ORIGINAL ARTICLE

A preliminary phylogeny of *Zapoteca* (Fabaceae: Caesalpinioideae: Mimosoid clade)

Julia Ferm[1]

Abstract

The legume genus *Zapoteca* is separated from the genus *Calliandra*, i.e., by having pollen arranged in 16-grained polyads (compared to 8-grained polyads in *Calliandra*) and in chromosome number (13 vs. 8 or 11). As currently circumscribed, *Zapoteca* contains 22 species and 13 subspecies placed in five subgenera. This study included 20 species and 11 subspecies representing all subgenera. Representative species from the closely related genera *Calliandra*, *Havardia*, *Pithecellobium* and *Viguieranthus* were also included, as the more distantly related species *Senegalia senegal* and *Vachellia farnesiana*. The aims of this study were to test the monophyly of *Zapoteca* and investigate phylogenetic relationships within the genus. Total DNA was extracted from leaf material and the nuclear ETS and ITS, and plastid *trn*L–*trn*F regions were amplified. Additional sequence data were downloaded from GenBank, and the data sets were analyzed using Bayesian inference. Results show that *Zapoteca* is monophyletic and that the monospecific subgenera (subg. *Nervosa* and subg. *Aculeata*) are resolved as separate lineages within the genus, subg. *Nervosa* (containing *Z. nervosa*) as sister to all remaining taxa of *Zapoteca*. Subgenera containing more than one species (subg. *Amazonica*, subg. *Zapoteca* and subg. *Ravenia*) are shown to be non-monophyletic. Two subspecies of *Z. caracasana*, subsp. *caracasana* and subsp. *weberbaueri*, are found together in a clade. Furthermore, *Zapoteca tehuana* and *Z. portoricensis* subsp. *portoricensis*, *Z. formosa* subsp. *gracilis* and *Z. formosa* subsp. *schottii* are shown to be monophyletic. However, the other subspecific taxa of *Z. formosa* and of *Z. portoricensis* are not supported as monophyletic.

Keywords Bayesian inference · Leguminosae · Neotropics · Phylogenetic analyses · Taxonomy

Introduction

The mimosoid genus *Zapoteca* H.M.Hern. (Fabaceae) mostly comprises erect or scandent shrubs up to 3–4 m tall with the main woody stems 1–2 cm in diameter, although individuals of *Z. tetragona* (Willd.) H.M.Hern. and *Z. portoricensis* (Jacq.) H.M.Hern. are reported to have stems up to 20 cm in diameter (Hernández 1989). *Zapoteca* is further characterized by stipulate, bipinnate leaves with opposite leaflets that range from 0.5 cm (*Z. alinae* H.M.Hern. and *Z. media* (M.Martens & Galeotti) H.M.Hern.) to 22 cm (*Z. amazonica* (Benth.) H.M.Hern.) in length, the number of

✉ Julia Ferm
   julia.ferm@ebc.uu.se

[1] Department of Organismal Biology, Systematic Biology, Uppsala University, Norbyv. 18D, 75236 Uppsala, Sweden

leaflets on each leaf being inversely related to leaflet size (Hernández 1989). The members of *Zapoteca* have axillary inflorescences with densely assembled flowers, each with ca. 30–60 stamens with long-exserted, white, pink, purple or bicolored filaments. The pods are dry and straight with thickened margins, the valves dehiscing explosively from the apex to the base. The seeds are hard and arranged in one series (Hernández 1989).

Zapoteca has its highest species diversity in southern Mexico but has a total distribution ranging from southwestern USA to northern Argentina, including the West Indies. They can be found from sea level up to ca. 2850 m altitude (Tropicos.org 2018). Most species grow in tropical dry forest and disturbed habitats in seasonally dry areas, but some species occur in montane wet forest and lowland rainforest (Hernández 1989).

Zapoteca was described by Hernández (1986). He considered the members of *Calliandra* ser. *Laetevirentes* Benth. to be morphologically different from all other

species of *Calliandra* Benth. and described *Zapoteca* to accommodate the approximately 25 species of this series. However, he only provided new combinations in *Zapoteca* for seven of them. The newly recognized genus *Zapoteca* was characterized by having pollen arranged in 16-grained polyads, thin and membranous (rarely coriaceous) leaflets and membranous to coriaceous pods, whereas the genus *Calliandra* has pollen in 8-grained polyads, chartaceous to coriaceous leaflets and rigid pods. Also, the basic chromosome number of *Zapoteca* is 13 (shared with the other members of Ingeae), while in *Calliandra* the basic chromosome number is 8 or 11 (Hernández 1986).

Hernández (1986) also discussed the possibility that two species of *Calliandra* ser. *Macrophyllae* Benth., *C. amazonica* Benth. and *C. aculeata* Spruce ex Benth., were misplaced in *Calliandra*, but was uncertain if they should be placed in *Zapoteca*. He pointed out that these two species and *Zapoteca* share the same polyad and inflorescence structures, but that the small number and larger size of the leaflets distinguishes them from all other species of *Zapoteca*. Therefore, Hernández (1986) considered further studies needed before any change in their taxonomic content or placement should be formally proposed.

A few years later Hernández (1989) presented a monograph of *Zapoteca* and provided new combinations in the genus for the remaining species of *Calliandra* ser. *Laetevirentes*, as well as those of ser. *Macrophyllae* mentioned above. By doing this, Hernández (1989) expanded *Zapoteca* to contain a total of 17 species and 11 subspecies (Table 1). Furthermore, in that work he recognized four subgenera, viz. subg. *Nervosa* H.M.Hern., subg. *Zapoteca*, subg. *Aculeata* H.M.Hern. and subg. *Amazonica* H.M.Hern. (Table 1), based on variation in vegetative characters such as leaf morphology and venation patterns of the leaflets. Following this classification, subg. *Zapoteca* has a distribution from Mexico throughout the Neotropics, while subg. *Aculeata* and subg. *Amazonica* are restricted to South America and subg. *Nervosa* to the West Indies.

Since the work by Hernández (1989) one additional subgenus, subg. *Ravenia* H.M.Hern., as well as five species and one subspecies have been described (Hernández 1990, 2015; Hernández and Campos 1994; Hernández and Hanan-Alipi 1998) (Table 1). Furthermore, one additional species of *Calliandra*, *C. socorrensis* I.M.Johnst., has been transferred to *Zapoteca* and placed as a subspecies of *Z. formosa* (Kunth) H.M.Hern. (Levin and Moran 1989). Thus, at present, *Zapoteca* consists of 21 species in five subgenera, with *Z. formosa*, *Z. caracasana* (Jacq.) H.M.Hern. and *Z. portoricensis* divided into eight, two and three subspecies, respectively (Table 1). *Zapoteca sousae* H.M.Hern. & A.Campos has not yet been placed in any subgenus although Hernández and Campos (1994) emphasized the resemblance

of this species to *Z. portoricensis* subsp. *portoricensis*, indication of a placement in subg. *Zapoteca*.

Previous phylogenetic studies of mimosoid legumes (Brown et al. 2008; Souza et al. 2013; Ferm et al. in prep.) have shown *Zapoteca* to be monophyletic. However, these studies included only a limited number of species of *Zapoteca*. DNA regions used in these studies were ETS and ITS (Brown et al. 2008), ITS and *trn*L–*trn*F (Souza et al. 2013), and ETS, ITS, *psbA*–*trn*H and *trn*L–*trn*F (Ferm et al. in prep.). Therefore, an analysis including a higher number of sampled species and subspecies of *Zapoteca* was considered necessary in order to thoroughly investigate the monophyly of *Zapoteca*, and currently recognized subgenera, as well as other phylogenetic relationships within the genus.

In this study, I present a phylogenetic analysis of *Zapoteca*, based on nuclear and plastid DNA sequence data, as a first step in investigating the evolutionary history of the genus. I sampled from all subgenera of *Zapoteca* and also included eight species from other genera closely related to *Zapoteca* (Souza et al. 2013; Ferm et al. in prep.), as well as the more distantly related, mimosoid species *Senegalia senegal* (L.) Britton and *Vachellia farnesiana* (L.) Wight & Arn. The study was carried out in order to (1) test the monophyly of *Zapoteca*; (2) test the monophyly of the subgenera in *Zapoteca*; and (3) evaluate phylogenetic relationships within *Zapoteca*.

Materials and methods

Taxon and DNA region sampling

This study is based on DNA sequence data from 20 species and 11 subspecies of *Zapoteca* (Table 1), representing all presently recognized subgenera (Hernández 1989, 1990). In order to test the monophyly of the genus, eight species from genera previously shown to be closely related to *Zapoteca* (Souza et al. 2013; Ferm et al. in prep.) were included in the analyses. In addition, *Senegalia senegal* and *Vachellia farnesiana* were included to represent more distantly related species (Kyalangalilwa et al. 2013). When available, several specimens of the same taxon were included in order to test the coherence of species and subspecies of *Zapoteca* as currently circumscribed (Hernández 1986, 1989, 1990, 2015; Levin and Moran 1989; Hernández and Campos 1994; Hernández and Hanan-Alipi 1998). Newly generated DNA sequences and previously published DNA sequence data (downloaded from GenBank) from a total of 58 specimens were included in the analyses. Voucher information and GenBank accession numbers are listed in Appendix.

The amplified DNA regions comprise the nuclear external transcribed spacer (ETS), the nuclear internal transcribed spacer (ITS) and the plastid region *trn*L–*trn*F

Table 1 Subgenera, species and subspecies of *Zapoteca* according to Hernandez ([1989](#), [1990](#), [2015](#)), Levin and Moran ([1989](#)), Hernández and Campos ([1994](#)) and Hernández and Hanan-Alipi ([1998](#))

Subgenus	Species	Subspecies
Acuelata H.M.Hern.[b]	*Zapoteca aculeata* (Spruce ex Benth.) H.M.Hern.[b]	–
Amazonica H.M.Hern.[b]	*Zapoteca amazonica* (Benth.) H.M.Hern.[b]	–
	Zapoteca microcephala (Britton & Killip) H.M.Hern.[b]	–
	Zapoteca quichoi H.M.Hern. & A.M.Hanan[f]	–
Nervosa H.M.Hern.[b]	*Zapoteca nervosa* (Urb.) H.M.Hern.[b]	–
Ravenia H.M.Hern.[d]	*Zapoteca ravenii* H.M.Hern.[d]	–
	Zapoteca tehuana H.M.Hern.[b]	–
Zapoteca H.M.Hern.[b]	*Zapoteca alinae* H.M.Hern.[b]	–
	Zapoteca andina H.M.Hern.[b]	–
	Zapoteca balsasensis H.M.Hern.[g]	–
	Zapoteca caracasana (Jacq.) H.M.Hern.[a]	*caracasana*[b]
		weberbaueri (Harms) H.M.Hern.[b]
	Zapoteca costaricensis (Britton & Rose) H.M.Hern.[b]	–
	Zapoteca cruzii H.M.Hern.[g]	–
	Zapoteca filipes (Benth.) H.M.Hern.[b]	–
	Zapoteca formosa (Kunth) H.M.Hern.[a]	*formosa*[b]
		gracilis (Griseb.) H.M.Hern.[b]
		mollicula (J.F.Macbr.) H.M.Hern.[b]
		rosei (Wiggins) H.M.Hern.[b]
		salvadorensis (Britton & Rose) H.M.Hern.[b]
		schottii (Torr. ex S. Watson) H.M.Hern.[b]
		sinaloana H.M.Hern.[g]
		socorrensis (I.M.Johnst.) G.A.Levin, H.M.Hern. & Moran[c]
	Zapoteca lambertiana (G.Don) H.M.Hern.[a]	–
	Zapoteca media (M.Martens & Galeotti) H.M.Hern.[a]	–
	Zapoteca mollis (Standl.) H.M.Hern.[a]	–
	Zapoteca portoricensis (Jacq.) H.M.Hern.[a]	*flavida* (Urb.) H.M.Hern.[b]
		portoricensis[b]
		pubicarpa H.M.Hern.[b]
	Zapoteca scutellifera (Benth.) H.M.Hern.[b]	–
	Zapoteca tetragona (Willd.) H.M.Hern.[b]	–
Not specified	*Zapoteca sousae* H.M.Hern. & A.Campos[e]	–

Species and subspecies included in this study are given in bold type

[a]Hernández ([1986](#)); [b]Hernández ([1989](#)); [c]Levin and Moran ([1989](#)); [d]Hernández ([1990](#)); [e]Hernández and Campos ([1994](#)); [f]Hernández and Hanan-Alipi ([1998](#)); [g]Hernández ([2015](#))

(including the *trn*L intron and the *trn*L–*trn*F spacer). These regions have previously been used in phylogenetic studies of genera closely related to *Zapoteca* (including also species of *Zapoteca*) where they show a high number of variable sites (Brown et al. [2008](#); Souza et al. [2013](#); Ferm et al. in prep.).

DNA extraction, amplification and sequencing

Total DNA was extracted from leaf material obtained from the herbaria at New York Botanical Garden (NY), the Swedish Museum of Natural History (S), Missouri Botanical Garden (MO) and the National Herbarium of Mexico (MEXU) following the protocol listed in Ferm et al. (in prep.). One additional DNA sample was obtained from Centro de Investigación Científica de Yucatán (CICY).

PCRs included 1.5 µl 10× Dream taq buffer with 20 µM $MgCl_2$, 0.3 µl $MgCl_2$ (25 µM), 0.75 µl DNTP (10 µM each), 0.15 µl of each primer (20 µM), 0.15 µl BSA 1%, and 0.075 µl Dream Taq DNA polymerase (5 U/µl) with 1 µl DNA template and 10.925 µl sterilized H_2O giving a total volume of 15 µl. ETS was amplified using the primers AcR2 (Ariati

et al. 2006) and 18S IGS (Baldwin and Markos 1998). ITS and *trn*L–*trn*F were amplified in two separate reactions. For ITS primers P17 (Popp and Oxelman 2001) with ITS 491 (Ferm et al. in prep.) and ITS 493 (Ferm et al. in prep) with 26S–82R (Popp and Oxelman 2001) were used. For *trn*L–*trn*F, the universal primers c, d and e were used (Taberlet et al. 1991), c with d and e with jf1 (Ferm et al. in prep.). The primers used are listed in Table 2.

PCRs were run on an Applied Biosystems Veriti™ 96-Well Thermal Cycler. Amplifications were carried out as follows: 3-min initial denaturation at 95 °C followed by 35 cycles of 30-s denaturation at 95 °C, 1-min annealing at 55 °C and 1-min extension at 72 °C, and completed by a final extension of 7 min at 72 °C.

The products were purified using Illustra ExoProStar 1-Step (GE Healthcare, Little Chalfont, UK) following the instructions from the manufacturer and sent to Macrogen Europe in Amsterdam, the Netherlands, for sequencing. The same primers were used for sequencing as for PCR (Fig. 1).

Multiple sequence alignment and phylogenetic analyses

Complementary strands of the sequences were assembled and edited using Geneious v.10.1.2 (https://www.geneious.com, Kearse et al. 2012). For each region, multiple alignments of the sequences were performed using MUSCLE (Edgar 2004), and corrected by eye, in AliView v.1.19-beta-3 (Larsson 2014).

Before analysis, the best-fitting nucleotide substitution models for each of the data sets were determined using MrAic v.1.4.6 (Nylander 2004). The GTR-I-G model was selected for ETS and the GTR-G model was selected for ITS and *trn*L–*trn*F. Bayesian inference analyses were performed for each data set using MrBayes v.3.2.6 (Ronquist

et al. 2011). Two parallel MCMC chains were run for 1 million generations with a sampling frequency of 1000. The average standard deviation of split frequencies was below 0.01 at the end of each of the runs, indicating that the Markov chains had converged on the stationary distribution. Ten percent of the sampled trees were discarded as burn-in after evaluation of the output parameters generated by the Bayesian analysis in Tracer v.1.6 (Rambaut et al. 2014). Majority-rule 50% consensus trees were calculated based on the remaining trees and inspected in FigTree v.1.4.3 (Rambaut 2006).

The trees were rooted using *Vachellia farnesiana*, a species phylogenetically distant from the members of *Zapoteca* (Kyalangalilwa et al. 2013). I consider nodes with a posterior probability (PP) of ≥ 0.95 as strongly supported. The resulting topologies from each of the single-region data sets were compared manually to detect potential conflicting topologies. No incongruences were discovered and the data sets of the individual regions were therefore concatenated using Abioscripts (Larsson 2010). The combined data set was analyzed using the same methods and settings as the single-region data sets. Newly obtained sequences were deposited in GenBank (Appendix). The aligned data sets and the phylogenetic trees are available in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S23432).

Results

Characteristics of each DNA sequence region are summarized in Table 3

The results of the three separate analyses of the single-region data sets show that *Zapoteca* is strongly supported as monophyletic (PP 1 in all analyses) and that *Z. nervosa*

Table 2 Primers used in this study

DNA region	Primer	Sequence 5′–3′	Reference
Forward			
ETS	AcR2	GGG CGT GTG AGT GGT GTT TGG	Ariati et al. (2006)
ITS (part 1)	P17	CTA CCG ATT GAA TGG TCC GGT GAA	Popp and Oxelman (2001)
ITS (part 2)	ITS 493	ATG CGA TAC TTG GTG TGA AT	Ferm et al. (in prep)
trnL–trnF	c	CGC GCA TGG TGG ATT CAC AAA TC	Taberlet et al. (1991)
trnL–trnF	e	GGT TCA AGT CCC TCT ATC CC	Taberlet et al. (1991)
Reverse			
ETS	18S-IGS	GAG ACA AGC ATA TGA CTA CTG GCA GGA TCA ACC AG	Baldwin and Markos (1998)
ITS (part 1)	ITS 491	TCA CAC CAA GTA TCG CAT TT	Ferm et al. (in prep.)
ITS (part 2)	26S-82R	TCC CGG TTC GCT CGC CGT TAC	Popp and Oxelman (2001)
trnL–trnF (part 1)	d	GGG ATA GAG GGA CTT GAA CC	Taberlet et al. (1991)
trnL–trnF (part 2)	Jf1	ATT TGA ACT GGT GAC ACG AGG	Ferm et al. (in prep.)

Fig. 1 **a–c** Three species of *Zapoteca* showing inflorescences and bipinnate leaves. **a** *Zapoteca portoricensis* subsp. *portoricensis.* Photo: S.M. Silvestrini. **b** *Zapoteca formosa* subsp. *gracilis.* Photo: P.G. Gutiérrez. **c** *Zapoteca caracasana*. Photo: G. Lewis

(Urb.) H.M.Hern. is the sister to the remaining species in the genus (supported by PP 1 for ETS; PP 1/PP 0.99 for ITS; PP 1 for *trn*L–*trn*F for the two nodes involved). Trees from the separate analyses are available in Treebase (http://purl.org/phylo/treebase/phylows/study/TB2:S2343 2).

The 50% majority-rule consensus tree derived from the Bayesian analysis of the combined data set (ETS, ITS and *trn*L–*trn*F) is presented in Fig. 2. The results show that *Zapoteca* is monophyletic (PP 1). The monospecific subg. *Nervosa* (Table 1) is sister to all the remaining species of *Zapoteca* (PP 1). The other monospecific subgenus, subg. *Aculeata* (Table 1), is also found as a separate lineage (PP 1/PP 0.99). In contrast, none of the subgenera containing more than one species, namely subg. *Zapoteca*, subg. *Amazonica* and subg. *Ravenia* (Table 1), are supported as being monophyletic in this study. Furthermore, many

nodes within *Zapoteca* are strongly supported, but relationships at species and subspecies levels are for the most part not strongly supported.

The results show that the two specimens of *Zapoteca nervosa* form a clade (PP 1), which is sister to a clade containing the remaining species of *Zapoteca* (PP 1). Within the latter clade, three monophyletic groups including more than one species or subspecies are found. These are referred to as clades I–III (Fig. 2).

Clade I (PP 1) is sister to a clade consisting of *Z. aculeata* (Spruce ex Benth.) H.M.Hern and *Z. amazonica*, plus clades II and III (PP 1). Clade I includes *Zapoteca filipes* (Benth.) H.M.Hern. and *Z. scutellifera* (Benth.) H.M.Hern. However, *Zapoteca filipes* A is sister to *Z. scutellifera* (PP 0.89), and they are together sister to *Zapoteca filipes* B (PP 1).

Table 3 Number of accessions, number of characters, number/percentage of variable characters and substitution model for data sets analyzed with Bayesian inference

Data set	# Accessions	# Characters	Variable characters #/(%)	Substitution model
ETS	38	467	281/(60%)	GTRIG
ITS	57	863	303/(35%)	GTR-G
trnL–trnF	41	1329	223/(17%)	GTR-G
Combined data set	60	2635	812/(31%)	–

Zapoteca aculeata is monophyletic (PP 1) and sister to the remaining taxa consisting of *Z. amazonica* plus clades II and III (PP 1/PP 0.99). *Zapoteca amazonica* in turn is sister to clades II and III together (PP 0.99/PP 1).

The results show that clade II and clade III are sisters (PP 1/PP 1). Clade II (PP 1) contains the monophyletic *Z. caracasana* (PP 1) with *Z. caracasana* subspecies *weberbaueri* shown to be monophyletic (PP 1) and as sister to *Z. caracasana* subsp. *caracasana* (PP 1).

Most of the taxa of *Zapoteca* included in this study are found in clade III (PP 1). Here, two well-supported subclades are found, in Fig. 2 marked with X (PP 0.99) and Y (PP 0.99). In subclade X, *Zapoteca formosa* subsp. *gracilis* (Griseb.) H.M.Hern. and *Z. portoricensis* subsp. *portoricensis* are both monophyletic (PP 1/PP 1) and found as sisters (PP 0.94). However, *Zapoteca portoricensis* is shown to be non-monophyletic since *Z. portoricensis* subsp. *pubicarpa* H.M.Hern. is found in a poorly supported position, excluded from subclade X. The two specimens of *Z. tetragona* are both found in subclade X, but the species is not supported as being monophyletic. Furthermore, *Z. quichoi* H.M.Hern. & A.M.Hanan, *Z. mollis* (Standl.) H.M.Hern., *Z. costaricensis* (Britton & Rose) H.M.Hern. and *Z. formosa* A are found in this subclade, but the phylogenetic relationships between these species and to the other species in subclade X are not resolved.

In subclade Y, two monophyletic groups are found as sisters (PP 0.99/PP 0.98). One of these groups contains *Z. formosa* subsp. socorrensis (I.M.Johnst.) G.A.Levin et al. and *Z. formosa* subsp. *rosei* (Wiggins) H.M.Hern. (PP 1) and the other contains the remaining taxa in subclade Y, viz., *Z. lambertiana* (G.Don) H.M.Hern., *Z. media*, *Z. formosa* subsp. *schottii* (Torr. ex S.Watson) H.M.Hern., *Z. formosa* subsp. *mollicula* (J.F.Macbr.) H.M.Hern. and *Z. alinae* (PP 0.98). *Zapoteca formosa* subsp. *schottii* is shown to be monophyletic (PP 1) based on three collections. The three specimens of *Z. lambertiana* included in this work are found in subclade Y, but they are not resolved as being monophyletic nor shown to be non-monophyletic since none of their positions within this clade are supported. Two out of three specimens of *Z. alinae* are found in subclade Y, but not as sisters, and one is found outside subclade Y, showing that this species is non-monophyletic.

Zapoteca sousae and *Z. formosa* B are found outside subclades X and Y and shown to be sisters (PP 1), as are *Z. ravenii* H.M.Hern. and *Z. cruzii* H.M.Hern. (PP 1). *Zapoteca tehuana* H.M.Hern. is shown to be monophyletic based on two terminals (PP 1), but its position in relation to the other taxa in clade III is unresolved.

Finally, all specimens of *Z. formosa* are found in clade III, but this species is not shown to be monophyletic since subspp. of *Z. formosa* are intermixed with other taxa in clade III. Subspecies *Z. formosa* subsp. *schotti* (PP 1) and subsp. *gracilis* (PP 1) are the only subspecies of *Z. formosa* shown to be monophyletic.

Discussion

In this study, both the separate analyses of each DNA region (trees not presented) and the combined analysis (ETS, ITS and *trn*L–*trn*F) (Fig. 2) show that *Zapoteca* is strongly supported as monophyletic. These results are in accordance with previous work based on much fewer taxa (Brown et al. 2008; Souza et al. 2013; Ferm et al. in prep.). The results of the combined analysis (Fig. 2) are discussed below.

Monophyly of subgenera of *Zapoteca*

The monospecific subgenera, subg. *Nervosa* (containing *Z. nervosa*) and subg. *Aculeata* (containing *Z. aculeata*), are recovered as early diverging and distinct lineages within *Zapoteca*, whereas subg. *Amazonica*, represented by two species, is shown to be non-monophyletic (Fig. 2). One of the species of subg. *Amazonica*, *Z. amazonica*, is found as sister to clades II and III, whereas the other one, *Zapoteca quichoi*, is found to clade III (Fig. 2). The third, and in this study missing, member of subg. *Amazonica*, *Z. microcephala* (Britton & Killip) H.M.Hern., is a rare and seldom collected species (Tropicos.org 2018), but must be included in any future analysis in order to further test the status of subg. *Amazonica*. Subgenus *Ravenia* is shown to be non-monophyletic. The two species included in subg. *Ravenia*, *Z. ravenii* and *Z. tehuana* (Hernández 1990), are both found in clade III, but not as sisters (Fig. 2). The species placed in subg. *Zapoteca* (Hernández, 1989, 2015; Levin and Moran 1989) are found in clades I, II and III,
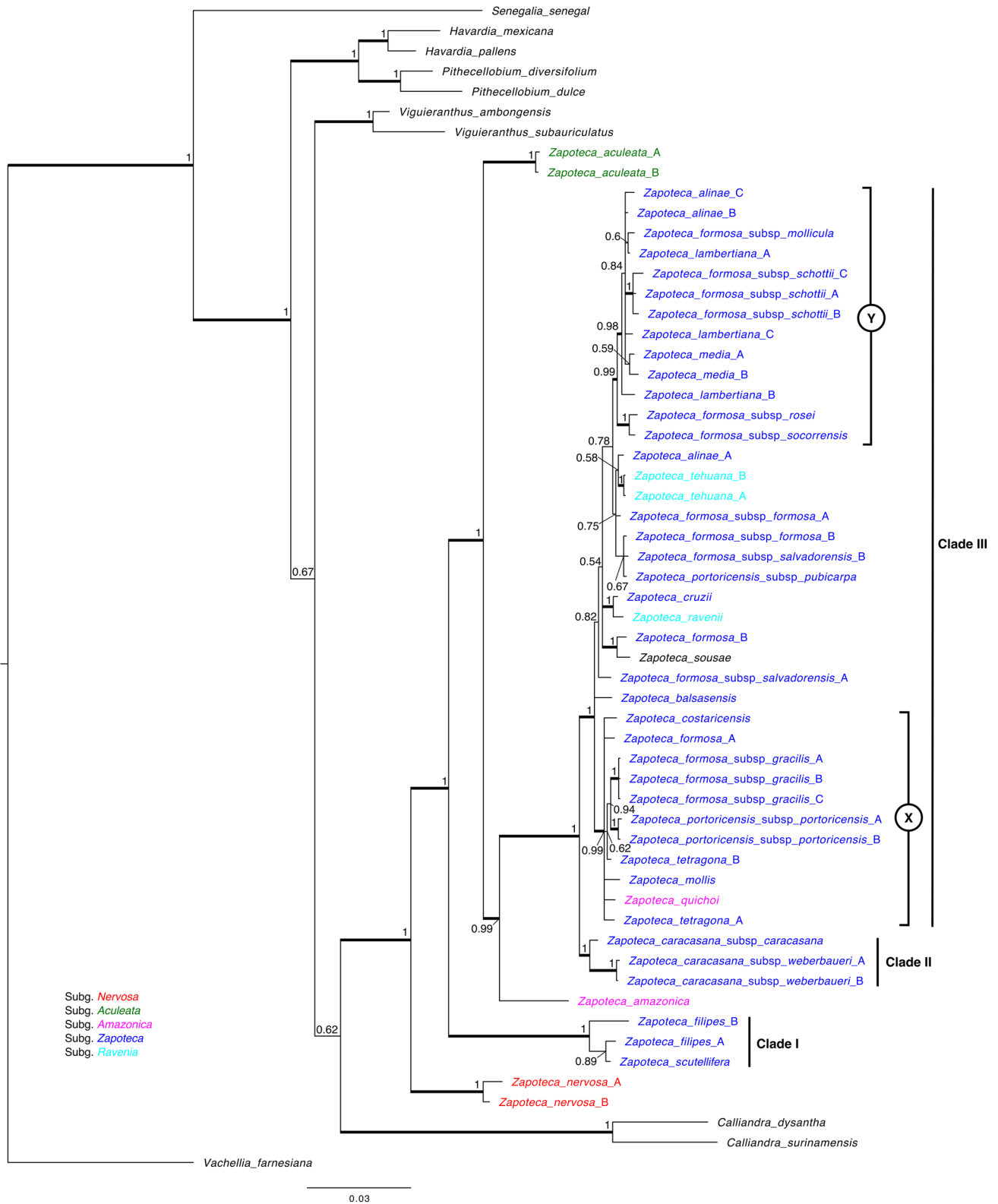
Fig. 2 Majority-rule (50%) consensus tree of 900 trees sampled at stationarity from the Bayesian analysis of the combined data set (ETS, ITS and *trn*L–*trn*F). Clades I–III are indicated with lines, and subclades Y and X within clade III are indicated with bracts. Numbers at nodes represent posterior probabilities, and thick lines indicate strong support (PP ≥ 95). Current subgeneric classification is indicated with colors

but since species of both subg. *Amazonica* and subg. *Ravenia* also are found in clade III, subg. *Zapoteca* is non-monophyletic (Fig. 2). As a consequence of the results in this study, a new subgeneric classification for the taxa in subg. *Zapoteca*, subg. *Ravenia* and subg. *Amazonica* is necessary, but further studies of both molecular and morphological data are needed before any taxonomic changes should be formally proposed.

Phylogenetic relationships within *Zapoteca*

The results of this study show that *Zapoteca nervosa* is the strongly supported sister to a group consisting of all the other species of *Zapoteca* (Fig. 2). This species is endemic to Hispaniola and was placed in subg. *Nervosa* as its only species by Hernández (1989). *Zapoteca nervosa* is distinguished from the other species of *Zapoteca* in having fissured bark and large, gray, coriaceous leaflets with a venation pattern not seen in any other member of the genus. Hernández (1989) suggested that *Z. nervosa* had diverged early from the other members of *Zapoteca* particularly evidenced by the absence of a lens-shaped structure in the central cells of the 16-celled pollen polyads (present in all other members of *Zapoteca*) (Guinet and Hernández 1989). The position of *Z. nervosa* as sister to all remaining species of *Zapoteca*, as shown in this study (Fig. 2), supports this idea of a long separate history of this species and seemingly merits the placement of *Z. nervosa* in a separate subgenus (Hernández 1989). Another way of treating this species would be to raise subg. *Nervosa* to genus level, thus creating a monospecific genus containing *Z. nervosa* only. A parallel case from the Caribbean is met with in *Clavija* Ruiz & Pav. (Theophrastaceae), a chiefly South and Central American genus with a single, morphologically and phylogenetically isolated species, *C. domingensis* Urb. & Ekman, endemic to Hispaniola (Källersjö and Ståhl 2003; Ståhl 2010), and similar patterns in other neotropical groups should be expected. Nevertheless, *Z. nervosa* has a combination of characters typical for *Zapoteca* (polyad structure of pollen and fruit type, as well as overall inflorescence structure) and it seems for now best treated in *Zapoteca*.

Clade I is strongly supported as monophyletic and as sister to *Z. aculeata* + *Z. amazonica* and clades II and III (Fig. 2). Clade I contains two terminals of *Z. filipes* and one of *Z. scutellifera*, but *Z. filipes* is not shown to be monophyletic since *Zapoteca filipes* A is the sister to *Z. scutellifera*, although this node is not strongly supported (Fig. 2). Both species were placed in subg. *Zapoteca* by Hernández (1989). Extrafloral glands are only found in these two species and in *Z. nervosa* (and occasionally in *Z. lambertiana*). *Zapoteca filipes* is distinguished by having cylindrical glands between the pinnae and the leaflets placed close to the base of the petioles and *Z. scutellifera* is distinguished by having

scutelliform glands between the pinnae close to the base of the petioles and sometimes also between the distal pairs of leaflets (Hernández 1989). The position of clade I as the closest diverging lineage to *Z. nervosa* could indicate that the occurrence of extrafloral glands is a primitive character in *Zapoteca*. Both *Z. filipes* and *Z. scutellifera* are distributed in Amazonian Brazil, with seemingly overlapping distributions; *Z. scutellifera* also occurs in Bolivia. The results in this study indicate that *Z. filipes* and *Z. scutellifera* are closely related, but the relationship between them remains unresolved (Fig. 2). Additional material of both species is needed in order to further investigate the phylogenetic relationship of these two species. A new subgenus is possibly needed to accommodate the species of clade I in order to retain subg. *Zapoteca* (with some adjustments), subg. *Aculeata* and subg. *Amazonica*. One possibility is to assign clade II and III to subg. *Zapoteca* and describe a new subgenus to accommodate clade I.

Zapoteca aculeata is strongly supported as sister to a clade consisting of *Z. amazonica* + clades II and III and as monophyletic based on two collections (Fig. 2). The present results show that *Z. aculeata* diverged from the main line of descent earlier than *Z. amazonica* and the taxa found in clades II + III, i.e., the taxa placed in subg. *Zapoteca* (except for *Z. filipes* and *Z. scutellifera* of clade I), subg. *Amazonica* and subg. *Ravenia* (Table 1) (Hernández 1989, 1990, 2015; Hernández and Hanan-Alipi 1998; Levin and Moran 1989), and thus support a placement of this species in a separate monospecific subgenus, subg. *Aculeata* (Hernández 1989). *Zapoteca aculeata* is distinguished from the other species of *Zapoteca* by having spinescent stipules. It is distributed in the Ecuadorian Andes (Hernández 1989) and appears to be the species of *Zapoteca* growing at the highest elevations (up to 2800 m according to Tropicos.org 2018).

The results of this study show *Zapoteca amazonica* strongly supported as sister to clades II + III (Fig. 2). Hernández (1989) placed *Zapoteca amazonica* in subg. *Amazonica* together with *Z. microcephala* (not included in this study). He considered these two species to represent a distinct evolutionary line within *Zapoteca* since they were distinguished from all other species of *Zapoteca* by having one pair of pinnae with few, large, chartaceous to coriaceous leaflets and flower heads arranged in long, simple or compound pseudopanicles. Whereas *Z. amazonica* is known from Amazonian Peru and adjacent Ecuador, *Z. microcephala* is confined to the Magdalena valley in central Colombia. The position of *Z. amazonica* in this study indicates that this species diverged from the main line of descent earlier than the species found in clade II + III, thus agreeing with the view of Hernández (1989) that *Z. amazonica* (and *Z. microcephala*) separated early from the other species of *Zapoteca*. Whether or not *Z. microcephala*

is the closest relative to *Z. amazonica* is yet to be tested. One additional species, *Z. quichoi*, endemic to southern Tabasco in Mexico, was included in subg. *Amazonica* by Hernández and Hanan-Alipi ([1998]). They considered *Z. quichoi* to be more closely related to the species of subg. *Amazonica* than to the other Mexican species of *Zapoteca*, based on morphological features such as leaves with one pair of pinnae with few, large leaflets and inflorescence characters. However, based on the results in this study *Z. amazonica* and *Z. quichoi* are not closely related and *Z. quichoi* is shown to be more closely related to the taxa in subclade X (Fig. [2]). Since the relationships within subclade X are for the most part poorly resolved, the phylogenetic position of *Z. quichoi* needs further study. Also, the inclusion of *Z. microcephala* is needed in order to confirm that *Z. quichoi* is not closely related to any species of subg. *Amazonica*.

Clade II, including the two subspecies of *Zapoteca caracasana*, is strongly supported as sister to clade III and shown to be monophyletic (Fig. [2]). Furthermore, *Z. caracasana* subsp. *weberbaueri* (Harms.) H.M.Hern. is strongly supported as monophyletic based on two specimens, and as sister to subsp. *caracasana*.

Phylogenetic relationships within clade III

Clade III is strongly supported as monophyletic (Fig. [2]) and contains most of the species of *Zapoteca* included in this study. Relationships within this clade are generally poorly resolved, but there are some strongly supported nodes.

In subclade X, *Zapoteca formosa* subsp. *gracilis* and *Z. portoricensis* subsp. *portoricensis* are both shown to be strongly supported as monophyletic, and moderately supported as sisters (Fig. [2]). According to Hernández ([1989]) collections of *Z. formosa* subsp. *gracilis* from the Bahamas can be confused with Z. *portoricensis* subsp. *portoricensis*, but can be distinguished from the latter in having smaller and narrower stipules and thicker leaflets. Two of the collections of *Z. formosa* subsp. *gracilis* (A+C) in this study are from the Bahamas, the third (B) was collected in Cuba, whereas the two specimens of *Z. portoricensis* subsp. *portoricensis* were collected in Hispaniola. The placement of the three collections of *Z. formosa* subsp. *gracilis* confirms that they represent the same taxon (Fig. [2]). The positions of *Z. formosa* subsp. *gracilis* and *Z. portoricensis* subsp. *portoricensis*, although only moderately supported, suggest a relationship between these two taxa (Fig. [2]). It is possible that *Z. formosa* subsp. *gracilis* should be treated at the specific level and not as a subspecies, especially since *Z. formosa* is shown to be non-monophyletic. *Zapoteca portoricensis* is also shown to be non-monophyletic in this study and *Z. portoricensis* subsp. *pubicarpa* should possibly be treated at the specific level or at least not as a subspecies of

Z. portoricensis. In any case, the status of the subspecific classifications of *Z. formosa* and *Z. portoricensis* needs to be investigated further. Also, studies including more samples of both *Z. formosa* subsp. *gracilis* and *Z. portoricensis* subsp. *portoricensis* from more localities are needed in order to investigate their relationship further.

Zapoteca formosa subsp. *rosei* and *Z. formosa* subsp. *socorrensis*, found in subclade Y, are strongly supported as sister taxa (Fig. [2]). They together are in turn strongly supported as sister to a group consisting of the remaining taxa in subclade Y. *Zapoteca formosa* subsp. *rosei* is a Mexican taxon known from Baja California and distributed south along the pacific slope while *Zapoteca formosa* subsp. *socorrensis* is known only from Socorro and Clarión islands, off the west Mexican coast. Hernández ([1989]) included the island populations in subspecies *Z. formosa* subsp. *rosei*. Levin and Moran ([1989]) considered the island populations to be morphologically distinct and recognized *Z. formosa* subsp. *socorrensis*, an endemic to the Socorro and Clarión islands. However, they considered these two subspecies to be closely related because of similarity in morphology. One morphological trait distinguishing the two subspecies from each other is filament color, which in subsp. *socorrensis* is white while it is bicolored (white basally and pink, purple or red distally) in subsp. *rosei*. Furthermore, subsp. *socorrensis* has diurnal flowers, otherwise unknown in *Zapoteca*, but this feature has only been observed in cultivation (Levin and Moran [1989]). The results of this study confirm that these subspecies are more closely related to each other than to any other species of *Zapoteca* (Fig. [2]). Whether the collections included represent distinct taxa or variations of the same taxon, and if they should be treated at subspecific or specific level, is yet to be tested with more samples from both subsp. *rosei* and subsp. *socorrensis*.

The two specimens of *Z. tehuana* included in this study are found in a position outside subclades X and Y, and the species is strongly supported as monophyletic (Fig. [2]). *Zapoteca tehuana* was originally placed in subg. *Zapoteca* by Hernández ([1989]), but when *Z. ravenii* was discovered he (Hernández [1990]) considered these two species to be more closely related to each other than to the other species of subg. *Zapoteca*. This conclusion was based on similarities in the leaf morphology, including venation and texture, and Hernández ([1990]) established a new subgenus, subg. *Ravenia*, to accommodate the two species. In contradiction to this, based on the results of this study, *Z. tehuana* and *Z. ravenii* are not closely related. Instead, *Z. ravenii* is a strongly supported as sister to *Z. cruzii* (Fig. [2]).

Zapoteca ravenii is known from montane rain forest in northern Mexico and from the Cayo District in Belize, occurring at altitudes of 400–900 m (Hernández [1990]). By contrast, *Zapoteca cruzii* has a limited distribution in northeastern Guerrero (southwestern Mexico), where it grows in tropical

deciduous forest at around 1000 m altitude (Hernández 2015). Furthermore, *Z. cruzii* has a scandent habit and the ability to produce adventitious roots, an attribute not known in any other taxon of *Zapoteca,* and these are traits which also clearly distinguish them from *Z. ravenii*. According to the results in this study, these two species are closely related, but further phylogenetic studies should include collections from Belize and additional morphological studies would be instructive.

Zapoteca formosa B and *Z. sousae* are strongly supported sisters found in clade III (Fig. 2). *Zapoteca sousae* was described by Hernández and Campos (1994), but its subgeneric placement has not been addressed. *Zapoteca sousae* is distinguished from other taxa of *Zapoteca* by its foliaceous stipules with tawny hairs and calyces with uneven teeth. It is known from two widely separated localities in two Mexican states, Oaxaca and Colima. Further phylogenetic and morphological studies of *Zapoteca* are needed before any subgeneric placement of *Z. sousae* can be made. It would also be interesting to test the monophyly of *Z. sousae* by including collections from both known localities. Furthermore, its relationship to *Z. formosa* in its broad circumscription also needs further study.

The non-monophyletic species *Zapoteca formosa*

All subspecies of *Zapoteca formosa* are found in clade III, but they are not found grouped together, thus rendering this species non-monophyletic. *Zapoteca formosa* is distributed throughout the Neotropics, with a northern extension to Arizona, USA, occurring from sea level up to 2000 m altitude (Tropicos.org 2018). *Zapoteca formosa* is recognized as an erect shrub up to 5 m tall with leaves lacking extrafloral nectaries and stamens that are white, greenish white, red–purple or bicolored (with filaments that are white basally and pink or red–purple distally) (Hernández 1989). The species is divided into eight subspecies (Hernández 1989, 2015; Levin and Moran 1989), and all except one (*Zapoteca formosa* subsp. *sinaloana* H.M.Hern.) are represented in this study. The subspecies are distinguished morphologically primarily based on a variation in leaf and floral characters, but intermediate forms are known (Hernández 1989). The results of this study clearly indicate that the subspecific classification of *Z. formosa* should be revised given that the subspecies of *Z. formosa* occur intermixed with other taxa in clade III. Since many nodes in clade III are poorly supported (Fig. 2), it is impossible to fully evaluate the monophyletic status of *Z. formosa* without more extensive phylogenetic analyses.

The non-monophyletic species in clade III

In clade III, several species are shown to be non-monophyletic. Two collections of *Zapoteca tetragona* are found in subclade X, but the species is not supported as monophyletic (Fig. 2).

Zapoteca portoricensis is shown to be non-monophyletic, subsp. *portoricensis* being found in subclade X and subsp. *pubicarpa* in a position outside subclade X (Fig. 2). Since subsp. *portoricensis* (discussed above) and subsp. *pubicarpa* are not closely related, these two taxa might not even represent the same species. The third subspecies of *Z. portoricensis*, subsp. *flavida* (Urb.) H.M.Hern., needs to be included in any future phylogenetic analyses in order to test the monophyly of *Z. portoricensis* further. Two collections of *Zapoteca alinae* are found in subclade Y and one collection is found outside of that subclade (Fig. 2), rendering this species non-monophyletic. All three specimens of *Z. lambertiana* included in this study are found in subclade Y, but they are not found clustered together. No conclusions can be drawn about the monophyly of *Z. lambertiana* since none of the three specimens are found in strongly supported positions; thus, more studies are needed in order to investigate this species further. Hernández (1989) concluded that intermediate forms exist for many taxa in subg. *Zapoteca* but that hybrids are rare in the genus, although they do occasionally occur. Possible hybrids mentioned by him are between *Z. tetragona* and *Z. portoricensis* subsp. *portoricensis*, between *Z. media* and *Z. formosa* and between *Z. formosa* subsp. *formosa* and subsp. *rosei*, but we cannot rule out the possibility of hybridization between other taxa of *Zapoteca*. The occurrence of naturally occurring hybrids could partly explain the non-monophyletic status of some taxa in this study. Another possible explanation is the misidentification of some specimens, both in herbaria and in the field, especially because diagnostic characters do not always exist.

Conclusions

Zapoteca is a well-defined genus based on morphological characters (Hernández 1986, 1989) and its monophyly is strongly supported in this study based on DNA sequence data (Fig. 2). However, the results of this study show that the classification of subgeneric taxa within *Zapoteca* needs to be revised and the monophyly of species and subspecies, as well as phylogenetic relationships between taxa, needs further study before any well-supported taxonomic rearrangements can be proposed, including the correct subgeneric placement of *Z. sousae*.

Compliance with ethical standards

Conflict of interest The author declares that there is no conflict of interest.

Appendix

Taxon names and GenBank accession numbers of DNA sequences (ETS, ITS, *trn*L–*trn*F) included in this study. Voucher data are given for accessions for which DNA sequences were newly obtained, using the following format: taxon name, country, collector and collection number, herbarium acronym; GenBank accession numbers (ETS, ITS, trnL–trnF)

Calliandra dysantha Benth.; EF638121.1, JX870684.1, JX870813.1 ◆ *Calliandra surinamensis* Benth.; -, JX870747.1, JX870865.1 ◆ *Havardia mexicana* (Rose) Britton & Rose; KF921655.1, KF933276.1, JX870878.1, ◆ *Havardia pallens* (Benth.) Britton & Rose; KF921656.1, KF921698.1, AF522955.1 ◆ *Pithecellobium diversifolium* Benth.; KF921666.1, JX870768.1, JX870884.1 ◆ *Pithecellobium dulce* (Roxb.) Benth.; EF638143.1, JX856483.1, KC479268.1, ◆ *Senegalia senegal* (L.) Britton; EF638152.1, HQ605075.1, AF522976.1 ◆ *Vachellia farnesiana* (L.) Wight & Arn.; EF638128.1, AF360728.1, AY574119.1◆ *Viguieranthus ambongensis* (R.Vig.) Villiers; KR997873.1, JX870773.1, JX870890.1◆ *Viguieranthus subauriculatus* Villiers; KR997876.1, JX870778.1, - ◆ *Zapoteca aculeata* (Spruce ex Benth.) H.M.Hern. (A) Ecuador, Delinks 332 (NY); MK622329*, MK638924*, MK622373* (B) Ecuador, Rose 22365 (NY); MK622330*, -, MK622363* ◆ *Zapoteca amazonica* (Benth.) H.M.Hern., Peru, Mexia 8295 (S); MK622344*, MK638946*, MK622377* ◆ *Zapoteca alinae* H.M.Hern. (A) Mexico, Pascual 1492 (NY); MK622336*, MK638925*, MK622368* (B) Mexico, Gomez 91-7-7 (NY); -, MK638926*, - (C) -, JX870779.1, JX870893.1 ◆ *Zapoteca balsasensis* H.M.Hern., Mexico, Contreras & Thomas 1735 (NY); -, MK638928*, - ◆ *Zapoteca caracasasa* (Jacq.) H.M.Hern. subsp. *caracasana*, Hispaniola, Ekman 16527 (S); MK622335*, MK638949*, MK622370* ◆ *Zapoteca caracasasa* subsp. *weberbaueri* (Harms.) H.M.Hern. (A) Ecuador, Asplund 15982 (S); MK622345*, MK638947*, MK622374* (B) Ecuador, Asplund 15503 (S); MK622333*, MK638948*, MK622376* ◆ *Zapoteca costaricensis* (Britton & Rose) H.M.Hern., Costa Rica, Morales & Hammel 9051 (MO); -, MK638961*, - ◆ *Zapoteca cruzii* H.M.Hern., Mexico, Gual 272 (MEXU); MK622328*, MK63896*2, MK622375* ◆ *Zapoteca filipes* (Benth.) H.M.Hern.; (A) Brazil, Teixeira 476 (NY); -, MK638927*, MK622367* (B) -, JX870780.1, JX870896.1 ◆ *Zapoteca formosa* (Kunth.) H.M.Hern. subsp. not assigned; (A) Novara & Bruno 8865 (S); -, MK638950*, MK622356* (B) -, JX870781.1, JX870897.1 ◆ *Zapoteca formosa* subsp. *formosa*, (Kunth.) H.M.Hern. (A) Mexico, McVaugh 20327 (NY); -, MK638929*, - (B) Mexico, McVaugh 19857 (NY); -, MK638930*, - ◆ *Zapoteca formosa* subsp. *gracilis* (Griseb.) H.M.Hern. (A) Bahamas, Howard 10021 (S); MK622346*, MK638951*, MK622362* (B) Cuba, Ekman 8198 (S); MK622347*, MK638952*, MK622359* (C) Bahamas, Webster, Samule & Williams 10511A (S); MK622348*, MK638953*, MK622372* ◆ *Zapoteca formosa* subsp. *mollicula* (J.F.Macbr.) H.M.Hern., Mexico, Hughes 1804 (NY); MK622337*, MK638931*, MK622362* ◆ *Zapoteca formosa* subsp. *rosei* (Wiggins) H.M.Hern., Mexico, Mexia 8731 (NY); MK622353*, MK638932*, - ◆ *Zapoteca formosa* subsp. *salvadorensis* (Britton & Rose) H.M.Hern. (A) Guatemala, G.J. & M.E. Breckon 2118 (NY); MK622352*, MK638933*, - (B) Guatemala, Williams & al. 22456 (NY); MK622338*, MK638934*, MK622355* ◆ *Zapoteca formosa* subsp. *schottii* (Torr. ex S.Watson) H.M.Hern. (A) US/Arizona, Parker 5861 (NY); -, MK638935*, - (B) US/Arizona, Kearney & Peebles 14960 (NY); MK622339*, MK638936*, MK622357* (C) Semillas cultivadas XDL89-0405D (CICY) MK638923*, -, MK622379* ◆ *Zapoteca formosa* subsp. *socorrensis* (I.M.Johnst.) G.A.Levin, H.M.Hern. & Moran, Mexico, Moran 25546 (NY); MK622340*, MK638937*, - ◆ *Zapoteca lambertiana* (G.Don) H.M.Hern.; (A) Mexico, Breedlove 36732 (NY); MK622331*, MK638938*, MK622360* (B) Mexico, Ton 3042 (NY); MK622332*, MK638939*, - (C) -, JX870782.1, JX870894.1 ◆ *Zapoteca media* (M. Martens & Galeotti) H.M.Hern.; (A) Mexico, Moore Jr. 3986 (NY); MK622341*, MK638940*, MK622365* (B) Mexico, Johnston 12043 (NY); MK622351*, MK638941*, MK622366* ◆ *Zapoteca mollis* (Standl.) H.M.Hern., Costa Rica, Rodriguez 2420 (NY); MK622342*, MK638942*, - ◆ *Zapoteca nervosa* (Urb.) H.M.Hern. (A) Hispaniola, Ekman 8959 (S); MK622349*, MK638954*, - (B) Hispaniola, Ekman 15423 (S); -, MK638955*, MK622358* ◆ *Zapoteca portoricensis* (Jacq.) H.M.Hern. subsp. *portoricensis*; (A) Hispaniola, Ekman 10924 (S); MK622350*, MK638956*, MK622371* (B) Hispaniola, Ekman 13376 (S); MK622334*, MK638957*, - ◆ *Zapoteca portoricensis* subsp. *pubicarpa* H.M.Hern., Mexico, Purpus 2668 (NY); -, MK638943*, - ◆ *Zapoteca ravenii* H.M.Hern., Mexico, Martinez 23967 (NY); MK622343*, MK638944*, MK622369* ◆ *Zapoteca scutellifera* (Benth.) H.M.Hern., Brazil, Amaral 1231 (NY); -, MK638945*, - ◆ *Zapoteca sousae* H.M.Hern. & A.Campos; -, JX870783.1, JX870898.1 ◆ *Zapoteca tehuana* H.M.Hern. (A) Mexico, A.Campos 4108 (MEXU) MK622326*, MK638963*, MK622378* (B) Mexico, Torres Colín 8934 (MEXU); -, MK638959*, MK622354* ◆ *Zapoteca tetragona* (Willd.) H.M.Hern.; (A) Colombia, Cuatrecasas 22400 (S); -, MK638958*, - (B) -, JX870784.1, JX870899.1 ◆ *Zapoteca quichoi* H.M.Hern & A.M.Hanan, Mexico, Calónico 21109 (MEXU); MK622327*, MK638960*, MK622364*

- missing data; * newly generated sequence

References

Ariati SR, Murphy DJ, Udovicic F, Ladiges PY (2006) Molecular phylogeny of three groups of acacias (*Acacia* subgenus *Phyllodineae*) in arid Australia based on the internal and external transcribed spacer regions of nrDNA. Syst Biodivers 4:417–426. https://doi.org/10.1017/S1477200006001952

Baldwin BG, Markos S (1998) Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: Congruence of ETS and ITS trees of *Calycadenia* (Compositae). Molec Phylogen Evol 10:449–463

Brown GK, Murphy DJ, Miller JT, Ladiges PY (2008) *Acacia s.s.* and its relationship among tropical legume tribe Ingeae (Leguminosae: Mimosoideae). Syst Bot 33:739–751. https://doi.org/10.1600/036364408786500136

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl Acids Res 32:1792–1797

Guinet P, Hernández HM (1989) Pollen characters in the genera *Zapoteca* and *Calliandra* (Leguminosae, Mimosoideae) and their systematics and phylogenetic relevance. Pollen Spores 31:5–22

Hernández HM (1986) *Zapoteca*: a new genus of neotropical Mimosoideae. Ann Missouri Bot Gard 73:755–763

Hernández HM (1989) Systematics of *Zapoteca* (Leguminosae). Ann Missouri Bot Gard 76:781–862

Hernández HM (1990) A new subgenus and a new species of *Zapoteca* (Leguminosae). Syst Bot 15:226–230

Hernández HM (2015) New taxa of *Zapoteca* (Leguminosae, Mimosoideae) from Mexico. Phytotaxa 239:233–241

Hernández HM, Campos A (1994) A new species of *Zapoteca* (Leguminosae, Mimosoideae) from Mexico. Novon 4:32–34

Hernández HM, Hanan-Alipi AM (1998) *Zapoteca quichoi* (Leguminosae, Mimosoideae), a new species from southeastern Mexico. Brittonia 50:211–213. https://doi.org/10.2307/2807853

Källersjö M, Ståhl B (2003) Phylogeny of Theophrastaceae (Ericales s.l.). Int J Pl Sci 164:579–591. https://doi.org/10.1086/375317

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649

Kyalangalilwa B, Boatwright JS, Daru BH, Maurin O, van der Bank M (2013) Phylogenetic position and revised classification of *Acacia* s.l. (Fabaceae: Mimosoideae) in Africa, including new combinations in *Vachellia* and *Senegalia*. Bot J Linn Soc 172:500–523. https://doi.org/10.1111/boj.12047

Larsson A (2010) Abioscripts. Available at: http://ormbunkar.se/phylogeny/abioscripts/

Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large data sets. Bioinformatics 30:3276–3278

Levin GA, Moran R (1989) The vascular flora of Isla Socorro, Mexico. Mem San Diego Soc Nat Hist 16:37

Nylander JAA (2004) MrAIC.pl. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. Available at: https://github.com/nylander/MrAIC

Popp M, Oxelman M (2001) Inferring the history of the polyploid *Silene aegae* (Caryophyllaceae) using plastid and homoeologous nuclear DNA sequences. Molec Phylogen Evol 20:474–481

Rambaut A (2006–2016) FigTree, version 1.4.3. Institute of Evolutionary Biology, University of Edinburgh. Available at: http://tree.bio.ed.ac.uk/software/figtree/

Rambaut A, Suchard M, Drummond A (2014) Tracer, version 1.6. Institute of Evolutionary Biology, University of Edinburgh. Available at: http://tree.bio.ed.ac.uk/software/tracer/

Ronquist F, Huelsenbeck JP, Teslenko M (2011) Draft MrBayes version 3.2 Manual. Tutorials and Model Summaries. http://mrbayes.sourceforge.net/download.php

Souza ER, Lewis GP, Forest F, Schnadelbach AS, van der Berg C, Paganucci de Queiros L (2013) Phylogeny of *Calliandra* (Leguminosae: Mimosoideae) based on nuclear and plastid molecular markers. Taxon 62:1200–1219

Ståhl B (2010) Theophrastaceae. Fl Neotrop. Monogr 105:1–161

Taberlet P, Gielly L, Patou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Pl Molec Biol 17:1105–1109. https://doi.org/10.1007/BF00037152

Tropicos.org (2018) Tropicos: botanical information system. Missouri Botanical Garden, Saint Louis. Available at: http://www.tropicos.org/. Accessed 17 Apr 2018