

## SYSTEMATICS AND PHYLOGENY

# Phylogeny of the Neotropical legume genera *Zygia* and *Marmaroxylon* and close relatives

Julia Ferm,<sup>1</sup> Petra Korall,<sup>1</sup> Gwilym P. Lewis<sup>2</sup> & Bertil Ståhl<sup>3</sup>

<sup>1</sup> Department of Organismal Biology, Uppsala University, 75236, Uppsala, Sweden

<sup>2</sup> Comparative Plant and Fungal Biology Department, Royal Botanic Gardens, Kew, Richmond, TW9 3AB, United Kingdom

<sup>3</sup> Department of Organismal Biology, Uppsala University Campus Gotland, 62167, Visby, Sweden

Address for correspondence: Julia Ferm, [julia.ferm@ebc.uu.se](mailto:julia.ferm@ebc.uu.se)

DOI <https://doi.org/10.1002/tax.12117>

**Abstract** The Neotropical legume genera *Zygia* and *Marmaroxylon* have traditionally been considered closely related and are sometimes treated as congeners. They have been referred to the mimosoid tribe Ingeae based on their possession of flowers with basally fused stamen filaments. However, their systematic status and position have not been analysed in a phylogenetic context. This study provides the first molecular phylogeny of *Zygia* and *Marmaroxylon* and closely related mimosoid legume genera, particularly from the tribe Ingeae. DNA sequence data were derived from the nuclear external transcribed spacer (ETS) and the internal transcribed spacer (ITS), and from the chloroplast *psbA-trnH* and *trnL-trnF* regions, and were analysed using Bayesian inference. In all, 29 species of *Zygia* and 7 species of *Marmaroxylon* were included in the study along with representative species from the closely related genera *Abarema*, *Acacia* s.str., *Archidendropsis*, *Calliandra*, *Chloroleucon*, *Cojoba*, *Faidherbia*, *Havardia*, *Hydrochorea*, *Inga*, *Leucochloron*, *Macrosamanea*, *Pithecellobium*, *Pseudosamanea*, *Samanea*, *Senegalia*, *Vachellia* and *Zapoteca*. The results show that neither *Zygia* nor *Marmaroxylon* are monophyletic as presently circumscribed. Furthermore, these two genera are not monophyletic together. None of the nine presently recognized sections of *Zygia* that contain more than one species are shown to be monophyletic. Two of the monospecific sections of *Zygia*, sect. *Ingopsis* (*Z. inundata*) and sect. *Pseudocojoba* (*Z. sabatieri*), were found in a clade together with species of *Inga*. *Marmaroxylon ocumarense* and *M. magdalanae*, previously treated as synonyms (as *Zygia*), are not conspecific, and are found in a clade with *Macrosamanea* and in a clade with species of *Abarema* and *Hydrochorea*, respectively. Our results also show the *Inga* alliance to be non-monophyletic. In correspondence with the results presented, all species referred to *Marmaroxylon*, except for *M. ocumarense* and *M. magdalanae*, are here included in *Zygia*, and *Z. inundata* is treated in *Inga*, in which genus it was first described.

**Keywords** Fabaceae; Ingeae; Leguminosae; phylogenetic analyses; taxonomy

## ■ INTRODUCTION

The mimosoid genera *Zygia* P.Browne and *Marmaroxylon* Killip (Fabaceae) include some 50 and 9–13 species, respectively, as currently circumscribed (Rico Arce, 1991; Barneby & Grimes, 1997). They consist of small or sometimes medium-sized, cauliflorous and/or ramiflorous trees or shrubs (Fig. 1A–E) and are distributed throughout much of the Neotropics, being prevalent in moist or wet lowland forest (Barneby & Grimes, 1997). The two genera are considered closely related and were treated as congeners by Barneby & Grimes (1997), but the monophyly of each of the two genera has hitherto not been tested. Furthermore, their phylogenetic relationships with closely related taxa have so far only been partly investigated (e.g., Brown & al., 2008; Kyalangalilwa & al., 2013; Souza & al., 2013; Iganci & al., 2016).

Traditionally, *Zygia* and *Marmaroxylon* have been placed in the Ingeae tribe due to their synandrous flowers, i.e., with stamens having the filaments basally united into a tube (Fig. 1C) (Bentham, 1865, 1875). Phylogenetic analyses,

however, have shown Ingeae to be non-monophyletic with respect to the genus *Acacia* Mill. s.str. (tribe Acacieae), also rendering Acacieae non-monophyletic (e.g., Miller & al., 2003; Lavin & al., 2005; Brown & al., 2008). These findings are not yet reflected in any formal tribal classification of the mimosoid clade as defined by the LPWG (2017).

In general, ingoid species have flowers with numerous showy stamens and bipinnate leaves. However, there are a few exceptions. Species in the genus *Inga* Mill. all have pinnate leaves, along with at least one other species of another genus, *Cojoba rufescens* (Benth.) Britton & Rose (Mohlenbrock, 1963). Among the unarmed, bipinnately leaved members of the Ingeae tribe, *Zygia* and *Marmaroxylon* have traditionally been distinguished by their cauliflorous and/or ramiflorous habit (Barneby & Grimes, 1997; Lewis & Rico Arce, 2005). However, this character is not ubiquitous; *Zygia ocumarenensis* (Pittier) Barneby & J.W.Grimes and *Z. pithecolobioides* (Harms) Barneby & J.W.Grimes are non-cauliflorous according to Barneby & Grimes (1997). In addition, rami- and cauliflory is occasionally reported in species of *Inga* (Pennington, 1997).

**Article history:** Received: 26 Nov 2018 | returned for (first) revision: 13 Feb 2019 | (last) revision received: 14 May 2019 | accepted: 24 May 2019

**Associate Editor:** Hervé Sauquet | © 2019 International Association for Plant Taxonomy



**Fig. 1.** Leaves, fruits and flowers of *Zygia* and *Marmaroxylon*. **A**, Bipinnate leaves of *Marmaroxylon basijugum*; **B**, A plant of *Zygia longifolia*, showing bipinnate leaves with one pair of pinnae, and cauliflory; **C**, A flower with showy stamens and flower buds of *Marmaroxylon basijugum*; **D**, A plant of *Marmaroxylon basijugum* with pods on the stem; **E**, The fruit of *Zygia heteroneura*. — Photos: Julia Ferm (A, C); Bertil Ståhl (B, D, E).

The Ingeae tribe has a taxonomic history of new genera being proposed and, less frequently, genera being merged (e.g., Nielsen, 1981; Barneby & Grimes, 1996; Lewis & Rico Arce, 2005; Brown, 2008). Bentham (1865) established the tribe Ingeae to comprise nine genera primarily distinguished by differences in pod characteristics. He later recognized “15 genera or subgenera” (Bentham, 1875), and since then the number of genera has continued to increase. Nielsen (1981) recognized 21 genera in Ingeae based on a combination of vegetative, floral and fruit characters. In the most recent classification, Lewis & Rico Arce (2005) followed Barneby & Grimes’s (1996, 1997) circumscription of the tribe and recognized 36 genera arranged in seven informal alliances. *Zygia* and *Marmaroxylon* are both found in the *Inga* alliance, together with genera including *Inga*, *Calliandra* Benth., *Cojoba* Britton & Rose and *Macrosamanea* Britton & Rose ex Britton & Killip.

*Zygia* was described by Browne (1756) based on a plant from Jamaica, now treated as *Z. latifolia* (L.) Fawc. & Rendle (Fawcett & Rendle, 1920). However, following Bentham’s (1875) classification, *Zygia* was for many years mostly included in a broadly defined *Pithecellobium* Mart. (e.g., Macbride, 1943; Standley & Steyermark, 1946; Woodson & Schery, 1950), although it was treated as a separate genus by Britton & Rose (1928). The genus *Zygia* has been more widely used in more recent studies (e.g., Nielsen, 1981; Barneby & Grimes, 1997).

*Marmaroxylon* was originally described by Killip (in Record, 1940) to accommodate the Amazonian species *Pithecellobium racemosum* Ducke. Rico Arce (1991) listed eight additional species of *Marmaroxylon* previously included in *Abarema* Pittier, *Klugiodendron* Britton & Killip, *Macrosamanea* and *Pithecellobium*. According to Rico Arce (1991), members of *Marmaroxylon* have leaves with several pairs of

pinnae, a feature differentiating them from species of *Zygia*, which have leaves with one pair of pinnae only.

In the latest monographic treatment of *Zygia* and allied genera, Barneby & Grimes (1997) included all species of *Marmaroxylon* in a broadly defined *Zygia*. They described several new species of *Zygia*, of which *Z. lathetica* Barneby & J.W.Grimes, *Z. palustris* Barneby & J.W.Grimes and *Z. tetragona* Barneby & J.W.Grimes have leaves with several pairs of pinnae. Thus, if following Rico Arce's (1991) concept of *Marmaroxylon*, these species should instead have been treated in *Marmaroxylon*. Barneby & Grimes (1997) divided *Zygia* into nine sections, five of which include, or consist entirely of, representatives of species referred to *Marmaroxylon* by Rico Arce (1991). Despite this, and likely because Barneby & Grimes (1997) themselves expressed an uncertainty about their classification, *Marmaroxylon* has continued to be recognized in the literature (e.g., Pennington & al., 2004; Lewis & Rico Arce, 2005; Ståhl & al., 2015); but see LPWG (2017).

To further complicate the classification, there are also some morphologically odd members of *Zygia* included in the treatment of Barneby & Grimes (1997). These include *Z. inundata* (Ducke) H.C.Lima ex Barneby & J.W.Grimes, which has simple pinnate leaves; *Z. ocumarensis*, with axillary, non-cauliflorous inflorescences; *Z. eperuetorum* (Sandwith) Barneby & J.W.Grimes, with exceptionally long leaflet pulvinules and pedicellate flowers; and *Z. sabatieri* Barneby & J.W.Grimes, with flowers arranged in heads on long, slender peduncles. Barneby & Grimes (1997) also pointed out that the lack of information, particularly about mature fruits, hindered any attempt to reach a good understanding of phylogenetic relationships within the group.

Previous phylogenetic studies of the Ingeae tribe have included, among other genera, a few representatives of *Zygia* and *Marmaroxylon* (e.g., Brown & al., 2008; Kyalangalilwa & al., 2013; Souza & al., 2013; Iganci & al., 2016). Two of these studies (Kyalangalilwa & al., 2013; Iganci & al., 2016) show, with strong support, that *Zygia* plus *Marmaroxylon* is sister to *Inga*, based on a single species and five species, respectively, whereas in the other studies the positions within Ingeae retrieved for *Zygia/Marmaroxylon* have very low support (Brown & al., 2008; Souza & al., 2013). Thus, neither the monophyly of *Zygia* and *Marmaroxylon*, nor their sister-group relationships within Ingeae have been thoroughly tested.

Our study presents a phylogenetic analysis of *Zygia* and *Marmaroxylon* based on nuclear and plastid DNA sequence data. We sampled broadly, with representatives from all alliances in the Ingeae tribe (Lewis & Rico Arce, 2005), and included two species of *Acacia* s.str., four species of *Senegalia* Raf., and *Vachellia farnesiana* (L.) Wight & Arn. to (1) test the monophyly of *Zygia*, the monophyly of the sections within *Zygia* and the positions of the morphologically odd species as listed by Barneby & Grimes (1997); (2) test the monophyly of *Marmaroxylon* as circumscribed by Rico Arce (1991); (3) test the monophyly of several species of both *Zygia* and *Marmaroxylon*;

and (4) test the relationships of *Zygia/Marmaroxylon* species to other ingoid taxa and closely related *Acacia* species (e.g., Brown & al., 2008).

## ■ MATERIALS AND METHODS

**Nomenclature.** — The nomenclature of *Zygia* follows Barneby & Grimes (1997), except for those species with combinations in *Marmaroxylon* recognized by Rico Arce (1991).

**Taxon and DNA region sampling.** — A total of 36 *Zygia/Marmaroxylon* species were included in this study. From *Zygia*, 28 species included in Barneby & Grimes (1997) and 1 more recently described species, *Z. nubigena* B.Ståhl & al. (Ståhl & al., 2010), were analysed, and from *Marmaroxylon* (Rico Arce, 1991), 7 species were included (Table 1).

Species from seven of the nine sections of *Zygia* recognized by Barneby & Grimes (1997) were included in the study. Furthermore, for 25 species multiple accessions were included to evaluate the monophyly of these species as presently circumscribed. Material was obtained from herbarium specimens at AAU, K, NY, P and S, from silica-dried leaf material collected in the field by the first author, or as DNA samples from the RBG Kew DNA Bank (<http://apps.kew.org/dnabank/>). To evaluate the phylogenetic relationships of the species of *Zygia* and *Marmaroxylon*, previously published sequence data (available in GenBank) for 29 species from 16 other genera were included in this study. The selected species represent all alliances of Ingeae as defined by Lewis & Rico Arce (2005) as well as the *Acacia* clade previously found to be nested within Ingeae (Brown & al., 2008). At least two genera from each alliance and two species from each genus were included, with the exceptions of *Faidherbia* A.Chev. (monospecific), *Hydrochorea* Barneby & J.W.Grimes, *Macrosamanea* and *Pseudosamanea* Harms, each represented by one species. Four species of *Senegalia* and *Vachellia farnesiana* were also included to represent species that are closely related to, but not included in, Ingeae. *Vachellia farnesiana* was used to root the trees (Kyalangalilwa & al., 2013). Voucher information and GenBank accession numbers are listed in Appendix 1.

Amplified DNA-regions were the nuclear external transcribed spacer (ETS) and the internal transcribed spacer (ITS) and the plastid regions *psbA-trnH* intergenic spacer and *trnL-trnF* (including the *trnL* intron and the *trnL-trnF* spacer). These regions have previously been used in phylogenetic studies of genera within, and closely related to, Ingeae, and show a high number of variable sites (Luckow & al., 2003; Miller & al., 2003; Brown & al., 2008; Souza & al., 2013).

**DNA extraction, amplification and sequencing.** — Total DNA was extracted from herbarium material or silica-dried leaf material using a modified Carlson-Yoon protocol (Yoon & al., 1991). If a polymerase chain reaction of the total DNA did not yield any product, 30 µl of the DNA sample were purified using the Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Little Chalfont, U.K.) following the protocol of the manufacturer. The primers used are listed

**Table 1.** Species of *Marmaroxylon* and *Zygia* included in this study, with synonyms and sectional placement in *Zygia* following Barneby & Grimes (1997).

Species	Synonym	Section in <i>Zygia</i>
<i>Marmaroxylon basijugum</i> (Ducke) L.Rico	<i>Zygia basijuga</i> (Ducke) Barneby & J.W.Grimes	<i>Zygiopsis</i>
<i>Marmaroxylon claviflorum</i> (Spruce ex Benth.) L.Rico	<i>Zygia claviflora</i> (Spruce ex Benth.) Barneby & J.W.Grimes	<i>Zygiopsis</i>
<i>Marmaroxylon collinum</i> (Sandwith) L.Rico	<i>Zygia collina</i> (Sandwith) Barneby & J.W.Grimes	<i>Parazygia</i>
<i>Marmaroxylon dinizii</i> (Ducke) L.Rico	<i>Zygia ramiflora</i> (Benth.) Barneby & J.W.Grimes <sup>a</sup>	<i>Parazygia</i>
<i>Marmaroxylon magdalenae</i> Killip ex L.Rico	<i>Zygia ocumarensis</i> (Pittier) Barneby & J.W.Grimes	<i>Nothellobium</i>
<i>Marmaroxylon ocumarensis</i> (Pittier) L.Rico	<i>Zygia ocumarensis</i> (Pittier) Barneby & J.W.Grimes	<i>Nothellobium</i>
<i>Marmaroxylon racemosum</i> (Ducke) Killip	<i>Zygia racemosa</i> (Ducke) Barneby & J.W.Grimes	<i>Marmaroxylon</i>
<i>Zygia biflora</i> L.Rico		<i>Zygia</i>
<i>Zygia bisingula</i> L.Rico		<i>Zygia</i>
<i>Zygia brenesii</i> (Standl.) L.Rico		<i>Zygia</i>
<i>Zygia cataractae</i> (Kunth) L.Rico		<i>Zygia</i>
<i>Zygia coccinea</i> (G.Don) L.Rico		<i>Zygia</i>
<i>Zygia coccinea</i> var. <i>coccinea</i>		<i>Zygia</i>
<i>Zygia coccinea</i> var. <i>macrophylla</i> (Spruce ex Benth.) Barneby & J.W.Grimes		<i>Zygia</i>
<i>Zygia coccinea</i> var. <i>oriunda</i> (J.F.Macbr.) Barneby & J.W.Grimes		<i>Zygia</i>
<i>Zygia confusa</i> L.Rico		<i>Zygia</i>
<i>Zygia konzattii</i> (Standl.) Britton & Rose		<i>Zygia</i>
<i>Zygia cupirensis</i> (C.Barbosa) L.Rico		<i>Zygia</i>
<i>Zygia dissitiflora</i> Barneby & J.W.Grimes		<i>Zygia</i>
<i>Zygia heteroneura</i> Barneby & J.W.Grimes		<i>Zygia</i>
<i>Zygia inaequalis</i> (Humb. & Bonpl. ex Willd.) Pittier		<i>Zygia</i>
<i>Zygia inundata</i> (Ducke) H.C.Lima ex Barneby & J.W.Grimes		<i>Ingopsis</i>
<i>Zygia juruana</i> (Harms) L.Rico		<i>Zygia</i>
<i>Zygia lathetica</i> Barneby & J.W.Grimes		<i>Zygiopsis</i>
<i>Zygia latifolia</i> (L.) Fawc. & Rendle		<i>Zygia</i>
<i>Zygia latifolia</i> var. <i>communis</i> Barneby & J.W.Grimes		<i>Zygia</i>
<i>Zygia latifolia</i> var. <i>controversa</i> Barneby & J.W.Grimes		<i>Zygia</i>
<i>Zygia latifolia</i> var. <i>latifolia</i> (L.) Fawc. & Rendle		<i>Zygia</i>
<i>Zygia longifolia</i> (Humb. & Bonpl. ex Willd.) Britton & Rose		<i>Zygia</i>
<i>Zygia macbridei</i> (C.Barbosa) L.Rico		<i>Zygia</i>
<i>Zygia morongii</i> Barneby & J.W.Grimes		<i>Zygia</i>
<i>Zygia multipunctata</i> Barneby & J.W.Grimes		<i>Zygia</i>
<i>Zygia nubigena</i> Ståhl, L.Rico & G.P.Lewis		— <sup>b</sup>
<i>Zygia palustris</i> Barneby & J.W.Grimes		<i>Zygiopsis</i>
<i>Zygia paucijugata</i> (Lundell) L.Rico		<i>Zygia</i>
<i>Zygia peckii</i> (B.L.Rob.) Britton & Rose		<i>Zygia</i>

(Continues)

Table 1. Continued.

Species	Synonym	Section in <i>Zygia</i>
<i>Zygia pithecolobioides</i> (Harms) Barneby & J.W.Grimes		<i>Zygia</i>
<i>Zygia sabatieri</i> Barneby & J.W.Grimes		<i>Pseudocojoba</i>
<i>Zygia selloi</i> (Benth.) L.Rico		<i>Zygia</i>
<i>Zygia tetragona</i> Barneby & J.W.Grimes		<i>Parazygia</i>
<i>Zygia trunciflora</i> (Ducke) L.Rico		<i>Zygia</i>
<i>Zygia unifoliolata</i> (Benth.) Pittier		<i>Zygia</i>

a Treated as *Zygia ramiflora* (Benth.) Barneby & J.W.Grimes by Barneby & Grimes (1997). However, the name is illegitimate as it is a later homonym of *Zygia ramiflora* (F.Muell.) Kosterm. (Neill & al. in Jørgensen & León-Yáñez, 1999).

b Described by Ståhl & al. (2010) and thus not placed in any section of *Zygia* by Barneby & Grimes (1997).

in Table 2. PCRs were run on an Applied Biosystems Veriti 96-Well Thermal Cycler. Protocols were adjusted for the specific region being amplified and the primers used. Amplifications were carried out as follows: ETS, 3 min initial denaturation at 94°C followed by 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C and 2 min extension at 72°C, and completed by a final extension of 7 min at 72°C; ITS, 1 min initial denaturation at 97°C followed by 40 cycles of 10 s denaturation at 97°C, 90 s annealing at 55°C and 1 min extension at 72°C, and completed by a final extension of 7 min at 72°C; *psbA-trnH*, 3 min initial denaturation at 94°C followed by 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 55°C and 10 s extension at 72°C, and completed by a final extension of 7 min at 72°C; *trnL-trnF*, 3 min initial denaturation at 94°C followed by 35 cycles of 1 min denaturation at 94°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.

ITS and *trnL-trnF* were amplified in two reactions. The products were purified using Illustra ExoProStar 1-Step (GE Healthcare) following the manufacturer's instructions, and sent to Macrogen Europe in Amsterdam, the Netherlands, for sequencing. The same primers were used for sequencing as for PCR.

**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 & al., 2012). For each region, multiple alignments of the sequences were performed using MUSCLE v.3.8.31 (Edgar, 2004), and adjusted by eye in AliView v.1.19-beta-3 (Larsson, 2014). Only specimens with sequences from at least two of the DNA regions were included in this study. Before analysis, the best-fitting nucleotide substitution models for each of the datasets were determined based on the corrected Akaike information criterion using

Table 2. Primers used in this study.

DNA region	Primer	Sequence 5' → 3'	Reference
<b>Forward</b>			
ETS	AcR2	GGG CGT GTG AGT GGT GTT TGG	Ariati & al. (2006)
ITS (part 1)	P17	CTA CCG ATT GAA TGG TCC GGT GAA	Popp & Oxelman (2001)
ITS (part 2)	ITS 493	ATG CGA TAC TTG GTG TGA AT	Ferm, this paper
<i>psbA-trnH</i>	psbAF	GTT ATG CAT GAA CGT AAT GCT	Sang & al. (1997)
<i>trnL-trnF</i> (part 1)	c	CGC GCA TGG TGG ATT CAC AAA TC	Taberlet & al. (1991)
<i>trnL-trnF</i> (part 2)	e	GGT TCA AGT CCC TCT ATC CC	Taberlet & al. (1991)
<b>Reverse</b>			
ETS	18S-IGS	GAG ACA AGC ATA TGA CTA CTG GCA GGA TCA ACC AG	Baldwin & Markos (1998)
ITS (part 1)	ITS 491	TCA CAC CAA GTA TCG CAT TT	Ferm, this paper
ITS (part 2)	26S-82R	TCC CGG TTC GCT CGC CGT TAC	Popp & Oxelman (2001)
<i>psbA-trnH</i>	trnHR	CGC GCA TGG TGG ATT CAC AAA TC	Sang & al. (1997)
<i>trnL-trnF</i> (part 1)	d	GGG ATA GAG GGA CTT GAA CC	Taberlet & al. (1991)
<i>trnL-trnF</i> (part 2)	jfl	ATT TGA ACT GGT GAC ACG AGG	Ferm, this paper

MrAic v.1.4.6. (Nylander, 2004). The HKY+ $\Gamma$  model was selected for *trnL-trnF*, GTR+I+ $\Gamma$  was selected for ITS and GTR+ $\Gamma$  for ETS and *psbA-trnH*. Bayesian inference analyses were performed for each dataset using MrBayes v.3.2.6. (Ronquist & al., 2011). Two parallel MCMC chains were run for 1 million generations at a time, with a sampling frequency of 1000, until the average standard deviation of split frequencies was below 0.01, indicating that the Markov chains had converged on the stationary distribution. For ETS and ITS, this was achieved after 1 million generations, and for *psbA-trnH* and *trnL-trnF* after 2 million generations. Ten percent of the sampled trees were discarded as burn-in after evaluation of the output parameters generated by the Bayesian analysis using Tracer v.1.6 (Rambaut & al., 2014). The resulting topologies from each of the single-region datasets were manually compared in FigTree v.1.4.3 (Rambaut, 2006) to detect potential conflicting topologies. Only minor conflicting topologies were discovered, and the datasets of the individual regions were concatenated using Abioscripts v.0.9.4 (Larsson, 2010). Bayesian inference analysis of the combined dataset was performed using MrBayes v.3.2.6 (Ronquist & al., 2011) using the same settings as for the separate datasets. The average standard deviation of split frequencies was below 0.01 after 2 million generations, 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 using Tracer v.1.6 (Rambaut & al., 2014). A majority-rule consensus tree was then calculated based on the remaining trees and inspected in FigTree v.1.4.3 (Rambaut, 2006). We consider clades with a posterior probability (PP) of  $\geq 0.95$  as well supported.

Newly obtained sequences were deposited in GenBank (Appendix 1). The aligned datasets and the phylogenetic trees of single-region datasets are available in TreeBase (<http://purl.org/phylo/treebase/phyloids/study/TB2:S23455>).

## ■ RESULTS

**Phylogenetic analyses.** — Characteristics of each DNA sequence region used in this study are summarized in Table 3.

We identified four clades that include *Zygia* and *Marmaroxylon* species. For this paper, we refer to them as the *Abarema* clade, the *Inga* clade, the *Macrosamanea* clade and the *Zygia* clade (Fig. 2).

The results of the individual gene trees all show similar topologies although most nodes are poorly supported. In the ETS tree, the *Abarema* clade (PP 1), the *Zygia* clade (PP 0.99) and the *Macrosamanea* clade (PP 1) are recovered. In the ITS tree, the *Abarema* clade (PP 1) and the *Zygia* clade (PP 1) are recovered, but *Macrosamanea pubiramea* is found in a clade together with the species of *Inga* and *Zygia inundata* (PP 1). *Marmaroxylon ocumarense* is not found in the same clade. However, we consider it to be a minor incongruence since most nodes in both the ETS tree and the ITS tree are

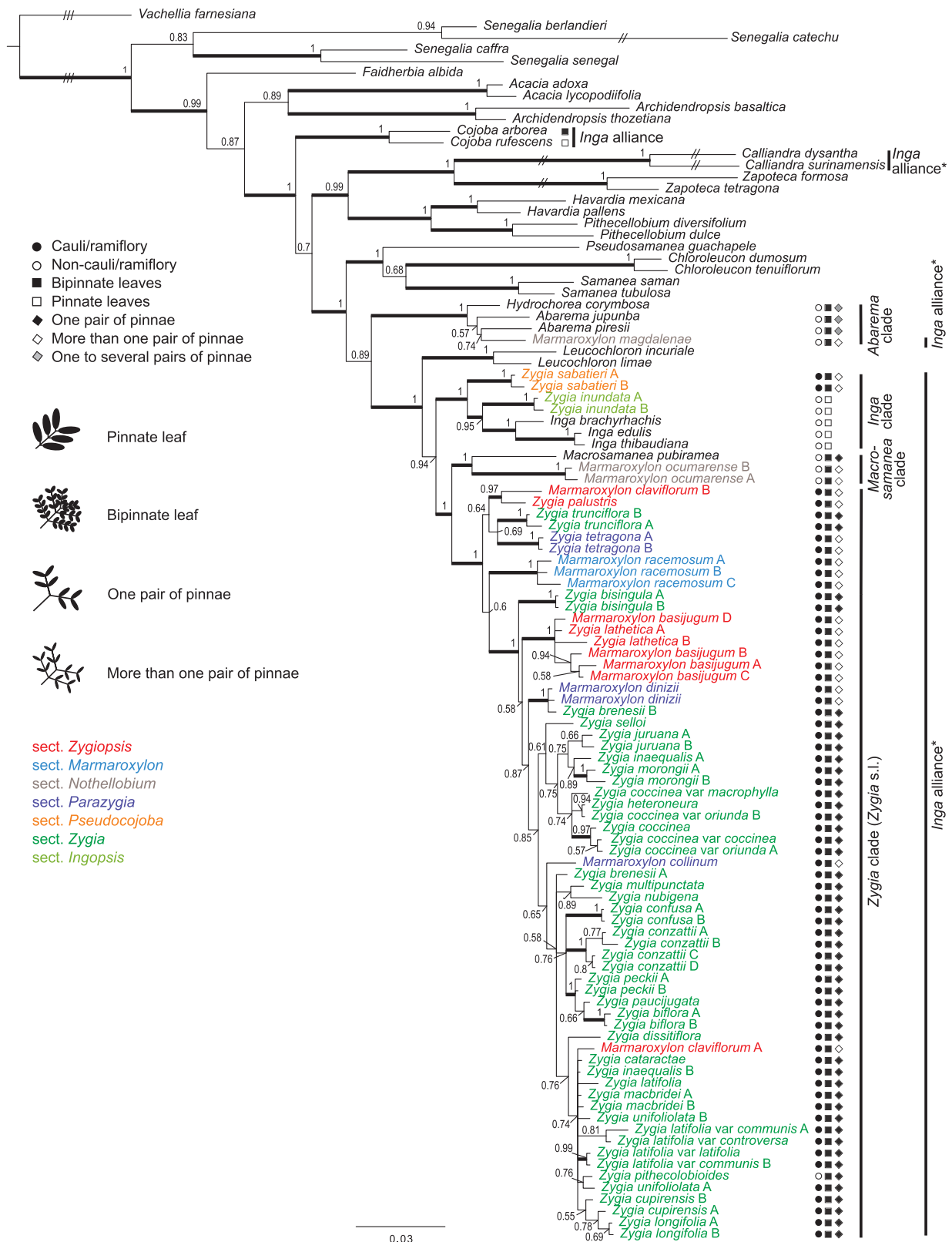
poorly supported, and the relationship between *Marmaroxylon ocumarense* and the clade consisting of the *Inga* species, *Zygia inundata* and *Macrosamanea pubiramea* in the ITS tree is not resolved. Moreover, the position of *Macrosamanea pubiramea* within this subclade is not resolved. Also, *Zygia sabatieri* is not included in the ITS tree, which could affect the results. In the *psbA-trnH* tree, the *Abarema* clade (PP 1) and the *Inga* clade (PP 1) are recovered. The *Zygia* clade, including *Marmaroxylon ocumarense* and *Macrosamanea pubiramea*, is strongly supported (PP 1) and found as sister to the *Inga* clade (PP 0.98), but relationships within the *Zygia* clade (including *Marmaroxylon ocumarense* and *Macrosamanea pubiramea*) are not resolved. In the *trnL-trnF* tree, the *Abarema* clade is recovered (PP 1). Phylogenetic trees of the individual gene trees are available in TreeBase (<http://purl.org/phylo/treebase/phyloids/study/TB2:S23455>). The 50% majority-rule Bayesian consensus tree based on the combined dataset (ETS, ITS, *psbA-trnH* and *trnL-trnF*) (Fig. 2) is in general robustly supported. Neither *Zygia* as circumscribed by Barneby & Grimes (1997) nor *Marmaroxylon* according to the concept of Rico Arce (1991) are monophyletic. Furthermore, the results show that *Zygia* and *Marmaroxylon* are not monophyletic together (Fig. 2). The *Inga* alliance (Lewis & Rico Arce, 2005) is shown to be non-monophyletic, and none of the sections of *Zygia* (Barneby & Grimes, 1997) containing more than one species are shown to be monophyletic.

### Clades including *Zygia* and *Marmaroxylon* species. —

The *Abarema* clade (PP 1) includes a single *Marmaroxylon* species, *M. magdalenae*, together with *Abarema piresii*, *A. jupunba* and *Hydrochorea corymbosa*. The clade is found, with moderate support (PP 0.89), as sister to a clade including two *Leucochloron* species and the three additional *Zygia/Marmaroxylon* clades. In the *Inga* clade (PP 1), two *Zygia* species, *Z. inundata* and *Z. sabatieri*, are found together with the *Inga* spp. and all nodes are strongly supported (PP  $\geq 0.95$ ). The *Macrosamanea* clade (PP 1) includes *Marmaroxylon ocumarense* and *Macrosamanea pubiramea* and is well supported as sister to the *Zygia* clade (PP 1). The *Zygia* clade is strongly supported as monophyletic (PP 1) and includes the remaining *Zygia* and *Marmaroxylon* species, including the three specimens of *Marmaroxylon racemosum*, the type of *Marmaroxylon*, strongly supported as monophyletic (PP 1). The five

**Table 3.** Number of accessions and characters, proportion of variable characters, and nucleotide substitution models used in the Bayesian inference analyses for the different datasets.

Dataset	No. of acc.	No. of char.	No. of var. char. (%)	Subst. model
ETS	87	521	325 (62%)	GTR+ $\Gamma$
ITS	93	872	329 (39%)	GTR+I+ $\Gamma$
<i>psbA-trnH</i>	59	606	130 (21%)	GTR+ $\Gamma$
<i>trnL-trnF</i>	76	1170	210 (18%)	HKY+ $\Gamma$
Combined	106	3169	(32%)	–



**Fig. 2.** Phylogeny of *Zygia*, *Marmaroxylon* and allies. Majority-rule (50%) consensus tree of 900 trees sampled at stationarity from the Bayesian analysis of the combined dataset (ETS, ITS, *psbA-trnH* and *trnL-trnF*). Numbers at nodes are posterior probabilities (PP) and thick lines indicate a PP of 0.95 or higher. Colours indicate which section in *Zygia* (Barneby & Grimes, 1997) the species are assigned to, and symbols show leaf type and cauliflory-ramiflory. \* *Inga* alliance according to Lewis & Rico Arce (2005). A double slash indicates branches shortened by half; a triple slash indicates branches shortened to one third.

collections of *Zygia latifolia*, the type of *Zygia*, are also found in the *Zygia* clade but not shown to be monophyletic. Furthermore, other relationships within the *Zygia* clade are only partly supported.

**Non-monophyletic species.** — Our results show five species to be non-monophyletic (Fig. 2): *Marmaroxylon claviflorum* A and B, *Zygia brenesii* A and B, as well as *Z. inaequalis* A and B, and *Z. unifoliolata* A and B are found in the *Zygia* clade but, assuming that the original specimen identifications are correct, none of these species are found to be monophyletic. All five terminals of *Z. latifolia* are found in the *Zygia* clade, but only *Z. latifolia* var. *latifolia* and *Z. latifolia* var. *communis* B are strongly supported as monophyletic together (PP 0.99). Furthermore, the results show all subspecific taxa of *Z. coccinea* and *Z. heteroneura* in a poorly supported subclade (PP 0.74) within the *Zygia* clade.

**Non-monophyly of the *Inga* alliance and sections in *Zygia*.** — The results of this study show that the *Inga* alliance (Lewis & Rico Arce, 2005) is non-monophyletic. A clade consisting of the two *Cojoba* species is found in a strongly supported position separated from the rest of the taxa of the *Inga* alliance. Furthermore, the two *Calliandra* species are together found as sister to *Zapoteca* (PP 1), the latter not a member of the *Inga* alliance. Also worth noting is that the species of the remaining genera included in the *Inga* alliance, *Inga*, *Macrosamanea*, *Marmaroxylon* and *Zygia* (Lewis & Rico Arce, 2005) are only moderately supported as monophyletic (PP 0.94) (with the exception of *Marmaroxylon magdalenae*). The results of this study support none of the sections of *Zygia* (that include more than one species) proposed by Barneby & Grimes (1997) as monophyletic.

## ■ DISCUSSION

### Clades including *Zygia* and *Marmaroxylon* species. —

The results of our phylogenetic analysis (Fig. 2) show that *Marmaroxylon* (Rico Arce, 1991) and *Zygia* (Barneby & Grimes, 1997) are not monophyletic, but instead that the species of the two genera are found intermixed with each other and with species of *Abarema*, *Hydrochorea*, *Inga* and *Macrosamanea*. Furthermore, our results, discussed in detail below, show that the key morphological features (cauliflory and number of pairs of pinnae on leaves) traditionally used to distinguish genera in the past are homoplasious and do not provide unique diagnostic synapomorphies of clades. Nevertheless, the possession of pinnate leaves seems to distinguish species of the apparently monophyletic *Inga* (e.g., Brown & al., 2008) from nearly all other ingoid genera, with *Cojoba rufescens* seemingly being the only other ingoid species with pinnate leaves (Barneby & Grimes, 1997).

Our results show that *Marmaroxylon magdalenae* is found in the *Abarema* clade (Fig. 2) and thus should be placed in the *Abarema* alliance (Lewis & Rico Arce, 2005). *Marmaroxylon magdalenae* has bipinnate leaves with two pairs of pinnae and sessile flowers arranged in compact heads. Rico Arce (1991)

stated that it is the only non-cauliflorous species of *Marmaroxylon*, an observation that by itself indicates a different taxonomic placement of this species. According to Barneby & Grimes (1997), there are two non-cauliflorous species in *Zygia*, viz. *Z. pithecolobioides* and *Z. ocumarensis* (*Marmaroxylon ocumarensis*). They treated *M. magdalenae* as a synonym of the latter. Our results show, however, that *M. magdalenae* and *M. ocumarensis* are not closely related and thus not conspecific (Fig. 2), as was recognized by Rico Arce (1991). Killip (in sched.) referred *M. magdalenae* to *Klugiodendron*, the combination in the latter genus being cited as a synonym of *M. magdalenae* by Rico Arce (1991). *Klugiodendron* was synonymized with *Abarema* by Barneby & Grimes (1996), which implies that *M. magdalenae* is morphologically similar to species of *Abarema*. The similarity in leaves and inflorescences with species of *Abarema* is also seen in the type specimen used in this study (Appendix 1). However, a more extensive phylogenetic analysis including a larger sample of taxa with focus on the *Abarema* alliance s.l. and closely related taxa is needed before any taxonomic decision is taken, especially as Iganci & al. (2016) showed that *Abarema* itself is non-monophyletic and requires recircumscription.

*Zygia inundata* and *Z. sabatieri* are both found in the *Inga* clade (Fig. 2). *Zygia inundata* is strongly supported as sister to the *Inga* spp. (Fig. 2) and shown to be monophyletic, based on two specimens. *Zygia inundata* was first described by Ducke (1922) as a species of *Inga* and considered as a close relative of *Inga huberi* Ducke, described in the same work. When the massive, dehiscent pod was discovered (*Inga* has mostly indehiscent pods), Ducke (1925) transferred *I. inundata* to *Pithecellobium* and later placed it in *Pithecellobium* ser. *Coriacea* Benth. (Ducke, 1949). However, Barneby & Grimes (1996) treated this series as part of *Macrosamanea* and assigned *I. inundata* to its own section within *Zygia*, *Z. sect. Ingopsis*, based on similarities to *Zygia* in fruit characters (Barneby & Grimes, 1997). They particularly noted the similarity in fruit between *Z. inundata* and *Z. juruana* (Harms) L.Rico (recovered in the *Zygia* clade in this analysis; Fig. 2). *Zygia inundata* has pinnate leaves and is not strictly cauli- and/or ramiflorous (Barneby & Grimes, 1997), i.e., this species conforms to characters possessed by *Inga*. Thus, both the phylogenetic analyses of sequence data presented here and some morphological features suggest a placement of *Zygia inundata* in *Inga*, which is in line with the taxonomic history of the species. The species was not considered in Pennington's (1997) monumental monograph of *Inga*, but it seems clear that it belongs in that genus.

*Zygia sabatieri* is strongly supported as sister to *Z. inundata* and the remaining species of *Inga* (Fig. 2) and shown to be monophyletic based on two specimens. *Zygia sabatieri* has bipinnate leaves as evidenced by the type specimen (*Sabatier & Prévost 3896*, isotype at NY seen for this study) and other collections used in this study (Appendix 1). Nevertheless, as stated by Barneby & Grimes (1997), *Z. sabatieri* differs from other *Zygia* species in fruit and seed morphology and by having flowers with narrow corollas arranged in long-pendunculate capitulae, similar to conditions



in *Cojoba* (Barneby & Grimes, 1997). Because of the morphological isolation of *Z. sabatieri* from the other species of *Zygia*, Barneby & Grimes (1997) assigned it to its own section, i.e., *Z. sect. Pseudocojoba*. However, the results in this study show that *Z. sabatieri* is more closely related to *Inga* than to *Zygia*. Including *Z. sabatieri* in *Inga* would render the latter genus morphologically less homogenous as *Z. sabatieri* then would be the only species with bipinnate leaves among some 300 pinnately leaved species. Nevertheless, as seen in *Cojoba*, presence of pinnate/bipinnate leaves is a homoplastic character and we cannot rule out the possibility of the occurrence of bipinnately leaved mimosoid species nested within *Inga*. Another option would be to assign *Z. sabatieri* to its own genus. Before any taxonomic action is taken, however, additional phylogenetic studies including a larger sample of *Inga* species are needed in order to investigate if *Z. sabatieri* is sister to *Inga* (including *Z. inundata*) as shown here (Fig. 2), or nested within it. *Zygia sabatieri* is known from very few collections, and additional fieldwork to further study its morphology would certainly be rewarding.

The *Macrosamanea* clade includes two collections of *Marmaroxylon ocumarensense* strongly supported as monophyletic and as sister to *Macrosamanea pubiramea* (Fig. 2). *Marmaroxylon ocumarensense* has bipinnate leaves with more than one pair of pinnae and one pair of leaflets on each pinna, similar to many *Zygia* species. However, *Marmaroxylon ocumarensense* has axillary, non-cauliflorous inflorescences (Barneby & Grimes, 1997), which differentiates this species from the cauli- and/or ramiflorous members of *Zygia* and *Marmaroxylon*. Despite the non-typical inflorescence of *Marmaroxylon ocumarensense*, Barneby & Grimes (1997) considered the resemblance of its fruits to those of *Zygia racemosa* significant enough for it to be placed in *Zygia*, but assigned the species (as *Z. ocumarensis*) to a separate, monospecific section, viz. *Z. sect. Nothellobium*. Our results contradict the taxonomic conclusions drawn by Barneby & Grimes (1997). However, in order to further evaluate the phylogenetic position of *Marmaroxylon ocumarensense* relative to the species of *Macrosamanea*, a more extensive analysis including more species from *Macrosamanea* is needed.

The *Zygia* clade contains most species of *Zygia* and *Marmaroxylon* included in this study (Fig. 2). Within the *Zygia* clade, the three collections representing *Marmaroxylon racemosum* are strongly supported as monophyletic (Fig. 2). *Marmaroxylon racemosum* has leaves with several pairs of pinnae and numerous pairs of opposite leaflets on each pinna (Rico Arce, 1991). Our results (Fig. 2) do not support *Marmaroxylon* as a genus defined by the occurrence of several pairs of pinnae per leaf since species of *Zygia* and *Marmaroxylon* with several pairs of pinnae and species with one pair of pinnae are found scattered throughout the phylogenetic tree (Fig. 2). Including *Marmaroxylon* as a synonym of *Zygia* is the preferred taxonomic action based on our current state of knowledge. Recognizing *Marmaroxylon* as a distinct genus would render it monospecific, comprising the single species *M. racemosum*, and would necessitate a new genus name to be proposed to accommodate the species in the subclade consisting of

*M. claviflorum* B, *Z. palustris*, *Z. trunciflora* and *Z. tetragona* (Fig. 2), a clade which is, however, only poorly supported.

Within the *Zygia* clade (Fig. 2), all four specimens of *Marmaroxylon basijugum* and the two specimens of *Zygia lathetica* are found together in a strongly supported subclade (Fig. 2). It is currently unclear from our results if these species are reciprocally monophyletic, or rather conspecific. According to Barneby & Grimes (1997), *Z. lathetica* is distinguished from *M. basijugum* by its larger flowers, longer and more numerous stamens and by having a broader pod with larger seeds. However, they are hard to differentiate on leaf characters alone and are therefore often misidentified in herbaria. According to Barneby & Grimes (1997), both species are restricted to northern South America but are not sympatric, even though a distribution overlap certainly seems likely. By including a larger sample of these two species and including collections from a wider geographical area, it would be possible to further investigate species delimitations. Such a study may also be a good candidate to elucidate the diversification of west Amazonian taxa in general.

**Non-monophyletic species.** — In our analysis, four species represented by two collections each, namely *Marmaroxylon claviflorum*, *Zygia brenesii*, *Z. inaequalis* and *Z. unifoliolata*, and one species, *Z. latifolia*, represented by five collections, are not resolved as monophyletic (Fig. 2). This could be an indication of the presence of cryptic species, or simply the need for more detailed taxonomic studies at the species level in order to identify specific characters that can be used for identification. According to Pennington & Lavin (2016), non-monophyly in widespread rainforest species is common and could be explained by massive population sizes, long life spans and effective seed and pollen flow, which lead to preservation of ancestral genetic polymorphism since time to coalescence will be extensive. Following this line of reasoning, a larger sample of the non-monophyletic species in this study might help to investigate the monophyly of these species. However, in our study, *Zygia latifolia* var. *latifolia* and *Z. latifolia* var. *communis* B are strongly supported as sisters (Fig. 2), but the sampled specimens were collected in very distant localities. *Zygia latifolia* var. *latifolia* was collected in Amapá, Brazil, and *Z. latifolia* var. *communis* B was collected in Pando, Bolivia. Nevertheless, to further investigate species boundaries within these complexes, a thorough study of more specimens, using both morphological and DNA sequence data, including more specimens, is needed.

Furthermore, our results resolve all intraspecific taxa of *Zygia coccinea* in a poorly supported subclade within the *Zygia* clade (Fig. 2). Also, one of our own collections of *Z. heteroneura* is found among the five intraspecific taxa of *Z. coccinea* included in this study. This collection was made in the Yasuní National Park in Amazonian Ecuador. It was identified as *Z. heteroneura* based on resemblance in leaf and fruit characters to other material of this species collected in the same general area. According to Barneby & Grimes (1997), the pod of *Z. heteroneura* is not known, but the pods of *Z. coccinea* are large and similar to the pod of the plant

we collected (Fig. 1E). *Zygia heteroneura* and *Z. coccinea* var. *oriunda* occur sympatrically. Both have morphologically similar, white flowers, but differ according to Barneby & Grimes (1997) in, e.g., number of stamens (*Z. heteroneura* having flowers with twice as many stamens as *Z. coccinea*) and leaflet venation. We cannot rule out the possibility that our material of *Z. heteroneura* is in fact a sample of *Z. coccinea* var. *oriunda*, which would give us a monophyletic, although still poorly supported, *Z. coccinea* (Fig. 2).

**Non-monophyly of the *Inga* alliance and sections in *Zygia*.** — Most members of the *Inga* alliance (Lewis & Rico Arce, 2005) included in this study (species of *Inga*, *Macrosamanea*, *Marmaroxylon* and *Zygia*, but excluding *Marmaroxylon magdalenae*) are found in a moderately supported clade (Fig. 2). However, the positions of the other members of the *Inga* alliance included in this study (*Marmaroxylon magdalenae* and species of *Calliandra* and *Cojoba*) render the alliance non-monophyletic (Fig. 2), as previously shown (e.g., Souza & al., 2013, 2016; Iganci & al., 2016).

In our results none of the *Zygia* sections of Barneby & Grimes (1997) including more than one species is shown to be monophyletic (Fig. 2).

## ■ CONCLUSIONS

Our study shows that neither *Zygia* (Barneby & Grimes, 1997) nor *Marmaroxylon* (Rico Arce, 1991) is monophyletic. Furthermore, as currently circumscribed, they are not monophyletic when considered together (Fig. 2). The *Zygia* clade is treated as *Zygia* (Fig. 2), thus rendering *Zygia* a well-supported monophyletic genus recognized by cauli- and/or ramiflorous inflorescences and bipinnate leaves (*Z. pithecoloboides* then being the only exception because it does not display cauli- or ramiflory). No new combinations are needed for the species involved (*Marmaroxylon basijugum*, *M. claviflorum*, *M. collinum*, *M. dinizii*, and *M. racemosum*), all species of *Marmaroxylon* included in this study having previously been treated in *Zygia* (Table 1). Furthermore, *Z. inundata* should be transferred to *Inga*, the genus it was first assigned to, and treated as *Inga inundata* Ducke.

## ■ AUTHOR CONTRIBUTIONS

BS and JF conceived and planned the outline of the project with advise from PK and GPL. JF collected the material from herbaria and the field, and conducted the laboratory work and phylogenetic analyses under the supervision of PK and BS with input from GPL. JF, PK and BS wrote the manuscript with support from GPL. All authors provided critical feedback and helped shape the research and manuscript. — JF, <https://orcid.org/0000-0002-8762-3942>; PK, <https://orcid.org/0000-0002-6677-5234>; GP, <https://orcid.org/0000-0003-2599-4577>

## ■ ACKNOWLEDGEMENTS

This study was supported by funds from Stiftelsen Lars Hiertas minne, Knigges resestipendium, R. Sernanders stipendiestiftelse,

T. Tullbergs stipendiestiftelse, Ossian Dahlgrens botaniska stipendiestiftelse, Helge Ax:son Johnsons stiftelse and G. Thelins stipendiestiftelse. We thank the curators and the staff of AAU, K, NY and PS for making material in their care accessible and for providing samples for DNA extraction.

## ■ LITERATURE CITED

- Ariati, S.R., Murphy, D.J., Udovicic, F. & Ladiges, P.Y. 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(4): 417–426. <https://doi.org/10.1017/S147720006001952>
- Baldwin, B.G. & 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(3): 449–463. <https://doi.org/10.1006/mpev.1998.0545>
- Barneby, R.C. & Grimes, J.W. 1996. Silk tree, guanacaste, monkey's earring: A generic system for the synandrous Mimosaceae of the Americas; *Abarema*, *Albizia*, and allies. *Mem. New York Bot. Gard.* 74(1): 1–292.
- Barneby, R.C. & Grimes, J.W. 1997. Silk tree, guanacaste, monkey's earring: A generic system for the synandrous Mimosaceae of the Americas; *Pithecolobium*, *Cojoba*, and *Zygia*. *Mem. New York Bot. Gard.* 74(2): 1–149.
- Benthams, G. 1865. Ordo LVII. Leguminosae. Pp. 434–600 in: Benthams, G. & Hooker, J.D., *Genera plantarum*, vol. 1(2). Londini [London]: Lovell Reeve & Co. <https://doi.org/10.5962/bhl.title.747>
- Benthams, G. 1875. Revision of the suborder Mimoseae. *Trans. Linn. Soc. London* 30: 335–664. <https://doi.org/10.1111/j.1096-3642.1875.tb00005.x>
- Britton, N.L. & Rose, J.N. 1928. *North American flora*, vol. 23(1), *Mimosaceae*. New York: New York Botanical Garden. <https://www.biodiversitylibrary.org/bibliography/889>
- Brown, G.K. 2008. Systematics of the tribe Ingeae (Leguminosae-Mimosoideae) over the past 25 years. *Muelleria* 26(1): 27–42.
- Brown, G.K., Murphy, D.J., Miller, J.T. & Ladiges, P.Y. 2008. *Acacia* s.str. and its relationships among tropical legume tribe Ingeae (Leguminosae: Mimosoideae). *Syst. Bot.* 33: 739–751. <https://doi.org/10.1600/036364408786500136>
- Browne, P. 1756. *The civil and natural history of Jamaica*. London: printed for the author. <https://doi.org/10.5962/bhl.title.10826>
- Ducke, A. 1922. Plantes nouvelles ou peu connues de la region amazonienne. *Arch. Jard. Bot. Rio de Janeiro* 3: 2–269.
- Ducke, A. 1925. Plantes nouvelles ou peu connues de la région amazonienne. *Arch. Jard. Bot. Rio de Janeiro* 4: 1–208.
- Ducke, A. 1949. Árvores amazônicas e sua propagação. *Bol. Mus. Paraense Emilio Goeldi* 10: 81–92.
- Edgar, R.C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucl. Acids Res.* 32(5): 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Fawcett, W. & Rendle, A.B. 1920. *Flora of Jamaica: Containing descriptions of the flowering plants known from the island*, vol. 4, *Dicotyledons: Families Leguminosae to Callitrichaceae*. London: printed by order of the Trustees of the British Museum. <https://doi.org/10.5962/bhl.title.2760>
- Iganci, J.R.V., Soares, M.V., Guerra, E. & Morim M.P. 2016. A preliminary molecular phylogeny of the *Abarema* alliance (Leguminosae) and implications for taxonomic rearrangement. *Int. J. Pl. Sci.* 177(1): 34–43. <https://doi.org/10.1086/684078>
- 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(12): 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kyalangalilwa, B., Boatwright, J.S., Daru, B.H., 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(4): 500–523. <https://doi.org/10.1111/boj.12047>
- Larsson, A.** 2014. AliView: A fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics* 30(22): 3276–3278. <https://doi.org/10.1093/bioinformatics/btu531>
- Larsson, A.** 2010. Abioscripts. <http://ormbunkar.se/phylogeny/abioscripts/>
- Lavin, M., Herendeen, P.S. & Wojciechowski, M.F.** 2005. Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the tertiary. *Syst. Biol.* 54(4): 575–594. <https://doi.org/10.1080/10635150590947131>
- Lewis, G.P. & Rico Arce, M.D.L.** 2005. Tribe Ingeae. Pp. 193–213 in: Lewis, G.P., Lock, M., Mackinder, B. & Schrire, B. (eds.), *Legumes of the World*. Richmond: Royal Botanic Gardens, Kew.
- LPWG** 2017. A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. *Taxon* 66(1): 44–77. <https://doi.org/10.12705/661.3>
- Luckow, M., Miller, J.T., Murphy, D.J. & Livshultz, T.** 2003. A phylogenetic analysis of the Mimosoideae (Leguminosae) based on chloroplast DNA sequence data. Pp. 197–220 in: Klitgaard, B.B. & Bruneau, A. (eds.), *Advances in legume systematics*, part 10, *Higher level systematics*. Richmond: Royal Botanic Gardens, Kew.
- Macbride, J.F.** 1943. Flora of Peru. Family Leguminosae. *Publ. Field Mus. Nat. Hist., Bot. Ser.* 13(3): 1–506.
- Miller, J.T., Grimes, J.W., Murphy, D.J., Bayer, R.J. & Ladiges, P.Y.** 2003. A phylogenetic analysis of the Acaciae and Ingeae (Mimosoideae: Fabaceae) based on *trnK*, *matK*, *psbA-trnH*, and *trnL/trnF* sequence data. *Syst. Bot.* 28(3): 558–566.
- Mohlenbrock, R.H.** 1963. Reorganization of genera within tribe Ingeae of the Mimosoid Leguminosae. *Reinwardtia* 6(4): 429–442.
- Neill, D.A., Klitgaard, B.B. & Lewis, G.P.** 1999. *Zygia dinizii* (Ducke) D.A. Neill. P. 956 in: Jørgensen, P.M. & León-Yáñez, S. (eds.), Catalogue of the vascular plants of Ecuador. *Monogr. Syst. Bot. Missouri Bot. Gard.* 75: 1–1181.
- Nielsen, I.** 1981. Tribe 5. Ingeae. Pp. 173–190 in: Polhill, R.M. & Raven, P.H. (eds.), *Advances in legume systematics*, part 1. Richmond: Royal Botanic Gardens, Kew.
- Nylander, J.A.A.** 2004. MrAIC.pl, version 1.4.6. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. <https://github.com/nylander/MrAIC>
- Pennington, T.D.** 1997. *The genus Inga: Botany*. Richmond: Royal Botanic Gardens, Kew.
- Pennington, R.T. & Lavin, M.** 2016. The contrasting nature of woody plant species in different Neotropical forest biomes reflects differences in ecological stability. *New Phytol.* 210(1): 25–37. <https://doi.org/10.1111/nph.13724>
- Pennington, T.D., Reynel, C. & Daza, A.** 2004. *Illustrated guide to the trees of Peru*. Sherborne: David Hunt.
- Popp, M. & Oxelman, M.** 2001. Inferring the history of the polyploid *Silene aegae* (Caryophyllaceae) using plastid and homoeologous nuclear DNA sequences. *Molec. Phylog. Evol.* 20(3): 474–481. <https://doi.org/10.1006/mpev.2001.0977>
- Rambaut, A.** 2006–2016. FigTree, version 1.4.3. Institute of Evolutionary Biology, University of Edinburgh. <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. <http://tree.bio.ed.ac.uk/software/tracer/>
- Record, S.J.** 1940. Some new names for tropical American trees of the family Leguminosae. *Trop. Woods* 63: 1–6.
- Rico Arce, M.L.** 1991. New species, combinations and synonyms for *Zygia*, *Cojoba*, *Marmaroxylon* and *Pithecellobium* (Leguminosae-Mimosoideae, Ingeae). *Kew Bull.* 46: 493–521. <https://doi.org/10.2307/4110539>
- Ronquist, F., Huelsenbeck, J.P. & Teslenko, M.** 2011. Draft MrBayes version 3.2 Manual. Tutorials and Model Summaries. <http://mrbayes.sourceforge.net/download.php>
- Sang, T., Crawford, D.J. & Stuessy, T.F.** 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *Amer. J. Bot.* 84(9): 1120–1136. <https://doi.org/10.2307/2446155>
- Souza, E.R., Lewis, G.P., Forest, F., Schnadelbach, A.S., Van den Berg, C. & Paganucci de Queiroz, L.** 2013. Phylogeny of *Calliandra* (Leguminosae: Mimosoideae) based on nuclear and plastid molecular markers. *Taxon* 62: 1200–1219. <https://doi.org/10.12705/626.2>
- Souza, E.R., Krishnaraj, M.V. & de Queiroz, L.P.** 2016. *Sanjappa*, a new genus in the tribe Ingeae (Leguminosae: Mimosoideae) from India. *Rheedeia* 26(1): 1–12.
- Ståhl, B., Rico Arce, M. & Lewis, G.P.** 2010. *Zygia nubigena* sp. nov. (Leguminosae-Mimosoideae) from a submontane cloud forest in western Ecuador. *Nordic J. Bot.* 28(4): 453–456. <https://doi.org/10.1111/j.1756-1051.2010.00829.x>
- Ståhl, B., Lewis, G.P. & Klitgaard, B.B.** 2015. *Flora of Ecuador*, vol. 92, *Leguminosae – Key to genera*. Gothenburg: Botanical Institute, Göteborg University.
- Standley, P.C. & Steyermark, J.A.** 1946. Flora of Guatemala IV. *Fieldiana, Bot.* 24(4): I–VI, 1–493. <https://doi.org/10.5962/bhl.title.2233>
- 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>
- Woodson, R.E. & Schery, R.W.** 1950. Flora of Panama: Mimosoideae. *Ann. Missouri Bot. Gard.* 37: 266–280.
- Yoon, C.S., Glawe, A. & Shaw, P.D.** 1991. A method for rapid small-scale preparation of fungal DNA. *Mycologia* 83(6): 835–838. <https://doi.org/10.1080/00275514.1991.12026093>

#### Appendix 1. Species names and GenBank accession numbers included in this study.

Voucher data is given for accessions for which DNA sequences were newly obtained, using the following format: Taxon name, country, collector and collector number, herbarium code, GenBank accession numbers (ETS, ITS, *psbA-trnH*, *trnL-trnF*). – missing data; \* newly generated sequence.

*Abarema jupunba* (Willd.) Britton & Killip, EF638110.1, EF638166.1, GQ428675.1, HQ634601.1; *Abarema piresii* Barneby & J.W.Grimes, KF921624.1, JX870655.1, KF921820.1, JX870787.1; *Acacia adoxa* Pedley, EF638087.1, AF360715.1, JF420154.1, JF420480.1; *Acacia lycopodiifolia* A.Cunn. ex Hook., EF638091.1, AF360716.1, AF195715.1, –; *Archidendropsis basaltica* (F.Muell.) I.C.Nielsen, EF638141.1, EF638178.1, –; *Archidendropsis thozetiana* (F.Muell.) I.C.Nielsen, EF638140.1, EF638179.1, KM895048.1, –; *Calliandra dysantha* Benth., EF638121.1, JX870684.1, –; *Calliandra surinamensis* Benth., –; *JX870747.1*, AF532165.1, JX870865.1; *Chloroleucon dumosum* (Benth.) G.P.Lewis, KF921632.1, KF921680.1, KF921831.1, KF921757.1; *Chloroleucon tenuiflorum* (Benth.) Barneby & J.W.Grimes, KF921646.1, KF921691.1, KF921843.1, KF921769.1; *Cojoba arborea* (L.) Britton & Rose, EF638095.1, EF638186.1, KJ426672.1, JX870874.1; *Cojoba rufescens* (Benth.) Britton & Rose, –, –, GQ982193.1, –; *Faidherbia albida* (Delile)

## Appendix 1. Continued.

A.Chev., EF638163.1, –, AF522954.1; *Havardia mexicana* (Rose) Britton & Rose, KF921655.1, KF933276.1, KF921851.1, JX870878.1; *Havardia pallens* (Benth.) Britton & Rose, KF921656.1, KF921698.1, AF524974.1, AF522955.1; *Hydrochorea corymbosa* (Rich.) Barneby & J.W.Grimes, KF921657.1, JX870763.1, KF921852.1, JX870879.1; *Inga edulis* Mart., KF921658.1, JX870764.1, GQ118870.1, JX870880.1; *Inga brachyrhachis* Harms, Brazil, *Krukoff 7040* (S), MK681164\*, –, MK876342\*, MK903286\*; *Inga thibaudiana* DC., KF921659.1, GU013360, GQ118886.1, GQ118743.1; *Leucochloron incurviale* (Vell.) Barneby & J.W.Grimes, KF921662.1, KF921701.1, –, KF921778.1; *Leucochloron limae* Barneby & J.W.Grimes, KF921663.1, JX870766.1, –, JX870882.1; *Macrosamanea pubiramea* (Steud.) Barneby & J.W.Grimes, KF921665.1, JX870767.1, KF921860.1, JX870883.1; *Marmaroxylon basijugum* (Ducke) L.Rico (A), Ecuador, *Ferm 8* (UPS), MK681153\*, –, MK876351\*, MK903293\*, (B) Ecuador, *Ferm 21* (UPS), MK681154\*, MK641684\*, MK876330\*, MK903322\*, (C) *Ferm 22* (UPS), MK681155\*, MK641683\*, MK876352\*, MK903325\*, (D) Brazil, *Krukoff 8639* (S), MK681168\*, MK641679\*, –, MK903326\*; *Marmaroxylon claviflorum* (Spruce ex Benth.) L.Rico, (A) Brazil, *Ducke 20169* (S), MK681148\*, –, MK876357\*, –, (B) Brazil, *Ducke 35530* (S), MK681147\*, MK641685\*, MK876358\*, MK903289\*; *Marmaroxylon collinum* (Sandwith) L.Rico, Venezuela, *Carlos 1143* (NY), MK681167\*, MK641706\*, MK876333\*, –, *Marmaroxylon dinizii* (Benth.) L.Rico, (A) Brazil, *Krukoff 6872* (S), MK681178\*, –, MK903285\*, (B) Brazil, *Ferreira & al. 7430* (NY), MK681150\*, MK641717\*, –, –, *Marmaroxylon magdalenae* Killip ex L.Rico, Colombia, *Haught 2097* (S), MK681168\*, MK641671\*, MK876337\*, MK903306\*; *Marmaroxylon ocamarensis* (Pittier) L.Rico, (A) Venezuela, *Pittier 14099* (K), MK681180\*, MK641673\*, –, MK903297\*, (B) Venezuela, *Stergios 14780* (NY), MK681197\*, MK641674\*, MK876353\*, MK903317\*; *Marmaroxylon racemosum* (Ducke) Killip ex Record, (A) Brazil, *Souza 2458* (S), MK681163\*, MK641678\*, MK876344\*, MK903295\*, (B) Brazil, *Souza 2510* (S), –, MK641677\*, MK876356\*, MK903327\*, (C) Brazil, *Souza 2359* (S), –, MK641692\*, MK876346\*, MK903287\*; *Pithecellobium diversifolium* Benth., KF921666.1, JX870768.1, –, JX870884.1; *Pithecellobium dulce* (Roxb.) Benth., EF638143.1, JX856483.1, KJ426883.1, KC479268.1; *Pseudosamanea guachapele* (Kunth) Harms, KF921667.1, JX870769.1, AF524983.1, AF522964.1; *Samanea saman* (Jacq.) Merr., KF921668.1, JX870770.1, AF524984.1, AF522965.1; *Samanea tubulosa* (Benth.) Barneby & J.W.Grimes, EF638135.1, EF638212.1, –, –, *Senegalia berlandieri* (Benth.) Britton & Rose, EF638162.1, –, –, HM020797.1; *Senegalia caffra* (Thunb.) P.J.H.Hurter & Mabb., –, JQ265905.1, GQ872306.1, –, *Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb., –, KF532064.1, KF532003.1, –, *Senegalia senegal* (L.) Britton, EF638152.1, HQ605075.1, AF524996.1, AF522976.1; *Vachellia farnesiana* (L.) Wight & Arn., EF638128.1, AF360728.1, FJ808552.1, AY574119.1; *Zapoteca formosa* (Kunth) H.M.Hern., EF638134.1, JX870781.1, –, JX870897.1; *Zapoteca tetragona* (Willd.) H.M.Hern., EF638133.1, JX870784.1, AF524986.1, AF278515.1; *Zygia biflora* L.Rico, (A) Costa Rica, *Hammel 19615* (K), MK681173\*, MK641695\*, –, MK903330\*, (B) Costa Rica, *Aguiar 3638* (K) MK681174\*, MK641721\*, –, –, *Zygia bisingula* L.Rico, (A) Venezuela, *Berti 983-034* (NY), MK681182\*, MK641688\*, –, MK903284\*, (B) Venezuela, *Ortega 2561* (NY), MK681183\*, MK641687\*, MK876347\*, MK903321\*; *Zygia brenesii* (Standl.) L.Rico, (A) Costa Rica, *Hammel & Morales 2055* (K), –, MK641730\*, –, MK903298\*, (B) Costa Rica, *Rivera 1189* (K), –, MK641716\*, –, –, *Zygia cataractae* (Kunth) L.Rico, Venezuela, *Williams 11990* (S), MK681146\*, MK641724\*, –, –, *Zygia coccinea* (G.Don) L.Rico, Ecuador, *Asplund 20021* (S), MK681165\*, –, MK876332\*, MK903308\*; *Zygia coccinea* var. *coccinea*, Peru, *Woytkowski 34375* (S), –, MK641698\*, –, –, *Zygia coccinea* var. *macrophylla* (Spruce ex Benth.) Barneby & J.W.Grimes, Bolivia, *Krukoff 10216* (S), –, MK641723\*, –, –, *Zygia coccinea* var. *oriunda* (J.F.Macbr.) Barneby & J.W.Grimes, (A) Peru, *Tessman 4157* (S), MK681149\*, MK641700\*, –, –, (B) Ecuador, *Ståhl 3953* (AAU), MK681170\*, MK641701\*, –, MK903290\*; *Zygia confusa* L.Rico, (A) Guatemala, *Lundell & Contreras 20612* (K), MK681172\*, MK641693\*, –, MK903310\*, (B) Honduras, *Gentle 5252* (NY), MK681184\*, MK641696\*, –, –, *Zygia conzattii* (Standl.) Britton & Rose, (A) Belize, *Gentle 920* (S), –, MK641704\*, –, MK903332\*, (B) Guatemala, *Contreras 8338* (S), MK681171\*, MK641707\*, MK876364\*, MK903331\*, (C) Mexico, *Demetrio Alvarez & al. 10510* (Kew DNA and tissue bank), MK681203\*, MK641705\*, –, –, (D) Mexico, *Sousa, M. & al. 13032* (Kew DNA and tissue bank), MK681202\*, MK641703\*, –, –, *Zygia cupirensis* (C.Barbosa) L.Rico, (A) Venezuela, *Steyermark 91215* (NY), MK681185\*, MK641715\*, –, MK903313\*, (B) Venezuela, *Broadway 624* (NY), MK681186\*, MK641720\*, –, –, *Zygia dissitiflora* Barneby & J.W.Grimes, Colombia, *C. Feddema 1967* (NY), –, MK641689\*, MK876340\*, MK903301\*; *Zygia heteroneura* Barneby & J.W.Grimes, Ecuador, *Ferm 19* (UPS), MK681156\*, MK641699\*, MK876339\*, MK903311\*; *Zygia inaequalis* (Humb. & Bonpl. ex Willd.) Pittier, (A) Brazil, *Steward 379* (NY), MK681187\*, MK641690\*, MK876361\*, MK903302\*, (B) Brazil, *Prance 14994* (NY), MK681151\*, MK641676\*, –, MK903319\*; *Zygia inundata* (Ducke) H.C.Lima ex Barneby & J.W.Grimes, (A) French Guiana, *Oldeman B994* (NY), MK681188\*, –, –, MK903318\*, (B) French Guiana, *Poncy 361* (NY), MK681189\*, MK920273\*, MK876348\*, MK903307\*; *Zygia juruana* (Harms) L.Rico, (A) Brazil, *Ducke 16777* (S), –, MK641691\*, MK876349\*, –, (B) Peru, *Ruiz 9613* (AAU), –, MK641697\*, –, MK903288\*; *Zygia lathetica* Barneby & J.W.Grimes, (A) Ecuador, *Valencia 68430* (AAU), MK681161\*, MK641675\*, MK876334\*, MK903294\*, (B) Ecuador, *Freire 5093* (AAU), MK681162\*, MK641682\*, MK876335\*, MK903303\*; *Zygia latifolia* (L.) Fawc. & Rendle, Ecuador, *Ståhl 7109* (S), MK681160\*, –, MK876343\*, MK903291\*; *Zygia latifolia* var. *communis* Barneby & J.W.Grimes, (A) Brazil, *Ducke 280* (S), MK681166\*, MK641711\*, MK876338\*, MK903292\*, (B) Bolivia, *Prance 6100* (S), MK681169\*, MK641708\*, –, MK903305\*; *Zygia latifolia* var. *contro-versa* Barneby & J.W.Grimes, Brazil, *Krukoff 5798* (S), –, MK641712\*, –, MK903323\*; *Zygia latifolia* var. *latifolia*, Brazil, *Egler & Pires 47190* (S), –, MK641710\*, MK876355\*, –, *Zygia longifolia* (Humb. & Bonpl. ex Willd.) Britton & Rose, (A) Ecuador, *Ferm 24* (UPS), MK681157\*, MK641714\*, MK876341\*, –, (B) Ecuador, *Gentry 9929* (S), –, MK641713\*, –, –, *Zygia macbridei* (C.Barbosa) L.Rico, (A) Peru, *Diaz 172* (NY), MK681190\*, MK641729\*, –, –, (B) Peru, *Revilla 2133* (NY), –, MK641728\*, MK876354\*, MK903312\*; *Zygia morongii* Barneby & J.W.Grimes, (A) Paraguay, *Hassler 12635* (NY), MK681195\*, MK641694\*, MK876359\*, MK903315\*, (B) Argentina, *Pedersen 3881* (NY), MK681196\*, MK641702\*, –, MK903309\*; *Zygia multipunctata* Barneby & J.W.Grimes, Colombia, *Roldan 1375* (NY), MK681191\*, MK920271\*, MK876360\*, MK903329\*; *Zygia nubigena* B.Ståhl, L.Rico & G.P.Lewis, Ecuador, *Ståhl 7155* (S), MK681159\*, MK641672\*, MK876350\*, MK903316\*; *Zygia palustris* Barneby & J.W.Grimes, Venezuela, *Thomas 3394* (NY), MK681192\*, MK641686\*, –, –, *Zygia paucijugata* (Lundell) L.Rico, Mexico, *Rico 773* (P), MK681198\*, MK920272\*, –, MK903328\*; *Zygia peckii* (B.L.Rob.) Britton & Rose, (A) Mexico, *Aguiar 5622* (K), –, MK641709\*, MK876363\*, MK903296\*, (B) Mexico, *Demetrio Alvarez 6247* (K), MK681181\*, MK641725\*, MK876345\*, –, *Zygia pithecoloboides* (Harms) Barneby & J.W.Grimes, Paraguay, *Hassler 7180* (NY), MK681193\*, –, –, MK903314\*; *Zygia sabatieri* Barneby & J.W.Grimes, (A) French Guiana, *Sabatier & Molino 4838* (P), MK681199\*, –, MK876331\*, MK903304\*, (B) French Guiana, *Richard & Mambe 571* (P), MK681200\*, –, MK876336\*, MK903320\*; *Zygia selloi* (Benth.) L.Rico, Brazil, *Gehrt 28064* (NY), MK681194\*, MK641719\*, –, MK903322\*; *Zygia tetragona* Barneby & J.W.Grimes, (A) French Guiana, *Poncy 896* (P), MK681152\*, MK641680\*, –, –, (B) French Guiana, *Cremers G. & Crozier F. 15184* (Kew DNA and tissue bank), MK681201\*, MK641681\*, –, –, *Zygia trunciflora* (Ducke) L.Rico, (A) Brazil, *Oliveira, Assunção & Cardoso 171* (K), MK681175\*, MK641722\*, –, MK903300\*, (B) Brazil, *Freitas 668* (K), MK681179\*, MK641718\*, –, MK903299\*; *Zygia unifoliolata* (Benth.) Pittier, (A) Brazil, *A. da Silva & al. 4206* (K), MK681176\*, MK641726\*, –, MK903283\*, (B) Brazil, *Pereira-Silva 15012* (K), MK681177\*, MK641727\*, MK876362\*, –.