con respecto a *Bellizinca*, *Csdapodya*, *Edithea* y *Schenckia* (tratado como *D. blumenavensis*). No obstante, se recuperan algunos clados, p. ej. *Csdapodya* (PP 1.00, BS 100%), *Edithea* (PP 0.97, BS

64%) y otros con especies de *Deppea*. En el clado 3, *Pinarophyllon* resulta el grupo hermano del resto del clado sin resolución *Deppeopsis-Hoffmannia-Omiltemia-Plocaniophyllon-Pseudomiltemia-Renistipula* (PP 0.75, BS 68%). En este clado se recupera como monofilético el género *Hoffmannia* (PP 0.74, BS 65%), *Omiltemia* se recupera como hermano de *Renistipula* (PP 0.73) y *Plocaniophyllon* como hermano (PP 0.67) de un clado formado por dos especies de *Deppeopsis* (PP 1.00, BS 97%). De los géneros del complejo *Deppea*, el género *Deppeopsis* se recupera como polifilético, dos especies (*D. foliosa* y *D. tubaeana*) forman un clado hermano de *Plocaniophyllon*, pero, la posición de la especie tipo del género (*D. hernandezii*) es incierta en el clado 3.

Asimismo, los resultados de Stranzinger *et al.* (2014) reflejan la complejidad morfológica, dado que géneros anidados en el clado *Deppea* (e.g., *Bellizinca*) tienen morfología floral más similar a otros géneros que no comparten ancestro común inmediato con *Deppea* (e.g., *Pseudomiltemia*), mientras que otros géneros que no comparten ancestro común inmediato con *Deppea* (e.g., *Plocaniophyllon*) tienen morfologías florales similares a *Deppea*.

Los resultados presentados por Manns & Bremer (2010) muestran cuatro linajes en la tribu Hamelieae: 1) *Cosmocalyx*, 2) *Deppea* (PP 1.0), 3) *Hamelia-Syringantha* (PP 1.0) y, 4) *Hoffmannia-Omiltemia-Plocaniophyllon-Pinarophyllon* (PP 1.0). El análisis muestra a *Deppea* parafilético con respecto a *Csdapodya* (tratado como *D. splendens*) y *Schenckia* (tratado como *D. blumenavensis*), *Hoffmannia* se recupera como hermano de *Omiltemia* (PP 1.0), ambos géneros como grupo hermano de *Plocaniophyllon* (PP 1.0) y *Pinarophyllon* como el hermano de estos tres géneros (PP 1.0).

En síntesis, los antecedentes de filogenia molecular para el complejo *Deppea* son incompletos. Por su parte Manns & Bremer (2010) tienen una buena representación de marcadores moleculares, no obstante, el muestreo de terminales no permite tomar decisiones acerca de los géneros del complejo *Deppea*. Por otro lado, el análisis de Stranzinger *et al.* (2014) presenta una mayor representación de los géneros de la tribu Hamelieae y del complejo *Deppea*, sin embargo el uso de dos marcadores moleculares puede explicar la falta de resolución en los clados de Hamelieae.

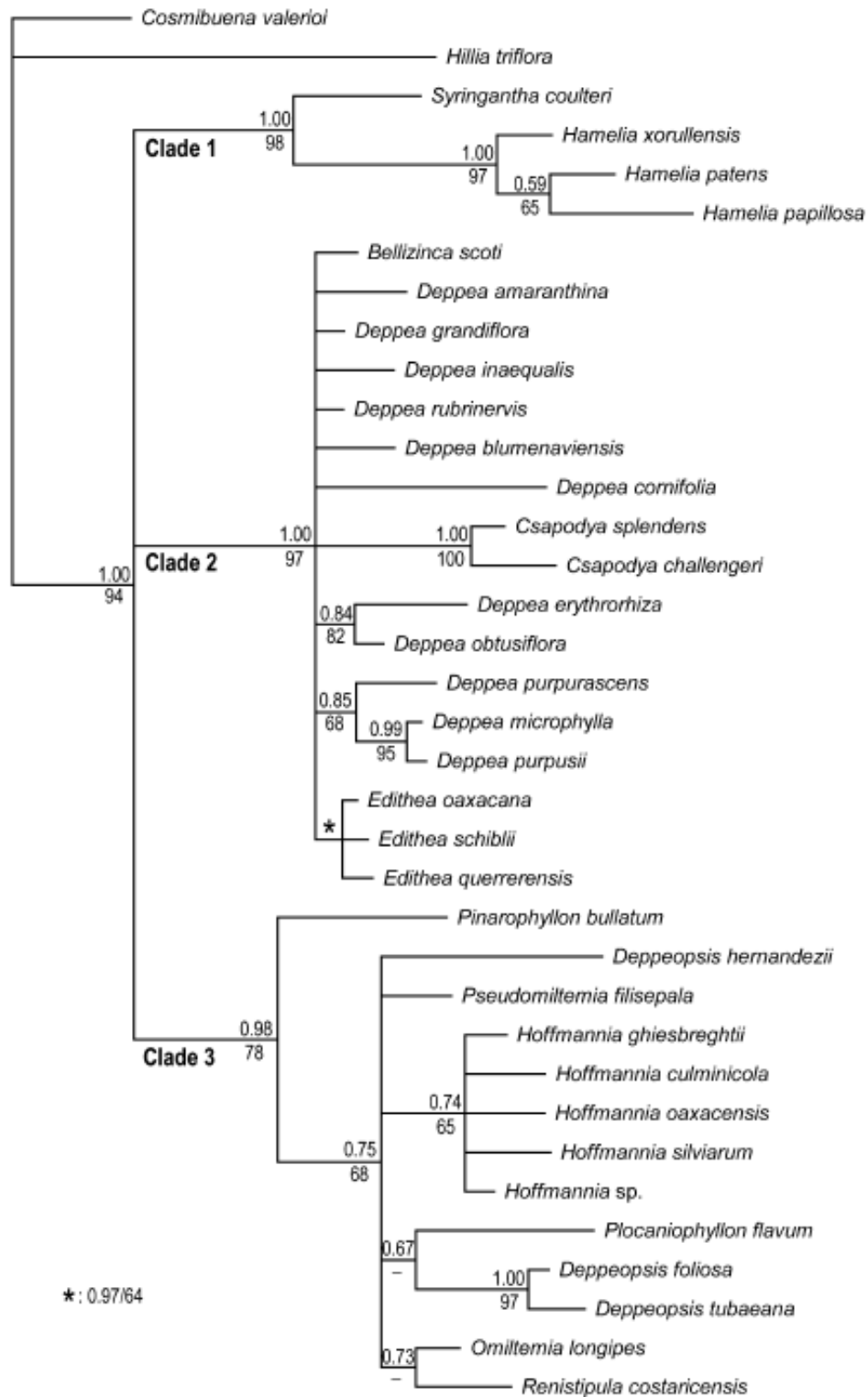


Figura 1. Árbol consenso de la tribu Hamelieae reconstruido por inferencia Bayesiana, tomado de Stranzinger *et al.* (2014). Los números arriba de las ramas representan las probabilidades posteriores (PP) y los números debajo de las ramas los valores de Bootstrap (BS) empleando Parsimonia.

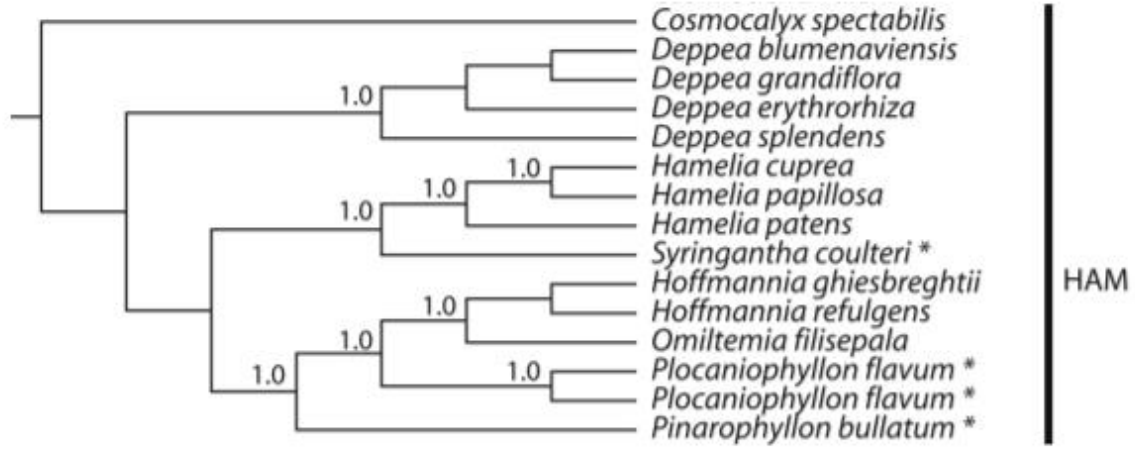


Figura 2. Clado correspondiente a la tribu Hamelieae tomado de Manns & Bremer (2010). Los números arriba de las ramas indican Probabilidades Posteriores (PP) de la inferencia Bayesiana.

II. OBJETIVOS

Objetivo general

- Poner a prueba la monofilia de *Deppea* así como su posición filogenética dentro de Hamelieae

Objetivos particulares

- Evaluar las relaciones filogenéticas entre las especies de *Deppea*.
- Estudiar la evolución de algunos caracteres morfológicos de potencial relevancia taxonómica (forma de vida, venación terciaria, tipo de inflorescencia, forma, tamaño y color de la corola, inserción de los estambres, así como forma y dehiscencia del fruto).
- Evaluar la morfología de la semilla y los caracteres que permitan la delimitación genérica.

III. JUSTIFICACIÓN

El género *Deppea* es un género muy diverso en México y muchas de sus especies son endémicas a este país (Borhidi, 2012; Lorence & Taylor, 2012). Su circunscripción genérica y la de algunas especies es controversial, lo que en gran parte se debe a la complejidad morfológica del género (o los géneros) y a su poco estudiada taxonomía.

El conflicto en la circunscripción genérica (Lorence & Dwyer, 1988; Lorence, 1999; Borhidi, 2006; Lorence & Taylor, 2012; Stranzinger *et al.*, 2014), y en las discrepancias en la circunscripción de algunas especies, carece de un respaldo filogenético robusto. Es necesaria una hipótesis filogenética que incluya el mayor número de géneros y especies de Hamelieae.

IV. MÉTODO GENERAL

El método general para abordar el estudio sistemático del complejo *Deppea* incluye cuatro ejes: 1) Trabajo en colecciones científicas y revisión bibliográfica, 2) Trabajo de campo, 3) Trabajo de laboratorio molecular y 4) Estudios morfológicos.

1) Colecciones científicas y revisión bibliográfica

A partir de la revisión bibliográfica, la consulta de bases de datos en línea y la consulta de herbarios, se generó una base de datos para los géneros y las especies asociados al complejo *Deppea*. La revisión bibliográfica incluye la consulta de literatura especializada (revisiones, floras, artículos), así como la recopilación de protólogos e imágenes de tipos de los taxones examinados.

Con la revisión morfológica y la base de datos se direccionó el trabajo de campo y se diseñó el muestreo de terminales para el trabajo de laboratorio posterior. La importancia de las colecciones científicas para este trabajo también se resalta con el uso de muestras colectadas recientemente y en buen estado de conservación para extraer ADN.

2) Trabajo de campo

El trabajo de campo tuvo dos propósitos principales, uno, proveer la materia prima para los estudios filogenéticos (muestras de hojas en silica gel), y, dos, el reconocimiento de las especies en vivo y su ambiente, fundamental para el entendimiento del grupo.

3) Trabajo de laboratorio molecular

Se representó *Deppea* con un número considerable de especies y todos sus géneros segregados, considerando el mayor número de especies posibles por género. Asimismo, se incluyeron representantes de los otros géneros de la tribu Hamelieae como grupo externo. Se realizó la extracción de ADN en el Laboratorio Genética y Ecología del Instituto de Ecología, UNAM y las extracciones fueron enviadas para la secuenciación genómica mediante captura de secuencias usando el kit Angiosperms353 (Johnson *et al.*, 2018).

4) Estudios morfológicos

Los estudios morfológicos se enfocaron en los caracteres empleados para la segregación de géneros, tales como forma, tamaño y color de la corola, inserción de los estambres, forma y dehiscencia del fruto. Asimismo, con base en los resultados de la filogenia, se tomaron las decisiones taxonómicas pertinentes a la aceptación o sinonimización de los géneros del complejo *Deppea*.

V. ARTÍCULOS GENERADOS A PARTIR DEL PROYECTO DE DOCTORADO

CAPÍTULO 1. Seed morphology of Hamelieae with emphasis on the *Deppea* complex

(Cinchonoideae, Rubiaceae)

Plant Ecology and Evolution 2023, 155(1): 51-69

doi: 10.5091/plecevo.84486

Seed morphology of Hamelieae with emphasis on the *Deppea* complex (Cinchonoideae, Rubiaceae)

María Aguilar-Morales^{1,2}, Helga Ochoterena^{2,*} & Teresa Terrazas²

¹Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, México, México

²Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, México, México

*Corresponding author: helga@ib.unam.mx

Background and aims – The neotropical tribe Hamelieae currently includes 16 genera mainly characterized by raphides, ebracteolate inflorescences, and 4-merous flowers with contorted corolla aestivation. Within this tribe, the circumscription of *Deppea* has been particularly controversial, as depending on the authors, several morphologically closely related genera are either treated as synonyms or accepted as such. This generic group, hereafter referred to as the *Deppea* complex, consists of up to 10 genera. Within Rubiaceae, seed morphology has proved to have taxonomic value for generic circumscriptions, however, it remained unexplored for Hamelieae and the *Deppea* complex.

Material and methods – We present a detailed study of the seed morphology of 37 species representing 15 out of the 16 genera recognized within Hamelieae, including all putative genera of the *Deppea* complex. Using scanning electron and light microscopy, we investigate 16 quantitative and qualitative seed characters that could have taxonomic value.

Key results – Our results show that the combination of some seed characters, such as shape and colour, dorsiventral compression, hilum position, and the periclinal microrelief, helps to distinguish some genera and most species, supporting or refuting the current taxonomic circumscription.

Conclusion – We conclude that the seed morphology within Hamelieae has taxonomic value but should be combined with other characters to achieve unequivocal delineation of the genera.

Keywords – Generic circumscription; LM; morphology; seed; SEM; systematics; testa.

INTRODUCTION

Hamelieae is a Neotropical tribe comprising 16 genera and approximately 225 species, notably diverse in Mexico where it has 14 genera and ca 100 species (Lorence & Dwyer 1988; Borhidi 2006, 2012). It is characterized by shrubs or treelets, presence of raphides, typically ebracteolate inflorescences, often yellow or yellowish 4-merous flowers, contorted corolla aestivation, and berries or capsular fruits (Bremer 1987; Borhidi et al. 2004a, 2004b; Manns & Bremer 2010; Stranzinger et al. 2014).

The tribe Hamelieae was traditionally classified in subfamily Rubioideae due to the presence of raphides (Bremekamp 1966). Nevertheless, molecular phylogenetic

analyses including relatively few species of Hamelieae have indicated that this tribe is more appropriately classified within Cinchonoideae (Bremer et al. 1995; Andersson & Rova 1999; Robbrecht & Manen 2006), even when using phylogenomic data, although this analysis only represented the tribe with one species (Antonelli et al. 2021).

Within the tribe, the generic circumscription has been controversial; the first classification systems for Rubiaceae (De Candolle 1830; Endlicher 1836) recognized eleven genera within Hamelieae: *Alibertia* A.Rich., *Axanthes* Blum, *Brignolia* DC., *Evosmia* Humb. & Bonpl., *Hamelia* Jacq., *Olostyla* DC., *Patima* Aubl., *Tepesia* Gartn., *Urophyllum* Jack ex Wall., *Sabicea* Aubl., and *Schradera* Vahl. Hooker (1873) considered only six genera based on the contorted

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Plant Ecology and Evolution is published by Meise Botanic Garden and Royal Botanical Society of Belgium
ISSN: 2032-3913 (print) – 2032-3921 (online)

and imbricate corolla aestivation plus fleshy fruits: *Bertiara* Aubl., *Bothriospora* Hook.f., *Gouldia* A.Gray, *Hamelia*, *Heinsia* DC., and *Hoffmannia* Sw. According to Schumann (1897), Hamelieae was considered a subtribe within Gardenieae, including five genera: *Bothriospora*, *Catesbaea* L., *Hamelia*, *Hoffmannia*, and *Phyllacantha* Hook.f. Later on, Verdcourt (1958) recognized in Hamelieae only *Bertiara*, *Hamelia*, and *Heinsia*, while Bremekamp (1966) restricted it even more, including only two genera, *Hamelia* and *Hoffmannia*, based on raphide presence plus fleshy fruits. Bremer (1987) recognized five genera within Hamelieae based on raphide presence and imbricate corolla aestivation: *Deppea* Schlttdl. & Cham., *Hamelia*, *Hoffmannia*, *Omittemia* Standl., and *Pinarophyllon* Brandege. Lorence & Dwyer (1988) considered the genus *Schenckia* K.Schum. to be a synonym of *Deppea*. The genus *Edithea* Standl. was included within Hamelieae by Robbrecht (1988), but treated as *Omittemia* by Kirkbride (1984), as *Deppea* by Lorence & Dwyer (1988), and resurrected by Borhidi et al. (2004a). Robbrecht & Bridson (1993) added *Eizia* Standl. and *Plocaniophyllon* Brandege to the tribe, while McDowell (1996) included the monotypic genus *Syringantha* Standl. Finally, Borhidi et al. (2004b) segregated *Bellizınca* Borhidi and *Csapodya* Borhidi from *Deppea*, both genera included in Hamelieae by the same authors.

Based on molecular evidence, Manns & Bremer (2010) recognized seven genera within Hamelieae: *Cosmocalyx* Standl., *Deppea*, *Hamelia*, *Hoffmannia*, *Omittemia*, *Pinarophyllon*, and *Plocaniophyllon*, plus two tentatively included (*Eizia* and *Patima*), while the genera *Bellizınca*, *Csapodya*, *Edithea*, and *Schenckia* were suggested as synonyms of *Deppea*.

In total, nine genera are morphologically closely related to *Deppea*, even treated as synonyms by some authors (Lorence & Dwyer 1988; Manns & Bremer 2010; Lorence 2012). These genera will be hereafter referred to as the *Deppea* complex: *Bellizınca*, *Csapodya*, *Deppea*, *Deppeopsis*, *Edithea*, *Omittemia*, *Pinarophyllon*, *Plocaniophyllon*, *Pseudomittemia* Borhidi, and *Schenckia* (fig. 1). Hence, the *Deppea* complex constitutes the majority of the Hamelieae tribe, including 10 out of the 16 genera and ca 50 species.

Molecular phylogenetic analyses retrieved a monophyletic and highly supported Hamelieae but did not solve the *Deppea* taxonomic problem: Manns & Bremer (2010) used a set of six markers (nrITS, *atpB-rbcL*, *ndhF*, *rbcL*, *rps16*, and *trnL-F*), but only studied six genera of the *Deppea* complex with a few species each, leaving many questions related to their generic circumscription open. Stranczinger et al. (2014), on the other hand, included representatives of all genera in the *Deppea* complex, but they only used two markers (ITS and *trnL-F*) resulting in a consensus tree with many polytomies that made the taxonomic decisions untenable. Their phylogenetic analysis showed that none of the genera in the *Deppea* complex were supported as monophyletic and that the genus *Renistipula* Borhidi should be included as a member of Hamelieae despite its lack of the diagnostic raphides. A phylogenetic analysis by Torres-Montúfar (2018) however placed *Renistipula* within Guettardeae and treated it as a synonym of *Arachmothyx* Planch. All morphological evidence indicates that *Renistipula* belongs in Guettardeae

and so we favour the results by Torres-Montúfar (2018), suggesting that the results by Stranczinger et al. (2014) are most likely an artifact of a methodological error.

In the literature, some morphological characters have been used to differentiate among the genera within Hamelieae and more precisely within the *Deppea* complex, such as the position of the stamen insertion on the corolla, calyx lobe form, and corolla length and shape, as well as fruit form and dehiscence (Borhidi et al. 2004a, 2004b; Borhidi 2006, 2012; Borhidi & Stranczinger 2012). Nevertheless, these characters display a complex overlapping mosaic and none of them is useful to clearly distinguish genera when not used in combination with other characters.

Therefore, the morphological and molecular data gathered so far does not provide convincing evidence for the adequate generic circumscription within Hamelieae, particularly among genera in the *Deppea* complex. Consequently, the number of genera that should be recognized within Hamelieae remains unclear and new sources of evidence should be incorporated to solve this problem.

Even though seed morphology has not been commonly used in Rubiaceae systematics, some studies indicate its taxonomic potential (e.g. Hayden & Dwyer 1969; Terrell et al. 1986; Robbrecht 1988; Khalik et al. 2008). Several seed characters have thus potential to be good taxonomic markers, such as seed shape (Breedlove & Lorence 1987), testa ornamentation (Hayden & Dwyer 1969), general form and hilum position (Terrell et al. 1986), seed size and anticlinal and periclinal cell wall boundary (Khalik et al. 2008). This information has helped the delimitation of some taxa at species level (e.g. Khalik et al. 2008) or at generic level (e.g. Hayden & Dwyer 1969). For the tribe Hamelieae, Robbrecht (1988) mentioned that the exotestal outer cells walls are granulate or tuberculate, while Martínez-Cabrera et al. (2014) observed unitegmic orthotropous ovules, horizontally oriented in the ovary locule. Other than that, information on the taxonomic value of the seed morphology within Hamelieae and among genera in the *Deppea* complex is scattered. It is important to mention that the seeds of species within the *Deppea* complex are in general very small, resembling dust, and this might be a reason why they have not been thoroughly studied. However, the seeds of *Plocaniophyllon* were described by Martínez-Cabrera et al. (2008) in a study of the genus including morphology and anatomy. In addition, studies by Borhidi et al. (2004a, 2004b) indicated the existence of seed differences in shape, hilum position, perpendicular wall thickness, and horizontal wall type and ornamentation among *Bellizınca*, *Csapodya*, *Deppea*, *Edithea*, *Omittemia*, and *Pseudomittemia*. Regardless of the evidence that seed morphology can provide taxonomically useful information within Hamelieae and the *Deppea* complex, there is no study using uniform terminology devoted to systematically comparing seed morphology among genera within the tribe.

Therefore, our aims in this work are to provide a thorough seed description for all genera of Hamelieae and to examine the potential utility of seed morphology for the generic circumscription in the tribe, mainly focusing on the genera within the *Deppea* complex.

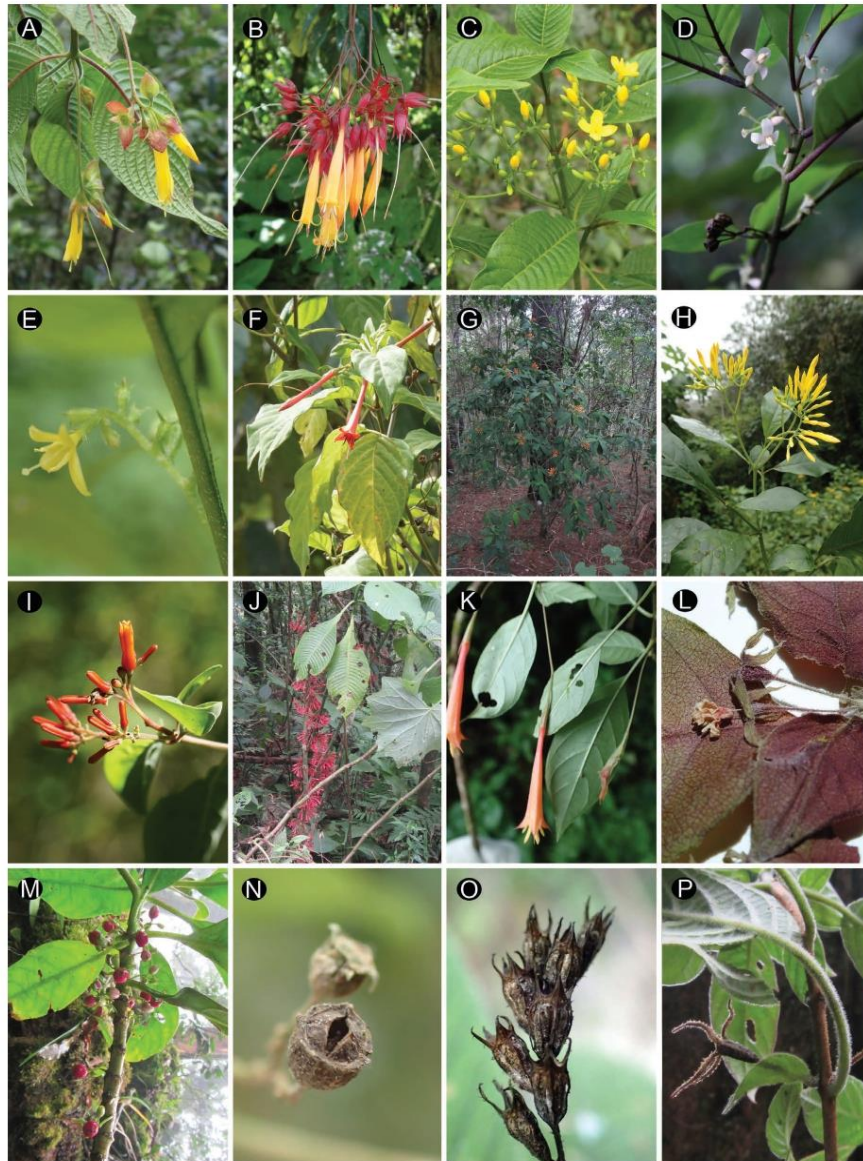


Figure 1—Different species representing the genera within Hamelieae. **A–L.** Flowers. **A.** *Bellizınca scoti* (Ochoterena et al. 1119). **B.** *Csapodya splendens* (Ochoterena et al. 1147). **C.** *Deppea cornifolia* (Ochoterena et al. 1120). **D.** *Deppea purpurascens* (Ochoterena et al. 1175). **E.** *Deppeopsis antsophylla* (Ochoterena et al. 1102). **F.** *Eizia mexicana* (Ochoterena et al. 1126). **G.** *Edithea miahuatlamica* (Ochoterena et al. 1095). **H.** *Edithea schiblii* (Ochoterena et al. 1112). **I.** *Hamelia patens* (Ochoterena et al. 1074). **J.** *Hoffmannia* sp. (Ochoterena et al. 1166). **K.** *Omiltemia parvifolia* (Ochoterena et al. 1105). **L.** *Schenckia blumenaviensis*. **M–P.** Fruits. **M.** *Hoffmannia* sp. (Ochoterena et al. 1164) **N.** *Deppea grandiflora* (Ochoterena et al. 1142). **O.** *Pinarophyllon flavum* (Ochoterena et al. 1133). **P.** *Pseudomiltemia davidsonii* (Ochoterena et al. 1162). All photographs by Helga Ochoterena, except G by Alejandro Torres.

MATERIAL AND METHODS

Our study includes all 16 genera within Hamelieae except for the South American *Patima* (with two species) for which we could not obtain seeds. This study includes all 10 genera within the *Deppea* complex. Seeds of at least one species of all the genera of the *Deppea* complex and five other genera in Hamelieae were examined. The voucher specimens of the 37 species are listed in supplementary file 1. Seeds were obtained from herbarium specimens at the Herbario Nacional de México (MEXU) and the Copenhagen herbarium (C). All seeds were chosen from mature fruits, with several seeds randomly selected, and the seeds were not treated or cleaned before microscopy due to their small size. For each sample, two groups of seeds were formed to be subsequently processed with different microscope techniques at the Laboratorio de Microscopía y Fotografía de la Biodiversidad at Instituto de Biología, UNAM. For scanning electron microscopy (SEM), the sampled seeds were sputter-coated with gold-palladium for 2–3 minutes with a HUMMER V Sputter Coater and they were observed and photographed using a Hitachi S-4000. For light microscopy (LM), the seeds did not receive any pre-treatment and stereoscopic photographs were taken using a Leica microscope equipped with a Leica Z16 APO A camera.

Seed colours were defined by placing the eyedropper tool of Photoshop (we used Photoshop CS3) on top of the central portion of the seed on the captured image and once the RGB colour palette was displayed, the colours were assigned to the standardized nearest colour: black, around brown as brownish, around red as reddish, or around yellow as yellowish.

Measurements were taken using the software Leica Application Suite v.2.8.1; at least five seeds per specimens were measured for size, length, and width. All measurements are presented in mm. Boxplots were created in Microsoft Excel and boxes represent the interquartile range, the horizontal line inside the box represents the median, the “X” represents the mean, and the circles are outliers.

The terminology proposed by Koch et al. (2009) and Barthlott & Hunt (2000) was adopted to describe the seed coat micromorphology observed using SEM, the other characters were defined here by the authors or follow Harris & Harris (2001). The micropyle position was defined assuming the orthotropous ovule nature described for the tribe by Martínez-Cabrera et al. (2014) or the anatropous position described for most Rubiaceae (Robbrecht 1988). A character matrix was constructed using Winclada (Nixon 1999) to show parallel descriptions of the 15 characters in the studied species. An illustrated overview of the 15 characters and character states is presented in supplementary file 2. Full seed descriptions for the 37 studied species in 15 genera are presented in the supplementary file 3, including the seed size that is not considered in the supplementary file 2 because it is a continuous character.

The seed morphology of the 15 studied genera is graphically summarized in fig. 2; we also compared the 28 species within the *Deppea* complex (fig. 3). The boxplots comparing seed length and width of the 15 studied genera

are shown in fig. 4. The SEM micrographs are shown in figs 5–10 and the LM micrographs are shown in figs 11–13.

RESULTS

In total, seeds from 15 genera and 37 species within Hamelieae were studied, among which 10 genera and 28 species belonging in the *Deppea* complex. Our results show high morphological overlap among the studied genera (fig. 2). Seed length and width (fig. 4) only allow for the recognition of *Cosmocalyx*, which has the largest seeds ($7.9\text{--}10.7 \times 4.8\text{--}5.8$ mm) of linear form (fig. 11C) and has the straight anticlinal wall boundaries (fig. 5F–H). The only other genus among Hamelieae that can be easily recognized by its seeds is *Syringantha*, because of the winged margins (figs 10A, 13Q, R).

The other 13 genera show a diversity in general shape, dorsiventral shape (figs 5–10), colour, anticlinal wall boundary, periclinal microrelief, and micropyle position (figs 11–13). Only a combination of these characters allows for taxonomic grouping, which is not necessarily similar to the current generic circumscription.

Outside the *Deppea* complex, the genus *Eizia* can be distinguished by the combination of black seeds (fig. 12S, T) with evidently depressed dorsiventral shape of cells (fig. 8I), and a sub-central micropyle position. These character states are each present in at least one species within the *Deppea* complex, but never in this combination. The studied *Hamelia* species share a rhomboidal seed shape, straight anticlinal wall boundaries, and papillose periclinal microrelief (figs 8M–X, 12U–Z). The rhomboidal seed shape is also present in *Deppea erythrorhiza* (figs 5Y, 11M) and *Omittemia longipes* (figs 9I, 13E), from which *Hamelia* is easily distinguished by the straight anticlinal wall boundaries and the evident hilum, in contrast to the U-undulate anticlinal wall boundaries and the inconspicuous hilum in the former two species (figs 5Z–AA, 9J–K, 11M, 13E). The genus *Hoffmannia* shows variation among the studied species and therefore is hard to characterize at generic level by its seeds (fig. 2). Among the evaluated species in this genus, *H. ghiesbreghtii* and *H. oaxacensis* are similar by the exotesta cells with verrucose periclinal microrelief (fig. 9D, H), a character state shared with *Bellizina* (fig. 5C–D) and *Deppea grandiflora*. This is in contrast to the concave outlines with sunken profile of the periclinal microrelief in *H. gesnerioides* (fig. 8AB), which is a character state shared with many other species within the studied genera.

Within the *Deppea* complex, dorsiventrally flattened seeds is one of the main character states useful for taxon grouping. The genera *Csapodya*, *Deppeopsis*, *Omittemia*, and *Pseudomittemia* have flattened seeds, in contrast to the polygonal seeds in *Bellizina*, *Deppea*, *Editheia*, *Pinarophyllon*, *Plocaniophyllon*, and *Schenckia* (fig. 2). Among the taxa with flattened seeds, *Csapodya* and *Pseudomittemia* have concave outlines with a sunken profile periclinal microrelief (fig. 5L, P), while it is papillose in *Deppeopsis* (fig. 7L, P, T), *Omittemia* (fig. 9L, P), *Pinarophyllon*, and *Plocaniophyllon*. Most of the character states of *Pseudomittemia* seeds overlap with those of at least one of the *Csapodya* species (fig. 2), however, both genera

can be distinguished by the shape of the cells next to the micropyle that differ from the shape of the lateral cells, in combination with the elongated lateral cells, and the straight anticlinal wall boundary in *Pseudomiltemia*. The seeds differ between *Deppeopsis* and *Omittemia* in that the latter has elongated lateral cells, and the micropyle in central/sub-central position (fig. 9I–P), isodiametric lateral cells, and lateral micropyle position of *Deppeopsis* (fig. 7I–T).

Among the genera which have polygonal seeds, differences in the periclinal microrelief are useful for grouping them. The genus *Bellizınca* is easily recognizable by the verrucose microrelief (fig. 5D), which is only shared with *Deppea grandiflora* (fig. 6D), but *Bellizınca* is distinguishable by the V-undulate anticlinal wall boundary and the elongated cells surrounding the micropyle (fig. 5A–C), in contrast to the U-undulate and isodiametric cells surrounding the micropyle in *D. grandiflora* (fig. 6A–C).

The genera *Pinarophyllon* and *Plocaniophyllon* share a papillose microrelief (fig. 9T, X), also found in several *Deppea* species (fig. 3). The genera can be distinguished by the elongated cells surrounding the micropyle in *Plocaniophyllon* (fig. 9U–V), in contrast to the isodiametric cells surrounding the micropyle in *Pinarophyllon* (fig. 9Q–R). The combination of seed colour, anticlinal wall boundary, and micropyle position is useful to differentiate both genera from *Deppea*, even though they share a papillose microrelief (fig. 3). The genus *Schenckia* shares the concave outline with a sunken profile microrelief (fig. 10E–H) with species of *Edithea* and *Deppea* (fig. 3), however, *Schenckia* is characterized by the V-undulate anticlinal wall boundary (fig. 10G), while the *Deppea* and *Edithea* species have straight anticlinal wall boundaries (figs 5Q–AB, 8A–H).

The genus *Deppea* is morphologically heterogeneous and shows overlap among the character states with all other genera: its seed shape, colour, and periclinal microrelief are variable. At species level, the rhomboidal seeds allow

for the recognition of *Deppea erythrorhiza* (figs 5Y, 11M) among the other studied species in the genus, since the other species have circular or ellipsoidal seeds. Among the species with ellipsoid seeds, *D. grandiflora* can be distinguished by the verrucose periclinal microrelief (fig. 6D) instead of the papillose or concave outlines with a sunken profile in the other species. The rest of the species with ellipsoid seeds can be distinguished by the colour: *D. amaranthina* has brown seeds (fig. 11I–J), *D. hamelioides* yellowish (fig. 11Q–R), *D. purpurascens* black (fig. 11AA–AB), and *D. purpusii* and *D. umbellata* have reddish seeds (fig. 12A–D). The latter two differ from each other by their testa cells: well-defined in *D. purpusii* (fig. 7B) but not well-defined in *D. umbellata* (fig. 7F). It is worth mentioning that for *D. umbellata*, the cells surrounding the micropyle could not be observed and therefore these character states were coded in the matrix with a question mark “?”; this is the only taxon that has this state.

Among the *Deppea* species with circular seeds (fig. 3), *D. obtusiflora* is distinguishable by the U-undulate anticlinal wall boundaries (fig. 6R–S), while the rest of the species in the genus have straight boundaries; among these, the seeds of *D. hintonii* have isodiametric lateral cells (figs 6I, 11S–T), while *D. cornifolia* and *D. microphylla* have elongated lateral cells (figs 5U, 6M, 11K, U). The differences between *D. cornifolia* and *D. microphylla* are the prominent anticlinal wall boundaries in the former in contrast to the only slightly prominent anticlinal wall boundaries in the latter (figs 5V–W, 6N–O).

For the genera sampled with more than one species, the examined *Csapodya* species are very different (figs 5I–P, 11E–H) from each other: brown seeds in *C. sousae* and reddish in *C. splendens*; elongated lateral cells in *C. sousae* and isodiametric in *C. splendens*; V-undulate anticlinal wall boundaries in *C. sousae* and straight in *C. splendens*; sub-central micropyle position in *C. sousae* and lateral in *C.*

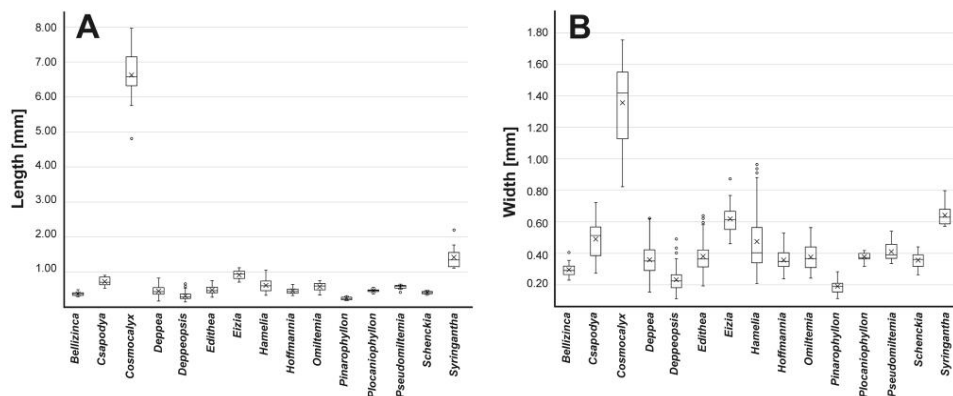


Figure 4 – Boxplots showing the variation in seed length (A) and width (B) per genera. Boxes represent the interquartile range; whiskers are drawn within the 1.5 IQR value; the “X” represents the mean; circles are outliers..

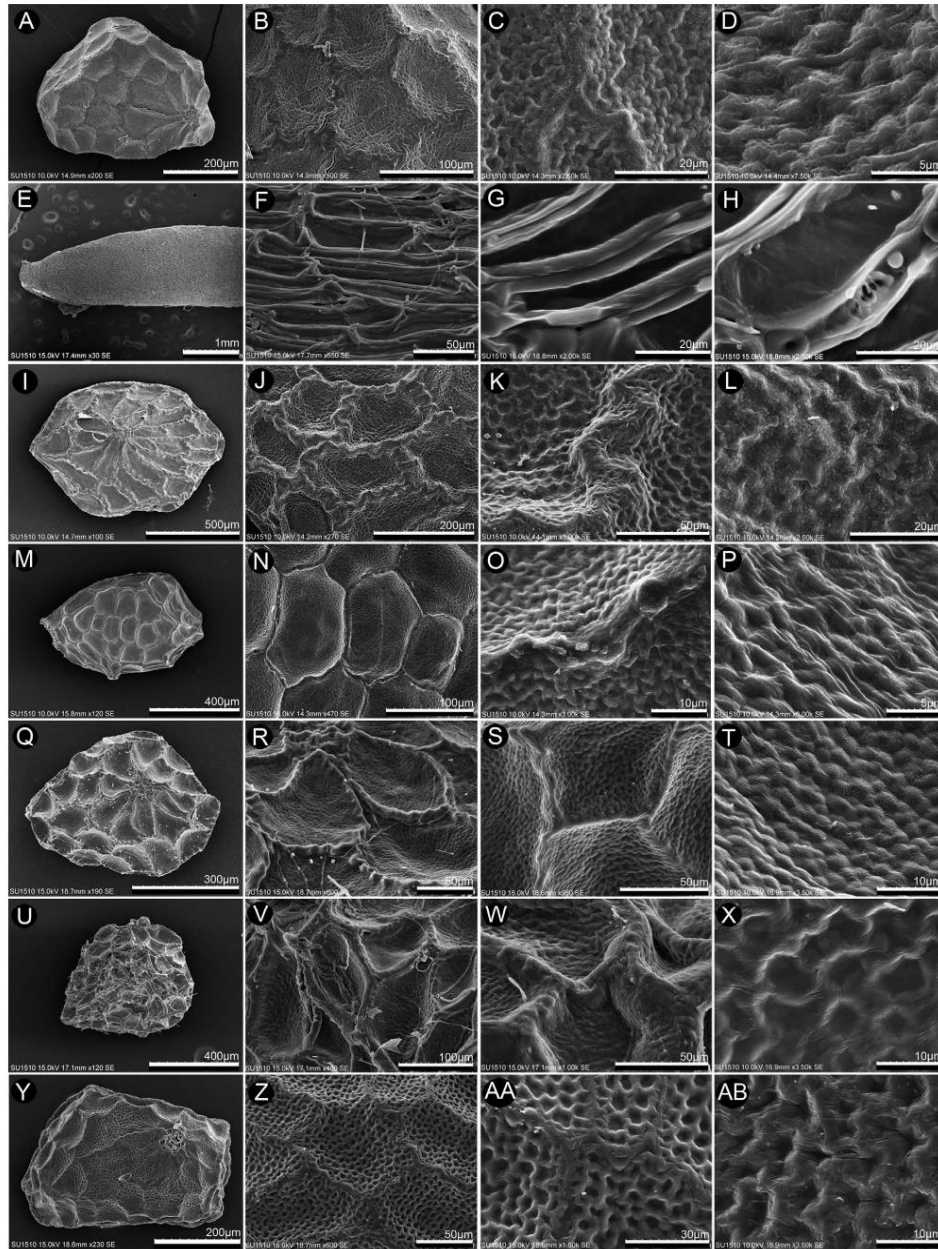


Figure 5 – SEM photographs showing, left to right, a general view of a seed, lateral cells, a detail of the anticlinal wall boundaries, and a detail of the periclinal microrelief. **A–D.** *Belliznca scotti*. **E–H.** *Cosmocalyx spectabilis*. **I–L.** *Csapodya sousae*. **M–P.** *Csapodya splendens*. **Q–T.** *Deppea amaranthina*. **U–X.** *Deppea cornifolia*. **Y–AB.** *Deppea erythrorhiza*.

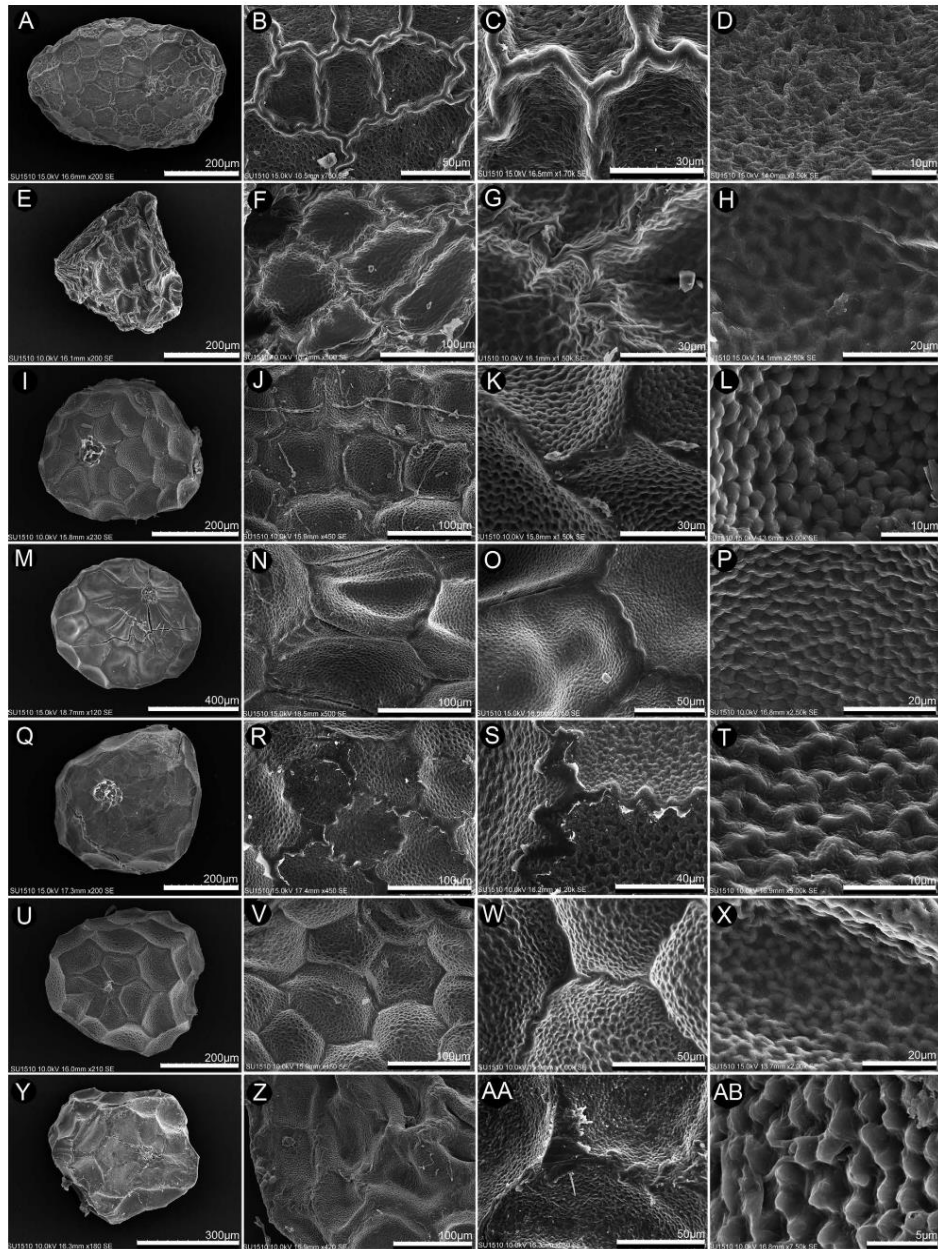


Figure 6 – SEM photographs showing, left to right, a general view of a seed, lateral cells, a detail of the anticlinal wall boundaries, and a detail of the periclinal microrelief. **A–D.** *Deppea grandiflora*. **E–H.** *Deppea hamelioides*. **I–L.** *Deppea hintonii*. **M–P.** *Deppea microphylla*. **Q–T.** *Deppea obtusiflora*. **U–X.** *Deppea pubescens*. **Y–AB.** *Deppea purpurascens*.

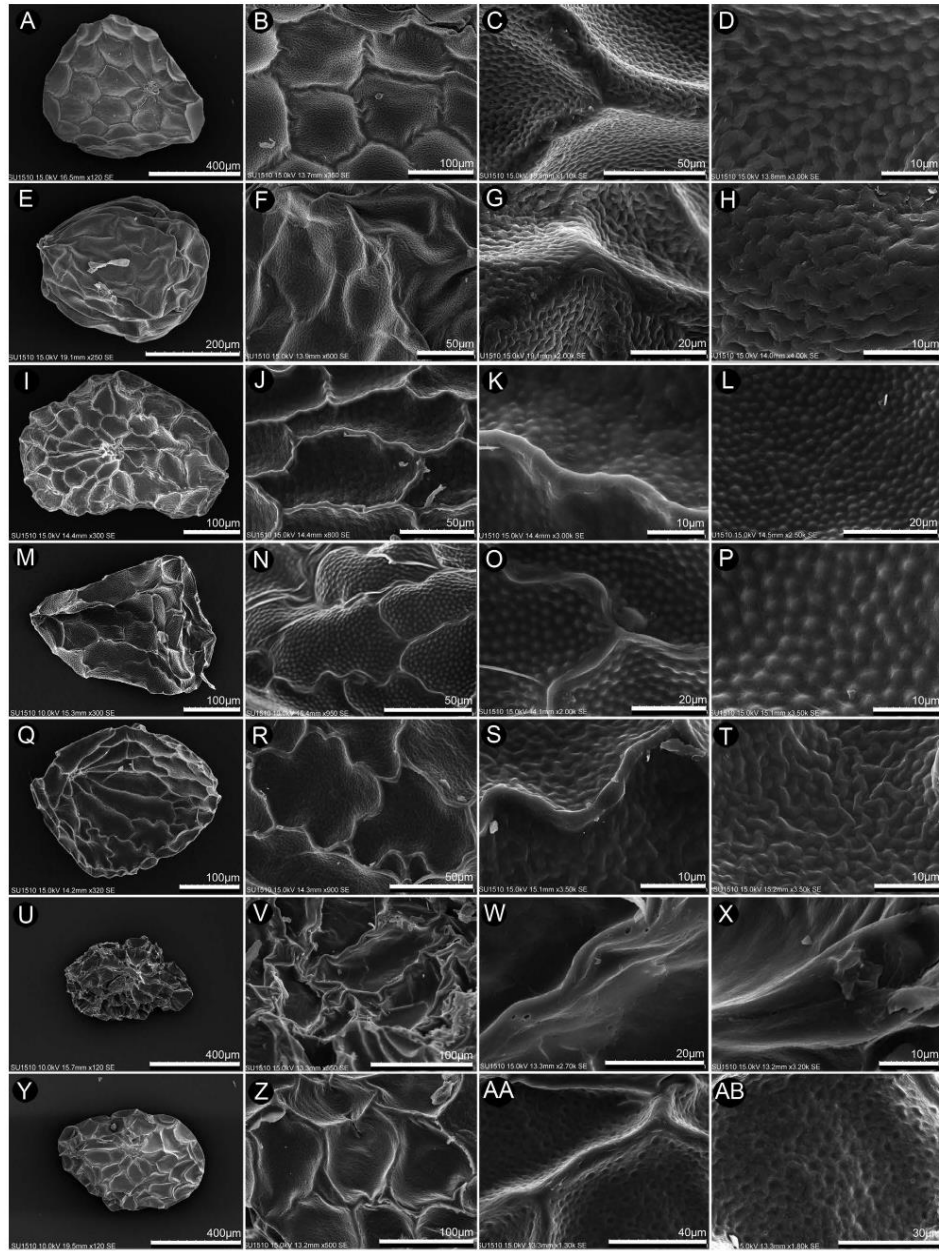


Figure 7 – SEM photographs showing, left to right, a general view of a seed, lateral cells, a detail of the anticlinal wall boundaries, and a detail of the periclinal microrelief. **A–D.** *Deppea purpusii*. **E–H.** *Deppea umbellata*. **I–L.** *Deppeopsis anisophylla*. **M–P.** *Deppeopsis hernandezii*. **Q–T.** *Deppeopsis tenuiflora*. **U–X.** *Editheia floribunda*. **Y–AB.** *Editheia guerrerensis*.

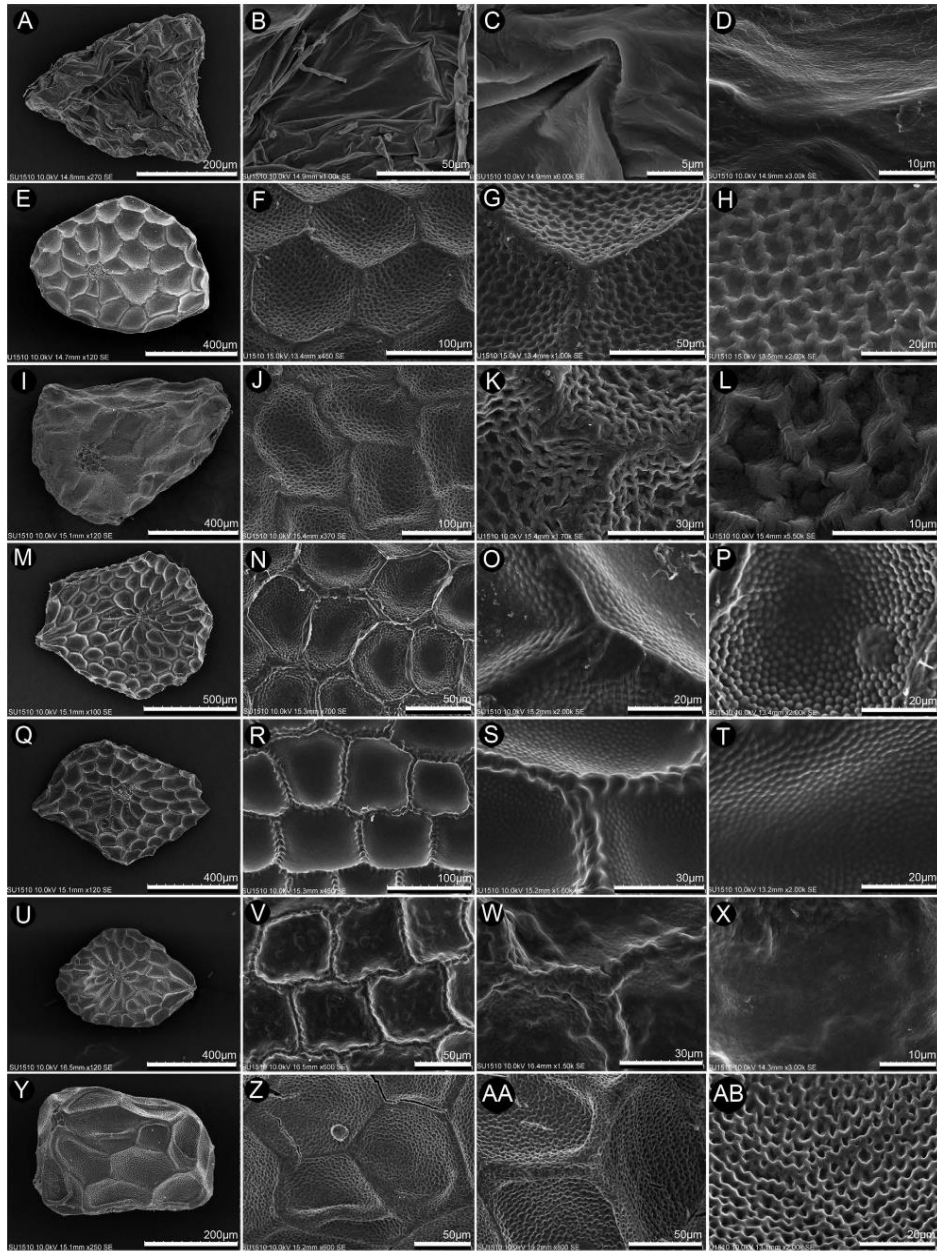


Figure 8 – SEM photographs showing, left to right, a general view of a seed, lateral cells, a detail of the anticlinal wall boundaries, and a detail of the periclinal microrelief. **A–D.** *Editheia miahuatlanica*. **E–H.** *Editheia schiblii*. **I–L.** *Eizia mexicana*. **M–P.** *Hamelia axillaris*. **Q–T.** *Hamelia patens*. **U–X.** *Hamelia xorullensis*. **Y–AB.** *Hoffmannia gesnerioides*.

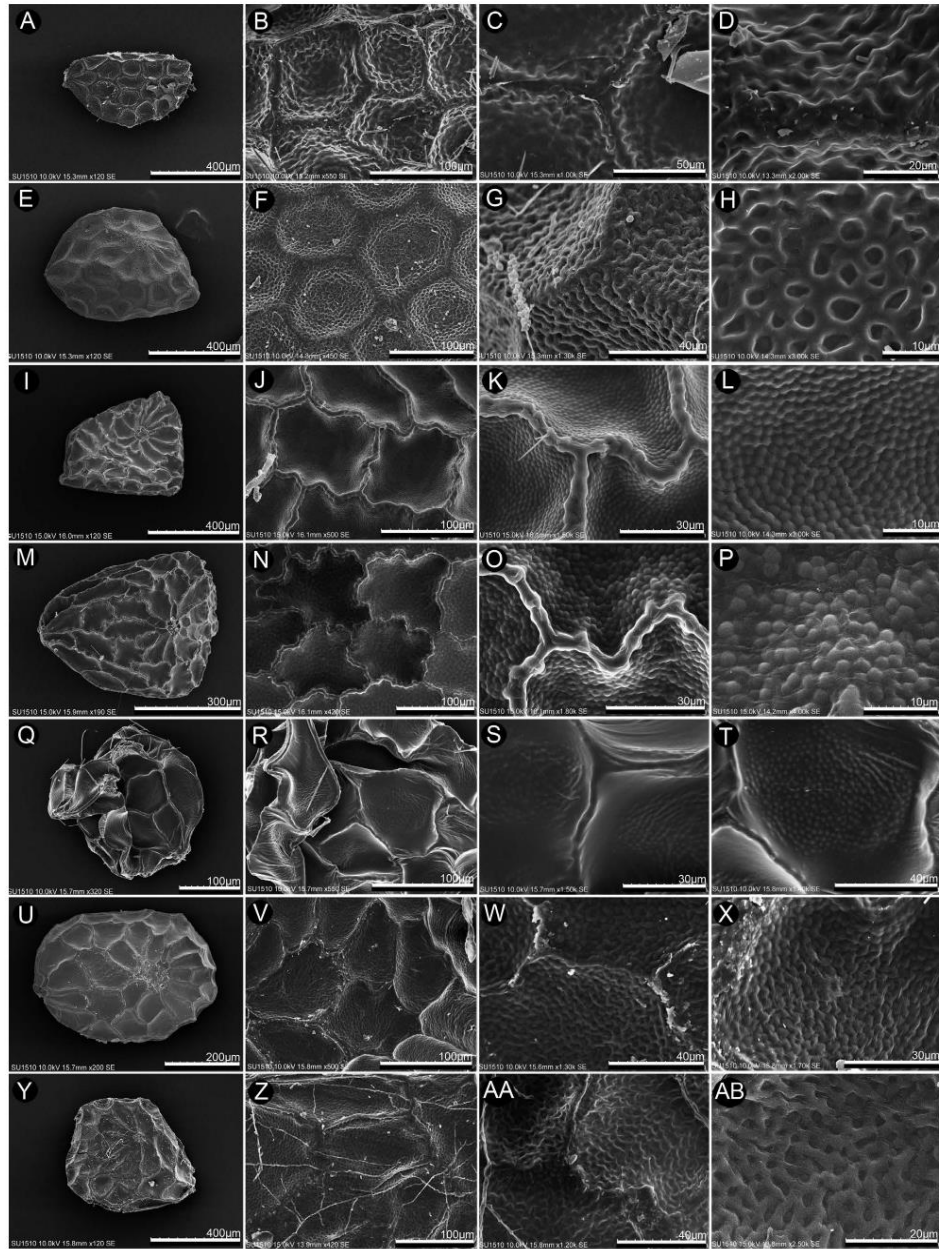


Figure 9 – SEM photographs showing, left to right, a general view of a seed, lateral cells, a detail of the anticlinal wall boundaries, and a detail of the periclinal microrelief. **A–D.** *Hoffmannia ghiesbreghtii*. **E–H.** *Hoffmannia oaxacensis*. **I–L.** *Omlitemia longipes*. **M–P.** *Omlitemia parvifolia*. **Q–T.** *Pinarophyllon flavum*. **U–X.** *Plocamophyllon flavum*. **Y–AB.** *Pseudomiltemia filisepala*.

splendens; inconspicuous hilum in *C. sousae* and evident in *C. splendens*.

Within *Deppeopsis* (figs 7I–T, 12G–J), *D. hernandezii* can be distinguished from the other species in the genus by the isodiametric cells surrounding the micropyle (figs 7M–O), in contrast to the elongated cells surrounding the micropyle of the other two species (figs 7I–L, Q–T), for which no seed characters were found to differentiate between them.

The *Omitelia* species can be distinguished by the general seed shape and hilum visibility: *O. longipes* has rhomboidal seeds and an inconspicuous hilum (figs 9I, 13E–F), while *O. parvifolia* has ellipsoidal seeds and an evident hilum (figs 9M, 13G–H).

The *Edithea* species are variable (figs 7U–AB, 8A–H): *E. mihuantlanica* can be distinguished from other species by the not well-defined testa cells, elongated lateral cells, and the lateral micropyle position (figs 8A–B, 12O–P). The species *E. guerrensis* and *E. schiblii* share the cells surrounding the micropyle different from the lateral ones, the elongated cells surrounding the micropyle plus the concave outline with a sunken profile microrelief (figs 7Y–AA, 8E–G); they differ by the evidently flattened seeds and the slightly anticlinal wall boundary in relief of *E. schiblii* (fig. 8F–G). The species *E. floribunda* and *E. guerrensis* differ mainly by the smooth periclinal microrelief of the former (fig. 7W–X) in contrast to the concave outline with a sunken profile of the latter (fig. 7AA–AB).

DISCUSSION

This study includes species from all genera in Hamelieae, except for the South American genus *Patima*, and it demonstrates the usefulness of seed morphology for the circumscription of genera and species, at least in most cases, but almost always using combinations of seed character states. Our sampling even included the monotypic genus *Eizia*, which was presumed extinct until it was rediscovered a few years ago (Martínez-Camilo et al. 2015).

Seed morphology in Hamelieae

Some Hamelieae genera are easily recognizable by unique character states such as the winged seeds in the monotypic *Syringantha* or the seed size and anticlinal wall boundaries in *Cosmocalyx*. Other genera have a unique combination of character states: e.g. *Hamelia* (with ca 16 spp) has rhomboidal seeds, straight anticlinal wall boundaries, and papillose microrelief. The genus *Hoffmannia*, the largest in the tribe (ca 100 spp.), requires more sampling to firmly make conclusions about the homogeneity or variability of its seed characters, given that only three species were included in our study. Nevertheless, there are several constant characters in the randomly sampled species (see supplementary file 3) and they might be constant at genus level as well. *Hamelia* and *Hoffmannia* have fleshy fruits, an uncommon character state in the tribe since all other genera have capsular fruits, although more species of *Hoffmannia* should be investigated to confirm if these are constant characters related to fruit texture.

For the genus *Patima* (not studied here), Delprete (1998) described the seeds as minute (ca 0.3–0.5 mm), globose, deeply reticulate, with regular shaped cells, with (4)5 or 6 sides per cell. Based on this description, we consider that the seeds of *Patima* are different from those of any other genus in Hamelieae.

Winged seeds are associated with some lineages in Rubiaceae, and this character state is generally constant at tribal or generic level. For example, Cinchoneae is identified by this character state (Andersson 1995), as well as *Bouvardia* Salisb. and *Manettia* Mutis ex L. (Terrell & Robinson 2004). The wings can be highly diverse: they can be equal and present all around the margin of the seed (e.g. *Bouvardia*), or unequal with edges at both poles of the seeds (e.g. *Cinchona*), or unilateral with only one pole developed (e.g. *Simira*) (Bremer & Eriksson 1992). Within Hamelieae, a winged margin is not a constant character state and, although it is only well-defined in one genus (i.e. *Syringantha*), in some species it is possible to see a more translucent or thinly

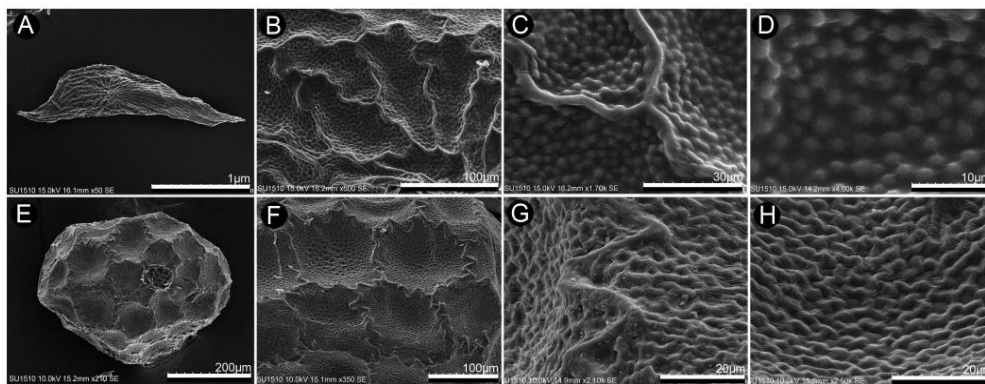


Figure 10 – SEM photographs showing, left to right, a general view of a seed, lateral cells, a detail of the anticlinal wall boundaries, and a detail of the periclinal microrelief. **A–D.** *Syringantha coulteri*. **E–H.** *Schenckia blumenaviensis*.

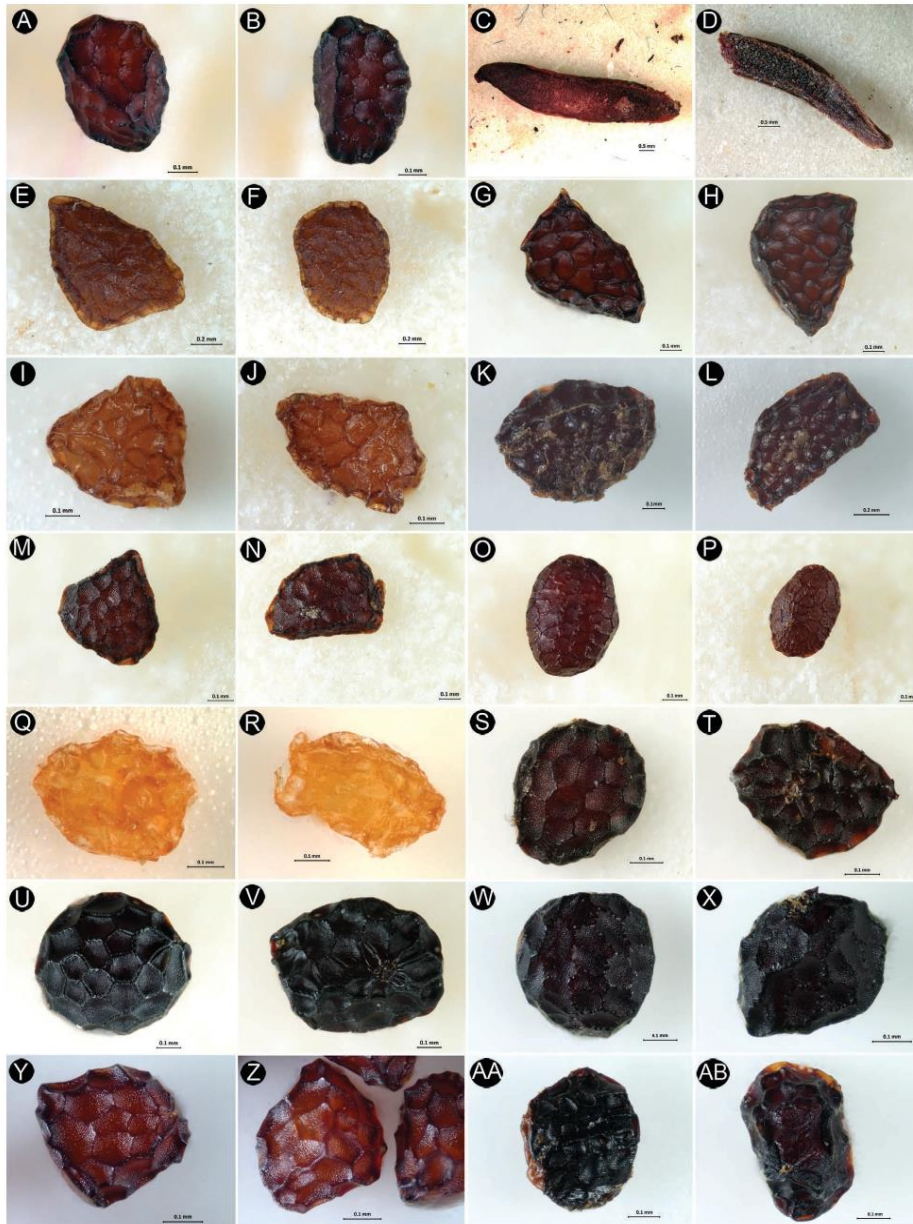


Figure 11 – LM photographs showing a dorsal (left) and a ventral (right) seed view. **A–B.** *Bellizica scoti*. **C–D.** *Cosmocalyx spectabilis*. **E–F.** *Csapodya sousae*. **G–H.** *Csapodya splendens*. **I–J.** *Deppea amarantina*. **K–L.** *Deppea cornifolia*. **M–N.** *Deppea erythrorhiza*. **O–P.** *Deppea grandiflora*. **Q–R.** *Deppea hamelioides*. **S–T.** *Deppea hintonii*. **U–V.** *Deppea microphylla*. **W–X.** *Deppea obtusiflora*. **Y–Z.** *Deppea pubescens*. **AA–AB.** *Deppea purpurascens*.

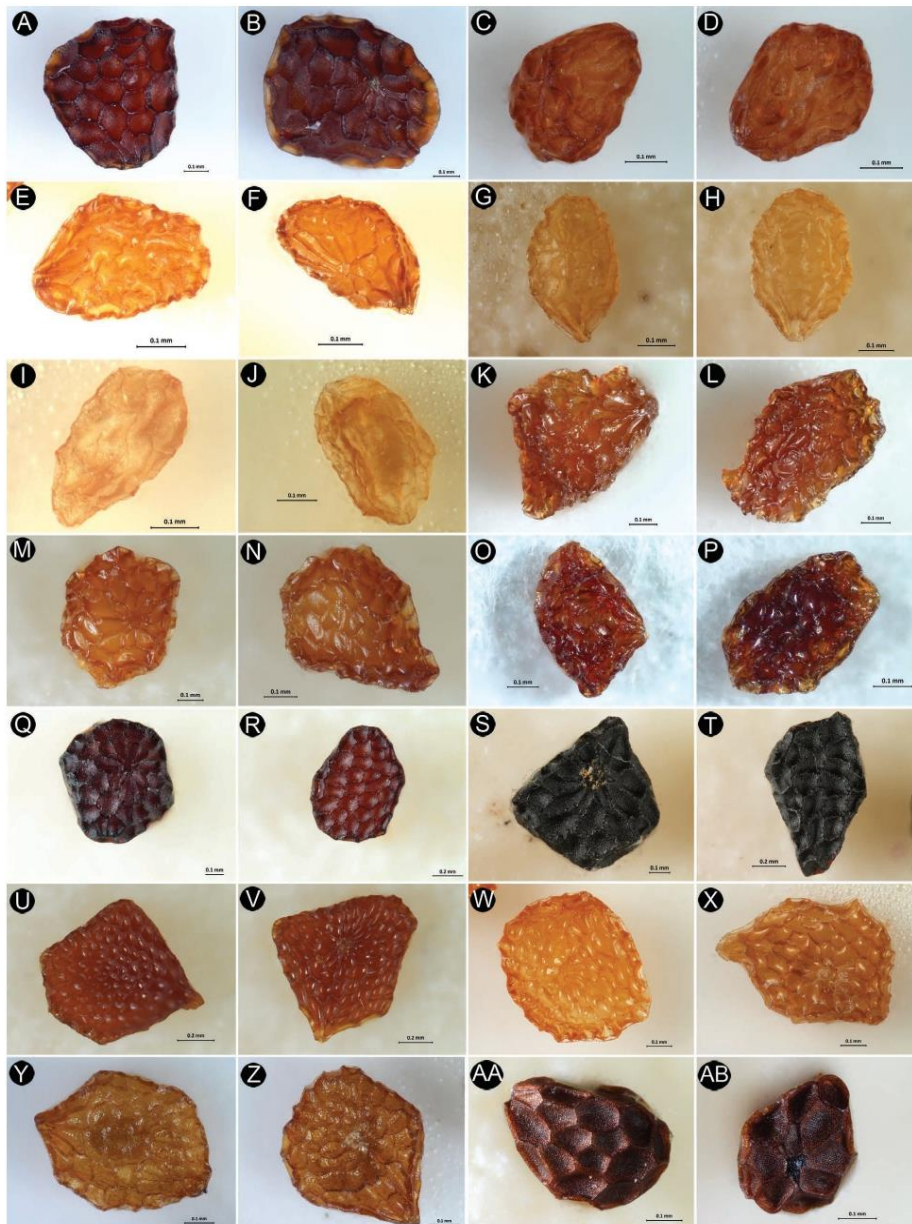


Figure 12 – LM photographs showing a dorsal (left) and a ventral (right) seed view. **A–B.** *Deppea purpusii*. **C–D.** *Deppea umbellata*. **E–F.** *Deppeopsis anisophylla*. **G–H.** *Deppeopsis hernandezii*. **I–J.** *Deppeopsis tenuiflora*. **K–L.** *Editheia floribunda*. **M–N.** *Editheia guerrenensis*. **O–P.** *Editheia mahuatlamica*. **Q–R.** *Editheia schiblii*. **S–T.** *Eizia mexicana*. **U–V.** *Hamelia axillaris*. **W–X.** *Hamelia patens*. **Y–Z.** *Hamelia xorullensis*. **AA–AB.** *Hoffmannia gesnerioides*.

slimmed edge (e.g. *Deppeopsis*; fig. 12I–J), but because it is very narrow we did not consider it as a true wing.

Seed shape could be related to the number of seeds per fruit or per locule, as discussed by Robbrecht (1988), who mentioned that seed shape can be dependent on available space, and small seeds with a convex abaxial side and angular radial sides are common in many-seeded fruits. However, this hypothesis is not corroborated by our observations, since the capsular fruits of the genera in the *Deppea* complex are many-seeded, and among them there are taxa with flattened seeds (e.g. *Deppeopsis*) and with polygonal seeds (e.g. *Deppea*), independent of fruit and seed size. Among

Hamelieae, there are two types of mature fruit according to texture: fleshy berries in *Hamelia*, *Hoffmannia*, and *Patima* (Delprete et al. 2005; Lorence 2012) and dry capsular fruits in the rest of the genera (Borhidi 2012; Lorence 2012). Nevertheless, there is no correlation between seed size or shape with fruit type as, for example, capsular fruits have seeds with different sizes and shapes. Typically, fleshy fruits are associated with zoochory (Bremer & Eriksson 1992) and in these cases, the seeds are sometimes covered by sclerified testa walls that protect the embryo from the digestive tract acids of the animals (e.g. Schaumann & Heinken 2002). We did not observe major differences between the testa walls

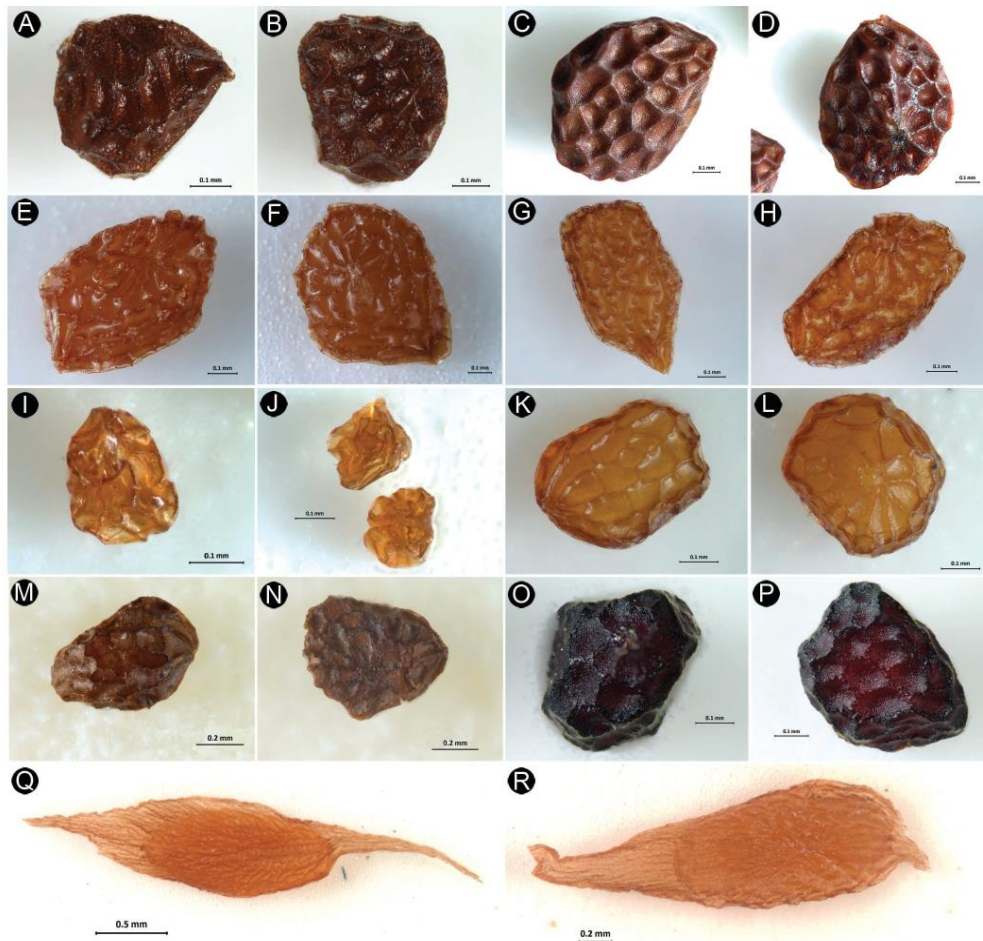


Figure 13 – LM photographs showing a dorsal (left) and a ventral (right) seed view. **A–B.** *Hoffmannia ghiesbreghtii*. **C–D.** *Hoffmannia oaxacensis*. **E–F.** *Omiletemia longipes*. **G–H.** *Omiletemia parvifolia*. **I–J.** *Pinarophyllon flavum*. **K–L.** *Plocaniophyllon flavum*. **M–N.** *Pseudomiletemia filisepala*. **O–P.** *Schenckia blumenaviensis*. **Q–R.** *Syringantha coulteri*.

from seeds in fleshy fruits (e.g. in *Hamelia*) as compared to those of seeds in dry fruits (e.g. in *Deppea*).

Systematics of the *Deppea* complex

The great generic diversity of Hamelieae is directly related to the *Deppea* complex, for which a phylogenetic framework is not yet conclusive. In the most complete phylogeny for the Hamelieae tribe, Stranzinger et al. (2014) included 13 genera and 33 species; their results showed that among the non-monotypic genera only *Hamelia* was retrieved as monophyletic, while all the genera in the *Deppea* complex were placed inside an unsolved clade or retrieved as paraphyletic. These results could reflect an artificial classification of the tribe, but they could also be an artifact of the few characters used to reconstruct the phylogenetic hypothesis (two molecular markers only). The seed morphological mosaic among the sampled taxa in this study does not solve the conflictive circumscription of *Deppea*, which depending on the author is synonymized with *Bellizınca*, *Csapodya*, *Deppeopsis*, *Editheia*, and *Schenckia* (Lorence & Dwyer 1988; Manns & Bremer 2010; Lorence 2012) or accepted as separate generic entities (Borhidi et al. 2004a, 2004b; Borhidi 2006, 2012). Either these genera are not monophyletic or the seed characters used here are in many cases highly homoplastic; only a robust phylogeny constructed with a diverse set of data will reveal which of these two explanations, if any, is more supported.

The phylogenetic hypothesis presented by Stranzinger et al. (2014) retrieved a clade without resolution in which *Bellizınca*, *Csapodya*, *Editheia*, and *Schenckia* are paraphyletic with respect to *Deppea*. This could support the recognition of a single large genus *Deppea*. Nevertheless, the flower morphology is very different in *Deppea* with respect to the other genera (fig. 1), not only regarding the colour of the petals, but also the corolla tube size. Morphologically, *Bellizınca*, *Csapodya*, and *Editheia* are different from *Deppea* by the corolla tube length being larger than 1 cm (Borhidi et al. 2004a). In addition, there are other differences in flower morphology among the genera: *Bellizınca* is recognized by the tubular corolla of 1.4 to 2.6 cm long, the foliaceous calyx lobes up to 1.8 cm long and the stamens attached at the base of the corolla tube (Borhidi et al. 2004a); *Csapodya* is characterized by the 5 to 5.5 cm tubular or salverform corolla, the foliaceous calyx lobes, 8 to 25 mm long, and the stamens attached at the middle portion of the corolla tube (Borhidi et al. 2004a; Borhidi & Reyes-García 2007), while *Editheia* is well characterized by the salverform corolla with the stamens attached near the corolla throat (Borhidi et al. 2004a). The corolla length and colour differences are interpreted as shifts in the pollination mechanism, with a bee pollination syndrome in *Deppea* and a hummingbird pollination syndrome in the other genera (Lorence & Dwyer 1988; Lorence 2012).

Taxonomic implications of seed morphology in the *Deppea* complex

Seed morphology for the *Deppea* complex was briefly studied by Borhidi et al. (2004a), who compared seed differences among *Bellizınca*, *Csapodya*, *Editheia*, and

Deppea mentioning shape, hilum position, and testa ornamentation differences. Our results partially agree with those of Borhidi et al. (2004a) in that the seed shape among these genera is different. However, we found discrepancies in certain characters, particularly regarding the hilum position: we treat as the micropyle what Borhidi and collaborators considered to be the hilum, based on our SEM microphotographs and on the orthotropous nature of the ovule in most of the studied genera (Martínez-Cabrera et al. 2014). Orthotropous ovules were described using anatomical methods for many Hamelieae members by Martínez-Cabrera et al. (2014) including *Deppea*, *Hamelia*, *Hoffmannia*, *Omitelia*, *Pimarophyllon*, *Plocaniophyllon*, and *Syringantha*. Nevertheless, for *Csapodya splendens*, the hilum and micropyle positions indicate a more likely anatropous ovule nature, a condition common in most Rubiaceae genera (Robbrecht 1988; Martínez-Cabrera et al. 2014). Further anatomical studies are however needed to corroborate the ovule nature in *Csapodya*.

Csapodya* and *Bellizınca – The seeds of *Csapodya* are easily recognized from those of *Deppea* by being flattened, a character state shared with *Deppeopsis*, *Omitelia*, and *Pseudomitelia*. *Csapodya* differs by the combination of the concave outline with a sunken profile microrelief plus V-undulate anticlinal wall boundaries and the evident hilum. It should be noted that in the phylogenetic framework presented by Stranzinger et al. (2014), *Csapodya* is placed in a different lineage with respect to *Deppeopsis*, *Omitelia*, and *Pseudomitelia*, which share a recent common ancestor. The seeds in the genus *Bellizınca* can be distinguished by the V-undulate anticlinal wall boundary, in contrast to the U-undulate or straight anticlinal wall boundary in *Deppea*. This character state, in addition to the corolla characters, could support the recognition of *Bellizınca* and *Csapodya* as separate generic entities. The genus *Csapodya* was segregated from *Deppea* based on *D. splendens* (Borhidi et al. 2004a) and currently could include two or three species (Borhidi & Reyes-García 2007). However, in the Rubiaceae treatment of the Mesoamerican Flora (Lorence 2012), *Csapodya* is treated as a synonym of *Deppea*, and only one species potentially belonging in *Csapodya* (i.e. *D. splendens*) is recognized, arguing that the other species were described based on immature inflorescences. Despite several attempts to collect *C. challengerii*, we failed as there appear to be errors in the type locality (the provided coordinates do not coincide with the vegetation or elevation description), however, our results support that *C. sousae* and *C. splendens* are different species, since they have several contrasting characters, including the colour, exotesta cell shape, anticlinal wall boundaries, micropyle position, and hilum visibility.

Editheia – The seed morphology of the genus *Editheia* is variable and overlaps with most of the other genera; some species are easily recognizable by the smooth periclinal microrelief (*E. floribunda* and *E. miahuatlanica*), a unique character state among all the Hamelieae taxa studied. However, the other species in the genus have concave outlines with a sunken profile (*E. guerrerensis* and *E. schiblii*), a common character state among the species in the *Deppea* complex. In contrast to *Csapodya* and *Bellizınca*, which in the Stranzinger et al. (2014) phylogeny formed

a clade within *Deppea* in an unsolved position, the *Edithea* species are placed inside a clade with other *Deppea* species, highlighting the possibility of a polyphyletic *Deppea*. Seed morphology may support the previously suggested synonymy of *Edithea* and *Deppea* by using other morphological characters and molecular evidence (Lorence & Dwyer 1988; Manns & Bremer 2010; Lorence 2012). However, further studies with other sources of evidence and broader taxon sampling are needed to reliably take decisions upon the synonymy of the names or the acceptance of these taxa.

Deppeopsis – Another genus sometimes synonymized with *Deppea* is *Deppeopsis*, which was primarily segregated by its cylindrical fruit shape (Borhidi & Stranzinger 2012). Currently, it includes five species from Mexico and Guatemala (Borhidi 2012; Borhidi & Stranzinger 2012). In the Mesoamerican Rubiaceae treatment (Lorence 2012), only two species of this genus are included, and they are treated under *Deppea*. Using seed morphology, *Deppeopsis* is easily distinguishable from *Deppea* by the flattened seeds, but other character states, such as the yellowish testa colour (only shared with *Deppea hamelioides*), U-undulate anticlinal wall boundaries, papillose periclinal microrelief, and the lateral position of the micropyle are shared with at least one species of *Deppea*. Based on our seed character observations, we support the separation of *Deppeopsis* from *Deppea*, as it was also suggested by the Stranzinger et al. (2014) phylogenetic hypothesis, in which *Deppeopsis* is clearly a separate lineage from *Deppea*, more closely related to other taxa with flattened seeds such as *Omitelia* and *Pseudomitelia*.

Pseudomitelia* and *Omitelia – The Mexican genus *Pseudomitelia* was segregated from *Omitelia* (Borhidi et al. 2004b) and has two species characterized by the stamen insertion near the corolla throat (vs near the base in *Omitelia*) and the funnelliform corolla (vs campanulate in *Omitelia*) (Martínez-Camilo et al. 2011; Borhidi 2012). The Mexican genus *Omitelia* was described by Standley (1918) based on the red and solitary flowers and has three species (Borhidi & Velasco-Gutiérrez 2010; Borhidi 2012; Lozada-Pérez & Rojas-Gutiérrez 2016). Both share flattened seeds, while the main difference is the periclinal microrelief, papillose in *Omitelia* and concave outlines with a sunken profile in *Pseudomitelia*. Borhidi et al. (2004b) also used seed morphology in conjunction with other evidence to justify the transfer of *Omitelia filisepala* to *Pseudomitelia* using differences in the hilum position (lateral in *Omitelia* and central in *P. filisepala*); perpendicular walls surface (slightly undulate in *Omitelia* and slightly tuberculated in *P. filisepala*), and horizontal wall microrelief (smooth in *Omitelia* and densely tuberculated in *P. filisepala*). Our study differs in the description of the micropyle position, presumably treated as hilum in Borhidi et al. (2004b), considered to be for both species as sub-central, as well as in the microrelief that was considered to be smooth in *Omitelia*, while our results evidently show a papillose microrelief. Based on the seed morphology, both genera, *Pseudomitelia* and *Omitelia*, should be recognized.

Pinarophyllon* and *Plocaniophyllon – The other genera of the *Deppea* complex are *Pinarophyllon* and *Plocaniophyllon*. The genus *Pinarophyllon* was recognized by the herbaceous habit and the turbinate fruits (Brandege 1914; Lorence

2012) and has two species in Mexico and Guatemala. The monotypic Mexican genus *Plocaniophyllon* was described based on the lineolate leaf venation (Brandege 1914; Lorence 2012). In both genera, the seeds are polygonal and ellipsoid with straight anticlinal wall boundaries. These are the same character states as in *Hoffmannia*, but they differ in that *Pinarophyllon* and *Plocaniophyllon* have smooth or papillose periclinal wall microrelief, while *Hoffmannia* has verrucose or concave outlines with a sunken profile. The seeds of *Plocaniophyllon* were described by Martínez-Cabrera et al. (2008) as ovoid, “irregular”, 0.4–0.6 mm long, with large polygonal shaped cells and foveolate multicellular sculpture, anticlinal walls boundaries slightly undulated, and outer periclinal walls concave with tuberculate micro-ornamentation. Our description agreed in the seed shape, size, and cell shape; however, Martínez-Cabrera et al. (2008) defined the cells boundaries as slightly undulated while we consider them to be straight, as well as the microrelief to be papillose instead of tuberculate. The seed character state to distinguish *Pinarophyllon* and *Plocaniophyllon* is the shape of the cells surrounding the micropyle: isodiametric in *Pinarophyllon* and elongated in *Plocaniophyllon*.

Schenckia – In the literature, there is consensus to treat *Schenckia* as a synonym of *Deppea* (Lorence & Dwyer 1988; Lorence 2012). Its inclusion gives the distribution of *Deppea* to a disjunct pattern since *Schenckia* is only known from Blumenau in Brazil and from the north of Argentina (Lorence & Dwyer 1988), whereas *Deppea* is practically restricted to the Mesoamerican region. The seed morphology, as the molecular evidence (Manns & Bremer 2010), supports its synonymy as there is no unique combination of characters to distinguish *Schenckia* from *Deppea*.

CONCLUSION

Our study reveals why the *Deppea* complex is so problematic from a taxonomic point of view and reflects the fact that a comprehensive approach with different lines of evidence, such as morphological, molecular, and phylogenetic characters, is needed to evaluate the generic circumscriptions within the *Deppea* complex in the Hamelieae tribe. Despite this, we addressed the importance of seed morphology as a source of characters in Hamelieae systematics and taxonomy and we highlight the broad diversity of seed morphological character states in this tribe.

Although the characters evaluated here show high overlap across the genera, there is some evidence that seed morphology can contribute to taking taxonomic decisions within the *Deppea* complex. Based on our study, we consider that there is support for the recognition of *Bellizina*, *Csapodya*, and *Deppeopsis*, currently considered to be synonyms of *Deppea* by some authors. Also, seed morphology supports the synonymy of *Edithea* and *Schenckia* with *Deppea*, and provided additional morphological evidence for the recognition of *Omitelia*, *Pinarophyllon*, *Plocaniophyllon*, and *Pseudomitelia*. However, for these taxonomic decisions to be definitive, an integrative approach using a robust phylogenetic context is still necessary. In particular to establish the generic limits within the *Deppea* complex inside the Hamelieae tribe, a full

perspective that includes micro- and macromorphology plus molecular evidence is desirable to unravel this conflictive group.

SUPPLEMENTARY FILES

Supplementary file 1 – Voucher information of the studied specimens, which are all deposited at MEXU except for *Schenckia blumenaviensis*, which is deposited at C.

<https://doi.org/10.5091/plecevo.84486.suppl>

Supplementary file 2 – Illustrated overview of the seed characters and character states.

<https://doi.org/10.5091/plecevo.84486.suppl2>

Supplementary file 3 – Full seed descriptions of the 37 studied species in 15 genera of the Hamelieae.

<https://doi.org/10.5091/plecevo.84486.suppl3>

ACKNOWLEDGEMENTS

The first author thanks the Programa de Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México (UNAM) for a graduate scholarship (CONACyT 320242). This paper is in fulfillment of the requirements of the Posgrado en Ciencias Biológicas, UNAM, for obtaining a Ph.D. degree in Systematics. We thank M.C. Berenit Mendoza for her assistance with the SEM, and Biol. Susana Guzmán Gómez for her assistance with the LM. Alejandro Torres assisted in the field and in the production of figures 2 and 3. Our deepest gratitude to Héctor Gómez (UNICACH) for his field assistance in Chiapas; Hilda Flores-Olvera and Carlos Gómez Hinostroza were also great field companions. We are grateful to the anonymous reviewers, to Petra De Block as communicating editor and to Brecht Verstraete as editor in chief for the suggestions to the manuscript that undoubtedly improved it.

REFERENCES

- Andersson L. 1995. Tribes and genera of the Cinchoneae complex (Rubiaceae). *Annals of the Missouri Botanical Garden* 82: 409–427. <https://doi.org/10.2307/2399891>
- Andersson L. & Rova H.E. 1999. The *rps16* intron and the phylogeny of the Rubioideae (Rubiaceae). *Plant Systematics & Evolution* 214: 161–186. <https://doi.org/10.1007/BF00985737>
- Antonelli A., Clarkson J.J., Kainulainen K., et al. 2021. Settling a family feud: a high-level phylogenomic framework for the Gentianales based on 353 nuclear genes and partial plastomes. *American Journal of Botany* 108: 1143–1165. <https://doi.org/10.1002/ajb2.1697>
- Barthlott W. & Hunt D. 2000. Seed-diversity in the Cactaceae subfamily Cactoideae. *Succulent Plant Research* vol. 5. David Hunt, Milborne Port.
- Borhidi A. 2006. Rubiaceae de México. Akadémiai Kiadó, Budapest.
- Borhidi A. 2012. Rubiaceae de México. Second edition. Akadémiai Kiadó, Budapest.
- Borhidi A. & Stranzinger S. 2012. *Deppeopsis*, un género nuevo (Hamelieae, Rubiaceae) de México y Guatemala. *Acta Botanica Hungarica* 54: 86–89. <https://doi.org/10.1556/abot.54.2012.1-2.10>
- Borhidi A., Darók J., Kocsis M., Stranzinger S. & Kaposvári F. 2004a. Critical revision of the *Deppea* complex (Rubiaceae, Hamelieae). *Acta Botanica Hungarica* 46: 77–89. <https://doi.org/10.1556/abot.46.2004.1-2.7>
- Borhidi A., Darók J., Kocsis M., Stranzinger S. & Kaposvári F. 2004b. Critical revision of the *Omittemia* complex (Rubiaceae, Hamelieae). *Acta Botanica Hungarica* 46: 69–76. <https://doi.org/10.1556/abot.46.2004.1-2.6>
- Borhidi A. & Reyes-García A. 2007. Estudios sobre Rubiaceae Mexicanas XI Revisión del género *Csapodya* Borhidi (Rondeletieae), endémico de Chiapas con tres especies. *Acta Botanica Hungarica* 49: 13–25. <https://doi.org/10.1556/abot.49.2007.1-2.2>
- Borhidi A. & Velasco-Gutiérrez K. 2010. Estudios sobre Rubiaceae Mexicanas XXV. Una especie nueva en el género *Omittemia* Standl. (Hamelieae). *Acta Botanica Hungarica* 52: 35–39. <https://doi.org/10.1556/abot.52.2010.1-2.4>
- Breedlove D.E. & Lorence D.H. 1987. New species of *Deppea* (Rubiaceae) from Chiapas, México. *Phytologia* 63: 43–47.
- Bremekamp C.E.B. 1966. Remarks on the position, the delimitation and the subdivision of the Rubiaceae. *Acta Botanica Neerlandica* 15: 1–33. <https://doi.org/10.1111/j.1438-8677.1966.tb00207.x>
- Bremer B. 1987. The sister group of the paleotropical tribe Argostemmatae: a redefined neotropical tribe Hamelieae (Rubiaceae). *Cladistics* 3: 35–51. <https://doi.org/10.1111/j.1096-0031.1987.tb00495.x>
- Bremer B., Andreassen K. & Olsson D. 1995. Subfamilial and tribal relationships in the Rubiaceae based on *rbcL* sequence data. *Annals of the Missouri Botanical Garden* 82: 383–397. <https://doi.org/10.2307/2399889>
- Bremer B. & Eriksson O. 1992. Evolution of fruit characters and dispersal modes in the tropical family Rubiaceae. *Biological Journal of the Linnean Society* 47: 79–95. <https://doi.org/10.1111/j.1095-8312.1992.tb00657.x>
- Brandege T.S. 1914. *Plantae Mexicanae Purpusianae* VI. *University of California Publications in Botany* 6: 71.
- De Candolle A.P. 1830. *Prodromus systematis naturalis regni vegetabilis*. Treuttel & Würtz, Paris.
- Delprete P.G. 1998. Notes on calycophyllous Rubiaceae. Part III. Systematic position of the monotypic Mexican genus *Cosmocalyx* and notes on the calycophyll development. *Brittonia* 50: 309–317. <https://doi.org/10.2307/2807774>
- Delprete P.G., Nee M. & Koek-Noorman J. 2005. Resurrection and revision of *Patima* (Rubiaceae, Cinchonoideae, Hamelieae). *Annals of the Missouri Botanical Garden* 92: 103–112. <https://www.jstor.org/stable/3298650>
- Endlicher S. 1836. *Genera Plantarum secundum ordines naturales disposita*. Apud Fr. Beck Universitatis Bibliopolam, Vinclobonae. <https://doi.org/10.5962/bhl.title.728>
- Harris J.G. & Harris M.W. 2001. *Plant identification terminology. An illustrated glossary*. Second edition. Spring Lake Publishing, Spring Lake.
- Hayden M.V. & Dwyer J.D. 1969. Seed morphology in the tribe Morindeae (Rubiaceae). *Bulletin of the Torrey Botanical Club* 96: 704–710. <https://doi.org/10.2307/2483550>
- Hooker J.D. 1873. Rubiaceae. In: Bentham G. & Hooker J.D. (eds) *Genera Plantarum* vol. 2(1): 7–151. Reeve & Co., London.

- Khalik K.A., El-Ghani M.A. & El Kordy A. 2008. Fruit and seed morphology in *Galium* L. (Rubiaceae) and its importance for taxonomic identification. *Acta Botanica Croatica* 67: 1–20.
- Kirkbride H.J. 1984. Manipulus Rubiacearum III. Deppeae, a new tribe of Rubioideae (Rubiaceae). *Brittonia* 36: 317–320. <https://doi.org/10.2307/2806533>
- Koch K., Bhushan B. & Barthlott W. 2009. Multifunctional surface structures of plants: An inspiration for biomimetics. *Progress in Materials Science* 54: 137–178. <https://doi.org/10.1016/j.pmatsci.2008.07.003>
- Lorence D.H. & Dwyer J.D. 1988. A revision of *Deppea* (Rubiaceae). *Allertonia* 4: 389–436.
- Lorence D.H. 2012. *Deppea*. In: Davidse G., Sousa M., Knapp S. & Chiang F. (eds) *Flora Mesoamericana* vol. 4(2): 75–78. Missouri Botanical Garden Press, St. Louis.
- Lozada-Pérez L. & Rojas-Gutiérrez J. 2016. Una nueva especie, *Omitelia guerrensis* (Rubiaceae) de Guerrero, México. *Novon* 24: 365–368. <https://doi.org/10.3417/2012041>
- Manns U. & Bremer B. 2010. Towards a better understanding of intertribal relationships and stable tribal delimitations within Cinchonoideae s.s. (Rubiaceae). *Molecular Phylogenetics and Evolution* 56: 21–39. <https://doi.org/10.1016/j.ympev.2010.04.002>
- Martínez-Cabrera D., Terrazas T., Flores H. & Ochoterena H. 2008. Morphology, anatomy, and taxonomic position of *Plocamiphyllon* Brandege (Rubiaceae): a monotypic genus endemic to Mesoamerica. *Taxon* 57: 33–42. <https://doi.org/10.2307/25065946>
- Martínez-Cabrera D., Terrazas T. & Ochoterena H. 2014. Morfología y anatomía floral de la tribu Hamelieae (Rubiaceae). *Brittonia* 66: 89–106. <https://doi.org/10.1007/s12228-013-9301-5>
- Martínez-Camilo R., Martínez-Meléndez N., Martínez-Meléndez M., Pérez-Farrera M.A. & Lorence D.H. 2011. Una nueva especie de *Pseudomitelia* (Rubiaceae) de Chiapas, México. *Brittonia* 63: 197–202. <https://doi.org/10.1007/s12228-010-9150-4>
- Martínez-Camilo R., Martínez-Meléndez N., Martínez-Meléndez M., Pérez-Farrera M.A. & Lorence D.H. 2015. Redescubrimiento de *Eizia mexicana*, una especie endémica de la familia Rubiaceae presumiblemente extinta. *Botanical Sciences* 93: 679–682. <https://doi.org/10.17129/botsci.242>
- McDowell T. 1996. *Syringantha coulteri* (Hooker f.) McDowell, and new combination and remarks on the relationships of the monotypic Mexican genus *Syringantha* Standley (Rubiaceae). *Novon* 6: 273–279. <https://doi.org/10.2307/3392093>
- Nixon K.C. 1999. WinClada ver. 1.0000. Published by the author, Ithaca, NY, USA.
- Robbrecht E. 1988. Tropical woody Rubiaceae. *Opera Botanica Belgica* vol. 1. National Botanic Garden of Belgium, Meise.
- Robbrecht E. & Bridson K.A. 1993. Nomenclatural notes on three Rubiaceae genera. *Opera Botanica Belgica* vol. 6: 199–200. National Botanic Garden of Belgium, Meise.
- Robbrecht E. & Manen J.F. 2006. The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms). Combined analysis (nDNA and cpDNA) to infer the position of *Coptosapelta* and *Luculia*, and supertree construction based on *rbcL*, *rps16*, *trnL-trnF* and *atpB-rbcL* data. A new classification in two subfamilies, Cinchonoideae and Rubioideae. *Systematics and Geography of Plants* 76: 85–146. <https://doi.org/10.2307/20649700>
- Schaumann F. & Heinken T. 2002. Endozoochorous seed dispersal by martens (*Martes foina*, *M. martes*) in two woodland habitats. *Flora - Morphology, Distribution, Functional Ecology of Plants* 197: 370–378. <https://doi.org/10.1078/0367-2530-00053>
- Schumann K. 1897. Rubiaceae. In: Engler A. & Prantl K. (eds) *Die natürlichen Pflanzenfamilien* 4(4): 1–156. Engelmann, Leipzig.
- Standley P.C. 1918. *Omitelia*, a new genus of Rubiaceae from Mexico. *Journal of the Washington Academy of Sciences* 8: 426–427. <https://www.jstor.org/stable/24521406>
- Stranczinger S., Galambos A., Szenas D. & Szalontai B. 2014. Phylogenetic relationships in the Neotropical tribe Hamelieae (Rubiaceae, Cinchonoideae) and comments on its generic limits. *Journal of Systematics and Evolution* 52: 643–650. <https://doi.org/10.1111/jse.12103>
- Terrell E.E., Lewis W.H., Robinson H. & Nowicke J.W. 1986. Phylogenetic implications of diverse seed types, chromosome numbers, and pollen morphology in *Houstonia* (Rubiaceae). *American Journal of Botany* 73: 103–115. <https://doi.org/10.1002/j.1537-2197.1986.tb09686.x>
- Terrell E.E. & Robinson H. 2004. Seed and capsule characters in *Arcytophyllum*, *Bouvardia*, and *Manettia* (Rubiaceae), with notes on *A. serpyllaceum*. *SIDA, Contributions to Botany* 21: 911–927.
- Torres-Montúfar A. 2018. Sistemática del complejo *Rondeletia* (Rubiaceae). PhD thesis, Universidad Nacional Autónoma de México, México. Available from <https://repositorio.unam.mx/contenidos/64735> [accessed 8 Sep. 2021].
- Verdcourt B. 1958. Remarks on the classification of the Rubiaceae. *Bulletin du Jardin botanique de l'État à Bruxelles* 28: 209–281. <https://doi.org/10.2307/3667090>

Communicating editors: Petra De Block & Brecht Verstraete.

Submission date: 13 Apr. 2021

Acceptance date: 8 Sep. 2021

Publication date: 30 Mar. 2022

**CAPÍTULO 2. The systematic significance of floral and fruit morphology in
Hamelieae tribe (Rubiaceae)**

The neotropical tribe Hamelieae comprises 10 to 16 genera and approximately 225 species, undoubtedly the center of diversity is Mexico, with 9 to 14 genera and ca. 100 species (Lorence & Dwyer, 1988; Borhidi, 2006, 2012). The tribe is diagnosed by the presence of raphides, ebracteate inflorescences, and usually yellow 4-merous flowers with imbricate aestivation (Bremer, 1987; Borhidi et al., 2004a, 2004b; Manns & Bremer, 2010; Stranzinger et al., 2014).

Although the tribe is retrieved as monophyletic (Manns & Bremer, 2010), the generic circumscription is debatable, particularly for the *Deppea* complex, a group of nine morphologically similar genera. Most of these genera were segregated from *Deppea*: *Bellizinca*, *Csapodya*, *Deppeopsis*, *Edithea*, *Omiltemia*, *Pinarophyllon*, *Plocaniophyllon*, *Pseudomiltemia*, and *Schenckia* based on some “diagnostic” reproductive characters, such as corolla form and size, stamen insertion, or fruit form and dehiscence (Borhidi et al. 2004a, 2004b, Borhidi 2006, 2012, Borhidi & Stranzinger 2012). Nevertheless, these characters are not hierarchical, showing overlapping variation that forms a mosaic and does not allow for the clear distinction among genera.

Molecular phylogenetic analyses have not provided a solution to this taxonomic problem. For example, Manns & Bremer (2010) used a set of six markers (nrITS, *atpB*, *rbcL*, *ndhF*, *rbcL*, *rps16*, and *trnL-F*) to test tribal circumscription and relationships within subfamily Cinchonoideae, but only six genera of the *Deppea* complex were represented with few species each, leaving many open questions related to the generic circumscription. On the other hand, Stranzinger et al. (2014) included representative species of all the genera in the *Deppea* complex, but they only used two molecular markers (nrITS and *trnL-F*) retrieving a consensus with little resolution and support, resulting in doubtful

taxonomic decisions. Both phylogenetic analyses pointed out the possibility that none of the genera in the *Deppea* complex is monophyletic, however, also stressed the need for a phylogenetic study including other sources of evidence, such as morphology.

The role of morphology has been relegated in modern systematic studies; its value has been widely discussed in the molecular phylogenetics age (Giribet, 2015, Lee & Palci, 2015). In the case of Rubiaceae, morphological characters have been repeatedly used alone in phylogenetic reconstructions (e.g. Andersson & Persson, 1991; Bremer, 1996; Persson, 1996), combined with molecular data in simultaneous analysis (e.g. Bremer & Struwe, 1992; Razafimandimbison & Bremer, 2001; Gustafsson & Persson, 2002; Barrabé et al., 2012) or mapped onto molecular phylogenies to study their evolution (e.g. Kainulainen et al., 2010; Löfstrand et al., 2019).

Reproductive traits have been a focus of many evolutionary and taxonomic studies aimed at explaining the observed diversity of angiosperms (e.g. Anderson et al., 2001). In particular, floral morphology can be interpreted as the result of concerted adaptation to selective pressures exerted by pollinators in interactions that vary in their degree of specialization (Faegri & van der Pijl, 1979; Fenster et al., 2004; Kephart et al., 2006), while fruit morphology, specifically fruit texture, is related to dispersal models (Bremer & Eriksson, 1992).

For Rubiaceae there are several studies that explore reproductive traits in a phylogenetic context: Motley et al. (2005) remarked that changes in floral morphologies in the Chiococceae-Catesbaeae tribes tend to correspond to cladogenesis among and within genera and that fleshy fruits allowed long-distance dispersal for this group; Soza & Olmstead (2010) studied the breeding systems polygamy, dioecy, and hermaphroditism that

occurs within the genus *Galium* in a phylogenetic framework. For fruits, Bremer & Eriksson (1992) studied their evolution in Rubiaceae, concluding that the fleshy fruits related to animal dispersion evolved 12 times in the family history; Torres-Montúfar et al. (2018) revised the fruit character definition in the tribes Guettardeae-Rondeletieae. For the tribe Hamelieae and the *Deppea* complex, although that the reproductive traits provide valuable comparative morphological, systematic, and evolutionary information, reproductive biology in the group has been poorly investigated.

Although a morphological mosaic may be responsible for uncertain generic delimitation in the *Deppea* complex, of the taxonomic characters have not been tested together in a cladistics framework and have been assumed to be highly homoplastic. The aim of this study was to analyze morphological reproductive traits such as the corolla form and color, and fruitform and dehiscence in the *Deppea* complex using the cladistic method. We aim to better understand and describe the morphological features as homology hypotheses, to explore their systematic value, and last but not least, to identify without bias, potential generic circumscriptions. Some questions are pertinent in this context: (1) Are the reproductive traits taxonomically used within *Deppea* complex supported as homology hypotheses? (2) Are there characters that can be postulated as synapomorphies at different hierarchical ranks? (3) Which characters are potentially useful to diagnose genera?

Material and methods

TAXON SAMPLING

The taxon set for Hamelieae included in Stranzinger et al. (2014) was extended with other Hamelieae members from other phylogenetic studies (Manns & Bremer, 2010). Some

genera representing other tribes of Cinchonoideae (Hillieae and Chiococceae), were used as outgroups. Taxon names with authorities based on Borhidi (2012), vouchers, and GenBank accession numbers are presented in Appendix 1.

CHARACTER SAMPLING

The plastid marker *trnL-F* and the nuclear ribosomal ITS region were downloaded from GenBank for our study because these markers are the most well represented for tribe Hamelieae. The resulting matrix was manually aligned using PhyDE v.0 995 (Müller et al., 2005).

Information on reproductive traits characters traditionally used to distinguish genera of Hamelieae was obtained from the literature (Lorence & Dwyer, 1988; Borhidi et al., 2004a, 2004b, Borhidi & Stranzinger, 2012; Lorence, 2012), through examination of voucher specimens collected in the field and deposited in several herbaria (CHAP, CHIP, C, ENCB, FCME, MEXU, SERBO and XAL) and of type images available at JStor (www.JStorPlants). A morphological character matrix of 24 characters (Table 1) was constructed and edited in Winclada (Nixon, 1999a).

PHYLOGENETIC ANALYSES

Maximum Parsimony (MP) analyses were performed using the concatenated matrix with nucleotides plus morphological matrix, only including potentially parsimony informative characters. A heuristic search with 10 000 replicates of Wagner trees constructed with random taxon addition followed by TBR branch swapping was performed in TNT version 1.1 (Goloboff et al., 2003); 10 trees were saved on each replicate and a further TBR was conducted to completion saving up to 10 000 trees. These trees were submitted to further

analysis using the “new technology” option, alternating ratchet parsimony (Nixon, 1999b), sectorial, drift, and tree fusion (Goloboff, 1999) options. One hundred initial sequences were used until the consensus was stabilized 10 times using a 100% factor. All the most parsimonious trees were collected and opened in WinClada (Nixon, 1999a) and summarized in a strict consensus tree. Additionally, a Jackknife analysis (JK) was executed in TNT version 1.1 (Goloboff et al., 2003), re-sampling the matrix 10 000 times with the same parameters used for the new technology searches; we considered a node be moderately supported if the JK value is 64-85% (Farris et al., 1996), while we treated the node as highly supported if it is above 85%. Nodes with JK below 64% are considered statistically not supported.

The morphological characters were optimized onto the MP trees using the Fitch optimization criterion (Fitch, 1971) as implemented in Winclada (Nixon, 1999a). The trees and figures were edited using the program CorelDraw v. 13.

Results

The aligned matrix with the two regions consisted of 2414 characters, of which 235 (9.8 %) were potentially informative for parsimony. The morphology matrix resulted in 24 characters that were potentially parsimony informative. Detailed matrix statistics for each marker contribution are given in Table 1. The combined matrix is presented in online Appendix 2.

Table 1. Contribution of the different genomic regions to the total matrix.			
Dataset	Sequences	Aligned positions	Potentially informative chars.
ITS	68	525	120 (22%)

<i>trnL-F</i>		1889	115 (6%)
Total		2414	235 (9.8%)

The addition of morphological characters to the molecular matrix improved the resolution of the phylogenetic hypothesis (full trees based only in the molecular dataset are presented in Appendix 1).

Figure 1 shows that the resulting consensus tree (L= 582 steps, Ci= 0.9, Ri= 0.85) from the 47 most parsimonious trees (L= 577 steps, Ci= 0.6, Ri= 0.86) retrieved a well-supported tribe Hamelieae (100% JK) formed by three main clades in unresolved relationships: clade A (99% JK) includes the monotypic *Syringantha* (100% JK) plus *Hamelia* (99% JK); clade B (99% JK) includes *Bellizinca*, *Csapodya* (100% JK), *Deppea*, *Edithea*, and *Schenckia*, and clade C includes *Deppeopsis*, *Hoffmannia* (94% JK), *Omiltemia*, *Plocaniophyllon*, *Pinarophyllon*, and *Pseudomiltemia* (78% JK). The genera of the *Deppea* complex are separated into the clades B and C, for which the internal relationships are unresolved. Of the genera represented with two samples, only *Csapodya* and *Pinarophyllon* are monophyletic.

The tribe Hamelieae is supported by two synapomorphies: absence of bracts and yellow flowers, plus the homoplastic dorsifixed anthers. Within the tribe, clade A is morphologically supported by the synapomorphic corymb inflorescence and the corolla tube length (1.1-2 cm) plus the homoplastic tubular corolla, also shared with *Bellizinca*, *Csapodya* and *Omiltemia*, basifixed anthers as in *Csapodya*, *Hoffmannia*, *Plocaniophyllon* and *Pseudomiltemia*, and the elliptic fruits, also shared with *Deppea amaranthina* (Fig. 1).

The genus *Syringantha* is supported by homoplastic stamen basal insertion, a character condition also in *Bellizinca*, *Omitemia*, and *Pinarophyllon*, and, winged applanate seeds, unique features among the sampled Hamelieae. The genus *Hamelia* is supported by the synapomorphic 5-locular ovary plus the fleshy indehiscent berry fruits also shared with *Hoffmannia* (Fig. 1).

Clade B is supported by several homoplasies: the glandular calyx sinus present in all samples except for *Csapodya*, *Edithea schiblii* and *Bellizinca*, also shared with some *Deppeopsis* in clade C. Other homoplasies are the short corolla tube (less than 0.5 cm), turbinate fruits less than 0.5 cm long with poricidal dehiscence, both character conditions with several reversions within the clade and shared with other Hamelieae genera. Within clade B there is no structure; all sampled are in an unresolved clade, except for *Deppea microphylla-Deppea purpusii* (95% JK), which are supported by the homoplastic scorpioid cyme inflorescence and the two species of *Csapodya* (100% JK) characterized by many homoplastic features plus three synapomorphies: yellow and red corollas with the tube expanded at medium portion and the medial stamen insertion (Fig. 1).

Clade C is supported by the homoplastic axillar inflorescences plus flowers disposed in scorpioid cymes and the applanate seeds, shared with many other Hamelieae. Within this clade there is a little more structure; *Deppeopsis foliosa-Deppeopsis tubaeana* form a clade (96% JK) supported by the corolla tube and fruit lesser than 0.5 cm long, the same homoplastic features that support *Plocaniophyllon-Pinarophyllon* (87% JK) plus the spheroidal fruits with poricidal dehiscence and polygonal seeds. The two sampled species of *Pinarophyllon* formed a group supported by the synapomorphic circular seeds and the homoplastic convolute corolla aestivation, with basal stamen insertion and turbinate fruits,

while *Plocaniophyllon* is characterized by the homoplastic hypocrateriform corolla (also shared with *Pseudomiltemia* and *Editheia*) and the racemose inflorescence and valvate aestivation, unique conditions for Hamelieae but shared with the outgroup genus *Chiococca*. *Hoffmannia* (94% JK) is monophyletic and well supported by the combination of several homoplasies: cymose inflorescence, basifixed anthers and the fleshy spheroidal fruits with polygonal seeds (Fig. 1).

INFLORESCENCES

The morphological characters form a mosaic in which all states among Hamelieae show high variation. For the Hamelieae, flowers disposed in inflorescences is the ancestral condition. Some species are characterized by solitary flowers (*Omiltemia longipes* and *Pinarophyllon bullatum*) or by a combination of solitary flowers and disposed in few-flowered inflorescences (*Deppea microphylla* and *Deppea purpusii*) (Figure 2). The terminal inflorescence is the ancestral condition. The axillar inflorescences characterize all taxa of clade C and evolved many times in taxa of clade B. The pendulous inflorescence evolved three times in clade B (*Bellizinca*, *Csapodya*, and *Deppea microphylla*) and once in clade C (*Omiltemia*). The corymb inflorescence is the ancestral condition from which evolved several other types of inflorescences, such as the scorpioid cymes (in some *Deppea* and *Deppeopsis*), paniculate (*Hamelia* and *Syringantha*), racemose (*Plocaniophyllon*) or cymes (*Csapodya*, *Hoffmannia* and *Omiltemia*). The ebracteated inflorescence is synapomorphic for the tribe Hamelieae (Figure 1, 2).

FLOWERS

The ancestral condition for the number of floral parts can be 4-merous or 5-merous; pentamerous flowers characterize clade A, while B and C share the tetramerous flowers. The calyx lobe sinus can be glandular or naked, the latter state is the ancestral condition for Hamelieae and characterized clades A and C; for the glandular calyx sinus evolved in clade B with several reversions to naked (*Bellizinca*, *Csapodya*, and *Edithea schiblii*), whereas for clade C the taxa *Deppeopsis hernandezii* and *Deppeopsis tubaeana* have glandular calyx sinus. For Hamelieae, the rotate corolla form is the ancestral condition, the tubular form evolved in *Syringantha* and *Hamelia* (clade A), *Bellizinca*, and *Csapodya* (clade B) and *Omitemia* (Clade C); the hypocrateriform corolla evolved in *Edithea* (clade B), *Omitemia*, and *Plocaniophyllon* (clade C) while an infundibuliform corolla is present in *Deppea cornifolia* (clade B). The yellow corolla is the ancestral condition for Hamelieae; red-colored flowers evolved in some *Hamelia* species (clade A) and *Omitemia* (clade C), purple-colored flowers evolved in *Deppea purpurascens* (clade B) and a bicolor corolla in *Csapodya* (clade B). The imbricate aestivation is the ancestral condition for the tribe, *Bellizinca* (clade B) shows valvate and convolute in *Schenckia* and *Csapodya* (clade B). An un-constrained tube is the ancestral condition for Hamelieae, the tube expanded at medium portion evolved in *Csapodya* (clade B) while *Omitemia* (clade C) has the tube constrained at base. Corolla tube length is very variable; the ancestral tube length is 0.6 to 1 cm long from which several lengths evolved many times in all the clades (Figure 1, 2).

ANDROECIUM/GYNOECIUM

The basal condition for the anther insertion to the filament can be basifixed or dorsifixed for Hamelieae, clade A has basifixed anthers, clade B has dorsifixed anthers except for *Csapodya* which have basifixed; in clade C, *Hoffmannia*, *Plocaniophyllon* and

Pseudomiltemia have basifixed anthers while *Deppeopsis*, *Omiltemia* and *Pseudomiltemia* have dorsifixed. The stamen inserted in the apex of the corolla tube (throat insertion) is the ancestral condition for Hamelieae; basal insertion evolved in *Syringantha* (clade A), *Bellizınca* (clade B), *Omiltemia*, and *Pinarophyllon* (clade C); the medially inserted stamens evolved in *Csapodya* (clade B). The 2-locular ovary is the ancestral condition for the tribe, only in *Hamelia* (clade A) is the ovary 5-locular (Figure 3).

FRUITS

The fleshy berry fruits evolved two times independently in the tribe Hamelieae: *Hamelia* (clade A) and *Hoffmannia* (clade C). Cylindrical is the ancestral fruit form for the tribe, elliptic fruits characterize *Syringantha*, *Hamelia* (clade A) and *Deppea amaranthina* (clade B), turbinate fruits are present in *Deppea*, *Csapodya*, *Edithea*, *Schenckia* (Clade B) and *Pinarophyllon* and *Pseudomiltemia* (clade C) while spheroidal form is present in the fruits of *Deppea inaequalis*, *Deppea erythrorhiza* (clade B), *Plocaniophyllon* and *Hoffmannia* (clade C). The ancestral reconstructed dehiscence can be septicidal or poricidal; *Hamelia* (clade A) and *Hoffmannia* (clade C) have indehiscent berry fruits; the capsules of *Syringantha* (clade A) and *Deppeopsis* (clade C) have septicidal dehiscence; *Bellizınca* (clade B) have loculicidal dehiscence while in the remaining genera the capsules are poricidal: *Deppea*, *Csapodya*, *Edithea*, *Schenckia* (clade B) plus *Omiltemia*, *Pinarophyllon*, *Pseudomiltemia*, and *Plocaniophyllon* (clade C). The fruit length is variable; the ancestral condition is fruits from 0.5 to 1 cm long, from which several lengths evolved many times in the three clades (Figure 3).

SEEDS

The wingless seed is the ancestral condition for Hamelieae; winged seeds evolved only in *Syringantha* (clade A). The polygonal seed is the ancestral condition for the tribe. Applanate seeds evolved in *Syringantha* (clade A); *Csapodya* (clade B); and *Deppeopsis*, *Omitemia*, and *Pseudomitemia* (clade C). The ellipsoid seeds are characteristic for all tribe members except *Pinarophyllon* (clade C), which has circular seeds (Figure 3).

Discussion

The protologue diagnosis and key to genera in taxonomic treatments remarks the utility of reproductive characters to distinguish genera of the *Deppea* complex. These characters include calyx lobe shape and color, corolla tube shape, size, and color, and fruit form. The combination of these characters permits differentiation among the genera (Lorence & Dwyer, 1988, Borhidi, 2004). In our phylogenetic tree, the *Deppea* complex was polyphyletic and none of its genera were retrieved as monophyletic, which reflects the intricate morphological complexity of the group.

The genera *Syringantha* and *Hamelia* were retrieved as sister groups and share paniculate inflorescences with flowers with tubes of 1.1-2 cm length. Within this clade evolved the fleshy berry fruits (*Hamelia*) from dry capsular ones (*Syringantha*). This shift from dry to fleshy fruits occurred also in *Hoffmannia* in clade C and is also relatively common in Rubiaceae (e.g. Terrell & Wunderlin, 2002; Torres Montúfar et al. 2017, 2020).

For clade B, *Deppea* resulted paraphyletic regarding to *Bellizinca*, *Csapodya*, *Edithea*, and *Schenckia*, same result obtained by Manns & Bremer (2010) and Stranzinger et al. (2014), the morphology supports this group by the combination of several homoplasies such as the glandular calyx sinus, the corolla tube lesser than 0.5 cm, turbinate

fruits up to 0.5 cm in length, conditions also present in taxa in clade C. The lack of resolution in addition to the morphological overlapping lead some authors to treat all these genera as *Deppea* synonyms (Lorence & Dwyer, 1988; Manns & Bremer, 2010; Lorence, 2012).

The monotypic Mexican genus *Bellizinca* was described based on “erect inflorescences”, basal stamen insertion and corolla tube 2.4–4 cm length (Borhidi et al., 2004a). However, for the inflorescence orientation our fieldwork rejected the erect inflorescence, which is undoubtedly an artifact of specimen mounting and after revisiting several specimens the corolla tube length at anthesis was redefined as greater than 3 cm. Our study includes only one sample of *Bellizinca*, which has several homoplasies: pendulous inflorescences, disposed on scorpioid cymes, naked calyx sinus, tubular corolla with tube 3.1 to 4 cm long, basal stamen insertion, and loculicidal capsules of 0.6 to 1 cm long; all these characters are shared with many other taxa in Hamelieae. The taxonomic history of *Bellizinca* exposes its morphological conflict; it was directly segregated from *Deppea scoti* (J.H. Kirkbr.) Lorence by Lorence & Dwyer (1988) and originally described as *Omiltemia scoti* J.H. Kirkbr. in 1984 and recently treated again as *Deppea* (Lorence, 2012).

Bellizinca resembles *Csapodya* by the large corolla tubes and the pendulous inflorescences. The genus *Csapodya* was segregated from *Deppea splendens* (Borhidi et al. 2004a) and currently includes three species distributed from Mexico to Guatemala (Borhidi & Reyes-García, 2007). Its segregation was based on the pendulous inflorescences (described as “scandent” by Borhidi et al. (2004a), the stamens inserted in the throat, and the corolla tube up to 5 cm long. Of these characters, we redefined the stamen insertion to

medially inserted, concordant with a corolla tube expansion. In the Rubiaceae treatment of Mesoamerican Flora, Lorence (2012) treats *Csapodya* as a synonym of *Deppea*, arguing that the large corolla tube is just an adaptation for bird pollinators (Lorence, 2012). However, this decision needs to be further evaluated since the *Csapodya* clade in our study is well-supported by several characters including three synapomorphies: bicolored flowers (yellow and red), the tube expanded at medium portion and medium stamen insertion.

Lorence (2012) for Mesoamerica only recognizes the species *Deppea splendens* (Breedlove & Lorence) Borhidi, mentioning that the other two species were described based on immature inflorescences. Our study includes three samples of the two species recognized as *Csapodya* by Borhidi & Reyes-García (2007). These taxa were retrieved in a well-supported clade but with unsolved positions, and there are no autoapomorphies for the species, therefore its synonymy is feasible.

The Mexican genus *Editheia*, described by Standley (1933), was synonymized with *Deppea* (Lorence & Dwyer, 1988), resurrected (Borhidi et al. 2004a), and again synonymized with *Deppea* (Lorence, 2012). The large corolla tube (compared with *Deppea*) with the stamens inserted at the throat were the characters used to separate both; however, with the description of many other *Editheia* species (Borhidi et al. 2004a; Borhidi, 2012) its circumscription became wider and with high overlapping with many other genera of the *Deppea* complex. Our study sampled three of the eight species currently recognized of *Editheia*, all of which were retrieved in a polytomy with the rest of taxa in clade B. This result makes it impossible to reach a taxonomic decision on *Editheia*.

The genus *Schenckia* is treated as a synonym of *Deppea* (Lorence & Dwyer, 1998; Manns & Bremer, 2010; Lorence, 2012). Its inclusion expands the distribution of *Deppea*

to a disjunct pattern since *Schenckia* is known from Blumenau in Brazil and northern Argentina (Lorence & Dwyer, 1988), whereas *Deppea* is practically restricted to the Mesoamerican region. Aside from the geographic position, *Schenckia* shares many morphological characters with *Deppea*, so its synonymy is corroborated.

For clade C, even though there are no synapomorphic conditions, *Hoffmannia* is well supported as monophyletic, and it is easily distinguished from other Hamelieae members by its berry fruits retrieved as homoplastic (shared with *Hamelia*). *Hoffmannia* also shares the cymose inflorescence and rotate flowers with many members of the *Deppea* complex, sometimes without fruits it is difficult differentiate from *Deppea* species.

The two species of the Mesoamerican genus *Pinarophyllon* are retrieved in a supported clade by the synapomorphic circular seeds plus the homoplastic basal stamen insertion. The genus *Pinarophyllon* was described based on herbaceous habit (Brandege, 1914; Lorence, 2012), unique among the tribe. It is retrieved as the sister group of the monotypic Mexican genus *Plocaniophyllon*, described based on its lineolate leaf venation (Brandege, 1914; Lorence, 2012). The diagnostic characters for these genera were not coded for our analysis, since they are vegetative; however, the clade is supported by the homoplastic flowers and fruits less than 0.5 cm long and corroborated the acceptance of both genera in the Rubiaceae treatments (Borhidi, 2012; Lorence, 2012).

The Mexican genus *Omiltemia*, described by Standley (1918) based on its red, large corolla, currently includes three species (Borhidi et al., 2004b; Borhidi & Velasco-Gutiérrez, 2010; Borhidi, 2012; Lozada-Pérez & Rojas Gutiérrez, 2016). Latter, the Mexican bitypic genus *Pseudomiltemia* was segregated from *Omiltemia* (Borhidi et al. 2004b) mainly based on its stamen insertion (apical in *Pseudomiltemia* vs. basal in

Omitelia). Our analysis represented one sample per genus, and these are retrieved in the unresolved clade C. The species *Omitelia longipes* is characterized by the synapomorphic tube constriction at base, diagnostic for the genus, coincident with the stamen insertion, also has many homoplastic features that are rare among Hamelieae such as the solitary flowers (shared with *Pinarophyllon bullatum*) disposed in pendulous pedicels, and red corolla (also in some *Hamelia* species). *Pseudomitelia filisepala* is characterized by a combination of homoplasies: cymose inflorescences, hypocrateriform corolla with tube of 3.1 to 4 cm long, and turbinate fruits longer than 1 cm. There is agreement in the acceptance of both genera.

The genus *Deppeopsis* was described by Borhidi & Stranzinger (2012) based on the cylindrical fruits and the septicidal dehiscence in contrast to the turbinated capsules with poricidal dehiscence of most *Deppea* species. It includes five species, of which four are endemic to Mexico and one is also shared with Guatemala. In the Mesoamerican Rubiaceae treatment only two species of this genus are included and treated under *Deppea* by its morphological similarity (Lorence, 2012). In the phylogeny of Stranzinger et al. (2014) *Deppeopsis* is polyphyletic, the species *Deppeopsis foliosa* and *Deppeopsis tubaeana* are the sister clade to *Plocaniophyllon* while *Deppeopsis hernandezii* is retrieved in the polytomy formed by *Pseudomitelia*, *Hoffmannia*, and *Omitelia-Renistipula*. Our combined matrix resulted in *Deppeopsis foliosa* and *Deppeopsis tubaeana* form a clade supported by the corolla tubes and fruits less than 0.5 cm long, both homoplastic, while *Deppeopsis hernandezii* is characterized by the glandular calyx sinus, also a homoplastic feature. The unresolved positions of the *Deppeopsis* species rises the hypothesis that with a broader character and taxon sampling a monophyletic genus will be retrieved and accepted.

Although the circumscription of *Deppeopsis* needs to be evaluated, it is evident that its synonymy with *Deppea* is not reliable and should be considered a separate entity or entities.

INFLORESCENCES

For Rubiaceae, the inflorescences are quite variable and not diagnostic for the lineages (Robbrecht, 1988). For the Hamelieae, the solitary flower condition evolved at least three times in the sampled taxa. There are some species characterized by solitary flowers (*Omitemia longipes* and *Pinarophyllon bullatum*) or by a combination of solitary flowers and disposed in few-flowered inflorescences (*Deppea microphylla* and *Deppea purpusii*). The flowers disposed in inflorescences are the ancestral condition for this character. Also, the inflorescence position (axillar vs terminal) and orientation (erect vs pendulous) are variable among Hamelieae, the terminal and erect inflorescences are the ancestral conditions, and the axillar inflorescence characterize all of clade C and evolved several times in clade B, even in many taxa (e.g., *Deppea purpurascens*) both axillar and terminal inflorescences are present. The inflorescence orientation is treated by some authors as a hummingbird adaptation (Silva et al., 2010), and it is correlated with the larger corolla tubes in Hamelieae (e.g. *Bellizinca*, *Csapodya*, and *Omitemia*); however, there are some *Edithea* or *Hamelia* species with large corolla tubes and erect inflorescences also visited by hummingbirds.

The inflorescences in Rubiaceae have been studied to delineate their architecture in detail (Weberling, 1977) and this architecture is used as a character to diagnose taxa, including infra-generic sections (Sohmer, 1978) and species (De Block, 1998). In general, Rubiaceae inflorescence has been studied only lightly in an evolutionary context (Delprete,

2001; Achille et al., 2006; Razafimandimbison et al., 2012) and its taxonomic value is underestimated. Here, we will not delve into the core architecture of the inflorescence, but we note its contribution and highlight the need of further studies for Hamelieae.

FLOWERS

The 4-merous corolla lobes are characteristic for clades B and C, while 5-merous flowers are only present in clade A in Hamelieae, due the unresolved relationships in these clades define the ancestral condition for the tribe is not clear. The naked calyx lobes are the ancestral condition for Hamelieae, for the clade B, a glandular calyx sinus evolved but also present many reversions in our cladogram (*Bellizinca*, *Csapodya* and *Edithea schiblii*). For clade C, *Deppeopsis hernandezii* and *Deppeopsis tubaeana* shares the glandular calyx sinus.

The corolla form is one of the most variable characters in literature for the Hamelieae. Clade A is well defined by the tubular corolla, a condition shared with *Bellizinca* and *Csapodya* (in clade B) and *Omiltemia* (in clade C). Most of the *Deppea* species shares the rotate corolla, except for *Deppea cornifolia* (infundibuliform). The rotate corolla form is also present in *Deppeopsis*, *Hoffmannia* and *Pinarophyllon* in clade C, while a hypocrateriform corolla is the condition of *Edithea*, *Plocaniophyllon*, and *Pseudomiltemia*. The corolla form deserves further study and relies on fieldwork. In pressed herbarium specimens it is often difficult to define the shape.

The corolla shape is related to the corolla tube length, rotate form is related to shortened tubes (less than 0.5 cm) while the rest are present in corollas with tubes conspicuous of more than 0.6 cm, except for *Deppea hernandezii*, which have a corolla

tube of 1 cm long and rotate corolla. The tube length was used to differentiate the genera of the *Deppea* complex, classifying them into large (*Bellizinca*, *Csapodya*, *Edithea*, *Omiltemia*, *Pseudomiltemia*), and shortened corollas (*Deppea*, *Deppeopsis*, *Plocaniophyllon*, *Pinarophyllon* and *Schenckia*); however, some *Deppea* species (e.g. *Deppea cornifolia*) can have tubes up to 1 cm long. The length of the tube is considered by Lorence & Dwyer (1988) as an extreme adaptation to hummingbird pollinators in *Bellizinca*, *Csapodya*, and *Edithea*, thus all are considered as *Deppea* synonyms. Also, large variation in corolla size is relatively common in genera of Rubiaceae as *Palicourea* (Taylor, 2012) or *Arachnothryx* (Torres-Montúfar, 2018); its correlation to pollinators should be studied.

The flower color has been widely used to differentiate genera, as *Omiltemia* with red colored flowers, or *Csapodya* with bicolor flowers ranging from red to yellow along a gradient. Yellow corolla is the ancestral condition for Hamalieae and is the predominant color in the tribe; purple corollas are rare, however are present in *Deppea purpurascens*, also white flowers are not common but present in some *Hoffmannia* species and in *Deppea densiflora* (not sampled).

The aestivation is another variable character within Hamelieae, with all members with imbricate petal disposition in bud, from this condition evolved the convolute in which the four petals are twisted together, in *Csapodya*, *Omiltemia*, *Pinarophyllon*, *Pseudomiltemia* and *Schenckia*. The valvate aestivation in *Bellizinca* is unique among Hamelieae but very common in Rubiaceae (e.g., Rubioideae).

Aside the corolla form, in some lineages there is a tube constriction that can be taxonomically useful to differentiate them. The ancestral condition is the tube not

constrained, for *Csapodya* the tube is expanded at the medium portion, coincidentally with the medial stamen insertion, while for *Omitemia* the tube is notably constrained at base also related to the basal stamen insertion; nevertheless, the corolla of other Hamelieae with basal stamen insertion as *Bellizinca* or *Pinarophyllon* is not constrained. Again, we highlighted the need of deeper studies on floral morphology for this group.

ANDROECIUM/GYNOECIUM

As we state above, stamen insertion in the Hamelieae ranges from basal, medium and apical. The ancestral condition for the tribe is throat insertion or apical, the basal insertion evolved in *Syringantha*, *Bellizinca*, *Omitemia* and *Pinarophyllon*. The stamen position is variable among Rubiaceae (Robbrecht, 1988) even in the same tribe as Rondeletieae (Torres-Montúfar et al., 2020). Furthermore, the anther insertion to the filament can be a useful character barely explored in Rubiaceae, for Hamelieae there is ambiguity to define if the basifixed or dorsifixed condition is the ancestral, both are widespread in the genera of the tribe and evolved many times.

As in Hamelieae, 2-locular ovaries are a very common feature in many Rubiaceae lineages (Robbrecht, 1988). The ovaries of the genus *Hamelia* is typically 5-locular, however there are some species with 4-locular (Pacheco-Trejo & Lorence, 2012), this condition can be related to the shift to fleshy fruits from capsular, as in *Arachnothryx-Gonzalagunia* (2-locular capsules to 4-locular drupes) (Torres-Montúfar et al., 2017).

FRUITS

For Hamelieae, the dry fruit (capsules) is retrieved as the ancestral condition, from which evolved the fleshy berry fruits two times in *Hamelia* and *Hoffmannia*. The shifts dry-fleshy

are not rare among Rubiaceae, Bremer & Eriksson (1992) mapped fruit evolution onto a Rubiaceae molecular phylogenetic tree suggesting that dry fruits have 12 shifts to fleshy in within Rubiaceae while Torres-Montúfar (2018) reported two shifts from dry to fleshy in Guettardeae.

The fruit form is very variable. The ellipsoidal fruits are characteristic for clade A (*Hamelia* and *Syringantha*) but also are present in *Deppea amaranthina*. The cylindrical fruits are the ancestral condition for this character, this fruit form was a diagnostic feature to segregate *Deppeopsis* from *Deppea* (turbinate) (Borhidi et al., 2004a) however it was not synapomorphic since *Deppeopsis* is polyphyletic and the cylindrical condition is also shared with *Omiltemia*. Except for *Deppeopsis* (septicidal) and *Bellizinca* (loculicidal), all the resting genera of the *Deppea* complex have poricidal fruit dehiscence. The ancestral condition is ambiguous, it can be poricidal or septicidal. The fruit size is related to corolla tube size, larger flowers produce larger fruits as in *Bellizinca*, *Csapodya*, *Omiltemia* and *Pseudomiltemia*.

SEEDS

Within Hamelieae, the winged seeds are diagnostic for *Syringantha* (McDowell, 1996), while the rest of the genera within Hamelieae have wingless seeds. The winged seed is a very variable character among Rubiaceae and diagnostic for many lineages such as Chiococceae (Motley et al., 2005). The seed compression is another useful character for Hamelieae, the applanate seeds are present in *Csapodya*, *Deppeopsis*, *Omiltemia*, *Pseudomiltemia* and *Syringantha*. For a deeply discussion of seed morphology in the Hamelieae see Aguilar-Morales et al. (2022).

Conclusions

The present study improves our understanding of the morphological characters and its utility on establishing generic limits in Hamelieae and the *Deppea* complex. The study combines chloroplast and nuclear data combined with morphological evidence. Concerning the *Deppea* complex, although our phylogenetic tree retrieved a polytomy, some relationships are delineated, as *Deppeopsis* accepted as a generic entity separated from *Deppea*. The genus *Deppea* resulted paraphyletic with *Bellizinca*, *Csapodya*, *Edithea* and *Schenckia*, corroborating the currently generic circumscription in which all are merged, however for *Bellizinca* and *Csapodya* there are many morphological characters that can support its acceptance in a more extensive sampling of molecular markers and taxa. Our study also highlighted the homoplastic nature of the main morphological characters used to split genera from *Deppea* as the corolla shape and size inviting to explore new characters and deeply revise others.

Acknowledgments

Literature cited

Achille, F., T. J. Motley, P.P. Lowry II & Jeremie, J. 2006. Polyphyly in Guettarda L. (Rubiaceae, Guettardeae), based on nrDNA ITS sequence data. *Annals of the Missouri Botanical Garden* 93: 103–120.

Anderson, G. J., G. Bernardello, T. Stuessy & D. Crawford. J. 2001. Breeding systems and pollination of selected plants endemic to the Juan Fernandez Islands. *American Journal of Botany* 88: 220–233.

Andersson, L. & C. Persson. 1991: Circumscription of the tribe Cinchoneae (Rubiaceae), a cladistic approach. *Plant Systematics and Evolution* 178: 65–94.

Barrabé, L., S. Buerski, A. Mouly, A. P. Davis, J. Munzingerand & L. Maggia. 2012. Delimitation of the genus *Margaritopsis* (Rubiaceae) in the Asian, Australasian and Pacific region, based on molecular phylogenetic inference and morphology. *Taxon* 61: 1251–1268.

Borhidi, A. 2006. *Rubiáceas de México*. Akadémiai Kiadó, Budapest.

Borhidi, A. 2012. *Rubiáceas de México*. Akadémiai Kiadó, Budapest.

Borhidi, A. & S. Stranzinger. 2012. *Deppeopsis*, un género nuevo (Hamelieae, Rubiaceae) de México y Guatemala. *Acta Botanica Hungarica* 54: 86–89. DOI:

Borhidi, A., J. Darók, M. Kocsis, S. Stranzinger & F. Kaposvári. 2004a. Critical revision of the *Deppea* complex (Rubiaceae, Hamelieae). *Acta Botanica Hungarica* 46: 77–81. DOI:

Borhidi, A., J. Darók, M. Kocsis, S. Stranzinger & F. Kaposvári. 2004b. Critical revision of the *Omiltemia* complex (Rubiaceae, Hamelieae). *Acta Botanica Hungarica* 46: 82–85. DOI:

Borhidi, A. & A. Reyes-García. 2007. Estudios Sobre Rubiáceas Mexicanas XI Revisión del género *Csapodya* Borhidi (Rondeletieae), endémico de Chiapas con tres especies. *Acta Botanica Hungarica* 49: 13–25. DOI:

Borhidi, A. & K. Velasco-Gutierrez. 2010. Estudios sobre Rubiáceas Mexicanas XXVI. *Paradeppea* sect. nova del género *Deppea* Cham. et Schltld. (Hamelieae) y dos especies nuevas de Oeste de Oaxaca. *Acta Botanica Hungarica* 52: 41–48. DOI:

Brandege, T.S. 1914. *Plantae Mexicanae Purpusianae*. University of California Publications of Botany 6(4): 69.

Bremer, B. 1987. The sister group of the paleotropical tribe Argostemmatae: a redefined neotropical tribe Hamelieae (Rubiaceae). *Cladistics* 3: 35–51. DOI:

Bremer, B. & O. Eriksson. 1992. Evolution of fruit characters and dispersal modes in the tropical family Rubiaceae. *Biological Journal of the Linnean Society* 47: 79–95. DOI: <https://doi.org/10.1111/j.1095-8312.1992.tb00657.x>.

Bremer, B. & L. Struwe. 1992. Phylogeny of the Rubiaceae and the Loganiaceae: congruence or conflict between morphological and molecular data? *American Journal of Botany* 79: 1171–1184. DOI:

Bremer, B. 1996. Combined and separate analyses of morphological and molecular data in the plant family Rubiaceae. *Cladistics* 12: 21–40. DOI:

De Block, P. 1998. The African species of *Ixora* (Rubiaceae-Pavetteae). *Opera Botanica Belgica* 9: 1–218.

Delprete, P. G. 2001. Notes on some South American species of *Psychotria* subgenus *Heteropsychotria* (Rubiaceae), with observations on rubiaceous taxonomic characters. *Brittonia* 53: 396–404.

Faegri, K. & L. van der Pijl. 1979. The principles of pollination ecology. Pergamon Press, Oxford, United Kingdom.

Farris, J.S., V. Albert, M. Källersjö, D. Lipscomb & A. G. Kluge, A.G. 1996. Parsimony Jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124. DOI: 10.1006/clad.1996.0008.

Fenster, C., W. Armbruster, P. Wilson, M. Dudash & J. Thomson. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology and Systematics* 35: 375–403. DOI:

Fitch, W. M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* 20: 406–416. DOI:

Giribet, G. 2015. Morphology should not be forgotten in the era of genomics, a phylogenetic perspective. *Zoologischer Anzeiger* 256: 96–103. DOI:

Goloboff, P. 1999. Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics* 15: 415–428. DOI: 10.1006/clad.1999.0122

Goloboff, P., S. Farris & K. C. Nixon. 2003. TNT (Tree analysis using New Technology) (BETA) ver. 1.1. Published by the authors, Tucumán, Argentina.

Gustafsson, C. & C. Persson. 2002. Phylogenetic relationships among species of the Neotropical genus *Randia* (Rubiaceae, Gardenieae) inferred from molecular and morphological data. *Taxon* 51: 661–674. DOI:

Kainulainen, K., C. Persson, T. Eriksson & B. Bremer. 2010. Molecular systematics and morphological character evolution of the Condamineae (Rubiaceae). *American Journal of Botany* 97: 1961–1981.

Kephart, S., R. J. Reynolds, M. T. Rutter, C. B. Fenster & M. R. Dudash. 2006. Pollination and seed predation by moths on *Silene* and allied Caryophyllaceae: evaluating a model system to study the evolution of mutualisms. *New Phytologist* 169: 667–680. DOI: <https://doi.org/10.1111/j.1469-8137.2005.01619.x>

Lee, M. S. Y. & A. Palci. 2015. Morphological phylogenetics in the genomic age. *Current Biology* 25: R922–R929. DOI: <https://doi.org/10.1016/j.cub.2015.07.009>.

Löfstrand, S. D., S. G. Razafimandimbison & C. Rydin. 2019. Phylogeny of Coussareeae (Rubiaceae, Rubiaceae). *Plant Systematics and Evolution* 305: 293–304. DOI: <https://doi.org/10.1007/s00606-019-01572-8>

Lorence, D.H. & J. D. Dwyer. 1988. A revision of *Deppea* (Rubiaceae). *Allertonia* 4: 389–436. DOI:

Lorence, D. H. 2012. *Deppea*. In: G. Davidse, M. Sousa, S. Knapp & F. Chiang (eds). *Flora Mesoamericana* 4: 260–262. Missouri Botanical Garden Press, St Louis.

Lozada-Pérez, L. & J. Rojas-Gutiérrez. 2016. Una nueva especie, *Omiltemia guerrerensis* (Rubiaceae) de Guerrero, México. *Novon* 24: 365–368.

Manns, U. & B. Bremer. 2010. Towards a better understanding of intertribal relationships and stable tribal delimitations within Cinchonoideae s.s. (Rubiaceae). *Molecular Phylogenetics and Evolution* 56: 21–39. DOI:

McDowell, T. 1996. *Syringantha coulteri* (Hooker f.) McDowell, and new combination and remarks on the relationships of the monotypic Mexican genus *Syringantha* Standley (Rubiaceae). *Novon* 6: 273–279. DOI:

Motley, T. J., K. J. Wurdack & P. G. Delprete. 2005. Molecular systematics of the Catesbaeeae-Chiococceae complex (Rubiaceae) flower and fruit evolution and biogeographic implications. *American Journal of Botany* 92: 316–329. DOI: <https://doi.org/10.3732/ajb.92.2.316>.

Müller, K., D. Quandt, J. Müller & C. Neinhuis. 2005. PhyDE 0.995. Phylogenetic data editor. Published at <http://www.phyde.de/>

Nixon, K. C. 1999a. Winclada (beta) ver. 0.9. Published by the author Ithaca, NY.

Nixon, K. C. 1999b. The Parsimony Ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 407–414. Doi: 10.1111/j.1096-0031.1999.tb00277.x

Pacheco-Trejo, J. & D. H. Lorence. 2012. *Hamelia*. In: G. Davidse, M. Sousa, S. Knapp & F. Chiang (eds). *Flora Mesoamericana* 4: 113–116. Missouri Botanical Garden Press, St Louis.

Persson, C. 1996. Phylogeny of the Gardenieae (Rubiaceae). Botanical Journal of the Linnean Society 121: 91–109. DOI: <https://doi.org/10.1111/j.1095-8339.1996.tb00746.x>.

Razafimandimbison, S. G. & B. Bremer. 2001. Tribal delimitation of Naucleae (Rubiaceae): Inference from molecular and morphological data. Systematics and Geography of Plants 71: 515–538.

Razafimandimbison, S. G., S. Ekman, T. D., McDowell & B. Bremer. 2012. Evolution of growth habit, inflorescence architecture, flower size, and fruit type in Rubiaceae: its ecological and evolutionary implications. PLoS ONE 7:1-10.

Robbrecht, E. 1988. Tropical woody Rubiaceae. Opera Botanica Belgica 1: 1–272.

Silva, C. A., M. F. Vieira & C. H. Amaral. 2010. Floral attributes, ornithophily and reproductive success of *Palicourea longepedunculata* (Rubiaceae), a distylous shrub in southeastern Brazil. Revista Brasileira de Botanica 33: 207–213.

Sohmer, S. H. 1978. Morphological variation and its taxonomic and evolutionary significance in the Hawaiian Psychotria (Rubiaceae). Brittonia 30:256–264.

Soza, V. L. & R. G. Olmstead. 2010. Molecular systematics of tribe Rubieae (Rubiaceae): Evolution of major clades, development of leaf-like whorls, and biogeography. Taxon 59: 755–771. DOI: 10.1002/tax.593008

Standley, P. C. 1918. *Omiltemia*, a new genus of Rubiaceae from Mexico. Journal of the Washington Academy of Sciences 8: 427.

Standley P. C. 1933. *Edithea*, a new genus of Mexican shrubs of the family Rubiaceae
Tropical Woods 34: 1–3.

Stranczinger, S., A. Galambos, D. Szenas & B. Szalontai. 2014. Phylogenetic relationships in the Neotropical tribe Hamelieae (Rubiaceae, Cinchonoideae) and comments on its generic limits. *Journal of Systematics and Evolution* 52: 643–650. DOI:

Taylor, C. M. 2012. *Palicourea*. In: G. Davidse, M. Sousa, S. Knapp & F. Chiang (eds). *Flora Mesoamericana* 4: 163–181. Missouri Botanical Garden Press, St Louis.

Terrell, E. E. & R. P. Wunderlin. 2002. Seed and fruit characters in selected Spermaceae and comparison with Hedyotideae (Rubiaceae). *SIDA* 20: 549–557. DOI:

Torres-Montúfar, A. 2018. Sistemática del complejo *Rondeletia* (Rubiaceae). Unpublished D. Phill. Thesis, Universidad Nacional Autónoma de México.

Torres-Montúfar, A., T. Borsch, S. Fuentes, T. Clase, B. Peguero & H. Ochoterena. 2017. The new Hispaniolan genus *Tainus* (Rubiaceae) constitutes an isolated lineage in the Caribbean biodiversity hotspot. *Willdenowia* 47: 259–270. DOI:

Torres-Montúfar, A., T. Borsch & H. Ochoterena. 2018. When homoplasy is not homoplasy: dissecting trait evolution by contrasting composite and reductive coding. *Systematic Biology* 67: 543–551. DOI:

Torres-Montúfar, A., T. Borsch, S. Fuentes, J. Gutiérrez & H. Ochoterena. 2020. It is not a disaster: molecular and morphologically based phylogenetic analysis of *Rondeletieae*

and the *Rondeletia* complex (Cinchonoideae, Rubiaceae). *Plant Systematics and Evolution* 306: 1–25. DOI:

Weberling, F. 1977. Beiträge zur Morphologie der Rubiaceen-Infloreszenzen. *Berichte der Deutschen Botanischen Gesellschaft* 90:191–209.

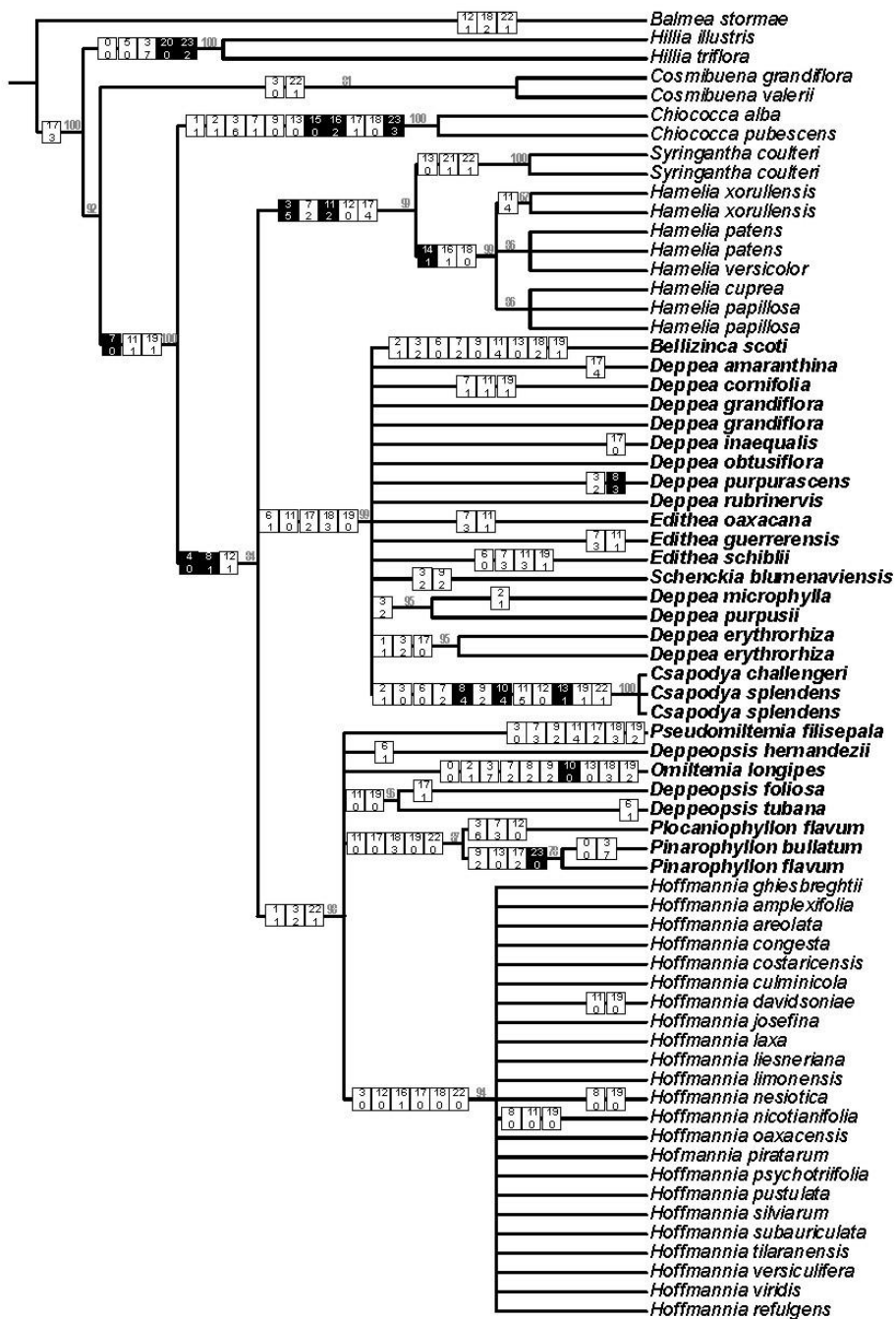


Figure 1. Parsimony consensus tree (L= 582 steps, Ci= 0.9, Ri= 0.85) of the combined matrix. Boxes include the character (number above) and character states (number below), black boxes indicate synapomorphies, white boxes are homoplasies. Numbers above branches represent Jackknife support.

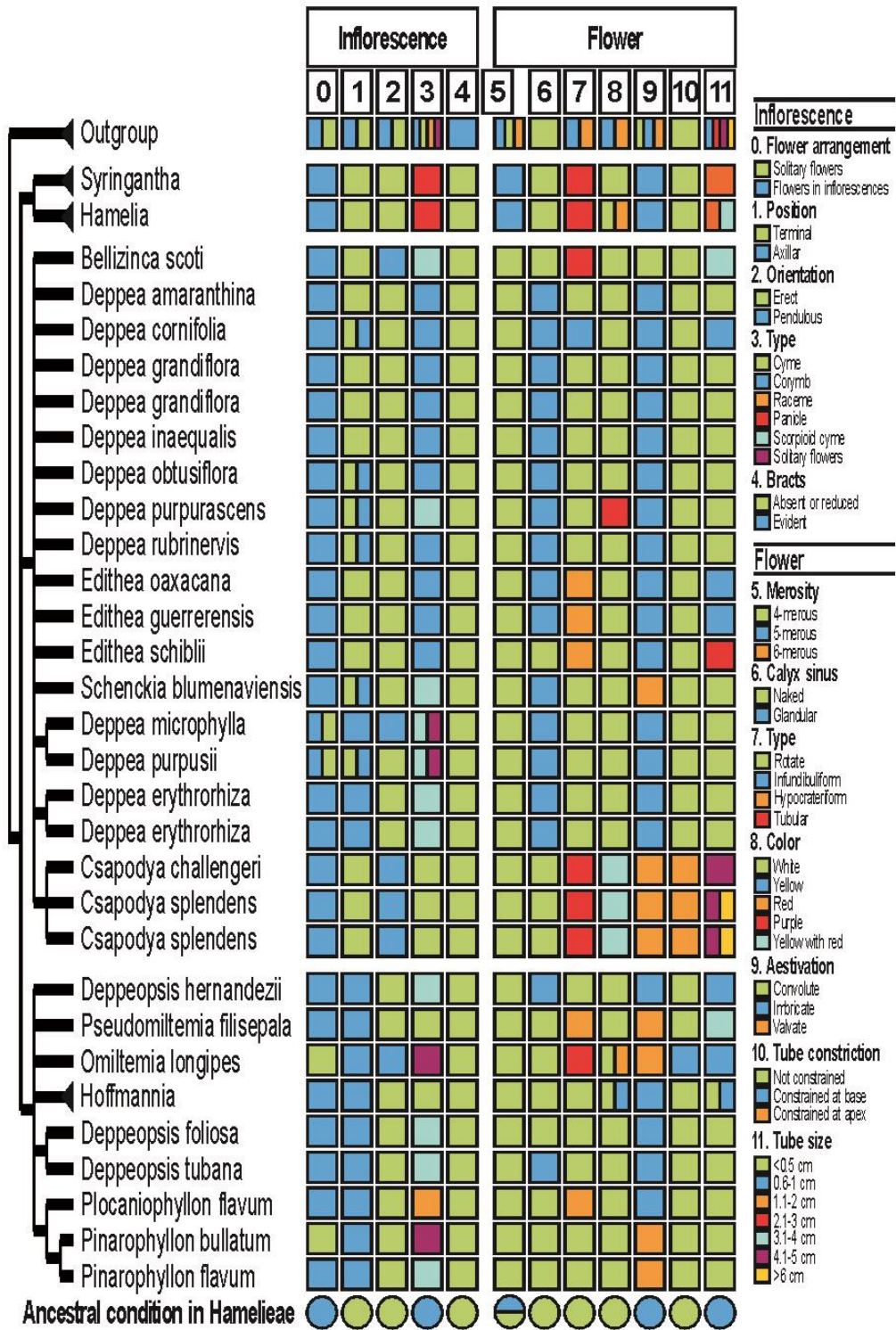


Figure 2. Parsimony consensus tree (left) with graphical representation of the morphological characters (right).

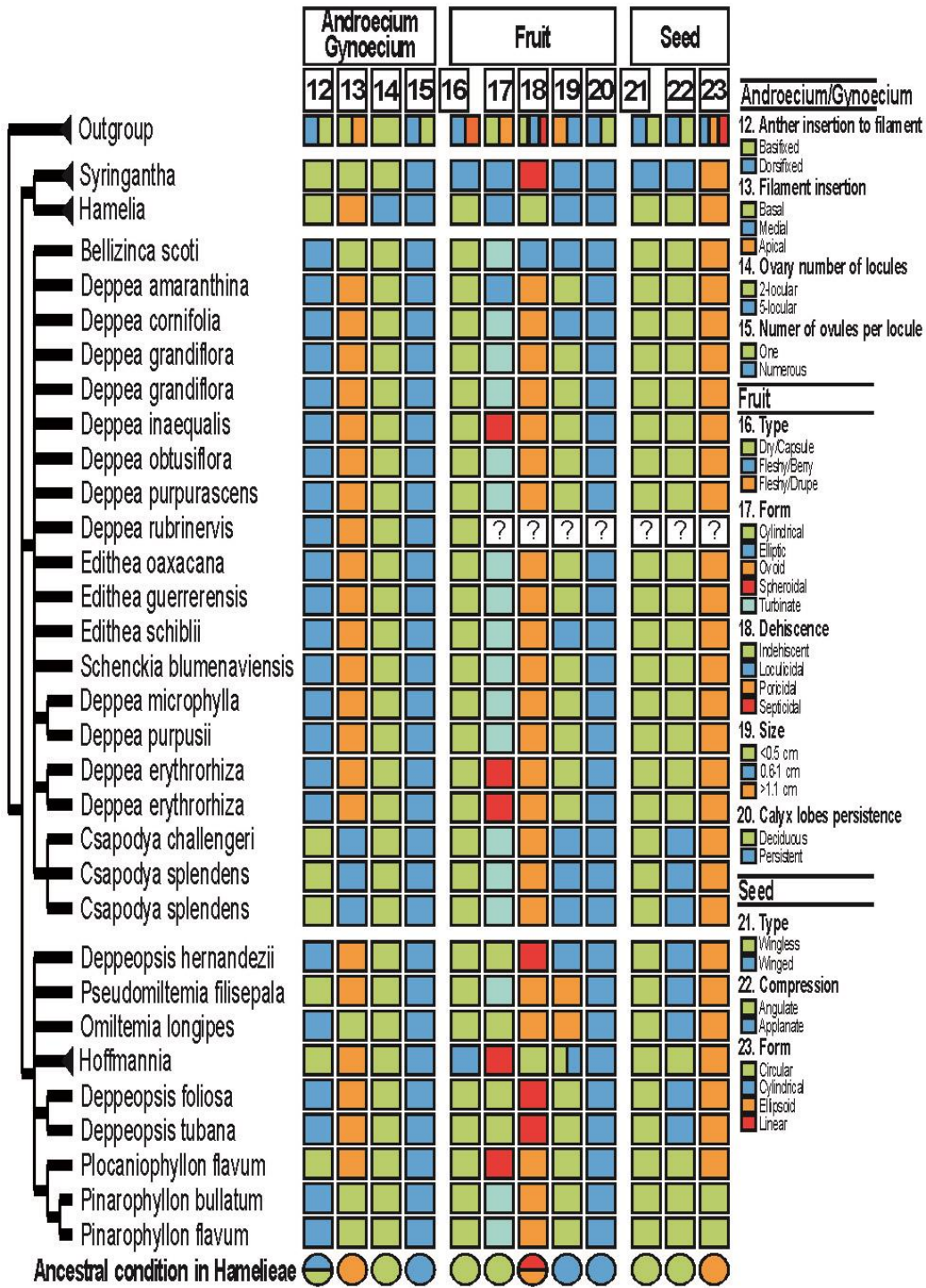


Figure 2. Continued.

CAPÍTULO 3. Nuclear phylogenomics and evaluation of the generic limits within the *Deppea* complex (Hameliaceae, Rubiaceae)

Abstract

Within the tribe Hamelieae (Rubiaceae), the *Deppea* generic complex has been taxonomically challenging due to its complex mosaic of morphologically important features. This generic complex includes 10 genera, some of which have been widely accepted, while others have been controversially synonymized to *Deppea*. Current molecular phylogenetic studies lack a representative sampling of taxa and/or molecular markers, resulting in unsatisfactory phylogenetic hypotheses relevant for taxonomic decisions regarding the generic limits within the *Deppea* complex. The aim of this study is to test the monophyly of *Deppea* and its morphologically related genera using the most extensive sampling of taxa for the *Deppea* complex (including 10 genera and 40 species) and an expanded number of molecular markers. Our phylogenetic analysis is based on 164 nuclear genes retrieved through the Angiosperms353 targeted enrichment probe for 94 taxa and is complemented with a morphological survey of 31 characters that were mapped onto the molecular phylogenetic hypothesis. All phylogenetic inference methods (BI, ML and P) consistently recovered equal topologies with strong support for most groupings. Based on the present results, the genera *Deppea*, *Deppeopsis*, *Omitemia*, *Pinarophyllon*, *Plocaniophyllon*, and *Pseudomitemia* are accepted, although the generic circumscription had to be modified to match the phylogenetic hypothesis. The genera *Bellizinca*, *Csapodya*, *Edithea*, and *Schenckia* are synonymized with *Deppea* based on both, molecular and morphological evidence. Two new genera, *Pseudodeppea* and *Pseudodeppeopsis* are here described.

Keywords

Angiosperms353, *Deppea*, Hamelieae, Target-capture, Taxonomy, Nuclear phylogenomics

Introduction

The neotropical tribe Hamelieae comprises 10 to 16 genera and approximately 225 species. Mexico is undoubtedly the center of diversity of the tribe with 9 to 14 genera and ca. 100 species (Lorence & Dwyer, 1988; Borhidi, 2006, 2012). This tribe is well diagnosed by the presence of raphides, the ebracteate inflorescences, and the usually yellow or yellowish 4-merous flowers with contorted aestivation (Bremer, 1987; Borhidi & al., 2004a,b; Manns & Bremer, 2010; Stranzinger & al., 2014). Although this is a unique combination of features, Hamelieae has not been conflict-free. It was originally classified in the subfamily Rubioideae by the presence of raphides (Verdcourt, 1958; Bremekamp, 1966; Kirkbride, 1984), but molecular phylogenetic analyses including few genera placed it within Cinchonoideae (Bremer & al., 1995; Andersson & Rova, 1999; Robbrecht & Manen, 2006).

The generic content of Hamelieae has changed over the time, ranging from two genera: *Hamelia* Jacq. and *Hoffmannia* Sw. (Verdcourt, 1958; Bremekamp, 1966) to eleven (De Candolle, 1830; Endlicher, 1836): *Alibertia* A. Rich., *Axanthes* Blum, *Brignolia* DC., *Evosmia* Humb. & Bonpl., *Hamelia*, *Olostyla* DC., *Patima* Aubl., *Tepesia* Gartn., *Urophyllum* Jack ex Wall, *Sabicea* Aubl., and *Schradera* Vahl. More recently, the morphology-based classification by Bremer (1987) included five genera: *Deppea* Schldl. & Cham., *Hamelia*, *Hoffmannia*, *Omittemia* Standl., and *Pinarophyllum* Brandege. Later, the genus *Edithea* Standl. was included by Robbrecht (1988) in the tribe, and *Eizia* Standl. and *Plocaniophyllum* Brandege were added by Robbrecht & Bridson (1993), while McDowell (1996) included the monotypic genus *Syringantha* Standl. Finally, Borhidi & al. (2004b) segregated *Bellizinca* Borhidi and *Csapodya* Borhidi from *Deppea*, both included in Hamelieae by the authors.

The current molecular-based classification by Manns & Bremer (2010), recognizes eight genera: *Cosmocalyx* Standl., *Deppea*, *Hamelia*, *Hoffmannia*, *Omittemia*, *Pinarophyllum*, *Plocaniophyllum*, and *Syringantha*, plus two tentatively included (*Eizia* and *Patima*). The genera *Bellizinca*, *Csapodya*, *Edithea*, and *Schenckia* were treated as *Deppea* synonyms.

The main taxonomic controversies within Hamelieae rely on the generic delimitation of genera closely related to *Deppea*. This generic group here called “the *Deppea* complex” includes *Bellizinca*, *Csapodya*, *Deppeopsis*, *Edithea*, *Omittemia*, *Pinarophyllon*, *Plocaniophyllon*, *Pseudomittemia*, and *Schenckia*. Some of these genera have been treated as synonyms to *Deppea* by some authors mainly based on morphological similarities (Lorence & Dwyer, 1988; Manns & Bremer, 2010; Lorence, 2012).

The *Deppea* complex encompasses the majority of the species in the Hamelieae tribe with 10 of the 16 genera. This generic complex can be characterized by the usually turbinate capsules with poricidal dehiscence, while the rest of the tribe includes mainly genera with fleshy fruit, or if having capsular fruits, they are cylindrical. The genera within the *Deppea* complex have been distinguished by different combination of the corolla stamen insertion, the calyx lobes form, and the corolla type and size (Standley, 1918; Borhidi & al., 2004a, b, Borhidi, 2006, 2012; Borhidi & Reyes-García, 2007; Borhidi & Stranczinger, 2012). However, these characters show a mosaic of highly overlapping features and therefore are not unequivocally useful for recognizing the genera.

Molecular phylogenetic analyses retrieved Hamelieae as a highly supported lineage but did not contribute to solve the *Deppea* complex taxonomic problem (Manns & Bremer, 2010), maybe because of the poor sampling of species or weak character sampling (few molecular markers). Manns & Bremer (2010) provided the most complete molecular phylogenetic hypothesis for the Cinchonoideae subfamily based on Bayesian analysis of a combined matrix of nuclear ribosomal ITS region and plastid markers (*atpB-rbcL*, *ndhF*, *rbcL*, *rps16*, *trnL-F*), but only represented few taxa from Hamelieae of which six genera and seven species are part of the *Deppea* complex. This analysis retrieved two well supported clades, one including the sampled species of *Deppea* (including those treated by some authors as *Csapodya* and *Schenckia*). The other clade included the genera *Pinarophyllon*, *Plocaniophyllon*, *Omittemia* and *Hoffmannia*, this last genus clearly different from the *Deppea* complex members by its fleshy fruits.

More recently, Stranczinger & al. (2014) focused more specifically on Hamelieae, provided by far the most extensive molecular phylogenetic study for this tribe, including

samples of all the genera in the *Deppea* complex. Nevertheless, they had a relatively poor marker sampling (nrITS combined with the plastid spacer *trnL-F*), which most likely led to the unsolved phylogenetic tree in which only *Hamelia* was retrieved as monophyletic.

In summary, the current molecular studies do not provide enough evidence for establishing reliable generic limits within the *Deppea* complex, hence new sources of evidence are needed including an extensive sampling of both, taxa and characters. Regarding character sampling, current phylogenetic studies have turned to next generation sequencing for large data sets (e.g., targeted sequencing). Particularly, targeted sequencing can yield hundreds of DNA loci, depending on the targeted sequencing kit used (e.g., Johnson & al., 2018; Couvreur & al., 2019), providing sequencing data suitable to address question in plant systematics at different levels (e.g., Nicholls & al., 2015; Stevens & al., 2015). Moreover, this technique is suitable for degraded DNA, such as that obtained from herbarium material (Hart & al., 2016). Therefore, targeted sequencing is becoming a good option for phylogenomic methods tackling difficult taxonomic problems (McKain & al., 2018).

The goal of our study is therefore to generate a comprehensive DNA character matrix using next generation protocols, including all genera of the *Deppea* complex and as many taxa as possible classified in Hamelieae. We specifically aimed to evaluate the monophyly of *Deppea* and its morphologically related genera for circumscribing taxonomic entities. Additionally, we will explore the efficiency of the Angiosperm353 target capture kit (Johnson & al., 2018) to resolve generic-level relationships and limits. Finally, we will explore the phylogenetic value of some taxonomically important morphological characters and establish hypotheses for their evolution.

Materials and Methods

Taxon Sampling

We sampled all the genera currently corroborated in Hamelieae according to several studies (Manns & Bremer, 2010, Stranzinger et al., 2014). We further increased the species sampling for several genera in comparison to previous authors, making our study the most

comprehensive for the entire tribe to date. Full names with authors, vouchers and GenBank accession numbers for all taxa included in the analyses are presented in Appendix 1. The only genera that were not included in the molecular analyses due to the lack of suitable samples are *Eizia* Standl., which was considered extinct until a few years ago (Martínez-Camilo & al., 2015), and *Patima* Aubl. endemic to Guiana with two species.

DNA Extraction

Silica gel dried leaves were used for DNA extraction when available, complemented with herbarium material. DNA was extracted using the Plant DNA Easy Kit (Qiagen Inc.) following the manufacturer's protocol or using the CTAB protocol (Doyle & Doyle, 1987). Extractions were quantified employing Qubit 2.0 (Invitrogen, Carlsbad, California, USA). DNA concentrations were standardized between 150 to 200 ng/uL either by diluting solutions with deionized water or performing additional DNA extractions for later concentration.

Genomic Library Preparation and bioinformatic Analyses

Approximately 50 uL of each sample was sent to Arbor Biosciences (Ann Arbor, Michigan, USA) for library preparation and DNA sequencing on an Illumina HiSeq 2000 sequencer (Illumina, San Diego, California, USA). The Angiosperm353 target capture kit (Johnson & al., 2018) was purchased from Arbor Biosciences and used for targeted enrichment of each sample.

The quality of the newly generated sequencing data was assessed using the FastQC software (freely available at <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Paired-end DNA sequencing reads were adapter-trimmed and quality filtered with the pipeline TrimGalore! v.0.6.5 (freely available at https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) using a Phred score quality threshold of 30 (flag -q), a minimum read length value of 20 (flag --length), and retaining only read pairs that passed all quality filtering thresholds. For each sample, the Angiosperms353 loci were retrieved through the pipeline HybPiper v.1.3.1 (Johnson & al., 2016) by mapping the clean reads against template sequences of the 353 low copy nuclear

genes (available at <https://github.com/mossmatters/Angiosperms353>) using the Burrows-Wheeler alignment (BWA) program v.0.7 (Li & Durbin, 2009) and then de-novo assembling mapped reads for each gene separately using the software SPAdes v. 3.13 (Bankevich & al., 2012), with a minimum coverage threshold of 8x.

HybPiper v. 1.3.1 (Johnson & al., 2016) was used to assemble and extract target regions. Read mapping, contig assembly, and coding sequence extraction were performed running the `reads_first.py` script. The `intronerate.py` script was run to extract introns and intergenic sequences flanking targeted exons. The `retrieve_sequences.py` script was run first with the “dna” argument to extract coding regions and subsequently with the “supercontig” argument to extract both coding and non-coding regions as a single concatenated sequence for each target gene.

The Gentianales reference for Angiosperms353 is *Pteleocarpa malaccensis* Oliv. A Gelsemiaceae. To accurately extract target regions, we designed a new reference specific for Rubiaceae with our own data. We selected seven terminals representing several lineages of our dataset and assembled the Rubiaceae350, which includes 350 genes from the original 353. With this Rubiaceae-specific reference we repeated the contig assembly and posterior steps.

From the genes retrieved using Rubiaceae350, samples with paralogs were investigated using the HybPiper scripts `paraloginvestigator.py` and `paralogretriever.py`. For each locus with paralog issues, we generated alignments using MAFFT v7.407 (Kato & Standley, 2013) followed by tree reconstruction using FastTree v2.1.7 (Price & al. 2010). This resulted in 189 loci being removed from further processing. The non-paralogous resulting genes were manually aligned using PhyDe (Müller & al., 2005) and concatenated for phylogenetic analyses.

Phylogenetic studies

Parsimony (P) analyses were performed only including potentially parsimony informative characters using the following parameters in TNT version 1.1 (Goloboff & al. 2003): 1000 random addition sequences, retaining 10 trees per replicate, and then submitting them to a

driven search with the option “New Technology Search” including the algorithms Ratchet (Nixon, 1999a), Tree Drifting (Goloboff, 1999) and Sectorial Searches (Goloboff, 1999) with the default options; the P trees were requested to be hit 1000 times. The most parsimonious trees were collected and opened in WinClada (Nixon, 1999b). Additionally, a Jackknife analysis (JK) was executed in TNT version 1.1 (Goloboff & al. 2003), re-sampling the matrix 10 000 times with the same parameters used for the new technology searches; we considered a node supported if the JK value is $\geq 64\%$ (Farris & al. 1996). The JK values were mapped onto the P tree using Winclada (Nixon, 1999b).

Maximum Likelihood (ML) analyses were performed with RAxML (Stamatakis, 2014) using the default parameters on the phylogenetic webserver CIPRES Gateway (<https://www.phylo.org/>) implementing the RAxML-HPC2 on XSEDE option with 10 000 bootstrap iterations with 25 distinct rate categories; estimate proportion of invariable sites (GTRGAMMA + I) activated and the rest of parameters were the default in the RAxML function. Phylogenetic trees were visualized using Figtree (Rambaut & Drummond 2010) and edited in TreeGraph2 (Stöver & Müller 2010). Nodes with Bootstrap (BS) values $\geq 85\%$ were considered here as well supported.

Bayesian inference (BI) analyses analyses were performed with MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001) in the phylogenetic webserver CIPRES gateway (<https://www.phylo.org/>). Four independent Markov Chain Monte Carlo (MCMC) runs were carried out, each with four parallel chains. Each chain was performed for 1 000 000 generations, saving one random tree every 1 000 generations. Phylogenetic trees were visualized using FigTree (Rambaut and Drummond 2010) and edited in TreeGraph2 (Stöver & Müller 2010). For posterior probabilities (PP) we considered a clade to be well supported if PP was equal or greater than 0.95 (Alfaro & Holder 2006), and moderately supported if PP was between 0.85 and 0.95. Nodes with PP below 0.85 were considered not supported.

We ran ASTRAL v. 5.15.4 on the CIPRES Gateway using a tree per gene and then calculating posterior probabilities and quartets for 164 nuclear loci from the Angiosperms353 probe set (Appendix 2)

Morphological data

A morphological matrix was constructed using WinClada (Nixon, 1999b) with data predominantly obtained from personal observations from the field and from specimens deposited at MEXU, but complemented with information from original descriptions and type specimens, terminology follows (Harrias & Harris, 2001). Publications with descriptive data (Lorence & Dwyer, 1988; Borhidi, 2006, 2012; Lorence, 2012) were used as a source to postulate characters and character states. All characters and character states are listed in Table 1. The morphological matrix was optimized onto the P trees using the Fitch optimization criterion (Fitch, 1971) as implemented in Winclada (Nixon 1999b).

Results

Of our 96 samples submitted for sequencing, two failed to return sufficient coverage (*Hamelia patens*-Hpat48 and *Pinarophyllon bullatum*-Pbull12) resulting in 94 satisfactory samples. The 94 samples included 14 of the 16 genera recognized in Hamelieae and 40 species representing all ten genera in the *Deppea* complex. The number of genes with sequences per sample ranged from 29 (*Schenckia*) to 344. From the Angiosperms353 a total of 352 genes were satisfactory retrieved, from these 350 genes were recovered for the Rubiaceae-specific reference (Rubiaceae350). Using Rubiaceae350 a total of 348 genes were retrieved from which 164 genes were used after removing loci with insufficient data and genes with paralogy concerns. The average gene recovery success was 87.8% (Fig. 2).

After alignment and trimming, the average gene alignment length was 1009 bp (min = 78, max = 4,796). The aligned matrix comprises 94 taxa with a total of 164 loci with a total of 166,259 bp of which 25,426 were potentially informative for parsimony. A data summary for each gene is presented in Appendix 2. The individual alignment files as well as the concatenated matrix are available on Digital Dryad.

The tree topologies are congruent independently of the inference method; there are only minor differences in resolution related to support values among the analyses. In Figure 2 we used as reference the ML tree obtained with the total dataset as it is the most resolved, and we indicate the clades that are presented in the other analysis including the BS, JK and

PP values for each clade. Also, the phylogenetic trees obtained from the ASTRAL III coalescent-based analysis and the Bayesian Inference/RAxML concatenated maximum likelihood/Parsimony analysis are identical in terms of phylogenetic clustering/topologies and node support.

This tree shows the tribes Hillieae (100% JK, 100% BS, 1.00 PP), Guettardeae (100% JK, 100% BS, 1.00 PP), and Hamelieae (100% JK, 100% BS, 1.00 PP) as monophyletic (tribes Paederieae and Chiococceae were represented by only one sample). Among the tribes, Hillieae showed one synapomorphic morphological character: the epiphytic habit. Guettardeae is supported by a combination of homoplasies: spheroidal fruits up to 0.5 cm long. The tribe Hamelieae is supported by the yellow corolla (although this condition is further changed to orange within the clade) plus the presence of raphides; this last is homoplastic character because it is shared by Paederieae (Figure 3).

Within Hamelieae most genera represented by more than one species, or at least two individuals in the case of monotypic genera, are supported as monophyletic: *Cosmocalyx*, *Hamelia*, *Hoffmannia*, *Omitemia*, and *Pseudomitemia*. The Mesoamerican monotypic genus *Cosmocalyx* is the earliest divergent taxon in the tribe, and it is sister to two main clades: Clade I (100% JK, 100% BS, 1.00 PP), including *Syringantha-Hamelia* plus *Deppea densiflora*, *Deppeopsis*, *Hoffmannia*, *Omitemia*, *Pinarophyllon*, *Plocaniophyllon*, and *Pseudomitemia*; Clade II (100% JK, 100% BS, 1.00 PP) includes *Bellizinca*, *Csapodya*, *Deppea*, *Edithea*, and *Schenckia*. The genera belonging in the *Deppea* complex are placed in both of these clades: *Deppeopsis*, *Omitemia*, *Pinarophyllon*, *Plocaniophyllon*, *Pseudomitemia*, and the species *Deppea densiflora* do not share a most recent common ancestor with the remaining *Deppea*.

The genus *Cosmocalyx* is supported by three synapomorphic morphological characters: reddish wood oxidation; asymmetric calyx lobes, with one calyx larger than the rest; and samaroid fruits. The sister relationship of Clades I and II is supported by the synapomorphic rotate (although further reverted within *Hoffmannia* and *Edithea* into hypoc crateriform) and the papillose seed ornamentation. Clade I can be recognized by the homoplastic inflorescences (cymes/monochasium) and Clade II by the combination of

homoplastic turbinate fruits with apical poricidal dehiscence (shared by genera belonging in the *Deppea* complex but placed within Clade I) (Figure 3).

Within Clade I, a subclade is formed by the monotypic Mexican genus *Syringantha* as sister to *Hamelia* (100% JK, 100% BS, 1.00 PP); this subclade is sister to the rest of the genera in Clade I (74% JK, 100% BS, 1.00 PP). The *Hamelia-Syringantha* clade shared several homoplastic characters such as the tubular corolla form, pentamerous flowers, and basal stamen attachment. Both genera are recognized by a combination of several homoplastic characters, plus the synapomorphic 5-locular ovary in the genus *Hamelia*, and the autoapomorphic elliptic fruit in *Syringantha* (Figure 3). The rest of the terminals in Clade I have the homoplastic axillary inflorescences.

Within the rest of the taxa of Clade I, *Deppeopsis hernandezii* is the earliest divergent taxon and it is sister to two subclades (100% JK, 100% BS, 1.00 PP). One subclade includes *Hoffmannia* (100% JK, 100% BS, 1.00 PP) which is the most specious and diverse genus in the tribe, plus a subclade (96% JK, 100% BS, 1.00 PP) including the remaining species of *Deppeopsis*, *Omiltemia*, *Pinarophyllon*, *Plocaniophyllon*, *Pseudomiltemia* and *Deppea densiflora*. The taxon *Deppeopsis hernandezii* is recognized by the ternate leaf disposition, also shared by several species in other genera (such as *Hamelia*). The genus *Hoffmannia* is recognized by spheroidal berry fruits and the polygonal seed form (Figure 3).

The Mexican genus *Pseudomiltemia*, which includes two species, is retrieved as monophyletic (100% JK, 100% BS, 1.00 PP) but the relationships among the individuals of the two species sampled is not solved. The Mexican genus *Omiltemia* is also retrieved as monophyletic (90% JK, 100% BS, 1.00 PP). Inside the genus the relationships of the individuals of the two species sampled are not well resolved.

The genus *Pseudomiltemia* is supported by the hypocrateriform corolla, and the seed ornamentation described as concave outline with sunken profile, both retrieved as homoplastic. *Omiltemia* is also supported by homoplasies (such as solitary flowers, tubular corolla with medially attached stamens, and cylindrical fruits) in addition to the synapomorphic corolla tube expanded at medium portion.

The monotypic genus *Plocaniophyllon* is sister to *Deppeopsis tenuiflora* (100% JK, 100% BS, 1.00 PP). Both share the lineolate-like third venation. This subclade is sister to the remaining *Deppeopsis* species plus *Pinarophyllon* and *Deppea densiflora* (100% JK, 100% BS, 1.00 PP). The monotypic genus *Pinarophyllon* is sister to *Deppea densiflora* (100% JK, 100% BS, 1.00 PP), both sister to the remaining three *Deppeopsis* species (100% JK, 100% BS, 1.00 PP).

The subclade formed by *Deppeopsis* spp.-*Deppea densiflora*-*Plocaniophyllon*-*Pinarophyllon* is supported by the homoplastic flowers tube and fruits less than 0.5 cm long. The genus *Plocaniophyllon* is easily recognized by its lineolate venation pattern, unique among the tribe, plus several homoplastic characters (such as hypocrateriform corolla and basal stamen attachment). *Deppeopsis tenuiflora* has a combination of homoplastic characters such as turbinate (not cylindrical as the rest of *Deppeopsis*), septicidal capsules, and persistent stipules. *Pinarophyllon* can be distinguished by its herbaceous habit. *Deppea densiflora* is easily recognized by its white corolla and pinkish wood oxidation. *Deppeopsis anisophylla*, *Deppeopsis foliosa*, and *Deppeopsis tubaeana* share cylindrical and septicidal capsules differing from *Deppeopsis hernandezii* and *Deppeopsis tenuiflora* by stipule persistence.

Within Clade II, the genera *Bellizinca*, *Csapodya*, *Edithea* and *Schenckia* are paraphyletic with respect to *Deppea*. Clade II is then divided into two major subclades (100% JK, 100% BS, 1.00 PP) without morphological support. One of these subclades includes *Deppea pauciflora*-*Deppea amaranthoides*-*Deppea cornifolia* (100% JK, 100% BS, 1.00 PP); in which *D. pauciflora* is the earliest divergent taxon (100% JK, 100% BS, 1.00 PP), sister to the clade formed by the unique sample of *D. amaranthoides* paraphyletic to *D. cornifolia* (100% JK, 100% BS, 1.00 PP).

The second major subclade of the *Deppea* complex includes the monophyletic Mesoamerican *Csapodya* (100% JK, 100% BS, 1.00 PP), sister to an unsolved group formed by clades including the remaining *Deppea* species plus *Bellizinca*, *Edithea*, and *Schenckia* (100% JK, 100% BS, 1.00 PP). Despite the little structure within this clade, five subclades are overall well supported. One of these includes *Bellizinca*, *Edithea* (except

Editheia rupicola), and several *Deppea* species (100% JK, 97% BS, 1.00 PP), the monotypic Mexican genus *Bellizinca* (100% JK, 100% BS, 1.00 PP) is sister to *Deppea purpurascens* (100% JK, 100% BS, 1.00 PP), and both are sister to *Editheia-Deppea* (99% JK, 100% BS, 1.00 PP). In the another subclade the monotypic South American genus *Schenckia* is nested within several *Deppea* species (100% JK, 100% BS, 1.00 PP), while the other includes *Editheia rupicola* nested in a clade with *Deppea* species (100% JK, 100% BS, 1.00 PP). The remaining subclades are exclusively formed by *Deppea* species.

Some morphological characters support the structure of some subclades. For *Csapodya*, the red-yellow corolla plus the tube 4.1-5 cm long are synapomorphies, while pendulous inflorescences and hypocrateriform corollas with stamens medially attached are retrieved as homoplasies. The monotypic genus *Bellizinca* shares the pendulous inflorescences and large hypocrateriform corollas, but can be distinguished by its calyx lobe size, with two lobes larger than the rest. The monotypic *Schenckia* has the homoplastic deciduous stipules and convoluted aestivation. The genus *Editheia* is not supported as monophyletic as its species are dispersed among different subclades without any morphological support.

Discussion

To date, our analysis includes the largest representation of Hamelieae genera and species. Our results partially agree with previous studies (Manns & Bremer, 2010; Stranzinger & al., 2014) in the monophyly of *Hamelia* and *Hoffmannia* and some general relationships.

The study by Manns & Bremer (2010) included nine out of the 16 genera in the tribe and 14 species (*Csapodya* as *Deppea splendens* and *Schenckia* as *Deppea blumenaviensis*). In that study only *Deppea*, *Hamelia*, and *Hoffmannia* represented by more than one species. The general topology of their tree coincides with our results in that *Cosmocalyx* was retrieved as the earlier divergent taxon in the tribe and that members of the *Deppea* complex were dispersed in two clades. Other than that, their analysis resulted in a group formed by *Deppea* (including *Csapodya* and *Schenckia*) and another group formed by *Omitelia-Plocaniophyllon-Pinarophyllon-Hoffmannia* and all these in turn as sister to *Hamelia-Syringantha*. However, these relationships were considered as provisional since

Deppeopsis, and *Pseudomiltemia* were not sampled. In our study including these genera the overall relationships are quite different. Also, the genus *Deppea* in the sampling of Manns & Bremer (2010) included four from the 20 species in the genus and the two mentioned above were treated as *Csapodya* and *Schenckia*. In our study the two *Csapodya* species are in a clade nested within a large clade formed by *Deppea*, *Edithea*, and *Bellizinca*.

The study by Stranczinger & al. (2014) included 12 out of the 16 genera and 33 species. Their result retrieved three major clades in an unresolved polytomy: (1) *Hamelia-Syringantha*, (2) *Bellizinca-Csapodya-Deppea-Edithea-Schenckia*, and (3) *Deppeopsis-Hoffmannia-Omiltemia-Plocaniophyllon-Pinarophyllon* plus *Renistipula*. In our results the group formed by *Hamelia-Syringantha* was retrieved as sister to *Deppeopsis-Hoffmannia-Omiltemia-Plocaniophyllon-Pinarophyllon*, both groups in turn sister to the clade with *Deppea*. The genus *Renistipula* was controversially included in Hamelieae by Stranczinger & al. (2014) because it clearly differs from the rest of Hamelieae by among other things the absence of raphides. It is placed in our analysis as sister to *Gonzalagunia*, in the tribe Guettardeae, as in Torres-Montúfar (2018).

Deppeopsis

Our results are in line with Stranczinger & al. (2014) in recovering *Deppeopsis hernandezii* outside the clade including most *Deppeopsis* species. In Stranczinger & al. (2014) it is placed in a clade lacking resolution, but there, *D. tubaeana* is sister to *D. foliosa* and both are the sister group of *Plocaniophyllon flavum* in a moderately supported clade.

Our study includes all five *Deppeopsis* species and this genus is retrieved as polyphyletic. *Deppeopsis anisophylla-D. foliosa-D. tubaeana* form a well-supported clade; however, *D. tenuiflora* is sister to *Plocaniophyllon* also in a well-supported clade, while *D. hernandezii* is the earlier divergent taxon in the clade also formed by *Hoffmannia*, *Pseudomiltemia*, and *Omiltemia*.

The genus *Deppeopsis* was described by Borhidi & Stranczinger (2012) based on the cylindrical fruits and the septicidal dehiscence in contrast to the turbinate capsules with poricidal dehiscence of *Deppea*. The type species (*D. hernandezii*) is found in cloud forest

of central Mexico (in the state of Hidalgo) while the remaining four species occur in the tropical rainforests or cloud forests from southern Mexico (Chiapas and Oaxaca) with one species (*D. tenuiflora*) shared with Guatemala.

Due to the biparental inheritance of nuclear DNA, the phylogenetic position of the *Deppeopsis* type species (*D. hernandezii*) could raise questions about possible hybridization. We performed an analysis of plastid *trnL-F* from Genbank data (results not shown here) in which this taxon also does not form part of a clade with the other *Deppeopsis* species. The chloroplast together with the morphological evidence, in which *D. hernandezii* is different from the other *Deppeopsis* by the ternate leaves, is used here to accept the isolated position of *D. hernandezii* and to concurrently recognize *Deppeopsis* as a monotypic genus.

As for *Deppeopsis tenuiflora*, our results place it in a well-supported clade together with *Plocaniophyllon flavum*. In Stranzinger & al. (2014) *Plocaniophyllon flavum* was retrieved as sister to a clade including *Deppeopsis foliosa* and *Deppeopsis tubaeana* while in Manns & Bremer (2010) it was retrieved as sister to the group formed by *Hoffmannia* plus *Omitelia*. The monotypic genus *Plocaniophyllon* is a Mexican-Guatemalan taxon and has been considered as an easily identifiable species by its lineolate venation disposition (Lorence, 2012), a character not common in Rubiaceae (Robbrecht, 1988). Based on our results, it is possible to transfer *D. tenuiflora* to *Plocaniophyllon*. However, while lineolate venation has been documented for *Plocaniophyllon* (Lorence 2012), for *Deppeopsis tenuiflora* the venation has only been described for the primary and secondary veins (described by Lorence 2012 as eucamptodromous to brochidrodromous and treated as *Deppea tenuiflora*). Martínez-Cabrera & al. (2007) explored the leaf architecture of Hamelieae members, and in that study the third venation of *Plocaniophyllon* was described as random reticulate while the quaternary venation was described as fingerprint pattern (an alternative denomination for what others consider lineolate). Unfortunately, Martínez-Cabrera & al. (2007) did not mention the leaf venation pattern of *D. tenuiflora*. Our observations, though not anatomical, indicate that the venation patterns of *D. tenuiflora* can be understood as lineolate-like. In addition, *D. tenuiflora* morphologically differs from the rest of the *Deppeopsis* species by its turbinate capsules and the persistent stipules, in

contrast to the cylindrical capsules and deciduous stipules in *Deppeopsis*. Based on our observations, we suggest below a new combination for *D. tenuiflora* as *Plocaniophyllon tenuiflorum*.

The remaining three *Deppeopsis* species (*D. anisophylla*, *D. foliosa*, *D. tubaeana*) are grouped in a well-supported clade characterized by the cylindrical fruit form with septicidal dehiscence as in the other *Deppeopsis* species, differing from *Deppeopsis hernandezii* by opposite leaf arrangement (vs. ternate) and from *Deppeopsis tenuiflora* by deciduous stipules (vs. persistent). For a natural classification this clade deserves to be included in a new genus (see taxonomic synthesis below).

Pinarophyllon

The genus *Pinarophyllon* currently includes two species (Lorence, 2012) ranging from southern Mexico to Guatemala. In the phylogeny by Manns & Bremer (2010) *P. bullatum* is the earlier divergent taxon in a clade formed by *Plocaniophyllon-Omiltemia-Hoffmannia*; in Stranzinger & al. (2014) it is sister to a group formed by *Deppeopsis-Hoffmannia-Omiltemia-Plocaniophyllon-Pseudomiltemia-Renistipula*. Our study includes only the type species of *Pinarophyllon* (*P. flavum*) supported by herbaceous habit. The two *Pinarophyllon* species share these features and therefore are expected to be retrieved as monophyletic and thus the genus is accepted. The species *P. flavum* was retrieved as sister to *Deppea densiflora*. The two differ by habit (shrub in *D. densiflora*), corolla color (white in *D. densiflora*), and fruit dehiscence (poricidal in *D. densiflora*).

Omiltemia

The genus *Omiltemia* was described by Standley (1918) based on the red and large corollas (Borhidi & Velasco-Gutierrez 2010, Borhidi 2012, Lozada-Pérez & Rojas Gutiérrez 2016). Later, *Pseudomiltemia* was segregated from *Omiltemia* by Borhidi & al. (2004b) mainly based on the stamen insertion (apical in *Pseudomiltemia* vs. medial in *Omiltemia*). Both genera are retrieved as monophyletic in our study. We sampled the two known species of *Pseudomiltemia* retrieved in a well-supported clade. The clade is morphologically supported by the homoplastic characters hypocateriform corolla, and seed ornamentation

defined as with concave outlines with a sunken profile. The genus *Pseudomiltemia* was represented by one sample in Stranzinger & al. (2014) and its position was uncertain regarding the other taxa in the clade.

Of the three described *Omiltemia* species, we could not include *O. guerrerensis*. The five samples of the two other species are grouped in a well-supported clade, but only the individuals from *O. longipes* share an exclusive most recent common ancestor (i.e., the species is supported as monophyletic). The genus *Omiltemia* is characterized by the synapomorphic corolla tube expanded at the medium portion, consistently with the medium stamen insertion, also the homoplastic solitary flower disposition, tubular corolla and cylindrical fruit form. In Stranzinger & al. (2014) *O. longipes* is weakly retrieved as sister to *Renistipula* which is here discarded as belonging in Hamelieae.

Bellizinca, Csapodya, Deppea, Edithea and Schenckia

In our results, Clade II includes the genera *Bellizinca, Csapodya, Edithea* and *Schenckia* as paraphyletic with respect to *Deppea*. The same result was obtained by Stranzinger & al. (2014). The study by Manns & Bremer (2010) treats *Csapodya* and *Schenckia* as *Deppea splendens* and *D. blumenaviensis*, respectively, but they only sampled two other *Deppea* species. The species *D. blumenaviensis* is included in a clade with *D. grandiflora*, while *D. splendens* is retrieved as the earlier divergent taxon in the *Deppea* clade, a result that could have been interpreted as the acceptance of *Csapodya*; however, the lack of a satisfactory sampling of *Deppea* in Manns & Bremer (2010) plus the existence of the segregated genera prevented this decision.

In our analysis, the subclade formed by *D. amaranthoides, D. cornifolia,* and *D. pauciflora* (within Clade II), does not have any morphological support. Regarding the internal relationships, *D. amaranthoides* is paraphyletic with respect to the widespread *D. cornifolia*, raising the need to update the taxonomic treatment of *Deppea*. The group including the *Csapodya* species is morphologically supported by the combination of several homoplasies including the tree lifeform, pendulous inflorescences (also shared with *Bellizinca*), hypocateriform corolla, convoluted corolla aestivation, corolla tube up to 5 cm long with medium stamen insertion, and the seed with concave outlines with a sunken

profile ornamentation. The subclade formed by *Bellizinca*, *Deppea*, *Edithea* and *Schenckia* is supported by the homoplastic persistent stipules.

There is consensus that *Schenckia blumenaviensis* is nested within *Deppea* by molecular evidence (Manns & Bremer 2010, Stranzinger & al. 2014), despite the geographical disjunction that this represents: *Schenckia* is distributed in Argentina and Brazil, while *Deppea* ranges from Mexico to Panama. In both Manns & Bremer (2010) and Stranzinger & al. (2014) *Schenckia* is treated as *Deppea blumenaviensis*, in the former it is retrieved as the sister taxon to *D. grandifolia*, while in the latter it is in an unsolved position with the rest of *Bellizinca*, *Csapodya*, *Deppea*, and *Edithea*. Despite the morphological similarities with *Deppea*, in our study we treated it initially as *Schenckia blumenaviensis* with the intention to explicitly evaluate its naturalness. Although the two *Schenckia* samples retrieved very few sequence base pairs, both are included in a well-supported clade as sister to *D. ehrenbergii*, a species endemic to Oaxaca, Mexico. The clade of *S. blumenaviensis* is morphologically supported by the homoplastic deciduous stipules and the convoluted corolla aestivation, conditions very variable and shared by other taxa in the clade, such as in *Csapodya* and many *Deppea* species. With the morphological evidence in conjunction with the phylogenetic position, we agree with the synonymy of *Schenckia* with *Deppea* as in several previous studies (Lorence & Dwyer, 1988; Manns & Bremer, 2010; Stranzinger & al., 2014).

The genus *Bellizinca* was first described as *Omitemia scoti* J.H. Kirkbr. based on the flower size. Later, Borhidi & al. (2014) described *Bellizinca* due to its pendulous inflorescence, calyx lobe morphology, and basal stamen insertion. In our study the *Bellizinca* samples are well-supported as monophyletic and as the sister group to the clade including the Mexican *Deppea purpurascens*, a species that is characterized, as the specific epithet suggests, by the purplish primary veins and purplish corolla (an atypical color within the tribe). In Manns & Bremer (2010) *Bellizinca* was not included, while in the study by Stranzinger & al. (2014) only one sample of this genus was included and retrieved in an unresolved clade with *Deppea*, *Csapodya* and *Edithea*, at the same time, *D. purpurascens* was recovered as sister to *D. microphylla* plus *D. purpusii*. The genus *Bellizinca* is well defined by the unequal calyx lobes with two lobes larger than the other

two retrieved as a synapomorphy, also the clade is supported by several homoplastic characters such as non-pinkish wood oxidation (as in *Deppea hamelioides*, *Editheia* and *Csapodya*), pendulous inflorescences (as *Csapodya*), tubular corolla with valvate aestivation (a unique condition in the tribe but shared with samples of the outgroup), large corolla tube of 3.1 to 4 cm long (as in some species of *Editheia*), basal stamen insertion (as in *Pinarophyllon* and *Plocaniophyllon* of Clade I), loculicidal capsule dehiscence (unique in the tribe but shared with *Renistipula* in our sampling), and verrucose seed ornamentation (as in *Deppea grandiflora*).

The genus *Editheia* was described by Standley (1933) based on the apical stamen insertion and large flowers; however, it was later treated under *Deppea* (Lorence & Dwyer, 1988), but Borhidi & al. (2004a) resurrected it including two species with corolla tube of 10-35 mm long and apical stamen insertion. Currently there are eight species recognized for *Editheia*, of which we included seven (only *Editheia serboi* (Borhidi & K.Velasco) Borhidi could not be included). Our analysis retrieved the genus as polyphyletic, with its species spread in three clades sharing the non-pinkish wood oxidation, but otherwise morphologically variable. In one subclade *E. rupicola* is sister to *Deppea purpusii* without any morphological character to support it; *E. rupicola* as the other *Editheia* species lacks the distinctive corolla tube expanded at the apex, although they have the non-pinkish wood oxidation. Another clade includes *Editheia oaxacana* plus *E. sousae*, both sister to *Deppea keniae*; this clade has no morphological support, but *E. oaxacana-E. sousae* is also defined by the homoplastic corolla with the tube expanded at the apex.

The other subclade includes the four species sampled in a clade defined by the mentioned non-pinkish wood oxidation, and corolla tube expansion at the apex but also by the fruits 0.6-1 cm long. The resolution at species level is not satisfactory in the cases of *Editheia miahuantlanica* and *E. floribunda-E. guerrerensis*, raising questions about potential hybridization. In Manns & Bremer (2010) *Editheia* was not included, while in the phylogeny by Stranzinger & al. (2014) the three sampled *Editheia* species (*E. guerrerensis*, *E. oaxacana* and *E. schiblii*) were retrieved in a moderately supported unresolved subclade within the unresolved clade formed by *Bellizinca*, *Csapodya*, and *Deppea*.

The paraphyletic relationship of *Deppea* and *Bellizinca*, *Csapodya* and *Editheia* was also retrieved by Stranzinger & al. (2014), the only other phylogenetic study that sampled all these genera. Also, the close relationship is supported by morphological studies as in the Rubiaceae treatment of mesoamerican flora in which the three genera are treated as *Deppea* synonyms (Lorence, 2012), as well as in the Hamelieae circumscription by Manns & Bremer (2010), where these three genera were included in *Deppea*.

The inclusion of morphological characters in our study helped to make taxonomic decisions. At the same time, our results also show that some morphological features can be misleading in the absence of a phylogenetic framework. For example, all the genera previously segregated from *Deppea* used flower size as an evident criterion to split the genus; however, a solid phylogenetic framework shows that the evolution of flower size is very plastic in the group. Hence, it is reasonable to include within *Deppea* species with large corolla tubes (e.g., *D. grandiflora*). The larger floral tube is explained as a recurrent pollinator shift (Lorence & Dwyer, 1988; Lorence, 2012). Although floral size is the most evident character that was used for the segregation of genera, there are other less evident characters that are equally homoplastic, such as the inflorescence type, corolla tube expansion, stamen insertion, and fruit dehiscence. This explains the morphological mosaic that caused great confusion and that now can be consistently translated into the reduction of *Bellizinca*, *Csapodya* and *Editheia* as synonyms to *Deppea*.

Conclusions

The phylogenetic understanding of the *Deppea* complex could be improved by increased individual and species sampling, as we sampled only one to three individuals per species. The solution of questions of species delimitation may require greater sampling. Additional molecular data (such as nuclear and plastid sequences) could also improve the phylogenetic inference for this generic complex.

TAXONOMIC SYNTHESIS

According to our results, the following genera are here included in Hamelieae: *Cosmocalyx* (monotypic), *Deppea* (20 species), *Deppeopsis* (monotypic), *Hamelia* (16 species),

Hoffmannia (ca. 100 species), *Omitelia* (three species), *Pinarophyllon* (two species), *Plocaniophyllon* (two species), *Pseudodeppea* (monotypic), *Pseudodeppeopsis* (three species), *Pseudomitelia* (two species), and *Syringantha* (monotypic). New genera and new combinations are presented below.

Key to the genera in Hamelieae

- 1. Fleshy fruits.....2
- 1. Dry fruits.....3

- 2. Ovary 5-locular; inflorescences mainly terminal..... *Hamelia*
- 2. Ovary 2-locular; inflorescences mainly axillar..... *Hoffmannia*

- 3. Fruits indehiscent; pterophyllous..... *Cosmocalyx*
- 3. Fruits variedly dehiscent; not pterophyllous.....4

- 4. Capsules septicidal.....5
- 4. Capsules poricidal.....7

- 5. Corolla tube longer than 1.4 cm; winged seeds..... *Syringantha*
- 5. Corolla tube up to 1 cm; wingless seeds.....6

- 6. Deciduous stipules; ternate leaves..... *Deppeopsis*
- 6. Stipules persistent; opposite leaves..... *Pseudodeppeopsis*

- 7. Herbaceous plants.....*Pinarophyllon*
- 7. Woody plants, shrubs or treelets.....8

- 8. Tertiary veins lineolate..... *Plocaniophyllon*
- 8. Tertiary veins reticulate..... 9

- 9. Fruits longer than 1 cm..... 10
- 9. Fruits up to 1 cm long.....11

- 10. Flowers solitary, red colored *Omitelia*
- 10. Flowers mainly disposed in inflorescences, yellow colored... *Pseudomitelia*

- 11. Corolla yellow, reddish, or purple (*D. purpurascens*) *Deppea*
- 11. Corolla white..... *Pseudodeppea*

***Pseudodeppeopsis* Aguilar & Ochoterena gen. nov.**

Type: *Deppeopsis anisophylla* (L.O. Williams) Borhidi & Stranzinger (basionym: *Deppea anisophylla* L.O. Williams).

Diagnosis: *Pseudodeppeopsis* is similar to *Deppeopsis* (as here delimited) in being shrubs with rotate flowers, the stamens apically inserted and cylindrical capsules with septicial dehiscence; the former differs from the latter in having persistent stipules (vs. deciduous in *Deppeopsis*), opposite leaf arrangement (vs. ternate in *Deppeopsis*), and the corolla tube less than 5 mm long (vs. 0.6 to 1 cm in *Deppeopsis*). *Pseudodeppeopsis* is also similar to *Plocaniophyllon* (as here delimited) in being shrubs with persistent stipules, opposite leaf arrangement, and rotate flowers with tube less than 0.5 cm long; the former differs from the latter in having reticulate venation (vs. lineolate-like in *Plocaniophyllon*) and cylindrical capsules (vs. turbinate in *Plocaniophyllon*). *Pseudodeppeopsis* is also similar to some species of *Deppea* in the opposite leaf arrangement and rotate flowers less than 0.5 mm long; the former differs from the latter in having cylindrical capsules with septicial dehiscence (vs. turbinate with poricidal dehiscence in *Deppea*).

Etymology: The generic name alludes to the close morphological resemblance to the genus *Deppeopsis*, from which it is segregated.

Pseudodeppeopsis anisophylla comb. nov. Basionym: *Deppea anisophylla* L.O. Williams.

Type: GUATEMALA, Finca Moca, 7 January 1935, *Skutch 2072* (holotype: F).

Pseudodeppeopsis foliosa comb. nov. Basionym: *Deppea foliosa* Borhidi, Salas-Mor. & E. Martínez. Type: MEXICO, Oaxaca, mpio. San Miguel del Puerto, distr. Pochutla, Copalilla, 30 July 1999, 15°54'18"N, 96°8'37"W, *Rivera 1739* (holotype: MEXU; isotypes: XAL).

Pseudodeppeopsis tubaeana comb. nov. Basionym: *Deppea tubaeana* Borhidi. Type: MEXICO, Oaxaca, mpio. Santiago La Galera, distr. Pochutla, 11 km al N de La Galera, carretera a Miahuatlan, 1 Aug 1988, 16°01'N, 96°32'W, *Campos 2106* (holotype: MEXU; isotypes: JPU)

***Pseudodeppea* Aguilar & Ochoterena gen. nov.**

Type: *Deppea densiflora* Borhidi & Reyes-García

Diagnosis: *Pseudodeppea* is similar to *Deppea* (as here delimited) in having pinkish wood oxidation and turbinate capsules with poricidal dehiscence; the former differs from the latter in having white flowers (vs. yellow or orange in *Deppea*, except for *D. purpurascens* with purple flowers). *Pseudodeppea* is also similar to *Pinarophyllon* in having a rotate corolla with tube less than 0.5 cm long, and turbinate capsules with poricidal dehiscence; the former differs from the latter in having shrub habit (vs. herbaceous in *Pinarophyllon*), pinkish wood oxidation (vs. not visible in *Pinarophyllon*), apical stamen insertion (vs. basal in *Pinarophyllon*), and white flowers (vs. yellow in *Pinarophyllon*).

Etymology: The generic name alludes to the close morphological relationship to the genus *Deppea*, from which is segregated.

***Plocaniophyllon tenuiflorum* (Benth.) Aguilar & Ochoterena comb. nov.**

Basionym: *Deppea tenuiflora* Benth., Plantas Hartwegianas imprimis Mexicanas 349. 1857. (1857). Type: MEXICO. In Mexici prov. Chiapas ad San Bartolo, Feb 1840, Linden 1644 (holotype: K, Number K000424316; isotypes: F, Number F0068979F, F0068978F [fragment]).

LITERATURE CITED

Andersson, L. & Rova, H.E. 1999. The *rps16* intron and the phylogeny of the Rubioideae (Rubiaceae). *Plant Syst. Evol.* 214: 161–186.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A., Dvorkin, M., Kulikov, A.S., Lesin, V., Nikolenko, S., Pham, S., Prjibelski, A., Pyshkin, A., Sirotkin, A., Vyahhi, N., Tesler, G., Alekseyev, M.A. & Pevzner, P.A. 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19: 455–477.

Borhidi, A. 2006. *Rubiáceas de México*. 1st edn. Budapest: Akadémiai Kiadó.

Borhidi, A. 2012. *Rubiáceas de México*. 2nd edn. Budapest: Akadémiai Kiadó.

Borhidi, A. & Stranczinger, S. 2012. *Deppeopsis*, un género nuevo (Hamelieae, Rubiaceae) de México y Guatemala. *Acta Bot. Hung.* 54: 86–89.

Borhidi, A., Darók, J., Kocsis, M., Stranczinger, S. & Kaposvári, F. 2004a. Critical revision of the *Deppea* complex (Rubiaceae, Hamelieae). *Acta Bot. Hung.* 46: 77–81.

Borhidi, A., Darók, J., Kocsis, M., Stranczinger, S. & Kaposvári, F. 2004b. Critical revision of the *Omiltemia* complex (Rubiaceae, Hamelieae). *Acta Bot. Hung.* 46: 82–85.

Borhidi, A. & Reyes-García, A. 2007. Estudios sobre rubiáceas mexicanas XI Revisión del género *Csapodya* Borhidi (Rondeletieae), endémico de Chiapas con tres especies. *Acta Bot. Hung.* 49: 13–25.

Borhidi A. & Velasco-Gutierrez, K. 2010. Estudios sobre Rubiáceas Mexicanas XXVI. *Paradeppea* sect. nova del género *Deppea* Cham. et Schldl. (Hamelieae) y dos especies nuevas de Oeste de Oaxaca. *Acta Bot. Hung.* 52: 41–48.

Bremekamp, C.E.B. 1966. Remarks on the position, the delimitation and the subdivision of the Rubiaceae. *Acta Bot. Neerl.* 15: 1–33.

Bremer, B. 1987. The sister group of the paleotropical tribe Argostemmateae: a redefined neotropical tribe Hamelieae (Rubiaceae). *Cladistics* 3: 35–51.

Bremer, B., Andreasen, K. & Olsson, D. 1995. Subfamilial and tribal relationships in the Rubiaceae based on *rbcL* sequence data. *Ann. Missouri Bot. Gard.* 82: 383–397.

Couvreur, T.L.P., Helmstetter, A.J., Koenen, E.J.M., Bethune, K., Brandão, R.D. & Little, S.A. 2019. Phylogenomics of the major tropical plant family Annonaceae using targeted enrichment of nuclear genes. *Front. Plant Sci.* 9: 1941.

De Candolle, A.P. 1830. *Prodromus systematis naturalis regni vegetabilis*. Paris.

Doyle J.J., Doyle J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19:11–15.

Endlicher, S. 1836. *Genera Plantarum secundum ordines naturali disposita*. Vienna: Apud Fr. Beck Universitatis.

Farris, J.S., Albert, V., Källersjö, M., Lipscomb, D. & Kluge, A.G. 1996 Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124. doi: 10.1006/clad.1996.0008

Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* 20: 406–416.

Goloboff, P. 1999. Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics* 15: 415–428. Doi: 10.1006/clad.1999.0122

Goloboff, P., Farris, S. & Nixon, K. 2003. TNT (Tree analysis using New Technology) (BETA) ver. 1.1. Published by the authors, Tucumán, Argentina.

Hart, M.L., Forrest, L.L., Nicholls, J.A. & Kidner, C.A. 2016. Retrieval of hundreds of nuclear loci from herbarium specimens. *Taxon* 65: 1081–1092.

- Johnson M.G., Gardner E.M., Liu Y., Medina R., Goffinet B., Shaw A.J., Zerega N.J.C. & Wickett N.J. 2016. HybPiper: Extracting coding sequence and introns for phylogenetics from high-throughput sequencing reads using target enrichment. *Appl. Plant Sci.* 4.
- Johnson, M.G., Pokorny, L., Dodsworth, S., Botigué, L.R., Cowan, R.S. & Devault, A. 2018. A universal probe set for targeted sequencing of 353 nuclear genes from any flowering plant designed using k-medoids clustering. *Syst. Biol* 68: 594–606.
- Katoh, K. & Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30:772–780.
- Kirkbride, H.J. 1984. Manipulus Rubiacearum III. Deppeae, a new tribe of Rubioideae (Rubiaceae). *Brittonia* 36: 317–320.
- Li, H. & Durbin, R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics.* 25:1754–1760.
- Lorence, D.H. & Dwyer, J.D. 1988. A revision of *Deppea* (Rubiaceae). *Allertonia* 4: 389–436.
- Lorence, D.H. 2012. *Deppea*. Pp. 260–262 in: Davidse, G., Sousa, M., Knapp, S., Chiang, F. (eds). *Flora Mesoamericana*. St Louis: Missouri Botanical Garden Press.
- Lozada-Pérez, L. & Rojas-Gutiérrez, J. 2016. Una nueva especie, *Omiltemia guerrerensis* (Rubiaceae) de Guerrero, México. *Novon* 24: 365–368.
- Manns, U. & Bremer, B. 2010. Towards a better understanding of intertribal relationships and stable tribal delimitations within Cinchonoideae s.s. (Rubiaceae). *Mol. Phylogenetics Evol.* 56: 21–39.
- Martínez-Cabrera, D. Terrazas, T. & Ochoterena, H. 2007. Leaf architecture of Hamelieae (Rubiaceae). *Feddes Repert.* 118: 286–310.

Martínez-Camilo, R., Martínez-Meléndez, N., Martínez-Meléndez, M., Pérez-Farrera, M.A. & Lorence, D.H. 2015. Redescubrimiento de *Eizia mexicana*, una especie endémica de la familia Rubiaceae presumiblemente extinta. *Bot. Sci.* 93: 679–682. Doi: <https://doi.org/10.17129/botsci.242>

McDowell, T. 1996. *Syringantha coulteri* (Hooker f.) McDowell, and new combination and remarks on the relationships of the monotypic Mexican genus *Syringantha* Standley (Rubiaceae). *Novon* 6: 273–279.

McKain, M.R., Johnson, M.G., Uribe-Convers, S., Eaton, D. & Yang, Y. 2018. Practical considerations for plant phylogenomics. *Appl. Plant Sci.* 6: e1038.

Müller, K., Quandt, D., Müller, J. & Neinhuis, C. 2005. PhyDE 0.995. Phylogenetic data editor. Published at <http://www.phyde.de/>

Nicholls, J.A., Pennington, R.T., Koenen, E.J.M., Hughes, C.E., Hearn, J. & Bunnefeld, L. 2015. Using targeted enrichment of nuclear genes to increase phylogenetic resolution in the neotropical rain forest genus *Inga* (Leguminosae: Mimosoideae). *Front. Plant Sci.* 6: 710.

Nixon, K.C. 1999a. The Parsimony Ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 407–414. Doi: 10.1111/j.1096-0031.1999.tb00277.x

Nixon, K.C. 1999b. Winclada (beta) ver. 0.9. Published by the author Ithaca, NY.

Price M.N., Dehal P.S. & Arkin A.P. 2010. FastTree 2, approximately maximum-likelihood trees for large alignments. *PLoS One* 5:e9490.

Robbrecht, E. 1988. Tropical woody Rubiaceae. *Opera Bot. Belg.* 1: 1–272.

Robbrecht, E. & Bridson, K.A. 1993. Nomenclatural notes on three Rubiaceae genera. *Opera Bot. Belg.* 6: 199–200

Robbrecht, E. & Manen, J.F. 2006. The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms). Combined analysis (nDNA and cpDNA) to infer the position of

Coptosapelta and *Luculia*, and supertree construction based on *rbcL*, *rps16*, *trnL-trnF* and *atpB-rbcL* data. A new classification in two subfamilies, Cinchonoideae and Rubioideae. *Syst. Geogr. Plants.* 76: 85–146.

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

Standley, P.C. 1918. *Omittemia*, a new genus of Rubiaceae from Mexico. *J. Wash. Acad. Sci.* 8: 427.

Stevens, J.D., Rogers, W.L., Heyduk, K., Cruse-Sanders, J.M., Determann, R.O. & Glenn, T.C. 2015. Resolving phylogenetic relationships of the recently radiated carnivorous plant genus *Sarracenia* using target enrichment. *Mol. Phylogenetics Evol.* 85: 76–87.

Stranzinger, S., Galambos, A., Szenas, D. & Szalontai, B. 2014. Phylogenetic relationships in the Neotropical tribe Hamelieae (Rubiaceae, Cinchonoideae) and comments on its generic limits. *J. Syst. Evol.* 52: 643–650.

Verdcourt, B. 1958. Remarks on the classification of the Rubiaceae. *Jard. Bot. État Bruxelles* 28: 209–281.

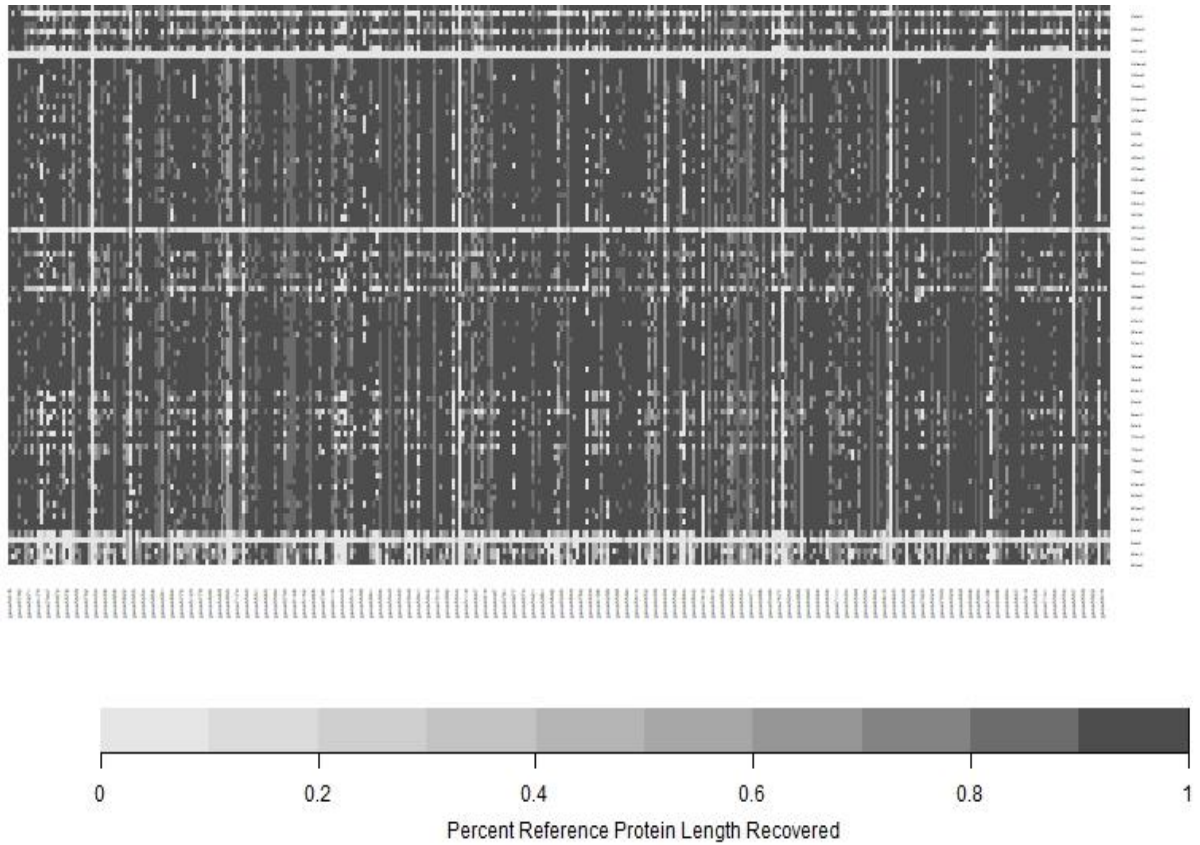


Fig. 1. Recovered sequence length heatmap for our final set of 164 genes. Each row corresponds to a taxonomic group, and each column corresponds to a gene. The shading of the bars represents the length of the recovered sequence relative to the reference gene. The percentage values for each taxonomic group represent the average sequence length recovered for the whole gene set.

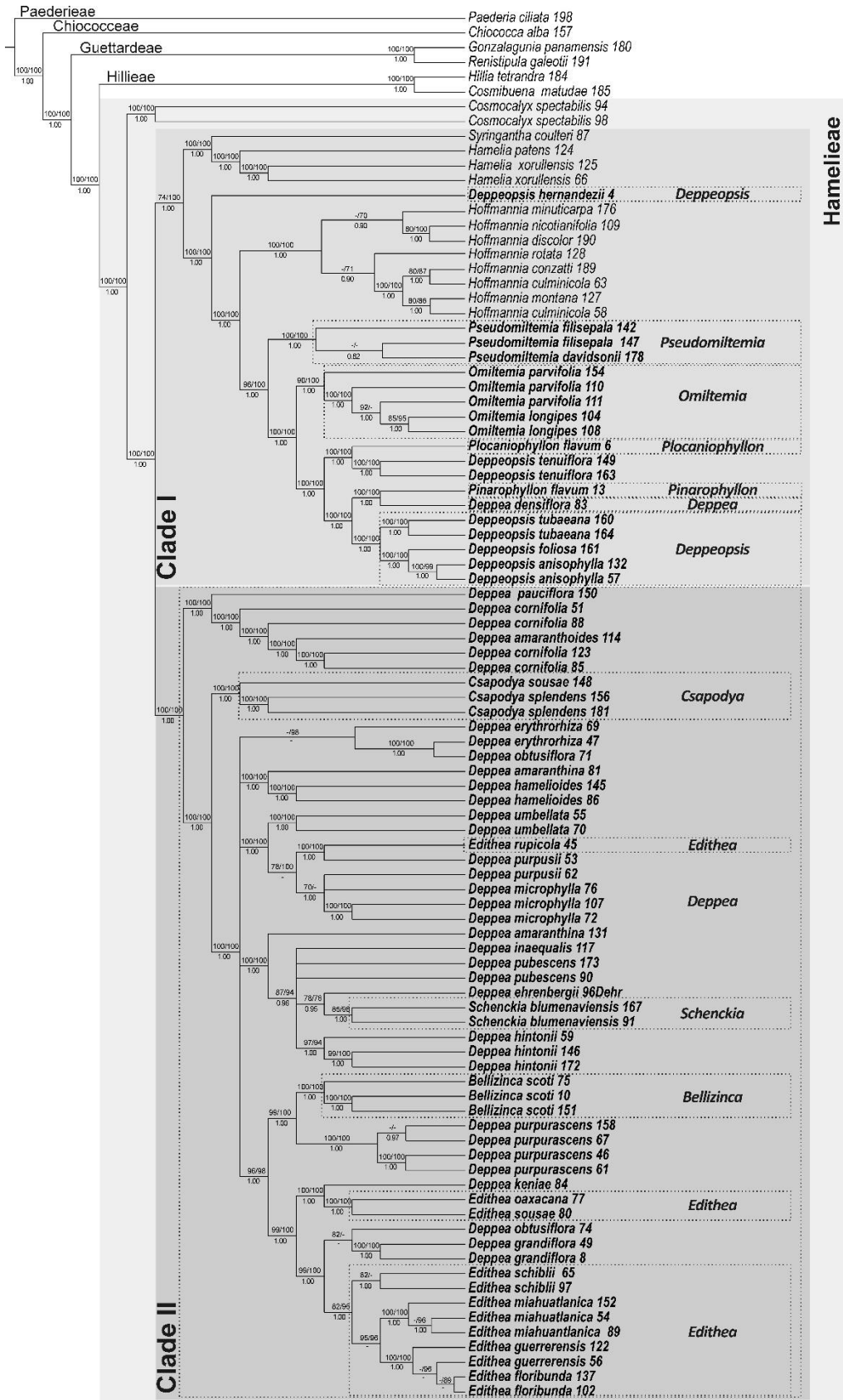


Fig. 2. Phylogenetic relationships as inferred from the concatenated dataset (94 taxa and 164 nuclear genes) showing the clades well supported in either the BI, MP (L= 82931, Ci= 0.41, Ri= 0.72), or ML (ln =8355.76187) topologies (trees are presented as online Appendix 3). Numbers above branches indicates Jackknife (JK) from Parsimony/Bootstrap (BS) support values from Maximum Likelihood; numbers below branches corresponds to posterior probability (PP) values from Bayesian Inference. Branches collapsed with JK values $\leq 64\%$, BS $\leq 50\%$ and PP ≤ 0.51 . Taxa belonging to the *Deppea* complex are marked in bold.



Fig. 3. Most parsimonious tree obtained from the concatenated analyses showing morphological characters (numbers above in the square) and their states (numbers below in the square). The synapomorphies (black squares) and homoplasies (white squares) were recognized using the Fitch optimization criterion in Winclada.

[Table 1. Morphological characters evaluated

Character	Character state
0. Plant life form	(0) Herbaceous; (1) Shrubs; (2) Treelets; (3) Trees; (4) Vines
1. Plant habit	(0) Terrestrial; (1) Epiphytic
2. Plant wood oxidation	(0) Not visible; (1) Pinkish; (2) Reddish
3. Plant raphides (stem and leaf)	(0) Absent; (1) Present
4. Stipule persistence	(0) Persistent; (1) Deciduous
5. Leaf disposition	(0) Opposite; (1) Ternate.
6. Leaf midrib color	(0) Greenish; (1) Purplish; (2) Yellowish; (3) Reddish.
7. Leaf tertiary veins disposition	(0) Reticulate; (1) Lineolate; (2) Tertiary veins inconspicuous
8. Leaf domatia	(0) Absent; (1) Present
9. Flower arrangement	(0) Solitary flowers; (1) Flowers arranged in inflorescences
10. Flower/Inflorescence position	(0) Terminal; (1) Axillar
11. Flower/Inflorescence disposition	(0) Erect; (1) Pendulous
12. Inflorescence type	(0) Cyme; (1) Monochasium; (2) Raceme; (3) Corymb; (4) Spiciform; (5) Solitary flowers
13. Calyx lobes size relation	(0) All equal to slightly unequal; (1) One lobe larger than the rest; (2) Two lobes larger than the rest.
14. Calyx lobes form	(0) Triangular; (1) Linear.
15. Corolla form	(0) Hypocrateriform; (1) Infundibuliform; (2) Rotate; (3) Tubular
16. Corolla color	(0) White; (1) Yellow; (2) Red; (3) Purple; (4) Yellow with red; (5) Orange
17. Corolla aestivation	(0) Valvated; (1) Imbricated; (2) Convolute
18. Corolla tube constriction	(0) Tube constrained at base; (1) Tube not constrained; (2) Tube expanded at apex; (3) Tube expanded at base; (4) Tube expanded at medium portion
19. Corolla tube external indumenta	(0) Glabrate; (1) Pubescent
20. Corolla tube size	(0) Tube lesser than 0.5 cm; (1) Tube 0.6-1 cm long; (2) Tube 1.1-2 cm long; (3) Tube 2.1-3 cm; (4) Tube 3.1-4 cm long; (5) Tube 4.1-5 cm long; (6) Tube 5.1-6 cm long; (7) Tube larger than 6 cm long
21. Corolla number of lobes	(0) 4; (1) 5
22. Stamen filament insertion	(0) Basally; (1) Medially; (2) Apically
23. Ovary locule number	(0) 2-locular; (1) 4-locular; (2) 5-locular
24. Fruit type	(0) Capsular; (1) Berry; (2) Drupe; (3) Samara
25. Fruit form	(0) Spheroidal; (1) Turbinate; (2) Cylindrical; (3) Elliptic; (4) Dorsiventrally flattened
26. Fruit dehiscence	(0) Indehiscent; (1) Septicidal; (2) Loculicidal; (3) Apically

		poricidal
27. Fruit size		(0) Fruits up to 0.5 cm long; (1) Fruits 0.6-1 cm long; (2) Fruits longer than 1 cm
28. Seed wing		(0) Wingless; (1) Winged
29. Seed dorsiventral compression		(0) Flattened; (1) Polygonal
30. Seed ornamentation		(0) Smooth; (1) Concave outlines with a sunken profile; (2) Papillose; (3) Verrucose

Appendix 1. Species voucher information

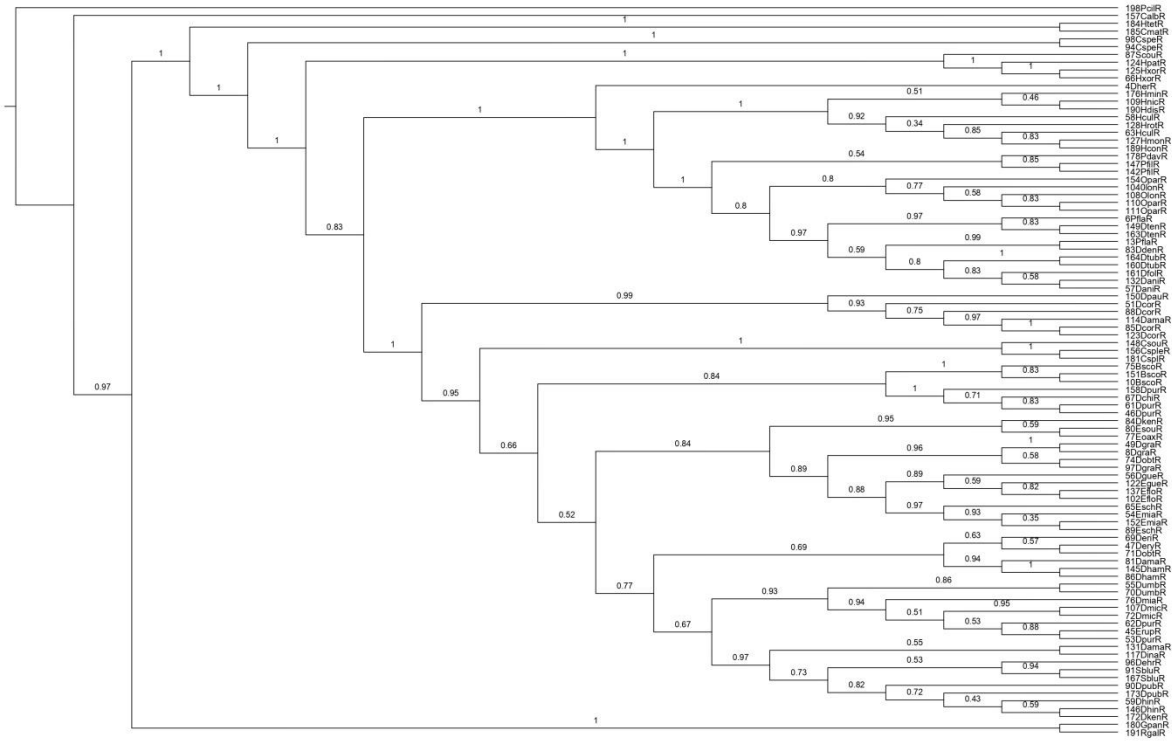
	Species	Tribe	Accession Genbank	Country	Colection	Herbarium
1	<i>Bellizinca scoti</i> (J.H. Kirkbr.) Borhidi	HAM		Mexico	<i>Ochoterena et al. 1119</i>	MEXU
2	<i>Bellizinca scoti</i> (J.H. Kirkbr.) Borhidi	HAM		Mexico	<i>Razo et al. 2668</i>	MEXU
3	<i>Bellizinca scoti</i> (J.H. Kirkbr.) Borhidi	HAM		Mexico	<i>Tenorio 20019</i>	MEXU
4	<i>Chiococca alba</i>	CHI		Mexico	<i>Ochoterena et al. 157</i>	MEXU
5	<i>Cosmibuena matudae</i>	HIL		Mexico	<i>Reyes-García et al. 7280</i>	MEXU
6	<i>Cosmocalyx spectabilis</i>	HAM		Mexico	<i>Álvarez et al. 1866</i>	MEXU
7	<i>Cosmocalyx spectabilis</i>	HAM		Mexico	<i>Álvarez 6000</i>	MEXU
8	<i>Csapodya sousae</i>	HAM		Mexico	<i>Maya 3892</i>	MEXU
9	<i>Csapodya splendens</i>	HAM		Mexico	<i>Ochoterena et al. 1053</i>	MEXU
10	<i>Csapodya splendens</i>	HAM		Mexico	<i>Ochoterena et al. 1053b</i>	MEXU
11	<i>Deppea pauciflora</i>	HAM		Mexico	<i>Medrano et al. 17033</i>	MEXU
12	<i>Deppea amaranthina</i>	HAM		Mexico	<i>Maya 1764</i>	MEXU
13	<i>Deppea amaranthina</i>	HAM		El Salvador	<i>Sandoval & Pérez 1225</i>	MEXU
14	<i>Deppea amaranthoides</i>	HAM		Mexico	<i>Barrera et al. 146</i>	MEXU
15	<i>Deppea cornifolia</i>	HAM		Mexico	<i>Aguilar et al. 41</i>	MEXU
16	<i>Deppea cornifolia</i>	HAM		Mexico	<i>Reyes 419</i>	MEXU
17	<i>Deppea cornifolia</i>	HAM		Mexico	<i>Steinmann 9491</i>	MEXU
18	<i>Deppea cornifolia</i>	HAM		Mexico	<i>Torres-Montúfar et al. 979</i>	MEXU
19	<i>Deppea densiflora</i>	HAM		Mexico	<i>Reyes-García & Gómez 4584</i>	MEXU
20	<i>Deppea ehrenbergii</i>	HAM		Mexico	<i>Velasco et al. 2438</i>	MEXU
21	<i>Deppea erythrorhiza</i>	HAM		Mexico	<i>Munn-Estrada & Kasey 1101</i>	MEXU
22	<i>Deppea erythrorhiza</i>	HAM		Mexico	<i>Ochoterena et al. 956</i>	MEXU
23	<i>Deppea grandiflora</i>	HAM		Mexico	<i>Ochoterena et al. 1025</i>	MEXU
24	<i>Deppea grandiflora</i>	HAM		Mexico	<i>Ochoterena et al. 699</i>	MEXU
25	<i>Deppea hamelioides</i>	HAM		Mexico	<i>Bye 7362</i>	MEXU
26	<i>Deppea hamelioides</i>	HAM		Mexico	<i>Téllez 9869</i>	MEXU
27	<i>Deppea hintonii</i>	HAM		Mexico	<i>Ochoterena et al. 332</i>	MEXU
28	<i>Deppea hintonii</i>	HAM		Mexico	<i>Torres et al. 5248</i>	MEXU
29	<i>Deppea hintonii</i>	HAM		Mexico	<i>Campos 1967</i>	MEXU
30	<i>Deppea inaequalis</i>	HAM		Mexico	<i>Breedlove 55760</i>	MEXU
31	<i>Deppea keniae</i>	HAM		Mexico	<i>Torres 17304</i>	MEXU
32	<i>Deppea microphylla</i>	HAM		Mexico	<i>Moreno 91</i>	MEXU
33	<i>Deppea microphylla</i>	HAM		Mexico	<i>Ochoterena et al. 678</i>	MEXU
34	<i>Deppea microphylla</i>	HAM		Mexico	<i>Sharp & Miranda 3397</i>	MEXU

35	<i>Deppea obtusiflora</i>	HAM	Mexico	<i>Ochoterena & Bailey</i> 329	MEXU
36	<i>Deppea obtusiflora</i>	HAM	Mexico	<i>Ochoterena et al.</i> 769	MEXU
37	<i>Deppea pubescens</i>	HAM	Mexico	<i>Ochoterena & Bailey</i> 333	MEXU
38	<i>Deppea pubescens</i>	HAM	Mexico	<i>Torres & Cedillo</i> 1403	MEXU
39	<i>Deppea purpurascens</i>	HAM	Mexico	<i>A. Torres Montúfar</i> 828	MEXU
40	<i>Deppea purpurascens</i>	HAM	Mexico	<i>Ochoterena et al.</i> 1016	MEXU
41	<i>Deppea purpurascens</i>	HAM	Mexico	<i>Ochoterena et al.</i> 1069	MEXU
42	<i>Deppea purpurascens</i>	HAM	Mexico	<i>Maya</i> 1345	MEXU
43	<i>Deppea purpusii</i>	HAM	Mexico	<i>Aguilar et al.</i> 47	MEXU
44	<i>Deppea purpusii</i>	HAM	Mexico	<i>Ochoterena et al.</i> 814	MEXU
45	<i>Deppea umbellata</i>	HAM	Mexico	<i>Ochoterena et al.</i> 836	MEXU
46	<i>Deppea umbellata</i>	HAM	Mexico	<i>Ochoterena et al.</i> 855	MEXU
47	<i>Deppeopsis anisophylla</i>	HAM	Mexico	<i>Ochoterena et al.</i> 792	MEXU
48	<i>Deppeopsis anisophylla</i>	HAM	Mexico	<i>Salas et al.</i> 4463	MEXU
49	<i>Deppeopsis foliosa</i>	HAM	Mexico	<i>Ochoterena et al.</i> 1102	MEXU
50	<i>Deppeopsis hernandezii</i>	HAM	Mexico	<i>Ochoterena et al.</i> 680	MEXU
51	<i>Deppeopsis tenuiflora</i>	HAM	Mexico	<i>Croat</i> 47794	MEXU
52	<i>Deppeopsis tenuiflora</i>	HAM	Mexico	<i>Ochoterena et al.</i> 1062	MEXU
53	<i>Deppeopsis tubaeana</i>	HAM	Mexico	<i>Ochoterena et al.</i> 1113a	MEXU
54	<i>Deppeopsis tubaeana</i>	HAM	Mexico	<i>Ochoterena et al.</i> 1113c	MEXU
55	<i>Edithea floribunda</i>	HAM	Mexico	<i>Calzada</i> 19772	MEXU
56	<i>Edithea floribunda</i>	HAM	Mexico	<i>Diego & R. de Santiago</i> 9575	MEXU
57	<i>Edithea guerrerensis</i>	HAM	Mexico	<i>Calzada</i> 19834	MEXU
58	<i>Edithea guerrerensis</i>	HAM	Mexico	<i>Ochoterena & Bailey</i> 335	MEXU
59	<i>Edithea miahuatlanica</i>	HAM	Mexico	<i>Ochoterena & Bailey</i> 331	MEXU
60	<i>Edithea miahuatlanica</i>	HAM	Mexico	<i>Ochoterena et al.</i> 1095	MEXU
61	<i>Edithea miahuatlanica</i>	HAM	Mexico	<i>Pascual</i> 2010	MEXU
62	<i>Edithea oaxacana</i>	HAM	Mexico	<i>Torres et al.</i> 2068	MEXU
63	<i>Edithea rupicola</i>	HAM	Mexico	<i>Sandoval et al.</i> 922	MEXU
64	<i>Edithea schiblii</i>	HAM	Mexico	<i>Trujillo</i> 195	MEXU
65	<i>Edithea schiblii</i>	HAM	Mexico	<i>Dominguez</i> 29	MEXU
66	<i>Edithea sousae</i>	HAM	Mexico	<i>Munn et al.</i> 261	MEXU
67	<i>Gonzalagunia panamensis</i>	GUE	Mexico	<i>Ochoterena et al.</i> 1101	MEXU
68	<i>Hamelia xorullensis</i>	HAM	Mexico	<i>Ochoterena & Bailey</i> 220	MEXU
69	<i>Hamelia patens</i>	HAM	Mexico	<i>Aguilar et al.</i> 66	MEXU
70	<i>Hamelia xorullensis</i>	HAM	Mexico	<i>Ochoterena & Bailey</i> 221	MEXU
71	<i>Hillia tetrandra</i>	HIL	Mexico	<i>Salazar</i> 344	MEXU

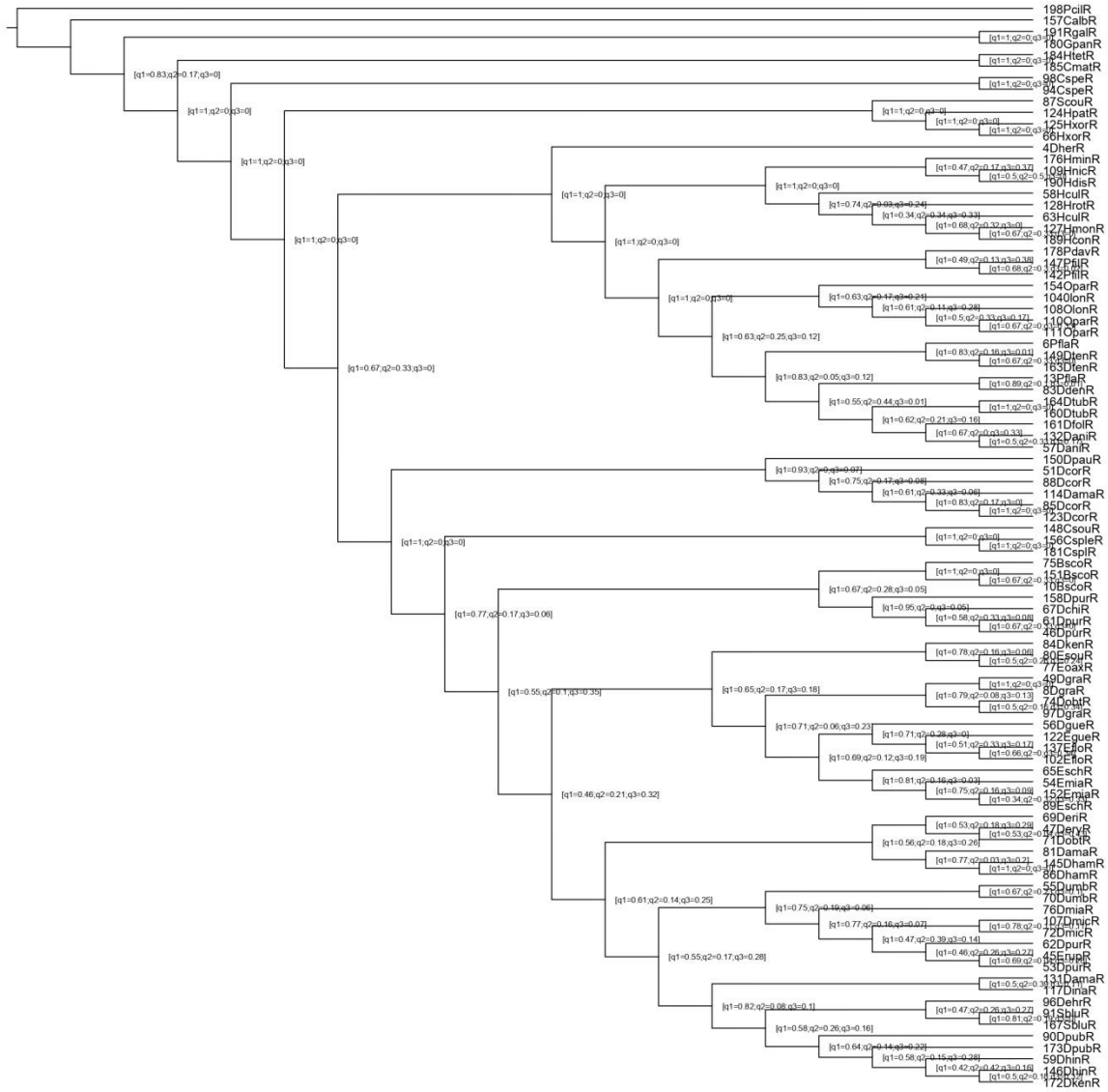
72	<i>Hoffmannia conzatti</i>	HAM	Mexico	<i>Aguilar et al. 48</i>	MEXU
73	<i>Hoffmannia culminicola</i>	HAM	Mexico	<i>Aguilar et al. 12</i>	MEXU
74	<i>Hoffmannia culminicola</i>	HAM	Mexico	<i>Aguilar et al. 59</i>	MEXU
75	<i>Hoffmannia discolor</i>	HAM	Mexico	<i>Ochoterena et al. 474</i>	MEXU
76	<i>Hoffmannia minuticarpa</i>	HAM	Mexico	<i>Perez & Orona 133</i>	MEXU
77	<i>Hoffmannia montana</i>	HAM	Mexico	<i>Aguilar et al. 25</i>	MEXU
78	<i>Hoffmannia nicotianifolia</i>	HAM	Mexico	<i>Ochoterena et al. 738</i>	MEXU
79	<i>Hoffmannia rotata</i>	HAM	Mexico	<i>Aguilar et al. 44</i>	MEXU
80	<i>Omiltemia longipes</i>	HAM	Mexico	<i>Fonseca 2 845</i>	MEXU
81	<i>Omiltemia longipes</i>	HAM	Mexico	<i>De Santiago 156</i>	MEXU
82	<i>Omiltemia parvifolia</i>	HAM	Mexico	<i>Carrada et al. 281</i>	MEXU
83	<i>Omiltemia parvifolia</i>	HAM	Mexico	<i>Carrada et al. 283</i>	MEXU
84	<i>Omiltemia parvifolia</i>	HAM	Mexico	<i>Ochoterena et al. 1105</i>	MEXU
85	<i>Paederia ciliolata</i>	PAE	Mexico	<i>Ochoterena et al. 886</i>	MEXU
86	<i>Pinarophyllon flavum</i>	HAM	Mexico	<i>Martínez 1 955</i>	MEXU
87	<i>Plocaniphyllon flavum</i>	HAM	Mexico	<i>Martínez 1 920</i>	MEXU
88	<i>Pseudomiltemia davidsonii</i>	HAM	Mexico	<i>Hapshire et al. 772</i>	MEXU
89	<i>Pseudomiltemia filisepala</i>	HAM	Mexico	<i>Heath & Long 842</i>	MEXU
90	<i>Pseudomiltemia filisepala</i>	HAM	Mexico	<i>Martínez et al. 1592</i>	MEXU
91	<i>Renistipula galeotii</i>	GUE	Mexico	<i>Corona & Campos 147</i>	MEXU
92	<i>Schenckia blumenaviensis</i>	HAM	Brasil	<i>Rambo 38 396</i>	
93	<i>Schenckia blumenaviensis</i>	HAM	Brasil	<i>Rambo 41682</i>	
94	<i>Syringantha coulteri</i>	HAM	Mexico	<i>Lorence 5043</i>	MEXU

CHI=Chiococceae; GUE=Guettardeae; HAM=Hamelieae; HIL=Hillieae; PAE= Paederieae

Appendix 2. A. ASTRAL-III species tree generated using 164 nuclear loci from the Angiosperms353 probe set. Numbers on branches denote local posterior probabilities (LPP). B. Quartet frequencies at branches are labeled on branches.



A. Astral tree. Posterior probabilities.



B. Astral tree. Quartets.

VI. DISCUSIÓN Y CONCLUSIONES GENERALES

A la fecha, el presente trabajo es el que incluye la mayor cantidad de géneros y especies para la tribu Hamelieae y para el complejo *Deppea* con 14 de los 16 géneros que conforman la tribu. Únicamente no fueron incluidas especies de los géneros *Eizia* (monotípico mexicano) y *Patima* (Sudamericano).

El primer reto que se enfrentó para el estudio del complejo *Deppea* fue encontrar caracteres morfológicos que sustentaran el reconocimiento de los géneros, que son inevitablemente necesarios en la práctica taxonómica por lo que todos los capítulos presentados en este trabajo tienen un componente morfológico.

Morfología de la semilla

En el complejo *Deppea*, los principales caracteres empleados para la segregación de géneros están relacionados con las flores y los frutos, es por ello que se decidió explorar nuevas fuentes de evidencia taxonómica, como el conocido valor taxonómico de la morfología de semilla para otros grupos de la familia (e.g., Terrel et al. 1986; Robbrecht 1988; Khalik et al. 2008). Dado el diminuto tamaño de las semillas en este grupo, el estudio empleó tanto microscopía estereoscópica como microscopía electrónica de barrido, lo que permitió observar y evaluar por primera vez la microestructura de las semillas para 15 géneros de Hamelieae y 37 especies, con todos los géneros del complejo *Deppea* incluidos y 29 especies.

Si bien existe un alto traslapamiento de las características observadas de la semilla, algunas permiten distinguir algunos géneros y especies dentro del complejo *Deppea*, tales como la forma de la semilla, el color de la testa, la compresión dorsiventral, la posición del hilum y el relieve periclinal.

En los géneros de Hamelieae, *Syringantha* se distingue entre todos los géneros de la tribu por los márgenes alados, por su parte, *Cosmocalyx* se caracteriza por la semilla de testa roja, aplanada y linear. La semilla de *Eizia* se distingue por la posición sub-central del micrópilo. En el género *Hamelia* tienen forma romboidal y la testa papilosa. Por otro lado

Hoffmannia no posee características distintivas a nivel genérico, ya que las especies estudiadas comparten características con otras especies muestreadas de *Deppea* y *Editheia*.

Dentro del complejo *Deppea* la compresión dorsiventral es una característica importante. Los géneros *Csapodya*, *Deppeopsis*, *Omiltemia* y *Pseudomiltemia* poseen semillas aplanadas, mientras que el resto tienen semillas poligonales. Otro carácter importante es la ornamentación de la testa, entre los géneros con las semillas aplanadas *Csapodya* y *Pseudomiltemia* tienen la testa papilosa, mientras que *Deppeopsis* y *Omiltemia* es verrucosa. En los géneros con semillas poligonales *Bellizinca* tiene la testa verrucosa (compartido con una especie de *Deppea*), en *Pinarophyllon* y *Plocaniophyllon* es papilosa. Se distinguen dado que *Pinarophyllon* tiene testa amarilla y células isodiamétricas del micrópilo y *Plocaniophyllon* semillas rojas y células del micrópilo son elongadas.

El género *Deppea*, el segundo más diverso en Hamelieae, es muy heterogéneo en la morfología de las semillas. Algunas tienen testa papilosa y otras tienen un contorno cóncavo con líneas hundidas, este último compartido con *Editheia* y *Schenckia*, de los cuales *Schenckia* posee el margen V-ondulado en las células anticlinales, mientras que *Deppea* y *Editheia* presentan margen recto.

Con base en la morfología de la semilla para el género *Deppea*, se podrían reconocer los géneros *Bellizinca*, *Csapodya*, *Deppea*, *Deppeopsis*, *Omiltemia*, *Pinarophyllon*, *Plocaniophyllon* y *Pseudomiltemia*, y únicamente los géneros *Editheia* y *Schenckia* podrían ser sinonimizados con *Deppea*.

Filogenia combinada: molecular (ITS+trnL-F) y morfología (flor+fruto+semilla)

Se realizó un ejercicio para evaluar la relevancia filogenética de los caracteres morfológicos a la luz de evidencia molecular disponible generando una reconstrucción filogenética realizada con 136 secuencias descargadas de GenBank y alineadas a ojo, del marcador de núcleo (ITS) y el plástido (*trnL-F*) en un análisis combinado con una matriz morfológica de 24 caracteres florales y de fruto, los cuales son los tradicionalmente empleados en la literatura para la distinción de los géneros.

Los resultados obtenidos recuperan al complejo *Deppea* en dos clados dentro de Hamelieae, en uno *Deppea* es parafilético con respecto a *Bellizinca*, *Csapodya*, *Editheia* y

Schenckia, mientras el otro clado hay una politomía que incluye a *Deppeopsis*, *Omiltemia*, *Plocaniophyllon*, *Pinarophyllon* y *Pseudomiltemia* donde también se incluye *Hoffmannia*.

Dos de los principales caracteres morfológicos empleados para la segregación de géneros son la forma de la corola y el largo de su tubo, los cuales fueron usados para segregar de *Deppea* a los géneros *Bellizinca*, *Caspodya* y *Edithea* (Borhidi et al., 2004; Borhidi & Reyes-García, 2007; Borhidi & Stranzinger 2012a; Borhidi & Stranzinger 2012b). Por su parte, Lorence & Dwyer (1988) consideran el tubo largo de la corola como una adaptación a la polinización por colibríes y como un extremo de variación. En el estudio molecular aquí presentado, estos géneros, junto con *Schenckia*, forman una politomía con *Deppea*, por lo cual su sinonimización es apoyada.

El resto de los géneros del complejo *Deppea* se ubican en un clado junto con *Hoffmannia*, el único otro género de Hamelieae con frutos carnosos además de *Hamelia*. Este clado carece de marcada resolución, pero la politomía implica a *Pinarophyllon* y *Hoffmannia* como monofiléticos, mientras que *Deppeopsis hernandezii* se recupera por fuera del clado *Deppeopsis*. De este clado, con respecto al complejo *Deppea*, se puede hipotetizar que *Pinarophyllon* y *Plocaniophyllon* deben ser aceptados; sin embargo, la baja representación de especies para estos géneros impide una resolución taxonómica más aproximada.

Si bien el pobre muestreo de terminales disponible en GenBank no permite proponer una hipótesis robusta sobre la relación de estos géneros y las decisiones taxonómicas subsecuentes, los caracteres morfológicos de la flor, fruto y semilla consideramos son buena fuente de evidencia para un análisis con un mayor número de taxones.

Filogenómica nuclear del complejo Deppea

Dado que el estudio filogenético empleando marcadores tradicionales no resultó en una filogenia robusta y dado que la perspectiva era un número limitado de caracteres potencialmente informativos, se optó por explorar secuenciación de nueva generación con una tecnología diseñadas para secuenciar gran cantidad de segmentos de ADN de forma masiva y en paralelo, en menor cantidad de tiempo y a un menor costo por base.

Particularmente se usó captura de secuencias mediante el kit Angiosperms353, el cual pretende funcionar para prácticamente cualquier grupo de plantas para la obtención de 353 loci. Nuestro muestreo de genes fue satisfactorio, ya que de los 353 posibles genes se obtuvieron para el análisis 164 genes sin problemas de paralogía potencial. Además, el muestreo de terminales incluye 14 de los 16 de los géneros de la tribu Hamelieae, incluidos los 10 géneros del complejo *Deppea*, asimismo se analizaron 40 especies de las aproximadamente 45 especies descritas para el complejo *Deppea*; con lo cual se tuvo un panorama más amplio para las decisiones taxonómicas.

En total se obtuvieron 166,259 pares de bases de las cuales 25,426 fueron potencialmente informativas para parsimonia. El análisis arrojó un resultado similar al obtenido con los marcadores descargados de GenBank, donde los géneros del complejo *Deppea* se encontraron en dos grandes clados, el primero incluye una politomía con *Bellizinca*, *Csapodya*, *Deppea*, *Edithea* y *Schenckia*, mientras el segundo clado, con el mayor muestreo de terminales y de caracteres, mostró mayor resolución. Dada la importancia observada de la morfología, en este análisis se mapeó una matriz morfológica que incluye caracteres vegetativos y reproductivos.

Este resultado apoya que *Bellizinca*, *Csapodya*, *Edithea* y *Schenckia* sean sinonimizados con *Deppea*, género donde el tamaño del tubo de la corola es ampliamente variable, pero que comparte los frutos turbinados con dehiscencia poricida. El género *Deppea* resultó polifilético, ya que *Deppea densiflora* se agrupó fuera del clado. Dado este resultado, y las flores blancas, carácter morfológico inusual en la tribu, proponemos la descripción de un nuevo género llamado *Pseudodeppea*.

Los géneros *Omitemia* y *Pseudomitemia*, resultaron monofiléticos, ambos con sustento morfológico, así como la única muestra de *Pinarophyllon flavum* que se recuperó como un linaje bien apoyado por la morfología.

El género *Deppeopsis*, con cinco especies, también resultó polifilético ya que su especie tipo, *D. hernandezii*, resultó hermana del clado formado por *Hoffmannia-Omitemia-Pinarophyllon-Plocaniophyllon-Pseudomitemia-Deppeopsis*, por lo cual se tomó la decisión de segregar la especie en un nuevo género monotípico sustentado en las hojas ternadas y las estípulas deciduas.

De igual manera la especie *Deppeopsis tenuiflora* se agrupó con *Plocaniophyllon flavum*, la única especie de este género; basados en la venación terciaria lineolada, se optó por proponer una nueva combinación para *Deppeopsis tenuiflora*: *Plocaniophyllon tenuiflorus*. Las especies restantes de *Deppeopsis* se agrupan en un clado, el cual conforma el nuevo género *Pseudodeppeopsis*, caracterizado por las hojas opuestas, estípulas persistentes y frutos cilíndricos con dehiscencia poricida.

En el presente trabajo se abordó la taxonomía del complejo *Deppea* desde el punto de vista morfológico y molecular, en este último aspecto se recuperaron hipótesis similares tanto teniendo el cloroplasto-núcleo en combinación, como con solo la filogenómica del núcleo, Secuenciación Sanger y Secuenciación de Nueva Tecnología, lo cual brinda cierta certeza a las decisiones taxonómicas propuestas.

El resultado de la filogenia abre otras preguntas, algunas relacionadas con la biogeografía, ya que *Deppea blumenaviensis* (tratada como algunos autores como *Schenckia blumenaviensis*) es un taxón que se distribuye en Brasil, mientras el resto de las especies de *Deppea* tienen centro de diversidad en México y se extienden hasta Centroamérica. También quedan algunas interrogantes a nivel de especie. Nuestro objetivo fue la delimitación genérica; sin embargo, en el análisis de filogenómica algunas especies con más de dos terminales, como *Omitelia parvifolia* o *Deppea amarantina*, no resultaron en especies monofiléticas, lo cual abre la posibilidad de explicaciones biológicas e históricas relevantes, como la posibilidad de hibridación ancestral o polimorfismo ancestral, o, del reconocimiento de nuevos taxones para ciencia. Por lo tanto, es deseable continuar con el estudio de esta tribu realizando tratamientos taxonómicos detallados para los géneros del complejo *Deppea* en los que se revise más material de herbario y se colecte en toda su área de distribución.

VII. REFERENCIAS BIBLIOGRÁFICAS GENERALES

- Andersson L. & Rova H. E. 1999. The rps16 intron and the phylogeny of the Rubioideae (Rubiaceae). *Pl. Syst. Evol.* 214: 161–186.
- Andreasen K. & Bremer B. 2000. Combined phylogenetic analysis in the Rubiaceae-Ixoroideae: morphology, nuclear and chloroplast DNA data. *Am. J. Bot.* 87(11): 1731-1748.
- Borhidi A. 2004. Critical revision of the *Omitemia* complex (Rubiaceae, Hamelieae) *Acta Bot. Hung.* 46(1–2): 82–85
- Borhidi A. & Reyes-García A. 2007. Estudios Sobre Rubiáceas Mexicanas XI Revisión del género *Csapodya* Borhidi (Rondeletieae), endémico de Chiapas con tres especies. *Acta Bot. Hung.* 49(1–2): 13–25.
- Borhidi A. 2006. Rubiáceas de México. Akadémiai Kiadó, Budapest. 608 pp.
- Borhidi A. 2012. Rubiáceas de México. Akadémiai Kiadó, Budapest. XXX pp.
- Borhidi A., Darók J., Kocsis M., Stranzinger Sz. & Kaposvári F. 2004. Critical revision of the *Deppea* complex (Rubiaceae, Hamelieae). *Acta Bot. Hung.* 46: 77–89.
- Borhidi A. & Stranzinger Sz. 2012a. Combinaciones nuevas en la familia Rubiaceae de la Flora de México. *Acta Bot. Hung.* 54(1–2): 81–84.
- Borhidi A. & Stranzinger Sz. 2012b. *Deppeopsis*, un género nuevo (Hamelieae, Rubiaceae) de México y Guatemala. *Acta Bot. Hung.* 54(1–2): 85–90.
- Borhidi A. & Velasco-Gutierrez, K. 2010. Estudios sobre Rubiáceas Mexicanas XXVI. *Paradeppea* sect. nova del género *Deppea* Cham. et Schltdl. (Hamelieae) y dos especies nuevas de Oeste de Oaxaca. *Acta Bot. Hung.* 52: 41–48.
- Brandege T. S. 1914. *Plantae Mexicanae Purpusianae*. Univ. Calif. Publ. Bot. 6(4): 69. 1914.
- Bremekamp C. E. B. 1966. Remarks on the position, the delimitation and the subdivision of the Rubiaceae. *Acta Bot. Neerl.* 15: 1-33.

- Bremer B. 1987. The sister group of the paleotropical tribe Argostemmatae: a redefined neotropical tribe Hamelieae (Rubiaceae, Rubioideae). *Cladistics* 3: 35–51.
- Bremer B. 1996. Combined and separate analyses of morphological and molecular data in the plant family Rubiaceae. *Cladistics* 12: 21–40.
- Bremer B. & Eriksson T. 2009. Time tree of Rubiaceae: phylogeny and dating the family, subfamilies, and Tribes. *Int. J. Plant Sci.* 170(6): 766–793.
- Bremer B., Andreasen K. & Olsson D. 1995. Subfamilial and tribal relationships in the Rubiaceae based on rbcL sequence data. *Ann. Mo. Bot. Gard.* 82(3): 383-397.
- Chamisso L. K. A. & Schlechtendal D. F. L. 1830 *Linnaea* 5: 167–168. 1830.
- Galambos A., Stranzinger Sz. & Borhidi A. (2009): Molecular phylogenetic studies in the Hamelieae section of Rubiaceae. Summaries, Poster-Session, (PG21), 8th Hungarian Genetic Congress XV. Cell and Evolution, Nyíregyháza. 109–110.
- Hooker J. D. 1873. Rubiaceae. In: G. Bentham & J. D. Hooker, editors *Genera Plantarum ad exemplaria imprimis in herbariis kewensibus servata defirmata*, 2: 7–151.
- Johnson M.G., Gardner E.M., Liu Y., Medina R., Goffinet B., Shaw A.J., Zerega N.J.C. & Wickett N.J. 2016. HybPiper: Extracting coding sequence and introns for phylogenetics from high-throughput sequencing reads using target enrichment. *Appl. Plant Sci.* 4.
- KEW. 2017. Royal Botanic Gardens <http://www.kew.org/science-conservation/research-data/science-directory/teams/rubiaceae> (Última consulta: 12/01/2017).
- Kirkbride H.J. 1984. Manipulus Rubiacearum III. Deppeae, a new tribe of Rubioideae (Rubiaceae). *Brittonia*, 36(3): 317-320.
- Lorence D. H. 1999. A nomenclator of Mexican and Central American Rubiaceae. *Mo. Bot. Gard. Monogr. Ser.* 73: 1-177.

- Lorence D. H. & Taylor C. M. 2012. In: Davidse, G., Sousa Sánchez M., Knapp S. & F. Chiang Cabrera. 2012. Rubiaceae a Verbenaceae. 4(2): i–xvi, 1–533. Fl. Mesoamer. Missouri Botanical Garden Press, St. Louis.
- Lorence D. & Dwyer J. 1988. A revision of *Deppea* (Rubiaceae). *Allertonia* 4(7): 389–436.
- Lozada-Pérez L. & Rojas-Gutiérrez J. 2016. Una nueva especie, *Omiltemia guerrerensis* (Rubiaceae) de Guerrero, México. *Novon* 24(4): 365–368.
- Martínez-Cabrera D., Terrazas T. & Ochoterena H. 2007. Leaf architecture of Hamelieae. *Feddes Repert.* 118: 286–310.
- Martínez-Cabrera D., Terrazas T. & Ochoterena H. 2009. Foliar and petiole anatomy of tribe Hamelieae and other Rubiaceae. *Ann. Mo. Bot. Gard.* 96: 133–145.
- Martínez-Cabrera D., Terrazas T., Ochoterena H. & López-Mata L. 2010. Bark and wood anatomy of the tribe Hamelieae (Rubiaceae). *Iawa Journal*, 31 (4): 425–442
- Martínez-Camilo, R., Martínez-Meléndes, N., Pérez-Farrera, M. & Lorence, D. 2011. Una nueva especie de *Pseudomiltemia* (Rubiaceae) de Chiapas, México. *Brittonia* 63(2): 198–199
- Manns U. & Bremer B. 2010 Towards a better understanding of intertribal relationships and stable tribal delimitations within Cinchonoideae s.s. (Rubiaceae). *Mol Phylogenet Evol* 56: 21–39.
- Manns U., Wikström N, Taylor C. M. & Bremer B. 2012 Historical Biogeography of the predominantly neotropical subfamily Cinchonoideae (Rubiaceae): into or out of America? *Int. J. Plant Sci.* 173(3):261–289.
- Morton C.V. 1968. A second species of *Omiltemia*. *Baileya* 16:98.
- Robbrecht E. 1988. Tropical woody Rubiaceae. *Opera Botanica Belgica* 1: 1–272.
- Robbrecht E. & Manen J.F. 2006. The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms). Combined analysis (nDNA and cpDNA) to infer the position of *Coptosapelta* and *Luculia*, and supertree construction based on *rbcL*,

- rps16, trnL-trnF and atpB-rbcL data. A new classification in two subfamilies, Cinchonoideae and Rubioideae. *Syst. Geogr. Pl.* 76: 85–146.
- Schumann K. M. 1889. Rubiaceae *Fl. Bras.* 6: 246.
- Standley P. C. 1918 Rubiaceae. *North American Flora* 32: 1–300.
- Standley P. C. 1933. *Edithea*, a new genus of Mexican shrubs of the family Rubiaceae *Tropical Woods* 34: 1-3.
- Stranzinger Sz., Galambos A., Szenas D. & Szalontai B. 2014. Phylogenetic relationships in the Neotropical tribe Hamelieae (Rubiaceae, Cinchonoideae) and comments on its generic limits. *J. Syst. Evol.* 52 (5): 643–650.
- Stresseman, E. 1954. Ferdinand's Deppe travels in Mexico, 1824–1829. *Condor* 56: 86–92.
- Taylor C. M. 2001 Rubiaceae Juss. In: Stewens, W. D. et al. (eds): *Flora de Nicaragua*. *Ann. Mo. Bot. Gard.* 85: 2206–2284.
- Torres-Montúfar J. A. 2018. Sistemática del complejo *Rondeletia* (Rubiaceae). Tesis de Doctorado, Instituto de Biología-Universidad Nacional Autónoma de México.
- Verdcourt B. 1958. Remarks on the classification of the Rubiaceae. *Bull. Jard. Bot. Etat, Brux.* 28: 209-281.
- Villaseñor J. L. 2016. Checklist of the native vascular plants of Mexico. *Revista Mexicana de Biodiversidad* 87: 559–902.