

Phylogenetic relationships of enigmatic *Sphingiphila* (Bignoniaceae) based on molecular and wood anatomical data

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Abstract *Sphingiphila* is a monospecific genus, endemic to the Bolivian and Paraguayan Chaco, a semi-arid lowland region. The circumscription of *Sphingiphila* has been controversial since the genus was first described. *Sphingiphila tetramera* is perhaps the most enigmatic taxon of Bignoniaceae due to the presence of very unusual morphological features, such as simple leaves, thorn-tipped branches, and tetramerous, actinomorphic flowers, making its tribal placement within the family uncertain. Here we combined molecular and wood anatomical data to determine the placement of *Sphingiphila* within the family. The analyses of a large *ndhF* dataset, which included members of all Bignoniaceae tribes, placed *Sphingiphila* within Bignoniaceae. A second, smaller *ndhF* and *pepC* dataset, which included only members of tribe Bignoniaceae, placed the genus within *Tanaecium*. Unlike most macro-morphological traits, *Sphingiphila* is not unusual within Bignoniaceae from a wood anatomical point of view. *Sphingiphila* shares the presence of narrow vessels and vasicentric to aliform confluent parenchyma with the rest of the family. In addition, *Sphingiphila* has several specific wood anatomical traits, such as vessels in a diagonal to tangential arrangement, small intervessel pits, and non-storied heterocellular rays with occasional perforated ray cells. These features suggest that the genus is best placed either in Tecomeae s.str. or Bignoniaceae, with a better placement in Bignoniaceae due to its abundant parenchyma, despite lacking the cambial variant synapomorphic for this group. *Sphingiphila* and *Tanaecium* form a clade that is strongly supported by molecular characters, including two indels that are molecular synapomorphies of this clade. In addition, careful morphological inspections show that *S. tetramera* shares with *Tanaecium* the subulate, bromeliad-like prophylls, the most evident synapomorphy of this genus apart from long, tubular, villose corollas, and a lepidote ovary. Given the molecular phylogenetic placement of *S. tetramera* and its macro-morphological and wood anatomical similarities with *Tanaecium*, we propose the new combination *Tanaecium tetramerum*.

Keywords Bignoniaceae; cambial variants; chaco; liana; perforated ray cells; *Tabebuia* alliance; Tecomeae

Supplementary Material Electronic Supplement (Figs. S1–S3) and DNA sequence alignments are available in the Supplementary Data section of the online version of this article at <http://www.ingentaconnect.com/content/iapt/tax>

■ INTRODUCTION

Sphingiphila A.H.Gentry is a monotypic genus, endemic to the Bolivian and Paraguayan Chaco, a hot, semi-arid lowland region (Gentry, 1990; Fuentes, 1998; Navarro & al., 2011). *Sphingiphila tetramera* A.H.Gentry, the sole species ever described in the genus, is perhaps the most unusual, enigmatic species within the Bignoniaceae (Gentry, 1990). In particular, the thorn-tipped branches, spiny stems, and tetramerous, actinomorphic flowers with sessile stamens are all features exclusive to this genus, making its tribal placement difficult (Gentry, 1990).

The septicidal fruit dehiscence and long white corolla tubes of *S. tetramera* greatly resemble those of *Millingtonia hortensis* L.f. (tribe Oroxyleae), a genus restricted to Asia (Zhang & Santisuk, 1998). However, *M. hortensis* has pinnately compound leaves, while *S. tetramera* has simple, occasionally 3-foliolate, leaves. In Bignoniaceae, spiny stems are only found in *Parmentiera* DC. (tribe Crescentieae), a Neotropical genus with indehiscent fleshy fruits (Gentry, 1980), and in *Phylloctenium* Baill. and *Catophractes* D.Don., both from Africa and members of the Paleotropical clade (Fischer & al., 2004; Olmstead & al., 2009). *Tabebuia nodosa* (Griseb.) Griseb. (*Tabebuia* alliance clade), another species from the Chaco

region (Gentry, 1992; Navarro & al., 2011), also resembles *Sphingiphila*, sharing simple leaves clustered on short-shoots and a treelet habit. However, *T. nodosa* has yellow tubular-campanulate flowers and loculicidal capsules (Gentry, 1992), while *Sphingiphila* has septicidal capsules and white actinomorphic flowers.

Given the various morphological similarities between *Sphingiphila tetramera* and several distantly related taxa, the phylogenetic placement and even tribal position of this genus has remained uncertain. The septicidal capsule dehiscence and Neotropical distribution of *Sphingiphila* led Gentry (1990) to hypothesize that the genus would be best placed within tribe Bignoniaceae. However, its treelet habit within a predominantly lianoid clade would be rather unusual, leading Gentry (1990) to suggest that *Sphingiphila* likely represented a derived lineage of shrubs within *Arrabidaea* DC. (a polyphyletic genus with species now recognized under *Cuspidaria* DC., *Fridericia* Mart., *Tanaecium* Sw. and *Xylophragma* Sprague; Lohmann & Taylor, 2014), where some species have a treelet habit (e.g., *Fridericia costaricensis* (Kraenzl.) L.G.Lohmann; Burger & Gentry, 2000). Given all the contradictory morphological information available, the placement of *S. tetramera* remains uncertain and unverified.

In the last decades, several researchers have reconstructed phylogenetic relationships among genera and tribes of the Bignoniaceae, mostly using molecular data (Spangler & Olmstead, 1999; Zjhra & al., 2004; Lohmann, 2006; Grose & Olmstead, 2007a; Olmstead & al., 2009). Such studies reconstructed well-supported clades that served as basis for new delimitations of genera and major clades within the family (Grose & Olmstead, 2007b; Olmstead & al., 2009; Lohmann & Taylor, 2014). The new phylogenies and classification systems available to date clearly illustrate that several morphological features traditionally used to delimit genera and tribes in the family were highly homoplasious (e.g., flower types; Lohmann, 2006; Alcántara & Lohmann, 2010) and not reliable indicators of relationships. Instead, other overlooked characters emerged as morphological synapomorphies of major clades in Bignoniaceae, such as stem cambial variants (Lohmann, 2006; Pace & al., 2015), trichome types (Lohmann, 2006; Nogueira & al., 2013), shape of the prophylls of the axillary buds (Lohmann, 2006; Lohmann & Taylor, 2014), corolla trichome structure (Grose & Olmstead, 2007a, b), and wood density (Grose & Olmstead, 2007a, b).

Wood anatomy provides extremely important taxonomic characters at higher hierarchical levels within Bignoniaceae. For instance, the storied structure is typical of the *Tabebuia* alliance (Pace & Angyalossy, 2013; Pace & al., 2015), diffuse axial parenchyma is exclusive of the Malagasy Coleeae (Pace & Angyalossy, 2013; Pace & al., 2015), and vessel-ray pits simple to semi-bordered is exclusive of Catalpeae (Pace & al., 2015). Furthermore, the presence of xylem furrowed by phloem wedges is a synapomorphy of Bignoniaceae, while its diversity provides important characters to delimit generic-level clades within the tribe (Dos Santos, 1995; Lohmann, 2006; Pace & al., 2009, 2015).

In this study we used molecular phylogenetic data and macro-morphological and wood anatomical characters to

determine the placement of *Sphingiphila tetramera* within Bignoniaceae. First, we used sequences of the *ndhF* and *pepC* genes as a basis to determine the phylogenetic placement of *Sphingiphila*. Second, we searched for morpho-anatomical synapomorphies that could provide further support for the phylogenetic placement of *S. tetramera* within the family. To that end, we conducted a thorough analysis of the secondary xylem of *Sphingiphila* and compared these data to those available for members from all major clades of Bignoniaceae (Olmstead & al., 2009; Pace & al., 2015). We also conducted a detailed macro-morphological study and compared these data to those available for other genera (e.g., Gentry, 1992; Lohmann & Taylor, 2014).

■ MATERIALS AND METHODS

Leaf material, DNA extraction, PCR amplification and sequencing. — Herbarium specimens and silica-dried leaves of two different individuals of *Sphingiphila tetramera* were collected in the Bolivian Chaco in the border of the Santa Cruz and Chuquisaca. Vouchers of these collections were deposited in SPF (Pace 31 and 32). Total DNA was extracted from silica-dried leaflets from one of those vouchers (Pace 32) and from a herbarium specimen of *S. tetramera* (*Antezana Valera 1327*, MO) using an Invisorb Plant Mini Kit (Invitex, Berlin, Germany). Both *ndhF* and *pepC* genes were amplified and purified following Zuntini & al. (2013). Automated sequencing was performed by Macrogen (Seoul, South Korea), and final sequences were deposited in GenBank under the accessions KU757039 to KU757045 (Appendix 1).

Molecular datasets. — The two newly generated sequences of *S. tetramera* were included in two separate datasets that were used to test the phylogenetic placement of *Sphingiphila*, as follows: (1) a broad *ndhF* dataset including members of all Bignoniaceae tribes and main clades and (2) a combined *ndhF* and *pepC* dataset including only members of tribe Bignoniaceae. The broad Bignoniaceae dataset was assembled using *ndhF* sequences from Olmstead & al. (2009), and at least two species from all major clades identified in that study were sampled. The Bignoniaceae dataset was assembled using all *ndhF* and *pepC* sequences from Lohmann (2006). For each dataset, sequences were downloaded from GenBank (Appendix 1) and aligned with MUSCLE v.3.5 (Edgar, 2004), using standard parameters, along with the newly generated sequences of *S. tetramera*. The alignment was subsequently checked by visual inspection and manually adjusted. Indels were not coded separately.

Phylogenetic analyses. — Both datasets were analyzed using parsimony (PA), maximum likelihood (ML) and Bayesian inference (BI). Each marker of the combined dataset was also analyzed individually. For PA, an initial run with 100 replicates was carried out in PAUP* v.4.0b10 (Swofford, 2002), with the shorter trees saved. Each of these was then used as a starting tree in a second round of swapping. Most parsimonious trees were summarized under a strict consensus rule; bootstrap support was analyzed with 10,000 replicates. For ML, a rapid hill-climbing search was conducted in RAxML

v.7.2.8 (Stamatakis, 2006) followed by 1000 replicates of rapid bootstrapping. For ML searches, the nucleotide substitution model used was GTR CAT, and data were partitioned for the Bignoniaceae dataset. For BI, nucleotide substitution models were evaluated for each dataset with jModelTest v.2.0 (Guindon & Gascuel, 2003; Darriba & al., 2012) using seven substitution schemes. The best-fit models were selected following Bayesian information criteria. TVM+G was identified as the best model of nucleotide substitution for both *ndhF* matrices and HKY+G for *pepC*. Phylogenetic reconstructions were run in MrBayes v.3.1 (Ronquist & Huelsenbeck, 2003) with the following setting: two independent runs, each with four chains, running for 10 million generations, sampling every 100th generation. To assess if the analyses had achieved stationarity, analysis logs were analyzed in Tracer v.1.6 (Rambaut & al., 2014). The support for clades was inferred by bootstrap for both PA (BPA) and ML (BML) and posterior probabilities (PP) for BI.

Anatomical studies/sampling. — Portions of the stem of *Sphingiphila tetramera* were collected from the same individuals from which silica-dried material for DNA extractions was collected (SPF; Pace 31 and 32). Samples were collected at the stem base of mature fertile individuals of about 2 m height, where their stems were thickest (about 4 cm diameter). Samples were immediately fixed in FAA 70 (Johansen, 1940) for 36 hours and then transferred to a preservative solution of 70% ethanol. The samples were embedded in polyethylene glycol 1500 and sectioned transversely and longitudinally with the aid of polystyrene resin (Barbosa & al., 2010) in a sliding microtome, double stained in astra blue and safranin (Bukatsch, 1972), dehydrated in ethanol and butyl acetate and mounted in Canada balsam to make permanent slides. Wood descriptions followed the IAWA Committee (1989) for both qualitative and quantitative data, and vessels per group were calculated according to Carlquist (2001). Quantitative data were obtained using ImageJ v.1.45s (Rasband, 2012) with a minimum of 30 repetitions per specimen.

Anatomical data collected for *Sphingiphila tetramera* were compared with wood descriptions for representatives of all other Bignoniaceae available from Pace & al. (2015). Only shrubby and arboreal species of Bignoniaceae were considered in the comparison since lianas tend to share anatomical features that reflect habit convergence rather than phylogenetic proximity, which could be misleading in the analyses (Gasson & Dobbins, 1991; Lens & al., 2008).

Macro-morphological studies. — A macro-morphological study of eight herbarium specimens of *Sphingiphila tetramera* (*Antezana Valera 619*, *Antezana Valera 1327*, *Fuentes 2107*, *Fuentes 2272*, *Fuentes 2564*, *Hahn 1600*, *Pace 31*, *Pace 32*) was conducted focusing on diagnostic features of taxa from tribe Bignoniaceae, where this species was placed in our phylogenetic analyses. Images of selected characters were made with a stereomicroscope with multiple shots with different focuses, which were posteriorly joined into a single, entirely focused image using photo stacking in Adobe Photoshop. Morphological features gathered in our macro-morphological analysis were compared to descriptions of all genera of Bignoniaceae available from Lohmann & Taylor (2014) and with the original

description of *Sphingiphila tetramera* available from Gentry (1990). We paid special attention to characters found in taxa that emerged closely related to *Sphingiphila tetramera* in our phylogenetic analyses.

■ RESULTS

Phylogenetic placement of *Sphingiphila*. — High-quality sequences were obtained for both *ndhF* and *pepC* for the two accessions of *Sphingiphila tetramera* sampled (*Pace 32*, *Antezana Valera 1327*). The *ndhF* sequences are 2096 and 2067 bp long, respectively, and identical in the overlapping region. The *pepC* sequences are 635 and 436 bp long, respectively, with 1% of nucleotide divergence. The broad Bignoniaceae *ndhF* dataset is 2102 bp long and includes 40 terminals, 36 of which are Bignoniaceae and 4 are outgroups. The combined *ndhF* and *pepC* dataset has 123 terminals, 121 of which represent Bignoniaceae members, while 2 are outgroups. This dataset includes 123 *ndhF* sequences and 105 *pepC* sequences. The *ndhF* alignment is 2129 bp long, whereas the *pepC* is 875 bp long.

In the analyses of the broad *ndhF* dataset, *S. tetramera* emerged within Bignoniaceae, a strongly supported clade in PA, ML and BI analyses (Fig. 1). In these analyses, the two accessions form a strongly supported clade (BPA: 97/BML: 100/PP: 100) that is poorly supported as sister to *Tanaecium pyramidalatum* (BPA: 60/BML: 60/PP: 82). The analyses of the second dataset (Bignoniaceae) retrieved similar results when the *ndhF* and *pepC* datasets were analyzed individually. Although no resolution was obtained within *Tanaecium* in any of the analyses, the placement of *S. tetramera* within *Tanaecium* was highly supported by both the *ndhF* dataset (BPA: 68/BML: 78/PP: 100) and the *pepC* dataset (BPA: 78/BML: 90/PP: 100; Electr. Suppl.: Figs. S1, S2). Since no incongruence was observed between *ndhF* and *pepC* analyses (considering bootstraps and posterior probabilities above 80% and 95%, respectively), both markers were combined. The two accessions also emerged together (BPA: 100/BML: 100/PP: 100), in a polytomy with all sampled species of *Tanaecium* (BPA: 88/BML: 97/PP: 100, Fig. 1). A visual inspection of the alignment revealed two indels that are shared by *S. tetramera* and *Tanaecium*. Both indels are located in the intron 4 of *pepC*, the first in the position 209 to 242 and the second from 350 to 358 (Electr. Suppl.: Fig. S3).

Wood anatomy of *Sphingiphila*. — All the qualitative and quantitative wood anatomical data gathered for the present study is presented in Table 1. For comparison, these data are presented in combination with data for the other Bignoniaceae tribes from Pace & al. (2015).

Diagnostic features. — *Sphingiphila* has a distinctive diagonal to radial/tangential vessel arrangement, narrow vessels that are usually in multiples of 3–7, small intervessel pits, and non-storied heterocellular rays with perforated ray cells.

Detailed description. — *Growth rings* distinct (Fig. 2A–D). Earlywood marked by a narrow to broad marginal band of axial parenchyma (Fig. 2A–C) and a ring of closely spaced vessels (Fig. 2A–C). Some growth rings lack the closely spaced vessels (Fig. 2A) and/or the band of axial parenchyma (Fig. 2D).

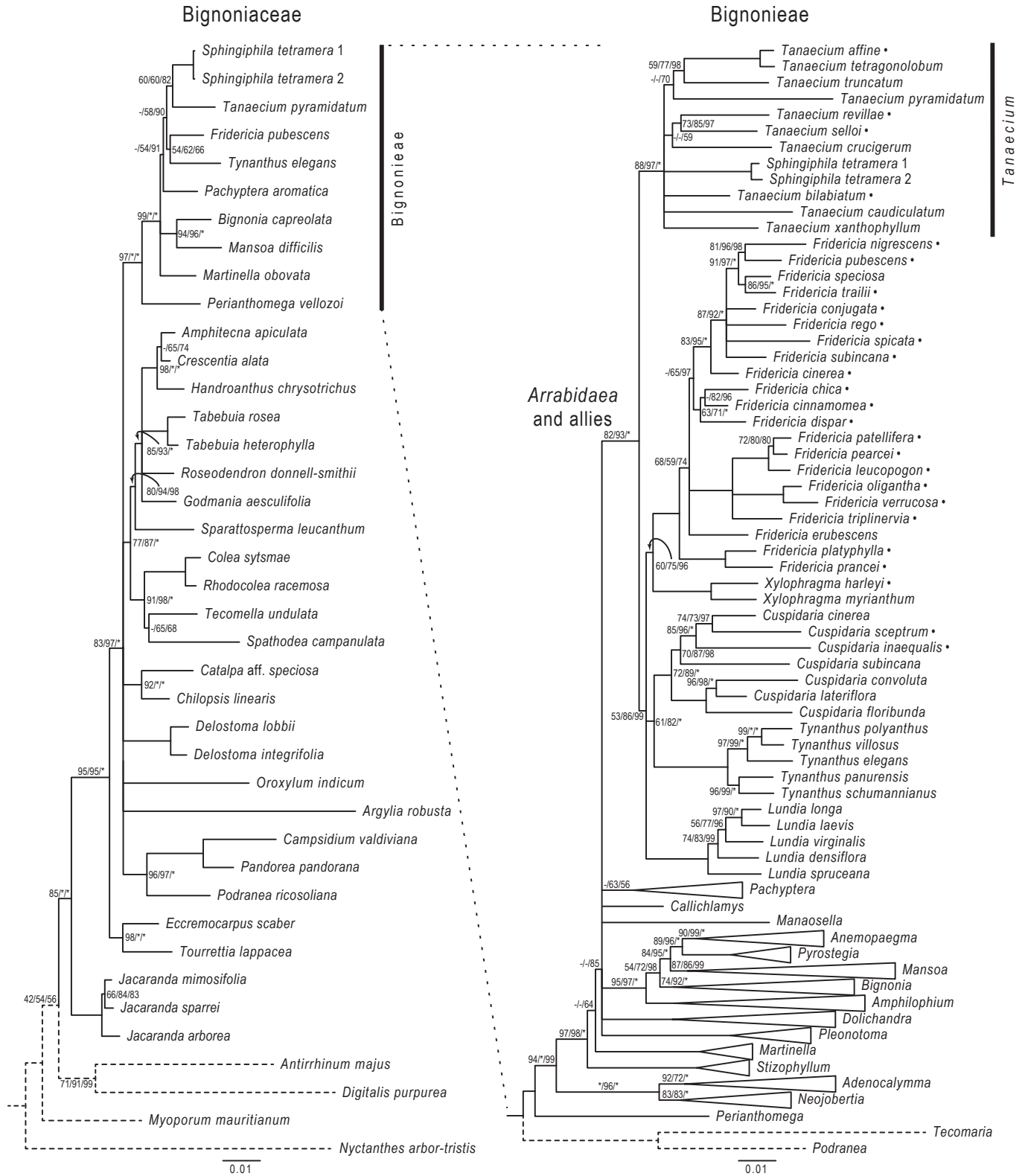


Fig. 1. Phylogenetic placement of *Sphingiphila*. Majority consensus trees from the broad dataset (entire Bignoniaceae, **left**) and from the combined dataset (focused on Bignoniaceae, **right**). Values next to branches indicate their support (PA bootstrap/ML bootstrap/BI posterior probabilities, in percentage), hyphens indicate clades not obtained in the ML analysis, asterisks denote maximum support, and absent values indicate maximum support in PA, ML and BI. Outgroups for each dataset are presented as dashed lines. Genera represented by two or more accessions were collapsed, except for those included in the “*Arrabidaea* and allies” clade. Species previously placed in *Arrabidaea* are marked with a dot.

Table 1. Synopsis of the qualitative and quantitative wood features of *Sphingiphila* and all other lineages (tribes or clades) in Bignoniaceae

Tribe or clade	Habit	Porosity	Growth ring markers		Vessels								
			Marginal parenchyma	Radially flattened fibers	Arrangement	Grouping	Vessel/group	Dimorphism	Frequency (per mm ²)	Diameter (µm)	Tyloses	Perforation plate	Helical thickening
<i>Sphingiphila</i> A.H.Gentry	Treelets to shrubs	Semi-ring porous	+	+	Diagonal to tangential pattern	Multiples of 3–7 and clusters	2.47	–	284 ± 34	35 ± 12	–	Simple	–
Jacarandaeae	Trees, and a few subshrubs in arid zones	Diffuse	+	+	Diffuse	Solitary to multiples of 2–3	1.23–2.11	–	10–21	68–75 (except for <i>J. copaia</i> with 300)	–	Simple	–
Tecomeae	Mostly lianas, with few trees and shrubs	Diffuse to ring-porous	±	+	Diffuse	Solitary to multiples of 2–3	1.93–5.32	+ in lianas	6–320	30–158	–	Simple	+ in species ring-porous
<i>Delostoma</i> D.Don	Trees	Diffuse	+	–	Radial pattern	Solitary to multiples of 2–3 & Radial multiples	2.93	–	46 ± 20	70 ± 12	–	Simple	–
Oroxyleae	Trees, a few lianas	Diffuse	+	+	Diffuse	Solitary to multiples of 2–3	1.24–1.94	–	4–27	80–179	–	Reticulate, foraminate and simple	–
Catalpeae	Trees	Semi-ring porous	+	+	Diffuse	Solitary to multiples of 2–3	1.33–1.56	–	6–34	131–204	+	Simple	+ in species semi-ring porous
Bignonieae	Liana, a few shrubs	Diffuse to semi-ring porous	+	+	Diffuse	Solitary to multiples of 2–3	1.31–4.73	+	14–236	45–293	–	Simple	–
<i>Tabebuia</i> alliance	Trees	Diffuse	+	–	Diffuse	Solitary to multiples of 2–3	1.24–2.22	–	12–51	44–125	–	Mostly simple	–
Paleotropical clade	Trees and shrubs	Diffuse	+	±	Diffuse	Solitary to multiples of 2–3	1.08–2.58	–	9–73	51–178	–	Mostly simple	–

Intervessel pit size (µm)	Axial parenchyma				Rays					Septate fibers	Stored structure	Crystals
	Patracheal parenchyma	Confluence	Diffuse parenchyma	Parenchyma strands	Ray height	Ray width (in number of cells)	Rays: cellular composition	Vessel-ray pitting	Perforated ray cells			
6 ± 0.4	Vasicentric to aliform	Short to long	–	Two (2) cells per strand	Short < 1 mm	2–3	Heterocellular with 1–2 rows of square cells	Similar to intervessel pits	+	+	–	Present in rays
7.2–10.3	Aliform	Short to long	–	Four (3–4) cells per strand	Short < 1 mm	1/2–3	Homocellular in <i>Jacaranda</i> sect. <i>Monolobos</i> DC. and heterocellular in <i>J</i> sect. <i>Dilobos</i> DC.	Similar to intervessel pits	–	–	–	Present in the rays of some species
4.3–9.4	Scanty to vasicentric	Absent from present	–	Mostly four (3–4) cells per strand	Short < 1 mm and high > 1 mm in lianas	2–3	Heterocellular	Similar to intervessel pits	+ in lianas	+	–	Present in the rays of some species
3.1	Scanty	Absent	–	Four (3–4) cells per strand	Short < 1 mm	3	Homo- and heterocellular with 1 row of square cells	Similar to intervessel pits	–	+	–	Present in rays
3.1–5.3	Vasicentric to aliform	Short	–	Four (3–4) cells per strand	Short < 1 mm	3	Homocellular	Similar to intervessel pits	–	±	–	–
4.1–11.1	Scanty to aliform	Absent to short	–	Four (3–4) cells per strand	Short < 1 mm	3	Homo- and heterocellular with 1 row of square cells	Simple to semi-bordered	–	±	–	Present in the rays of some species
2.6–12.4	Scanty to aliform	Absent to short	–	Four (3–4) cells per strand	Generally high > 1 mm, smaller in shrubs	1–9	Heterocellular mixed	Predominantly similar to intervessel pits	+	+	– present in but a few species	Present in the rays of some species
2.5–19.1	Aliform	Generally long, forming bands	–	2–4 cells per strand	Short < 1 mm	1–3	Homocellular	Similar to intervessel pits	–	–	+	When present, in both rays and axial parenchyma
2.2–10.7	Aliform	Short to long	+ in Coleaceae	Four (3–4) cells per strand	Short < 1 mm	1–3	Homo- and heterocellular with 1 row of square cells	Similar to intervessel pits	–	–	–	Present in the rays of some species

Latewood marked by radially flattened fibers, frequently associated with very narrow vessels (Fig. 2C–E). *Porosity* semi-ring-porous (Fig. 2A, B). *Vessels* arranged in a diagonal to radial pattern in one of the specimens (Fig. 2A; *Pace 31*) and diagonal to predominantly tangential in the other specimen (Fig. 2B; *Pace 32*). Vessels grouped in multiples of 3–7 (Fig. 2A–D), clusters present (Fig. 2C, D), 2.47 vessels/group (Table 1); narrow ($35 \pm 12 \mu\text{m}$), vessel density 284 ± 34 per mm^2 (Table 1). *Perforation plates* simple (Fig. 2E, H). *Intervessel pits* small ($6 \pm 0.4 \mu\text{m}$). *Vessel-ray pits* simple to semi-bordered. *Fibers* thick-walled (Fig. 2A–D), with simple to minutely bordered pits in both radial and tangential walls, septate and non-septate fibers present in the same sample (the majority non-septate). *Gelatinous fibers* present. *Parenchyma* vasicentric to aliform, with short to long unilateral confluent (Fig. 2A–C). Axial parenchyma bands ranging from narrow (1–2 cells wide) to broad (3–4 cells wide), delimit the growth rings (Fig. 2A–C). Parenchyma strands with 2 cells. *Rays* non-storied, 2–3 cells wide, short ($264 \pm 73 \mu\text{m}$; Fig. 2F, G). Homo- and heterocellular rays co-occur. Homocellular rays composed of procumbent cells, heterocellular rays with body cells procumbent and one or two marginal upright to square cells (Fig. 2H, I). *Perforated ray cells* present (Fig. 2I). *Navicular crystals* present in the ray cells. *Cambial variants* absent.

DISCUSSION

Phylogenetic placement of *Sphingiphila*. — In our phylogenetic reconstructions, *Sphingiphila tetramera* clearly emerges within Bignoniaceae within the clade “*Arrabidaea* and allies” (Lohmann, 2006) in a polytomy within *Tanaecium*. *Tanaecium* includes 17 species of lianas that are distributed from Mexico to Argentina in both dry and wet forests (Lohmann & Taylor, 2014). The closeness between *Sphingiphila* and *Tanaecium* is supported by all analyses conducted with the *ndhF* and *pepC* markers as well as by morphological features.

The placement of *Sphingiphila* within Bignoniaceae confirms Gentry’s (1990) earlier hypothesis that the genus might be best placed in this tribe. Gentry’s (1990) hypothesis was based on the Neotropical distribution, septicidal fruit dehiscence, and occasionally 3-foliolate leaves of the genus. The fruit morphology (Fig. 3G, H) also led Gentry (1990) to suggest that *S. tetramera* was likely closely related to *Arrabidaea*, a polyphyletic genus that was recognized at the time but whose

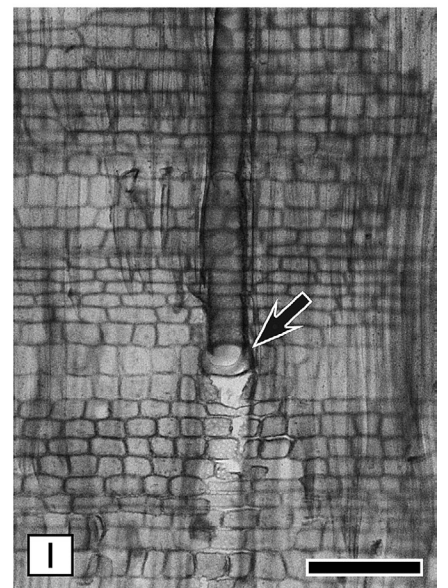
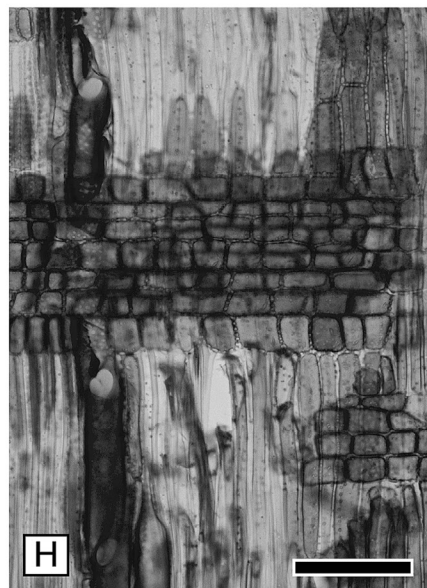
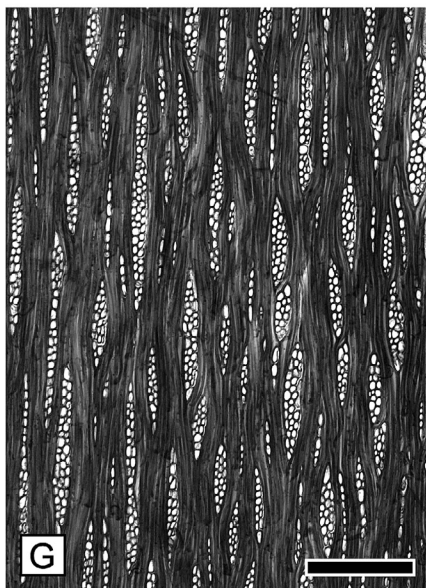
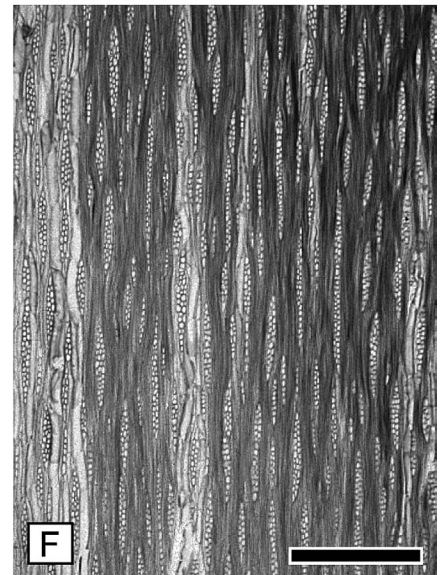
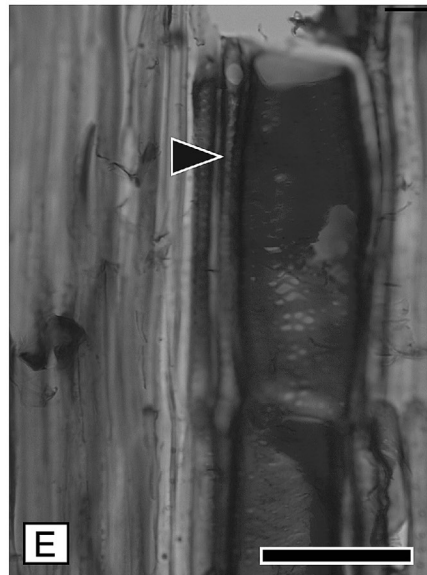
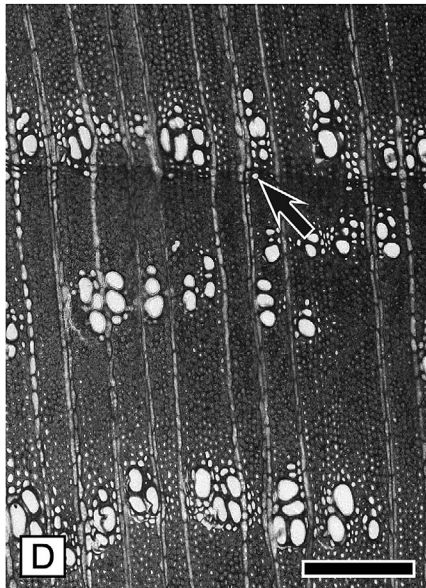
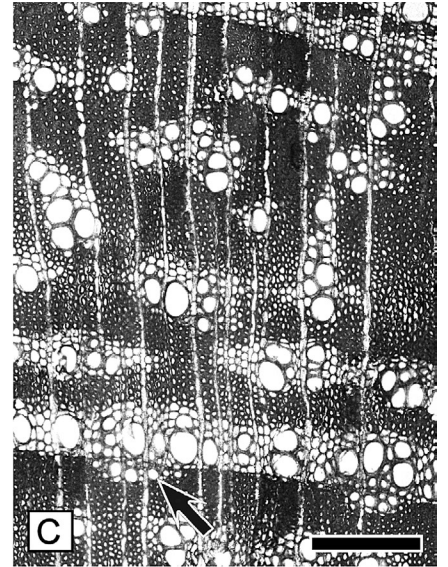
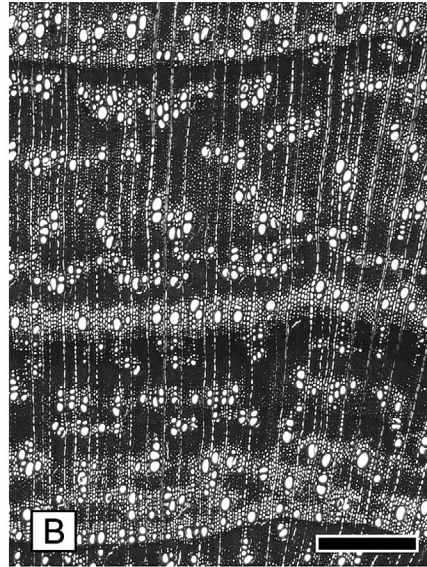
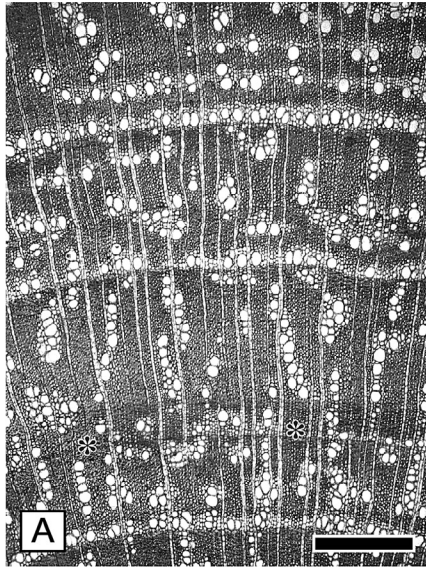
species are currently included in *Cuspidaria*, *Fridericia*, *Tanaecium*, and *Xylophragma* (Lohmann, 2006; Lohmann & Taylor, 2014).

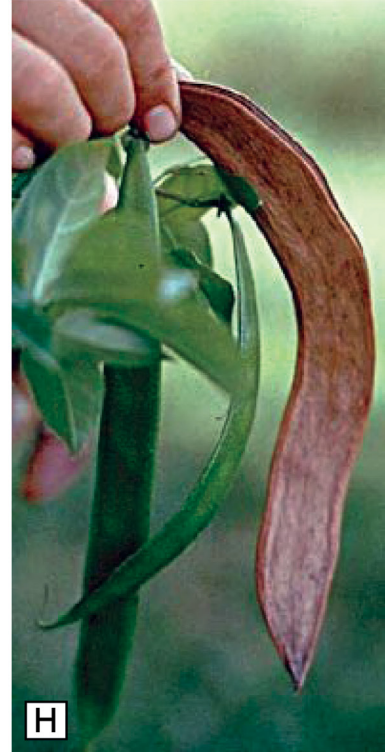
The flowers of *Sphingiphila* (Fig. 3D) are white with long corollas, pollinated by sphingid hawk-moths, and arranged in few-flowered inflorescences (Gentry, 1990). The morphologies of flower and inflorescence greatly resemble those of several species of *Tanaecium* (Gentry, 2009; Alcantara & Lohmann, 2010) (Fig. 3E), namely *Tanaecium crucigerum* Seem., *Tanaecium exitiosum* Dugand, and *Tanaecium jaroba* Sw. (Fig. 3E). The ancestral floral type of *Tanaecium* also likely had a *Tanaecium*-type morphology composed of white corollas with long tubes (Alcantara & Lohmann, 2010). This flower morphology evolved multiple times within Bignoniaceae (Alcantara & Lohmann, 2010) and is also found in *Bignonia nocturna* (Barb.Rodr.) L.G.Lohmann and *Pachyptera aromatica* (Barb. Rodr.) L.G.Lohmann.

In the most recent circumscription of *Tanaecium* (Lohmann & Taylor, 2014), five other genera were synonymized with it (*Ceratophyllum* Pittier, *Paragonia* Bureau, *Periarrabidaea* A.Samp., *Pseudocatalpa* A.H.Gentry, *Spathicalyx* J.C.Gomes) based on molecular phylogenetic data, a series of shared morphological features and one morphological synapomorphy, the presence of subulate, bromeliad-like prophylls (Fig. 3C) (Lohmann, 2006; Lohmann & Taylor, 2014). These prophylls are also found in *Sphingiphila* (Fig. 3B), although they are sometimes difficult to see in older interpetiolar nodes, given their caducous nature. Additional characters shared between *Sphingiphila* and *Tanaecium* are the calyx with glandular fields, the villose corolla (Fig. 3F), and the lepidote ovary (Gentry, 1990; Lohmann & Taylor, 2014). In addition to the morphological features shared between *Sphingiphila* and *Tanaecium*, *S. tetramera* also shares the two indels of *Tanaecium* reported by Lohmann (2006). These indels are synapomorphies of *Tanaecium* (Electr. Suppl.: Fig. S3).

On the other hand, the differences between *Sphingiphila* and *Tanaecium* mainly reflect features that have been lost in *S. tetramera*. For instance, the loss of the lianoid habit and acquisition of a shrubby to treelet habit (Fig. 3A) seen in *S. tetramera* is not unusual within Bignoniaceae, given that reversals to the shrubby or treelet habit occurred multiple times in the tribe (Lohmann, 2003) and even within *Tanaecium* in *T. exitiosum* (Dugand, 1942). In addition, the lack of tendrils, a synapomorphy of tribe Bignoniaceae, was also lost multiple times within the tribe, once in *Cuspidaria*, *Mansoa* DC. and

Fig. 2. Wood anatomy of *Sphingiphila tetramera*. **A–D**, Transverse sections; **E, H & I**, Radial longitudinal section; **F & G**, Tangential longitudinal sections. **A**, Semi-ring-porous. Growth rings marked by a marginal band of axial parenchyma and closely spaced vessels in tangential arrangement. Note the occasional absence of closely spaced vessels in one of the growth rings (asterisks). Vessels in diagonal to radial arrangement (*Pace 31*) in groups of 3–7. Parenchyma vasicentric to aliform confluent. **B**, Semi-ring-porous. Growth rings marked by a marginal parenchyma band and closely spaced vessels. Vessels in tangential to diagonal arrangement in groups of 3–4 (*Pace 32*). Parenchyma vasicentric to aliform confluent. **C**, Vessels in clusters, parenchyma vasicentric to aliform confluent, growth rings marked by a marginal band of axial parenchyma (*Pace 31*). Note narrow vessels as latewood markers (arrow). **D**, Growth ring marked sometimes only by latewood markers, such as radially flattened fibers and very narrow vessels (arrow). **E**, Vessels at the limit of a growth ring, showing the narrow vessels of the latewood (arrowhead) next to the larger vessels of the earlywood. Vessels with simple perforation plates. **F & G**, Rays non-storied 2–3 cells wide. **H**, Rays heterocellular with body cells procumbent and one marginal row of square cells. Vessels with simple perforation plates. **I**, Fused rays as seen in radial section showing a perforated ray cell (arrow). Rays heterocellular with two rows of marginal square cells. — Scale bars: A, B & F, 400 μm ; C, D & G, 200 μm ; E, 50 μm ; H & I, 100 μm .





Xylophragma, twice in *Adenocalymma* Mart. ex Meisn., and five times in *Fridericia* (Sousa-Baena & al., 2104). Similarly, the unusual loss of androecium parts is also observed in *Tanaecium caudiculatum* (Standl.) L.G.Lohmann, a species with only two anthers (Lohmann & Taylor, 2014). The tetramerous, actinomorphic flowers are undoubtedly the most unique characters of *Sphingiphila*. However, changes in the expression of single genes can alter each of these traits, either as nucleotidic mutations affecting the number of sepals/petals per whorl (Running & Meyerowitz, 1996), mutations mediated by transposable elements (Luo & al., 1996), or methylation (Cubas & al., 1999), the last two also affecting flower symmetry. In this case, mutations in a few genes could be responsible for the highly divergent flower morphology of *S. tetramera*.

Wood anatomy of *Sphingiphila*. — Regarding wood anatomy, *Sphingiphila tetramera* has a very typical Bignoniaceous wood, unlike its very unique and unusual macro-morphology. *Sphingiphila* shares several anatomical features with other shrubby to arboreal Bignoniaceae, namely narrow vessels, vasicentric to aliform parenchyma forming short to long unilateral confluent, growth rings marked by a marginal band of axial parenchyma and occasional crystals in rays. Distinctive features of *Sphingiphila* are the diagonally arranged vessels (also present in some species of *Zeyheria* Mart.), grouped in multiples of 3–7, quite frequent vessels (284 ± 34 vessels/mm², while most Bignoniaceae do not exceed 50 vessels/mm²), non-storied heterocellular rays and the presence of perforated ray cells.

Some of the distinctive features of *Sphingiphila*, such as the highly dense, multiple vessels arranged diagonally, are all features that can be attributed to its occurrence in xeric environment, something well established in ecological wood anatomy (Baas & al., 1983, 2003). Other Bignoniaceae which grow in xeric environments, such as *Zeyheria montana* Mart., abundant in the Cerrado, and *Catophractes alexandrii* D.Don from the African savannahs, also share these same features (Pace & Angyalossy, 2013).

The set of wood anatomical features of *Sphingiphila* suggests a placement either in tribe Tecomeae s.str. or Bignoniaceae (Pace & al., 2015). One of the main reasons is the presence of perforated ray cells in *Sphingiphila*. Perforated ray cells have only been recorded within lianoid lineages of Bignoniaceae, being particularly frequent in some lianas of both Tecomeae s.str. (e.g., *Campsis radicans* (L.) Bureau) and Bignoniaceae (*Stizophyllum riparium* (Kunth) Sandwith). Interestingly, perforated ray cells have also been found in shrubs with lianoid ancestors (Pace & Angyalossy, 2013; Pace & al., 2015).

On the other hand, the absence of a cambial variant, a synapomorphy of Bignoniaceae, would suggest that *Sphingiphila* would be best placed in Tecomeae s.str. In fact, all known species of Bignoniaceae share four to multiples of four phloem

wedges that furrow the xylem (Gentry, 1980; Dos Santos, 1995; Lohmann, 2006). Even the shrubby and treelet species of Bignoniaceae for which the wood anatomy is known (e.g., *Adenocalymma cladotrichum* (Sandwith) L.G.Lohmann, *Anemopaegma arvense* (Vell.) Stellfeld ex J.F.Souza, *Cuspidaria pulchra* (Cham.) L.G.Lohmann, and *Fridericia platyphylla* (Cham.) L.G.Lohmann), have a cambial variant, although reduced (Pace & al., 2009), and never lack it completely as does *S. tetramera*. Within Bignoniaceae, *Sphingiphila* may represent the only species to lack a cambial variant completely, thus representing a case of total loss of cambial variants associated with the evolution of liana to a self-supporting habit. On the other hand, no Tecomeae s.str. has as abundant parenchyma as *Sphingiphila*, whereas abundant parenchyma and the presence of vasicentric to aliform parenchyma with short confluent is common in the “*Arrabidaea* and allies” clade of the Bignoniaceae (Pace & Angyalossy, 2013; Pace & al., 2015), within which *S. tetramera* has here been placed based on molecular data.

Our molecular phylogenetic results indicate that *Sphingiphila* and *Tanaecium* form a strongly supported yet internally unresolved clade. Anatomically, although *Sphingiphila* lacks a very strong diagnostic feature of tribe Bignoniaceae, the cambial variant, it shares with *Tanaecium* the presence of abundant vasicentric to aliform confluent parenchyma, septate fibers, perforated ray cells and heterocellular non-storied rays 3 ± 1 cells in width (Pace & Angyalossy, 2013; Pace & al., 2015). *Tabebuia nodosa*, another Bignoniaceae from the Chaco, also has highly unique morphological and anatomical features, representing another rare example of a taxon that could not be placed in any of the three major wood anatomical groups described by Dos Santos & Miller (1992). The extreme isolation of these species in the Chaco, a hot semi-arid lowland region, may have been the driver of these remarkable cases of morphological and anatomical divergence. Further studies including more species from the Chaco are needed to test this hypothesis.

Following the criteria used by Lohmann & Taylor (2014: 358), who recognize genera as well-supported clades diagnosed by one or more morphological synapomorphies, we propose the synonymization of *Sphingiphila* with *Tanaecium*.

■ TAXONOMIC TREATMENT

Tanaecium Sw., Prodr.: 91. 1788, emend. L.G. Lohmann in Ann. Missouri Bot. Gard. 99: 463. 2014 – Type: *Tanaecium jaroba* Sw.

= *Sphingiphila* A.H.Gentry in Syst. Bot. 15: 277. 1990 – Type: *Sphingiphila tetramera* A.H.Gentry, **syn. nov.**

Tanaecium tetramerum (A.H.Gentry) Zuntini & L.G.Lohmann, **comb. nov.** ≡ *Sphingiphila tetramera* A.H.Gentry in Syst.

◀ **Fig. 3.** Macromorphological characterization of *Sphingiphila* and related taxa. **A, B, D & F**, *Sphingiphila tetramera* (Pace 31, SPF); **C**, *Tanaecium bilabiatum* (Centeno 151, SPF); **E**, *T. jaroba* (Gomes 598, SPF); **G**, *S. tetramera* (Pace 32, SPF); **H**, *T. caudiculatum* (Gentry 8216, MO). **A**, Shrubby habit; **B & C**, Interpetiolar region with bromeliad-like prophylls; **D**, Flowering branch; **E**, Flowers; **F**, Detail of the bud apex, showing the villose indument; **G & H**, Fruit. — Image credits: A, D & G, Marcelo R. Pace; B, C & F, Alexandre Zuntini; E, Beatriz Gomes; H, Alwyn Gentry from tropicos.org. Images B, C and F obtained from multiple shots in stereoscope and processed with focus stacking.

Bot. 15: 277–279, fig. 1. 1990 – Holotype: Paraguay: Alto Paraguay, Chovoreca, moist sandy soil along pond in open Cerrado vegetation, 19°20' S 59°05' W, 12 Aug 1983, *W. Hahn 1600* (MO barcode MO-077156! [No. 3208626]; isotypes: G barcode G00094221 [web image!], MBM barcode MBM117809, MO barcode MO-077155! [No. 3520050], NY barcode 00328929!, PY, US barcode 00432848 [No. 3289925] [web image!]).

Shrub or treelet up to 5 m tall, without dimorphic juvenile growth; stems without phloem wedges in cross section, without hollow pith; branchlets cylindrical to slightly angular, puberulous and lepidote, with or without lenticels, without interpetiolar glands, without interpetiolar ridge; prophylls of axillary buds bromeliad-like, caducous; the short-shoot of lateral branches conspicuously thorn-tipped. *Leaves* sessile or with petioles <2 mm, simple or 3-foliolate, terminal leaflets never replaced by tendrils; leaflets chartaceous to coriaceous, narrowly elliptic or oblong-elliptic or oblong-ob lanceolate, apex rounded or minutely apiculate, base cuneate, 1.0–4.2 cm long, 0.2–1.2 cm wide, without glands or pellucid punctations over blade, without domatia, lepidote on both sides, margin involute; petiole never modified into a tendril. *Inflorescence* an axillary, few-flowered fascicle, with the axis reduced. *Flowers* actinomorphic, tetramerous; calyx narrowly cupular, 4–5-denticulate, coriaceous, 0.3–0.6 cm long, 0.2 cm wide, lepidote or puberulous externally, with glands along ribs; corolla white, without nectar guides, narrow and tubular, straight, membranous, 7.2–14.3 cm long, 0.2–0.4 cm wide, glabrous externally, without glands, lobes imbricate; stamens without filaments, with anthers inserted directly into the corolla tube, partially included, anthers glabrous, thecae straight; pollen in monads, colpate, exine reticulate; ovary sessile, bilocular, with two series of ovules on each placenta, smooth and lepidote, occasionally with simple trichomes; disk annular. *Capsule* dehiscent parallel to the septum (septicidal), narrow and linear, flattened, straight, coriaceous, 4.2–10.3 cm long, 0.5–1.6 cm wide, valves lepidote, without lenticels, with glands, without ridges or wings, smooth, calyx deciduous; seeds winged, seed body smooth and glabrous, wings hyaline, rounded.

Habitat and distribution. – Only known from dry areas (Chaco) of Bolivia and Paraguay.

Specimens examined. – BOLIVIA. Cochabamba: Campesino. Bajada de Buena Vista al Río Grande, Grande (18°37'16" S, 63°32'52" W), 27 Jan 1993, *Antezana Valera 619* (BOLV, MO); 24 Nov 1999, *Antezana Valera 1327* (BOLV, MO). Santa Cruz: Cordillera, Tatiana, 50 km southwest from Robore, 30 Aug 1997, *Fuentes & Navarro 2107* (MO, USZ); Parque Nacional Kaa-Iya del Gran Chaco, Paleodunas, 25 km NE from Palmar de Las Islas (19°15'39" S, 60°25'30" W), 12 Feb 1998, *Fuentes & Navarro 2272* (MO, USZ); Parque Nacional Kaa-Iya del Gran Chaco, 11 km NW from Cerro Ustarez (19°56'44" S, 61°51'43" W), 15 Jul 1998, *Fuentes & Navarro 2564* (LPB, MO, USZ); Valle Grande, A 30 km do Pueblo de Pucará (18°42.88' S, 64°18.113' W), 19 Apr 2004, *Pace & al. 31* (SPF); Valle Grande, A 30 km do Pueblo de Pucará (18°42.88' S, 64°18.113' W), 19 Apr 2004, *Pace & al. 32* (MO, SPF).

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Appendix 1. Vouchers and GenBank accession numbers used in this study. Sequences used in both datasets are indicated with “+” and “na” indicates unavailable sequences. Sequences newly obtained in this study are marked with an asterisk (*).

Species, locality, voucher (herbarium): *ndhF*, *pepC*.

Tribe Bignoniaceae: *Adenocalymma adenophorum* (Sandwith) L.G.Lohmann, Brazil, Amazonas, Reserva Ducke, *Lohmann 30* (INPA, K, MG, MO, NY, RB, SP): DQ222608, DQ222766. *Adenocalymma bracteatum* (Cham.) DC., Brazil, São Paulo, Santa Cruz da Conceição, *Lohmann 719* (MO, SPF): DQ222527, DQ222649. *Adenocalymma bracteosum* (DC.) L.G.Lohmann, Brazil, Amazonas, Rio Negro, *Lohmann 290* (MO, NY, SPF, UNIP): DQ222609, DQ222767. *Adenocalymma campicola* (Pilg.) L.G.Lohmann, Brazil, Minas Gerais, Uberlândia, *Lohmann 266* (MO, SPF, U): DQ222610, DQ222770. *Adenocalymma cladotrichum* (Sandwith) L.G.Lohmann, Brazil, Acre, Rio Juruá, *Lohmann 389* (MO, NY, SPF, UFAC): DQ222611, na. *Adenocalymma cymbalum* (Cham.) Bureau & K.Schum., Brazil, Minas Gerais, PE do Rio Doce, *Lombardi 2495* (BHCB, MO): DQ222528, DQ222650. *Adenocalymma impressum* (Rusby) Sandwith, Brazil, Amazonas, Reserva Ducke, *Vicentini 1155* (INPA, K, MG, MO, NY, SP, U): DQ222529, DQ222652. *Adenocalymma magnificum* Mart. ex DC., Brazil, Pará, PE Moju, *Silva 30* (IAN, MO): DQ222612, DQ222771. *Adenocalymma moringifolium* (DC.) L.G.Lohmann, Brazil, Amazonas, Reserva

Appendix 1. Continued.

Ducke, *Lohmann 19* (INPA, K, MG, MO, NY, R, SP, SPF, U): DQ222613, DQ222773. *Adenocalymma salmoneum* J.C.Gomes, Brazil, Espírito Santo, Linhares, *Lohmann 658* (CVRD, MO): DQ222530, DQ222653. *Adenocalymma subincanum* Huber, Brazil, Amazonas, Reserva Ducke, *Lohmann 12* (INPA, MO): DQ222531, DQ222654. *Adenocalymma trichocladum* (DC.) L.G.Lohmann, Brazil, Bahia, Santa Maria da Vitória, *Hatschbach 50496* (MO): DQ222635, DQ222807. *Amphilophium aschersonii* Ule, Brazil, Acre, Rio Juruá, *Lohmann 390* (MO, NY, SPF, UFAC): DQ222532, DQ222655. *Amphilophium bauhinoides* (Bureau ex Baill.) L.G.Lohmann, Brazil, Espírito Santo, Linhares, *Lohmann 655* (CVRD, MO): DQ222586, DQ222734. *Amphilophium crucigerum* (L.) L.G.Lohmann, Brazil, Espírito Santo, Linhares, *Lohmann 685* (CVRD, MO): DQ222623, DQ222789. *Amphilophium elongatum* (Vahl) L.G.Lohmann, Brazil, Minas Gerais, PE do Rio Doce, *Lombardi 2433* (BHCB, MO): DQ222578, DQ222720. *Amphilophium frutescens* (DC.) L.G.Lohmann, Brazil, Paraíba, Rio Tinto, *Lohmann 695* (MO, SPF): DQ222581, DQ222724. *Amphilophium granulosum* (Klotzsch) L.G.Lohmann, Brazil, Acre, Rio Juruá, *Lohmann 470* (MO, NY, SPF, UFAC): DQ222582, DQ222726. *Amphilophium magnoliifolium* (Kunth) L.G.Lohmann, Suriname, Sipaliwini, Tafelberg Tepui, *Lohmann 214* (BBS, MO): DQ222579, DQ222722. *Amphilophium nunezii* (A.H.Gentry) L.G.Lohmann, Peru, Madre Dios, Manu National Park, *Lohmann 606* (MO, MOL): DQ222587, DQ222735. *Amphilophium paniculatum* (L.) Kunth, Peru, Madre Dios, Manu National Park, *Lohmann 609* (MO, MOL): DQ222533, DQ222656. *Amphilophium lohmanniae* (A. Pool) L.G.Lohmann, Brazil, Amazonas, Reserva Ducke, *Lohmann 20* (INPA, MO): DQ222580, DQ222723. *Amphilophium rodriguesii* (A.H.Gentry) L.G.Lohmann, Brazil, Acre, Rio Arara, *Lohmann 475* (MO, NY, SPF, UFAC): DQ222588, DQ222737. *Anemopaegma floridum* Mart. ex DC., Brazil, Amazonas, Reserva Ducke, *Lohmann 121* (INPA, MO, SPF): DQ222534, DQ222658. *Anemopaegma foetidum* Bureau & K.Schum., Brazil, Amazonas, Reserva Ducke, *Lohmann 35* (INPA, MO, SPF): DQ222535, DQ222659. *Anemopaegma laeve* DC., Brazil, Bahia, Chapada Diamantina, *Lohmann 253* (MO, SPF): DQ222536, DQ222661. *Anemopaegma oligoneuron* (Sprague & Sandwith) A.H.Gentry, Brazil, Amazonas, Reserva Ducke, *Apostolo 126* (BM, G, INPA, K, MG, MO, R, SP, US): DQ222537, na. *Anemopaegma robustum* Bureau & K.Schum., Brazil, Amazonas, Reserva Ducke, *Apostolo 126* (INPA, MO): DQ222538, DQ222663. *Bignonia aequinoctialis* L., Brazil, Amazonas, Rio Negro, *Lohmann 320* (MO, NY, SPF, UNIP): DQ222577, DQ222719. *Bignonia bracteomana* (K.Schum. ex Sprague) L.G.Lohmann, Peru, Madre Dios, Manu National Park, *Lohmann 614* (MO, MOL): DQ222634, DQ222806. *Bignonia callistegioides* Cham., U.S.A., Missouri, cultivated at MOBOT, *Lohmann 352* (MO): DQ222569, DQ222708. *Bignonia campanulata* Cham., Brazil, Minas Gerais, PE do Rio Doce, *Lombardi 2526* (BHCB, MO): DQ222570, na. *Bignonia capreolata* Kunth, U.S.A., Illinois, Johnson County, *Lohmann 356* (MO): DQ222566, DQ222706. *Bignonia corymbosa* (Vent.) L.G.Lohmann, Brazil, Espírito Santo, Linhares, *Lohmann 654* (MO): DQ222621, DQ222785. *Bignonia hyacinthina* (Standl.) L.G.Lohmann, Peru, Madre Dios, Manu National Park, *Lohmann 642* (MO, MOL): DQ222614, DQ222775. *Bignonia magnifica* W.Bull, Brazil, São Paulo, cultivated at Instituto Plantarum, *Lohmann 711* (MO, SPF): DQ222636, na. *Bignonia microcalyx* G.Mey., Suriname, Sipaliwini, Road between Blanche Marie and Paramaribo, *Evans 3198* (BBS, MO): DQ222629, DQ222797. *Bignonia nocturna* (Barb.Rodr.) L.G.Lohmann, Brazil, Acre, Rio Juruá, *Lohmann 451* (MO, NY, SPF, UFAC): DQ222641, DQ222813. *Bignonia prieurii* DC., Brazil, Espírito Santo, Linhares, *Lohmann 651* (INPA, MO): DQ222615, DQ222776. *Bignonia scuripabulum* (K.Schum.) L.G.Lohmann, Peru, Madre Dios, Manu National Park, *Lohmann 608* (MO, MOL): DQ222571, na. *Bignonia uleana* (Kraenzl.) L.G.Lohmann, Peru, Madre Dios, Manu National Park, *Lohmann 617* (MO, MOL): DQ222572, DQ222709. *Callichlamys latifolia* (Rich.) K.Schum., Brazil, Amazonas, Reserva Ducke, *Lohmann 1* (MO, SPF): DQ222567, na. *Cuspidaria cinerea* (Bureau ex K.Schum.) L.G.Lohmann, Brazil, Amazonas, Reserva Ducke, *Lohmann 34* (INPA, K, MG, MO, NY, SP, SPF, U, UB): DQ222631, DQ222801. *Cuspidaria convoluta* (Vell.) A.H.Gentry, Brazil, São Paulo, cultivated at Instituto Plantarum, *Lohmann 713* (MO, SPF): DQ222573, DQ222711. *Cuspidaria floribunda* (DC.) A.H.Gentry, Brazil, Acre, Rio Juruá, *Lohmann 418* (MO, NY, SPF, UFAC): DQ222574, DQ222713. *Cuspidaria inaequalis* (DC. ex Splitg.) L.G.Lohmann, Suriname, Sipaliwini, Tafelberg Tepui, *Lohmann 127* (BBS, MO): DQ222548, DQ222679. *Cuspidaria lateriflora* (Mart.) DC., Peru, Madre Dios, Manu National Park, *Lohmann 628* (MO, MOL): DQ222575, DQ222716. *Cuspidaria sceptrum* (Cham.) L.G.Lohmann, Brazil, São Paulo, Santa Cruz da Conceição, *Lohmann 717* (MO, SPF): DQ222557, DQ222698. *Cuspidaria subincana* A.H.Gentry, Brazil, Amazonas, Reserva Ducke, *Lohmann s.n. (Tree # 2638-24)* (INPA, MO): DQ222576, DQ222717. *Dolichandra cynanchoides* Cham., Argentina, Buenos Aires, cultivated, *Galleto 1019* (MO): DQ222583, DQ222728. *Dolichandra quadrivalvis* (Jacq.) L.G.Lohmann, U.S.A., Missouri, cultivated at MOBOT, *Lohmann 353* (MO): DQ222607, DQ222764. *Dolichandra steyermarkii* (Sandwith) L.G.Lohmann, Brazil, Acre, Rio Arara, *Lohmann 477* (MO, NY, SPF, UFAC): DQ222617, DQ222780. *Dolichandra unguis-cati* (L.) L.G.Lohmann, Brazil, Minas Gerais, PE do Rio Doce, *Lombardi 2432* (BHCB, MO): DQ222595, DQ222749. *Fridericia chica* (Bonpl.) L.G.Lohmann, Brazil, Amazonas, Reserva Ducke, *Lohmann s.n. (Tree # 2618-24)* (INPA): DQ222542, DQ222671. *Fridericia cinerea* (Bureau ex K.Schum.) L.G.Lohmann, Brazil, Bahia, Chapada Diamantina, *Lohmann 358* (MO, SPF): DQ222543, DQ222673. *Fridericia cinnamomea* (DC.) L.G.Lohmann, Brazil, Amazonas, Reserva Ducke, *Vicentini 809* (INPA, MO): DQ222544, DQ222674. *Fridericia conjugata* (Vell.) L.G.Lohmann, Brazil, Espírito Santo, Linhares, *Lohmann 650* (CVRD, MO): DQ222545, DQ222675. *Fridericia dispar* (Bureau ex K.Schum.) L.G.Lohmann, Brazil, Paraíba, Santa Rita, *Lohmann 694* (MO, SPF): DQ222546, DQ222676. *Fridericia erubescens* (DC.) L.G.Lohmann, Brazil, Bahia, Chapada Diamantina, *Lohmann 359* (MO, SPF): DQ222622, DQ222787. *Fridericia leucopogon* (Cham.) L.G.Lohmann, Brazil, São Paulo, Santa Cruz da Conceição, *Lohmann 714* (MO, SPF): DQ222549, DQ222681. *Fridericia nigrescens* (Sandwith) L.G.Lohmann, Brazil, Amazonas, Reserva Ducke, *Lohmann 78* (G, INPA, K, MG, MO, NY, RB, SP, U, UB): DQ222550, DQ222683. *Fridericia oligantha* (Bureau & K.Schum.) L.G.Lohmann, Brazil, Acre, Rio Juruá, *Lohmann 483* (MO, NY, SPF, UFAC): DQ222551, DQ222685. *Fridericia patellifera* (Schltdl.) L.G.Lohmann, Brazil, Acre, Rio Juruá, *Lohmann 412* (MO, NY, SPF, UFAC): DQ222552, DQ222687. *Fridericia pearcei* (Rusby) L.G.Lohmann, Peru, Madre Dios, Manu National Park, *Lohmann 637* (MO, MOL): DQ222553, na. *Fridericia platyphylla* (Cham.) L.G.Lohmann, Peru, Madre Dios, Manu National Park, *Lohmann 639* (MO, MOL): DQ222554, na. *Fridericia platyphylla* (Cham.) L.G.Lohmann, Brazil, São Paulo, cultivated at Instituto Plantarum, *Lohmann 709* (MO, SPF): DQ222541, DQ222669. *Fridericia prancei* (A.H.Gentry) L.G.Lohmann, Brazil, Amazonas, Reserva Ducke, *Sothers 460* (INPA, K, MBM, MG, MO, NY, RB, SP, U, UFMT): DQ222555, DQ222689. *Fridericia pubescens* (L.) L.G.Lohmann, Brazil, Minas Gerais, PE do Rio Doce, *Lombardi 2529* (BHCB, MO): DQ222556, DQ222690. *Fridericia rego* (Vell.) L.G.Lohmann, Brazil, Espírito Santo, Linhares, *Lohmann 660* (CVRD, MO): DQ222558, DQ222692. *Fridericia schumanniana* (Loes.) L.G.Lohmann, Brazil, Acre, Rio Xapuri, *Lohmann 547* (MO, NY, SPF, UFAC): DQ222565, na. *Fridericia speciosa* Mart., Brazil, Minas Gerais, PE do Rio Doce, *Lombardi 2521* (BHCB, MO): DQ222584, DQ222730. *Fridericia spicata* (Bureau & K.Schum.) L.G.Lohmann, Peru, Madre Dios, Manu National Park, *Lohmann 607* (MO, MOL): DQ222561, DQ222699. *Fridericia subincana* (Mart.) L.G.Lohmann, Brazil, Espírito Santo, Linhares, *Lohmann 659* (CVRD, MO): DQ222562, DQ222701. *Fridericia trailii* (Sprague) L.G.Lohmann, Brazil, Amazonas, Reserva Ducke, *Lohmann 29* (INPA, K, MG, MO, NY, SPF): DQ222563, DQ222703. *Fridericia triplinervia* (Mart. ex DC.) L.G.Lohmann, Brazil, Amazonas, Reserva Ducke, *Lohmann 18* (GH, IAN, INPA, K, MO, PEUFR, S, SPF, UEC): DQ222564, DQ222705. *Lundia densiflora* DC., Brazil, Amazonas, Reserva Ducke, *Lohmann 82* (INPA, MO): DQ222592, DQ222743. *Lundia laevis* Kaeherl, Brazil, Acre, Rio Abuna, *Lohmann 497* (MO, NY, SPF, UFAC): DQ222591, DQ222742. *Lundia longa* (Vell.) DC., Brazil, Espírito Santo, Linhares, *Lohmann 652* (CVRD, MO): DQ222590, DQ222741. *Lundia spruceana* Bureau, Peru, Madre Dios, Manu National Park, *Lohmann 610* (MO, MOL): DQ222593, DQ222745. *Lundia virginialis* DC., Brazil, Minas Gerais, PE do Rio Doce, *Lombardi 2530* (BHCB, MO): DQ222594, DQ222747. *Manaosella cordifolia* (DC.) A.H.Gentry, Brazil, Minas Gerais, PE do Rio Doce, *Lombardi 2546* (BHCB, MO): DQ222596, DQ222750. *Mansoa alliacea* (Lam.) A.H.Gentry, Brazil, Amazonas, Reserva Ducke, *Vicentini 672* (INPA, K, MG, MO, NY, RB, SPF): DQ222597, na. *Mansoa difficilis* (Cham.) Bureau & K.Schum., Brazil, Espírito Santo, Linhares, *Lohmann 662* (CVRD, MO): DQ222598, DQ222752. *Mansoa hirsuta* DC., Brazil, Bahia, Chapada Diamantina, *Lohmann 364* (MO, SPF): DQ222599, DQ222753. *Mansoa lanceolata* (DC.) A.H.Gentry, Brazil, Espírito Santo, Linhares, *Lohmann 661* (CVRD, MO): DQ222601, DQ222755. *Mansoa parvifolia* (A.H.Gentry) A.H.Gentry, Peru, Madre Dios, Manu National Park, *Lohmann 605* (MO, MOL): DQ222602, na. *Mansoa standleyi* (Steyerem.) A.H.Gentry, Peru, Madre Dios, Manu National Park, *Lohmann 638* (MO, MOL): DQ222603, DQ222757. *Mansoa verrucifera* (Schltdl.) A.H.Gentry, Peru, Madre Dios, Manu National Park, *Lohmann 612* (MO, MOL): DQ222604, DQ222759. *Martinellaquitoensis* A.Samp., Peru, Madre Dios, Manu National Park, *Lohmann 616* (MO, MOL): DQ222605, DQ222760. *Martinella obovata* (Kunth) Bureau & K.Schum., Suriname, Sipaliwini, Tafelberg Tepui, *Lohmann 126* (BBS, MO): DQ222606, DQ222762. *Nejobertia candolleana* (Mart. ex DC.) Bureau & K.Schum., Brazil, Bahia, Chapada Diamantina, *Lohmann 363* (MO, SPF): DQ222616, DQ222778. *Nejobertia mirabilis* (Sandwith) L.G.Lohmann, Brazil, Espírito Santo, Linhares, *Lohmann 681* (CVRD, MO): DQ222585, DQ222732.

Appendix 1. Continued.

Pachyptera aromatica (Barb.Rodr.) L.G.Lohmann, Brazil, Amazonas, Reserva Ducke, *Lohmann 28* (INPA, MO, SPF): DQ222589*, DQ222739. *Pachyptera kerere* (Aubl.) Sandwith, Brazil, Amazonas, Rio Negro, *Lohmann 336* (MO, NY, SPF, UNIP): DQ222600, na. *Perianthomega vellozoi* Bureau, Bolivia, Santa Cruz, Close to the Santa Cruz Botanical Garden, *Nee 35808* (LPB, MO): DQ222619, na. *Pleonotoma longiflora* B.M. Gomes & Proença, Brazil, Amazonas, Reserva Ducke, *Forzza 290* (G, INPA, K, MG, MO, NY, RB, SP, U, UB): DQ222624, DQ222791. *Pleonotoma jasminifolia* (Kunth) Miers, Brazil, Amazonas, Reserva Ducke, *Lohmann 122* (INPA): DQ222625, DQ222793. *Pleonotoma melioides* (S.Moore) A.H.Gentry, Peru, Madre Dios, Manu National Park, *Lohmann 643* (MO, MOL): DQ222626, na. *Pleonotoma stichadenia* K.Schum., Brazil, Espírito Santo, Linhares, *Lohmann 656* (CVRD, MO): DQ222627, DQ222795. *Pyrostegia venusta* (Ker Gawl.) Miers, Brazil, São Paulo, Santa Cruz da Conceição, *Lohmann 718* (MO, SPF): DQ222633, DQ222804. *Sphingiphila tetramera* A.H.Gentry 1, Bolivia, Santa Cruz, Vallegrande, *Pace 32* (MO, SPF): KU757039*, KU757041*. *Sphingiphila tetramera* A.H.Gentry 2, Bolivia, Cochabamba, Campero, *Antezana Valera 1327* (MO): KU757040*, KU757043*. *Stizophyllum inaequilaterum* Bureau & K.Schum., Brazil, Acre, Rio Juruá, *Lohmann 454* (MO, NY, SPF, UFAC): DQ222638, DQ222808. *Stizophyllum perforatum* (Cham.) Miers, Brazil, Minas Gerais, PE do Rio Doce, *Lombardi 2431* (BHCB, MO): DQ222639, DQ222809. *Tanaecium affine* (A.H.Gentry) L.G.Lohmann, Peru, Madre Dios, Manu National Park, *Lohmann 633* (MO, MOL): DQ222539, DQ222665. *Tanaecium bilabiatum* (Sprague) L.G.Lohmann, Brazil, Amazonas, Rio Solimões, *Lohmann 92* (MO, NY, SPF, UNIP): L.G.Lohmann, Brazil, Paraíba, Guarabira, *Lohmann 702* (MO, SPF): DQ222560, DQ222696. *Tanaecium tetragonolobum* (Jacq.) L.G.Lohmann, Peru, Madre Dios, Manu National Park, *Lohmann 619* (MO, MOL): DQ222568, DQ222707. *Tanaecium truncatum* (A.Samp.) L.G.Lohmann, Brazil, Amazonas, Reserva Ducke, *Lohmann 33* (INPA, K, MG, MO, NY, SPF): DQ222620, DQ222783. *Tanaecium xanthophyllum* (DC.) L.G.Lohmann, Bolivia, Beni, Jose Balivian, *Killeen 3590* (LPB, MO): DQ222637, na. *Tynanthus elegans* Miers, Brazil, Espírito Santo, Linhares, *Lohmann 663* (CVRD, MO): DQ222643, DQ222815. *Tynanthus panurensis* (Bureau ex Baill.) Sandwith, Brazil, Amazonas, Reserva Ducke, *Procopio 14* (G, INPA, K, MG, MO, NY, RB, SP, U, UB): DQ222644, DQ222817. *Tynanthus polyanthus* (Bureau ex Baill.) Sandwith, Brazil, Acre, Cruzeiro do Sul, *Lohmann 370* (MO, NY, SPF, UFAC): DQ222645, DQ222819. *Tynanthus schumannianus* (Kuntze) A.H.Gentry, Brazil, Acre, Rio Juruá, *Lohmann 452* (MO, NY, SPF, UFAC): DQ222646, na. *Tynanthus villosus* A.H.Gentry, Brazil, Acre, Rio Juruá, *Lohmann 413* (MO, NY, SPF, UFAC): DQ222647, DQ222820. *Xylophragma harleyi* (A.H.Gentry ex M.M.Silva & L.P.Queiroz) L.G.Lohmann, Brazil, Bahia, Chapada Diamantina, *Lohmann 362* (MO, SPF): DQ222547, DQ222678. *Xylophragma myrianthum* (Cham.) Sprague, Brazil, Espírito Santo, Linhares, *Lohmann 649* (CVRD, MO): DQ222648, DQ222822. — **Other Bignoniaceae:** *Amphitecna apiculata* A.H. Gentry, cult. MBG, *Spangler B1* (COLO): AF102624, na. *Argyria robusta* Sandwith, cult. RBGE #19951084, no voucher: FJ887853, na. *Campsidium valdiviana* (Phil.) Bull, cult. RBGE #1988-0922, *Gardner 4050* (E): EF104999, na. *Catalpa aff. speciosa* (Warder ex Barney) Warder ex Engelm., U.S.A., Michigan, cult. Colorado, *Olmstead 92-99* (WTU): L36397, na. *Chilopsis linearis* Sweet, U.S.A., Washington, Seattle, cult. UW campus, *Olmstead 96-139* (WTU): FJ887856, na. *Colea sytsmae* Zjhra, Madagascar, *Zjhra 917* (WIS): EF105001, na. *Crescentia alata* Kunth, Mexico, cult. UCB Bot. Gard. #89.1516, *Lomeli Sencion & V. Galindo s.n.*: FJ887857, na. *Delostoma integrifolium* D. Don, Ecuador, *Blanco 2155* (WTU): EF105004, na. *Delostoma lobbii* Seem., Peru, *Hutchison 5465* (UC): EF105005, na. *Eccremocarpus scaber* Ruiz & Pav., cult. In RBGK #1988-132, *Chase 2999* (K): AF102630, na. *Godmania aesculifolia* (HBK.) Standl., Costa Rica, *Grose 129* (WTU): EF105010, na. *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos, Brazil, UCB Bot. Gard. 85-0562, *H. Forbes s.n.* (JEPS, UC): EF105032, na. *Jacaranda arborea* Urban, Cuba, cult. Jard. Bot. Nac., *Olmstead 96-96* (WTU): FJ887866, na. *Jacaranda mimosifolia* D. Don, Brazil, São Paulo, cult. São Paulo, *Lohmann 369* (MO): EF105012, na. *Jacaranda sparrei* A.H. Gentry, cult. Waimea #82S772, no voucher (MO): AF102631, na. *Oroxylum indicum* Vent., cult. Waimea #79S51, no voucher: AF102635, na. *Pandorea pandorana* (Andrews) van Steenis, Australia, in cult. Austr. Nat. Bot. Gard., *Kelchner 219* (CANB): EF105016, na. *Podranea ricasoliana* (Tanfani) Sprague, Brazil, Minas Gerais, cultivated at Belo Horizonte, *Lombardi 2437* (BHCB, MO): DQ222628*, na. *Rhodocolea racemosa* (Lam.) H. Perrier, Madagascar, *Zjhra 943* (WIS): AY500443, na. *Roseodendron donnell-smithii* (Rose) Miranda, cult. Waimea #89P166, no voucher: AF102628, na. *Sparattosperma leucanthum* (Vell.) K. Schum., cult. Waimea #87-5446, no voucher: EF105022, na. *Spathodea campanulata* Beauv., cult. Waimea #78P1079, no voucher: AY500452, na. *Tabebuia heterophylla* (DC.) Britton, U.S.A., Missouri, cult. MOBOT, *Gentry s.n.*: L36416, na. *Tabebuia rosea* (Bertol.) DC., Mexico, *Grose 156* (WTU): EF105040, na. *Tecoma capensis* (Thunb.) Lindl., Suriname, Paramaribo, cultivated, *Lohmann 125* (BBS, MO): DQ222642, na. *Tecomella undulata* Seem., Pakistan, *Stewart 23756* (GH): FJ887875, na. *Tourretia lappacea* (L'Hérit.) Willd., Ecuador, *Dobson 1088* (US): FJ887876, na. — **Outgroups:** *Antirrhinum majus* L., cult., *C.W. dePamphilis s.n.* (PAC): L36413, na. *Myoporum mauritianum* A. DC., cult. RBGK #1984-4220, *Wallander 2000*: L36403, na. *Digitalis purpurea* L., cult. *Kim 13943* (YNUH): AF130150, na. *Nyctanthes arbor-tristis* L., cult. RBGK #099-86.00993, no voucher: U78708, na.