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A Toxic Story: Phylogeny and Classification of *Strychnos* L. (Loganiaceae)

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Abstract—*Strychnos* is a pantropical genus of Loganiaceae (Gentianales), with approximately 200 species, that lacks a detailed worldwide phylogenetic understanding until now. We investigated the global phylogeny of the majority of *Strychnos* species, and evaluated morphological and key character patterns to discuss congruence between phylogenetic clades and sectional classification systems. We included 147 ITS sequences across 12 genera, with 127 samples (103 species) of *Strychnos* and 20 outgroup accessions (19 species) in a Bayesian analysis. Tribes Antonieae, Loganieae, and Spigelieae were supported as monophyletic, but Strychneae was resolved as polyphyletic due to the positioning of *Gardneria* placed outside of the *Strychnos* + *Neuburgia* clade. *Strychnos* was supported as strongly monophyletic with 12 strongly supported clades, but the relationships among many of these clades were not well resolved. Most of the 12 sections in the current infrageneric classification system of *Strychnos* were resolved as non-monophyletic, indicating the need for a revision of the sectional divisions. Characters common in species placed within the relatively more nested clades include a non-climbing habit, invasion of non-rainforest habitats, absence of tendrils, absence of secondary phloem, and elongated corolla tubes, suggesting that these characters are relatively derived conditions in the genus. Inflorescence position, fruit size, and fruit wall thickness are extremely variable and were distributed among various clades in our phylogeny. Stamen, pistil, seed and seed coat, and phytochemical characters have figured prominently in the taxonomy of the genus, but are as yet incompletely described, thus preventing significant inference about their evolution. Most of the 12 well-supported clades within *Strychnos* are restricted to specific continents, sometimes with limited dispersion between neighboring continents, suggesting a history of repeated cross-oceanic dispersal or vicariance patterns. The Neotropical clades nested within the African clades have the shortest branches and the most unresolved topologies, probably indicating relatively recent radiation in the Neotropics.

Keywords—Asterids, curare, evolution, Gentianales, ITS, molecular systematics, strychnine.

Loganiaceae R.Br. ex Mart. (Gentianales: Asteridae) is a pantropical family of plants that comprises ca. 460 species in 16 genera distributed in four tribes: Antonieae Endl. (four genera; 14 species), Loganieae Endl. (eight; ∼150), Spigelieae Dumort. (one; 60–85), and Strychneae Dumort. (three; ∼220; Struwe et al. 2018). The last includes the pantropical genus *Strychnos* L. which is the largest genus of Loganiaceae with approximately 200 species. Species of *Strychnos* range from climber shrubs or lianas (1.5–45 m height × 4–120 m long, up to 50 cm in diam), subshrubs or shrubs (0.3–2 m height), and trees (2–40 m height, up to 100 cm in diam), with some species presenting more than one growth form according to the surrounding habitat (Leeuwenberg 1969; Krukoff 1972). Their habitats include forests (wet or dry, flooding or not), savannas, and grasslands, from sea level to 2000 m elevation. The geographical distribution of species richness in *Strychnos* is as follows: about 75 species in tropical Africa and Madagascar (Leeuwenberg 1969), 46 species in South and Southeast Asia (Leenhouts 1962; Tirel-Roudet 1972; Bisset et al. 1973; Khánh 1987), four species in northern and northeastern Australia (Conn et al. 1996), and 85 species in the Americas, from Mexico southwards to Argentina (Krukoff and Monachino 1942, and supplementary work after that; Huft 1988; Brant and Davidse 2009; McPherson 2011; Guimarães et al. 2015; Brandão and Rapini 2017).

From a morphological standpoint, *Strychnos* is a well-defined group, bearing relatively consistent generic characters throughout its distribution. Diagnostic features include simple, entire, and opposite leaves; acrodromous, 3–5(–7)-plinerved leaf venation; herbaceous or lignified, hook-shaped tendrils usually present instead of some of the leaves, these arranged solitary, paired, or in 2–3(–4) successive pairs along the stem; interpetiolar stipules reduced to an often ciliate and straight line connecting the petiole bases; cymose inflorescences (dichasial or thyrsoid); relatively small (0.3–2.0(–3 cm)), light-colored, (4–)5-merous and sympetalous flowers; (1–)2-celled ovaries; and small (1–2 cm) to large berries (6–10(–20) cm; Struwe et al. 2018). Other features that are commonly present in *Strychnos* are conspicuous nodal ‘leaf platforms’ formed by the thickened and woody petiole bases that support axillary branches after the leaves have fallen (Krukoff and Monachino 1942) and wood with interxylary phloem (Mennega 1980).

The chemical properties of *Strychnos* have been of great interest to naturalists since at least since the 16th century (see Buckingham 2007 for a cultural review). The genus was first described by Linnaeus (1753) based on the Asian species *Strychnos nux-vomica* L., the original source of the deadly toxin strychnine; indeed, the generic name was derived from a Greek word used for various solanaceous poisonous plants

(Gledhill 2002). Two major chemical groups of alkaloids produced by *Strychnos* species are the strychnine-like and curare-like types.

Strychnine is an indole alkaloid containing a tertiary ammonium and is associated mostly with Asian species of *Strychnos,* including *S. nux-vomica, S. ignatii* P.J.Bergius, and *S. wallichiana* Benth. (Bisset and Phillipson 1976), but also the African species *S. icaja* Baill. (Bisset 1980). When ingested, it presents convulsant, often lethal effects and is well known for its use as a poison to control rat and other vermin populations (CDC 2018); it also has been famously used to commit murder, in both historical and fictional accounts (e.g. Christie 1920; Bellandi 1990).

The curare-like alkaloids contain a quaternary ammonium and upon ingestion exhibit muscle-relaxant paralyzing effects (Bisset and Phillipson 1971). They are mostly found in American and African species of *Strychnos* (Bisset and Phillipson 1971). At least 21 such American species, most notably *S. toxifera* R.H.Schomb. ex Benth., are used as primary or secondary ingredients in the dart poison curare (Krukoff 1972). While tertiary alkaloids are mainly found in the branches, stem bark, fruits, and seeds, quaternary alkaloids occur mainly in the lower stem bark and roots (Marini-Bettòlo and Bisset 1972).

Infrageneric Classification—Multiple detailed taxonomic studies have been conducted in *Strychnos*, but nearly always from a regional perspective. In the last 150 yr, successive sectional classifications have erected a total of 27 sections, four subsections, and seven series to group the hundreds of species of the genus (see Table 1 for review).

Progel (1868) published the first infrageneric treatment of *Strychnos*, dividing the then-known 31 American species into three sections based mainly on the ratio of length of the corolla tube to the length of the corolla lobes: 1) *S.* sect. *Breviflorae* with proportionally short corolla tubes, 2) *S.* sect. *Longiflorae* with long corolla tubes, and 3) *S.* sect. *Rouhamon* with an intermediate, more equal, ratio. Solereder (1892–1895) expanded the geographical coverage of Progel's system by placing examples of 65 species from different continents into Progel's three sections (Table 1). Solereder also changed the name of *S.* sect. *Rouhamon* to *S.* sect. *Intermediae*, perhaps to highlight its intermediate condition with respect to corolla tube/lobe length ratio. In a monograph of American *Strychnos*, Krukoff and Monachino (1942) followed the systems of Progel and Solereder and assigned seven new species to *S.* sect. *Longiflorae* and one species to *S.* sect. *Breviflorae*.

Hill (1911) divided the known Asian species into two practical groups without formal names based on the same corolla criteria as used by Progel and Solereder. Later, he published an updated classification of the Asian *Strychnos* (Hill 1917), to which he added many new species, grouping them into four new sections (*S.* sect. *Brevitubae*, *S.* sect. *Lanigerae*, *S.* sect. *Penicillatae*, and *S.* sect. *Tubiflorae*) without using any of the existing American sectional names (Table 1). While still relying on corolla tube/lobe length ratio to separate *S.* sect. *Brevitubae* (short tube) from *S.* sect. *Tubiflorae* (long tube), species with intermediate ratios were separated by the indumentum type on the inside of the corolla. *Strychnos* section *Lanigerae* had long woolly hairs in the throat, while *S.* sect. *Penicillatae* had a line of erect bristle-like hairs either across the base or across the middle of the lobes.

Duvigneaud (1947) erected *Strychnos* sect. *Malacocladae* for eight African species with short corolla tubes and distinguished it from *S.* sects. *Breviflorae* and *Brevitubae* by differences in tendril arrangement, spine presence, inflorescence position, corolla indumentum, and filament size. He subsequently published a new classification system for *Strychnos* (Duvigneaud 1952) that included 21 sections (16 new) and seven new series, enlarging the circumscription of *S.* sects. *Penicillatae* (to include both Asian and African species) and *Breviflorae* (with the addition of one African species; Table 1). He also proposed a model of evolutionary trends in tendril and spine morphology, and flower and fruit organization, while criticizing the frequent use of the corolla tube/lobe length ratio to define sections.

Drawing on previous classifications, Leeuwenberg (1969) recognized 12 sections of *Strychnos*. In his classification, he expanded the geographic ranges of Asian *S.* sects. *Brevitubae* and *Lanigerae* by adding African species, standardized the description of comparative characters, and corrected some nomenclatural errors (Table 1). *Strychnos* section *Tubiflorae* was combined with *S.* sect. *Longiflorae*, and this large section was renamed *S.* sect. *Strychnos*, since it includes the type species of the genus, *S. nux-vomica*. Because of nomenclatural priority, he reinstated the sectional name *S.* sect. *Rouhamon* Progel over Solereder's *S.* sect. *Intermediae*. He also subsumed the genus *Scyphostrychnos* within *Strychnos*, as a monotypic section, and relegated several of Duvigneaud's sections to the synonymies of *S.* sect. *Rouhamon* and *S.* sect. *Breviflorae* (Table 1). Although his classification was based on a variety of characters, Leeuwenberg continued to use corolla tube/lobe length ratio as an important character to distinguish several large sections (*S.* sects. *Breviflorae*, *Brevitubae*, *Rouhamon*, and *Strychnos*). Of Leeuwenberg's 12 sections, 11 occur in Africa (*S.* sect. *Strychnos* is absent), and six are endemic to that continent (*S.* sects. *Densiflorae, Dolichanthae, Spinosae*, and the monotypic *S.* sects. *Aculeatae*, *Phaeotrichae*, and *Scyphostrychnos*). No section in Leeuwenberg's classification has species from three or more continents.

Working on the American species of *Strychnos*, Krukoff and Barneby (1969) erected two subsections within *S.* sect. *Breviflorae* based on the texture of the testa: *S.* subsect. *Breviflorae* (crustaceous and cartilaginous testa) and *S.* subsect. *Eriospermae* (fibrous testa). These were retained in Krukoff's (1972) revision of American *Strychnos*, which otherwise adopted Leeuwenberg's (1969) classification. However, in an update of that classification, Leeuwenberg and Leenhouts (1980) omitted Krukoff and Barneby's subsections of *S.* sect. *Breviflorae* (Table 1).

In his review of *Strychnos* for Flora of Vietnam, Khánh (1987) organized 19 species into five sections and two subsections using combined morphological and chemical characteristics. He erected the new monotypic *S.* sect. *Angustiflorae* based on the presence of alkaloids with yellow angustine-type bases in *S. angustiflora* Benth. (as opposed to the strychnine-type found in other Asian *Strychnos* species; see reviews by Bisset and Phillipson 1971, 1976). He also erected two subsections within *S.* sect. *Strychnos* (*S.* subsects. *Nitidae* and *Wallichianae;* the latter included the generic type species *S. nux-vomica* and therefore should have been called *S.* subsect. *Strychnos*; Table 1). Finally, he moved some species between sections (e.g. *S. polyantha* Pierre ex Dop from *S.* sect. *Lanigerae* to *S.* sect. *Brevitubae* and *S. umbellata* (Lour.) Merr. from *S.* sect. *Brevitubae* to *S.* sect. *Lanigerae*), and described two new species.

TABLE 1. Sectional classification systems in *Strychnos* from 1868 until current date. Bold names were accepted by the author of the publication; empty cells indicate sectional taxa not treated by the author. Note that section *Strychnos* should have been formed automatically as soon as sections were used in *Strychnos*, but it was not formally included as a section in a classification until Leeuwenberg (1969, marked with an asterisk). Leeuwenberg and Leenhouts (1980) republished Leeuwenberg (1969) without changes (subsections by Krukoff and Barneby (1969) were not expanded by the authors).

Progel (1868)	Solereder (1892–1895)	Hill (1917)	Duvigneaud (1947)	Duvigneaud (1952)	Krukoff and Barneby (1969)	Leeuwenberg (1969)	Khánh (1987)
				Aculeatae		***Aculeatae***	
							Angustiflorae
				Booneae		(included in *Rouhamon*)	
Breviflorae	***Breviflorae***		***Breviflorae***	***Breviflorae***		***Breviflorae*** (including *Ligustroides*, *Malacocladae*, *Micranthae*, *Mites*)	
					Breviflorae* subsect. *Breviflorae		
					Breviflorae* subsect. *Eriospermae		
		Brevitubae	***Brevitubae***	***Brevitubae***		***Brevitubae***	
				Densiflorae		***Densiflorae***	
				Dolichanthae		***Dolichanthae*** (including *Syringiflorae*)	
				Floribundae		(included in *Rouhamon*)	
				Heterodoxae		(included in *Rouhamon*)	
				Heterophyllae		(included in *Lanigerae*)	
	Intermediae (renamed section *Rouhamon*)		***Intermediae***	***Intermediae***		(included in *Rouhamon*)	
		Lanigerae	***Lanigerae***	***Lanigerae***		***Lanigerae*** (including *Heterophyllae*)	
				Ligustroides		(included in *Breviflorae*)	
Longiflorae	***Longiflorae***		***Longiflorae***	***Longiflorae***		(included in *Strychnos*)	
			Malacocladae	(included in *Micranthae*)		(included in *Breviflorae*)	
				Micranthae		(included in *Breviflorae*)	
				Mites		(included in *Breviflorae*)	
		Penicillatae	***Penicillatae***	***Penicillatae***		***Penicillatae***	
				Phaeotrichae		***Phaeotrichae***	
Rouhamon	(renamed as *Intermediae*)		(included in *Intermediae*)	(included in *Intermediae*)		***Rouhamon*** (including *Booneae*, *Heterodoxae*, *Floribundae*, *Intermediae*, *Variabiles*)	
				Sambae		(included in *Brevitubae*)	
						Scyphostrychnos	
				Spinosae		***Spinosae***	
						Strychnos* (including *Longiflorae*, *Tubiflorae*)	
							Strychnos* subsect. *Nitidae
							Strychnos* subsect. *Wallichianae
				Syringiflorae		(included in *Dolichanthae*)	
		Tubiflorae	***Tubiflorae***	***Tubiflorae***		(included in *Strychnos*)	
				Variabiles		(included in *Rouhamon*)	

Phylogeny—Although the taxonomic literature available for *Strychnos* is considerable, there has been relatively little published on the phylogeny of the genus, but genetic data were generated for an unpublished thesis (Frasier 2008). Adebowale et al. (2016b), using sequences from Frasier (2008) and newly generated data, was the first published phylogenetic study focused exclusively on *Strychnos*. In that study, the authors used nuclear internal transcribed spacer (ITS) and chloroplast DNA markers (*trnL-trnF*, *trnS-trnG*) to explore relationships among African species (~60% of the continent) on continental (48 species; ITS2 African dataset) and regional scales (14 species; ITS2 and/or plastid markers dataset) and investigated possible evolutionary trends in habitat and morphology. The monophyly of *Strychnos* was strongly supported, however, only *S.* sects. *Densiflorae* and *Spinosae* had their monophyly well supported in the southern African dataset, while *S.* sects. *Dolichanthae* and *Lanigerae* had their monophyly strongly supported in the ITS2 African dataset. In both the plastid and nuclear datasets, forest species with climber and/or shrub habits and small fruits were consistently grouped within the basally diverging clades, while savanna species with tree/shrub habit and large fruits placed within relatively more nested positions (Adebowale et al. 2016b).

It is clear that a much more densely sampled global phylogeny of *Strychnos* is needed to achieve a robust classification of this economically and ecologically important genus, and also as a critical basis to improve understanding of various aspects of its evolutionary diversification (e.g. morphology, anatomy, phytochemistry, and biogeography). Our goals were to: 1) substantially increase both the taxonomic and geographical scope of molecular sampling within *Strychnos*; 2) identify well-supported clades within the genus; 3) further test the monophyly of *Strychnos* and the sections of Leeuwenberg and other currently accepted infrageneric groups; 4) identify diagnostic characters of well-supported clades and develop preliminary hypotheses about the biogeography and morphological diversification of the genus.

Materials and Methods

Taxon Sampling—We sampled 147 herbarium accessions representing 122 species and 12 genera. Ingroup accessions represented 127 samples from 103 species of *Strychnos* (~50% of its species diversity) distributed among all 12 of Leeuwenberg's (1969) sections (see Appendix 1); 18 *Strychnos* species were represented by more than one accession. Geographic provenances of the ingroup accessions were as follows: 45 species from Africa (53 samples, one accession of *S. spinosa* cultivated in USA); 13 species from Asia (18 samples); four species from Australia and the Pacific (five samples); seven species from Madagascar (eight samples); and 38 species from the Neotropics (43 samples; including two varieties of *S. mitscherlichii*). Vouchers for the sequences JF937942 and JF937984 had their species determination changed by Adebowale et al. (2016a, 2016b) as *S. henningsii* and *S. angolensis*, respectively, based exclusively on their molecular and phylogenetic results. We checked both vouchers again and confirmed the determinations of the two specimens as *S. angolensis* and *S. henningsii* respectively, thus, not agreeing with Adebowale's suggestion (see Discussion for more details).

The outgroup included 19 sequences of 18 species from 10 other genera of Loganiaceae and one sequence of *Gelsemium sempervirens* (L.) J.St.-Hil. of the family Gelsemiaceae, providing good theoretical and practical support for a proper rooting of the phylogeny and testing of the monophyly of *Strychnos*. The sequences of *Gelsemium sempervirens* (DQ358881), *Geniostoma rupestre* (DQ499095, DQ499096), *Logania albiflora* (DQ358879), *Mitreola petiolata* (AF054635), and all *Spigelia* accessions (AF177992, AF178008, AF178000, AF177991, and AF178006) were from the studies of Gould and Jansen (1999), Robbrecht and Manen (2006), and Wright et al. (2006) and downloaded from GenBank (Appendix 1). Species identifications of sampled accessions were made by us using local and regional floras and revisions; we also sometimes relied on previous identifications made by taxonomic specialists of *Strychnos* (Leeuwenberg, Krukoff, etc.). Continental distributions and the sectional affiliations of sampled accessions of *Strychnos* were overlaid on the resulting phylogenetic tree to explore biogeographical patterns and to test the monophyly of the sections, respectively.

DNA Extraction, Amplification, and Sequencing—DNA was extracted from fresh leaf material dried in silica gel or from herbarium specimens (see Frasier 2008 for details). The PCR amplifications of the entire ITS region (ITS1, 5.8S, and ITS2) were performed in 25 µl reaction mixtures that contained (final concentration) 1× buffer, 2.5mM $MgCl_2$, 0.25mM dNTPs, 0.025 µg/µl BSA, 0.8 µM of each primer (Sigma, Saint Louis, Missouri), 0.2 units Taq polymerase, and 1 µl of DNA template (reagents from Fisher Scientific, Fair Lawn, New Jersey, unless otherwise noted). Primers described by Nickrent et al. (1994) were used to amplify the entire ITS region. When the entire ITS region could not be amplified, slightly modified internal primers corresponding to White et al.'s (1990) ITS2 and ITS3 were used in conjunction with the Nickrent primers. A different forward primer for *Strychnos aculeata* was used: 5′-GGAAGTAGAAGTCGTAACAAGG-3′; this is a slightly modified version of ITS5 (White et al. 1990). This primer was employed for *S. aculeata* because amplification of the first half of the ITS region for two different individuals produced a band that was slightly shorter than those of all the other *Strychnos* species and it sequenced poorly. Using this modified primer, combined with Nickrent's primers, produced a band of the expected length that was sequenced successfully, and, when run through BLAST against NCBI's GenBank nucleotide database, was a close match to *Strychnos* (and not potential contaminants). Some PCR reactions were also prepared using Taq ReadyMix (Sigma, Saint Louis, Missouri) according to the manufacturer's recommended concentration. The ITS thermocycling profile for all primer combinations was: 97°C for 1 min; 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min; extend for 4 min at 72°C; hold at 4°C. All cycling programs were run on an Applied Biosystems GeneAmp System 9700 (Waltham, Massachusetts).

Cycle sequencing reactions (10 µl) contained 3.2 pmol primer (same primers as for PCR), 2 µl of ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Waltham, Massachusetts), DNA template, and sterile water to obtain the final reaction volume. The amount of DNA template added was determined by the strength of the bands visualized on the gel containing the PCR products: 3 µl for weak bands, 2 µl for normal bands, and 4 µl of 1:10 diluted PCR products for strong bands. The cycle sequencing program was 95°C, 1 min; 32 cycles of: 96°C for 10 s, 50°C for 5 s, 60°C for 3 min; 4°C indefinitely. The products were cleaned using Centri-sep columns (Princeton Separations), dried in a Speedvac (Savant) for 20 min, and resuspended in 15 µl of formamide before being sequenced using an ABI 3100 Genetic Analyzer at the Biotech Center, Rutgers University, or outsourced to Genewiz (Plainfield, New Jersey). Raw sequence chromatograms were visualized and edited using Sequencher v. 4.1.4 to v. 4.7 (GeneCodes).

Sequence Alignment—The small (SSU) and large (LSU) ribosomal subunits flanking the ITS regions were excluded from the alignment and phylogenetic analyses (but not 5.8S region). Sequences were initially aligned in the online version of MAFFT v. 7.0 (Katoh et al. 2017) using the G-INS-i strategy (Katoh et al. 2005), which assumes that input sequences are globally alignable, with the option "try to align gappy regions anyway" selected (in alpha-testing). The G-INS-i alignment (with 706 bp) was then realigned in the MAFFT application within Geneious 7.1.9 (Biomatters Ltd., Auckland, New Zealand), allowing the program to automatically select the most suitable algorithm, which was FFT-NS-I (Katoh et al. 2002), resulting in an alignment of 683 bp.

Phylogenetic Analyses—To select the best-fitting model under the Bayesian information criterion (BIC), jModelTest 2.1.10 (Darriba et al. 2012) was used, which called for a GTR + I + G model. MrBayes v. 3.2.6 (Huelsenbeck and Ronquist 2001), as implemented in Geneious v. 11.1.5 (Biomatters Ltd.), was used to perform a Bayesian phylogenetic analysis, run for three million generations (four chains), with trees sampled every 500 generations, parameters set to apply the best-fitting model (GTR substitution model, rate variation=invgamma with four categories), and the burn-in = 25%. Only branches with posterior probability (PP) values ≥ 0.95 were considered strongly supported (Huelsenbeck and Rannala 2004). Trees were rooted with the sequence of *Gelsemium sempervirens* due to the close phylogenetic relationship of Gelsemiaceae to Loganiaceae, the former having been previously included within the latter (see Struwe et al. 1994, 2018; Yang et al. 2016 for a review of Gentianales relationships). Tracer v. 1.7 (Rambaut et al. 2018) was used to analyze trace files generated by Bayesian MCMC runs in MrBayes to assess convergence to the desired posterior distribution.

Results

ITS Alignment, Model Selection, and Bayesian Convergence Diagnostics—Sequences ranged in length from 233–621 bp (mean length 542 bp), with 50 accessions represented by partial sequences (either ITS1 or ITS2) due to poor sequence quality; G+C content of the alignment was 62.5%, with 10.2% gaps. The final alignment was 683 bp long. The standard deviation of split frequencies was ~0.01 after three million generations, the potential scale reduction factor (PSRF) for all parameters equal to one, and the effective sample sizes (ESS) > 300 for all parameters, all suggesting adequate convergence to and sufficient sampling of the posterior distribution (Ronquist et al. 2011). Trace plots for all sampled parameters did not show sharp fluctuations, indicative of good mixing.

Use of Partial Sequences in the Data Analysis—Generating sequence data for a global representation of *Strychnos* species mostly relied on the use of herbarium specimens collected between 1945 to 2005. Due to the condition of various samples, it was unavoidable that some species were only represented by partial sequences. The partial sequences were included in the phylogenetic analysis as any reduction in accuracy was predicted to be confined to the incomplete taxa and not affecting those with complete sequence data as was shown by Wiens (2003). Additionally, incomplete data were found to be problematic when too few phylogenetically informative characters were sampled (Wiens 2003). In our work, missing data were concentrated in either ITS1 or ITS2 (not randomly distributed throughout the dataset) and as both these gene regions have phylogenetically informative characters, having at least a partial ITS representation of a taxon should allow its placement in the tree to be reliably estimated.

To confirm the impact of the use of partial sequence data on the analysis, samples missing either the complete ITS1 or ITS2 sections were excluded from a second Bayesian analysis run with the same parameters as for the full dataset, but with 2 million generations. The topology and placement of taxa in the resulting tree (data and results not shown) were compatible with that generated by the full dataset including both complete and partial sequences. The outgroup relationships were congruent and the 12 *Strychnos* clades were present though some support values were lower, such as for Clade 10 (full dataset = 1.00 PP, partial data removed = 0.89 PP). The only differences of note were within Clade 6. In the tree from the reduced dataset, there was more resolution within Clade 6, with 6B (only represented by *S. innocua*) placed sister to the remaining subclades but with a low support value (0.86 PP).

Outgroup Relationships**—**The most basal branches within Loganiaceae were collapsed in a trichotomy of three strongly supported clades (Fig. 1). One clade (1.00 PP) comprises *Gardneria*, which is currently placed in tribe Strychneae; the second clade (1.00 PP) comprises all sampled representatives of the pantropical tribe Antonieae, with *Bonyunia* being resolved as paraphyletic (*Usteria* was not sampled). The third clade (0.94 PP) consists of the sampled representatives of tribes Loganieae, Spigelieae, and the remaining representatives of Strychneae, within which a marginally supported (0.92 PP) subordinate clade comprises tribes Loganieae and Spigelieae, while a strongly supported clade (0.99 PP) groups *Neuburgia* and *Strychnos*, which were resolved as sister genera. The monophyly of tribe Loganieae, dominated by Australian taxa, was weakly supported (0.87 PP); while that of the monogeneric tribe Spigelieae was strongly supported on a long branch (1.00 PP). Tribe Strychneae was resolved as non-monophyletic due to the placement of *Gardneria* (Fig. 1).

Strychnos Phylogeny and Distribution Patterns**—**The analysis strongly supported the monophyly of *Strychnos* (0.98 PP; Fig. 1), within which a basal dichotomy was resolved dividing two strongly supported clades, one (numbered here as Clade 1) containing eight American species (1.00 PP), the other with the remainder of *Strychnos* accessions (0.98 PP; Fig. 1). Within the latter clade, the remaining species were distributed among 11 strongly supported (0.98–1.00 PP) and mutually exclusive clades or branches with single terminals (numbered 2–12; Figs. 1–3). Relationships among these 11 clades or branches, which differed greatly in size, from a single species in Clade 3 to 35 species in Clade 6, were mostly poorly resolved. However, marginal support was obtained for a sister relationship between Clades 5 and 6 (0.91 PP; Fig. 2) and a clade grouping Clades 9–12 (0.93 PP; Fig. 3), while a sister relationship between Clades 11 and 12 was more strongly supported (0.96 PP).

Within Clade 1 (Neotropics; 1.00 PP), *S. brasiliensis* (1A), the type species of *S.* sect. *Breviflorae* subsect. *Breviflorae*, was placed sister to a well-supported clade (Clade 1B, 0.98 PP) comprising all of the sampled species of *S.* sect. *Breviflorae* subsect. *Eriospermae* (Fig. 1). The other sampled species of *S.* sect. *Breviflorae* were dispersed among Clades 4, 5, 6, 8, 9, and 10 (seven African species without subsectional assignments in Leeuwenberg and Leenhouts 1980) and Clade 12 (five Neotropical species classified in *S.* subsect. *Breviflorae*; Figs. 2–3).

Clade 2 (Africa; 1.00 PP) contains all the sampled species of *S.* sect. *Dolichanthae* and *S. camptoneura* of the monotypic *S.* sect. *Scyphostrychnos* (Fig. 1). Within Clade 2, the analysis placed *S. xantha* sister to a clade (0.96 PP) grouping the other species. The strongly supported nesting of *S. camptoneura* among several of the latter species (1.00 PP) renders *S.* sect. *Dolichanthae* paraphyletic. Clade 3 (Africa; 1.00 PP) consists of two accessions of *S. aculeata* of the monotypic *S.* sect. *Aculeatae* (Fig. 2).

Within Clade 4 (Africa, Asia, Australia; 0.98 PP), the African-Asian species *S. potatorum* of *S.* sect. *Rouhamon* and the African species *S. icaja* of *S.* sect. *Breviflorae* were positioned outside of a strongly supported (1.00 PP) clade comprising five Asian representatives (*S. lucida* also reaches Australia) of *S.* sect. *Strychnos* (Fig. 2). Relationships within this last clade, which includes well-known toxic species, such as *S. ignatii* and the generic type species, *S. nux-vomica*, were mostly unresolved, although *S. angustiflora* and *S. lucida* appear to be closely related.

Clade 5 (Africa; 1.00 PP) comprises six species, four of *S.* sect. *Rouhamon*, as well as *S. cuniculina* of *S.* sect. *Brevitubae* and *S. malchairii* of *S.* sect. *Breviflorae* (Fig. 2); all three sections appear to be non-monophyletic in our analysis. Within Clade 5, the analyses resolved two strongly supported clades (1.00 PP for each; Fig. 2): one comprising three species of three different sections, the other three species of *S.* sect. *Rouhamon*.

Clade 6 (Africa, Asia, Australia, Madagascar, and the Americas, 1.00 PP) has a pantropical distribution and comprises 35 species of nine different sections (Fig. 2), making it the largest and most broadly distributed of the 12 numbered clades. Within Clade 6, the analysis placed species among five mutually exclusive subordinate clades plus a branch with *S. phaeotricha* as its single terminal (lettered A–F in Fig. 2), which were resolved with marginal (6A; 0.91 PP) to strong support (6B, 6C, 6D + 6E + 6F; 1.00 PP). Relationships among these lettered clades were mostly unresolved, however, a strongly supported branch groups Clade 6F as sister to *S. phaeotricha* (6D) and Clade 6E.

Clade 6A includes two African species, *S. mitis* of *S.* sect. *Breviflorae* and *S. staudtii* of *S.* sect. *Densiflorae*. The other sampled species of *S.* sect. *Densiflorae*, four African and one Madagascan, were grouped in Clade 6B. Clade 6C comprises the four species of *S.* sect. *Spinosae*, thus supporting the monophyly of that section. “Clade 6D,” just a single accession of *S. phaeotricha*, corresponds to the monotypic African *S.* sect. *Phaeotrichae*. Clade 6E comprises four American species of *S.* sect. *Strychnos* and *S. panurensis* of *S.* sect. *Rouhamon*. Clade 6F comprises accessions of 19 species from Africa, Madagascar, Asia, and Australia. Most of the species in this clade are classified in *S.* sect. *Lanigerae*, but that section is rendered non-monophyletic by the nesting of species of *S.* sects. *Brevitubae*, *Penicillatae*, and *Strychnos* within it. However, a strongly supported clade groups the African species *S. ngouniensis*, *S.*

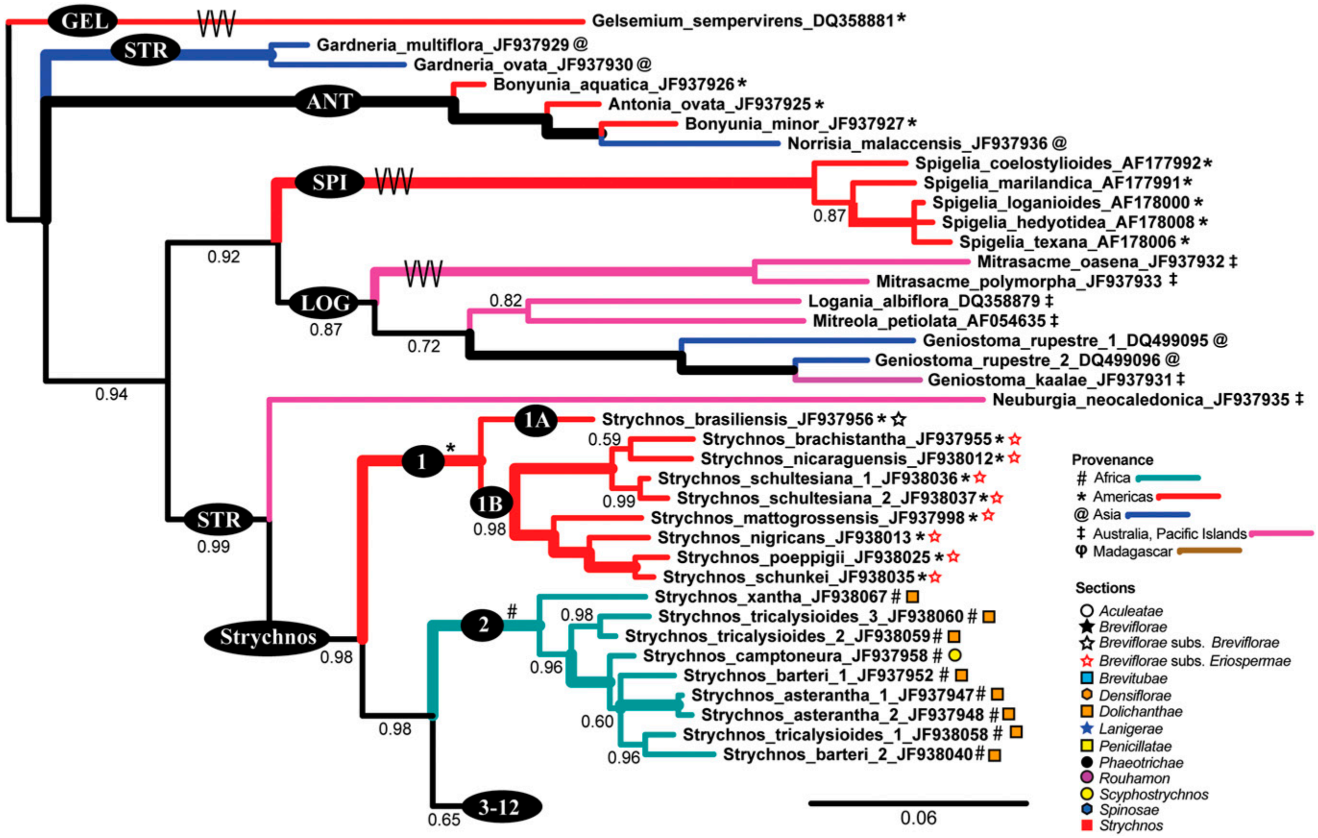

FIG. 1. Partial results from a phylogenetic reconstruction of *Strychnos* and other Loganiaceae genera from a 50% majority rule consensus tree based on ITS sequences from Bayesian analysis (see Figs. 1–3 for complete tree). Posterior probabilities (PP) above or equal to 0.50 are indicated at each branch; thickest branches have 1.00 PP. Symbols above branches indicate area mapping based on distribution of species included in the clade (note, this is not strict character mapping, just an aid in understanding general distributions of clades). Symbols after species names and branch colors indicate provenance of DNA samples and the most recent sectional classification (see legend for symbol explanations). Clade names are indicated in black ovals on branches: ANT = *Antonieae*, GELS = Gelsemiaceae, LOG = *Loganieae*, SPI = *Spigelieae*, STR = *Strychneae, Strychnos* for *Strychnos*, and Clades 1–12 within *Strychnos*. Shortened branches are indicated with a squiggle for Gelsemiaceae, *Spigelia*, and *Mitrasacme*, allowing them to fit on the page.

soubrensis, and *S. splendens*; a second groups the Asian species *S. borneensis* and *S. polytrichantha*; a third groups the Asian species *S. lanata*, *S. umbellata*, *S. vitiensis*, and one accession of *S. minor*; and a fourth groups the Asian species *S. axillaris*, *S. villosa*, *S. wallichiana*, and three other accessions of *S. minor*.

Clade 7 (Africa, 1.00 PP) comprises four species of *S.* sect. *Brevitubae* (Fig. 3). Clades 8–11 (all 1.00 PP) each contain only two species, except Clade 10 with three species: Clade 8 (Africa, Madagascar) comprises one accession of *S. angolensis* of *S.* sect. *Breviflorae* and the African/Madagascan species *S. decussata* of *S.* sect. *Rouhamon*; Clade 9 (Africa) *S. boonei* of *S.* sect. *Rouhamon* and *S. campicola* of *S.* sect. *Breviflorae*; Clade 10 (Africa) a second accession of *S. angolensis*, *S. malacoclados*, and the African/Madagascan species *S. henningsii*, all of *S.* sect. *Breviflorae*; and Clade 11 (Africa, Madagascar) the African/Madagascan species *S. myrtoides* and the Madagascan species *S. mostueoides*, both of *S.* sect. *Penicillatae*.

Clade 12 (Africa, Australia, Madagascar, Neotropics; 1.00 PP) is the second-most species-rich clade in our phylogeny (Fig. 3). It contains species classified in *S.* sects. *Penicillatae*, *Rouhamon*, *Strychnos*, and *S.* sect. *Breviflorae* subsect. *Breviflorae*. A strongly supported subordinate clade contains all species of Clade 12 except for the Madagascan species *S. diplotricha* and *S. pentantha* of *S.* sect. *Penicillatae*. The African species *S. afzelli* and *S. matopensis* plus the Australian *S. arborea*, all members of *S.* sect. *Penicillatae*, grouped together in a weakly supported clade (0.64 PP) that is sister to a larger Neotropical clade. The strongly supported Neotropical clade (1.00 PP) includes all 25 American species of Clade 12. Most species in this clade are classified in *S.* sect. *Strychnos*, but three species of *S.* sect. *Rouhamon* (including *S. guianensis*, type species of *S.* sect. *Rouhamon*) and five species of *S.* sect. *Breviflorae* are also included. The Neotropical clade displays mostly short internal branches, and relationships among its species were not well resolved by the analysis.

DISCUSSION

ITS Region—The high degree of sequence divergence in the ITS region of the nrDNA among closely related species has been a benefit to a broad range of phylogenetic studies (Baldwin et al. 1995). Within the Gentianales, the region provided resolution within many genera and tribes (e.g. Molina and Struwe 2009; Popovkin et al. 2011; Calió et al. 2017). Moreover, compensatory base changes (CBC) that maintain the well-conserved secondary structure of ITS2 have been found to correlate with species boundaries, resulting in the Compensatory Base Change species concept (Müller et al. 2007; Wolf et al. 2013). The CBC species concept was tested by Adebowale et al. (2016a) to resolve the *Strychnos madagascariensis* complex.

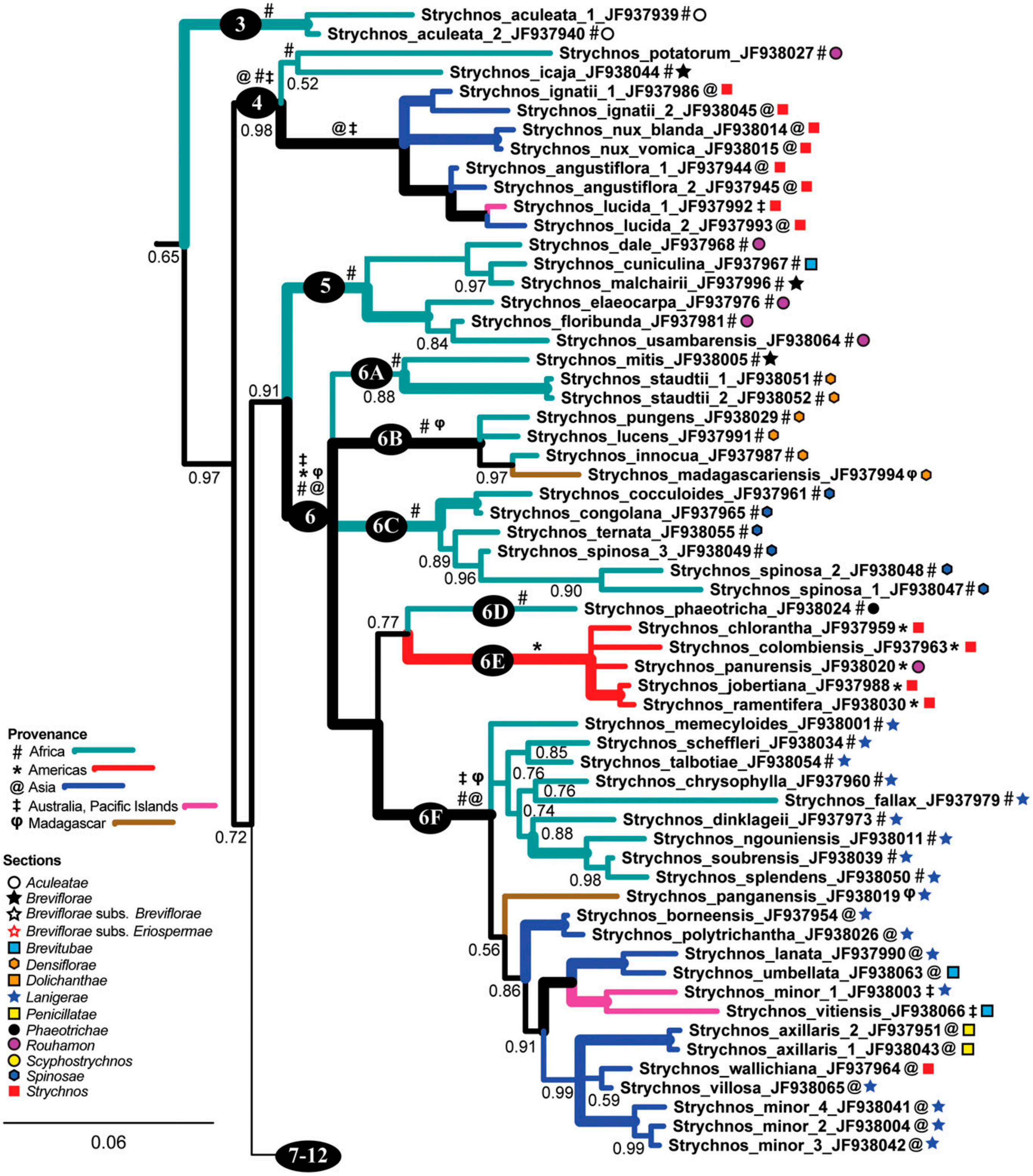

FIG. 2. Partial results from a phylogenetic reconstruction of *Strychnos* and other Loganiaceae genera from a 50% majority rule consensus tree based on ITS sequences from Bayesian analysis (see Figs. 1–3 for complete tree). Posterior probabilities (PP) above or equal to 0.50 are indicated at each branch; thickest branches have 1.00 PP. Symbols above branches indicate area mapping based on distribution of species included in the clade (note, this is not strict character mapping, just an aid in understanding general distributions of clades). Symbols after species names and branch colors indicate provenance of DNA samples and the most recent sectional classification (see legend for symbol explanations). Clade names within *Strychnos* are indicated in black ovals on branches.

Relative to coding regions, ITS is highly prone to indel mutations, requiring the insertion of gaps to maintain positional homology, which is critical for phylogenetic studies (Baldwin et al. 1995). As the sampling of a group of species expands, it can become difficult to align ITS sequences between species that are evolutionarily more distant. Goertzen et al. (2003) stressed the value of dense sampling to enhance the alignment process for ITS. By having a broad and dense data set, one has a better understanding of the variability of the gene region and the advantage of using

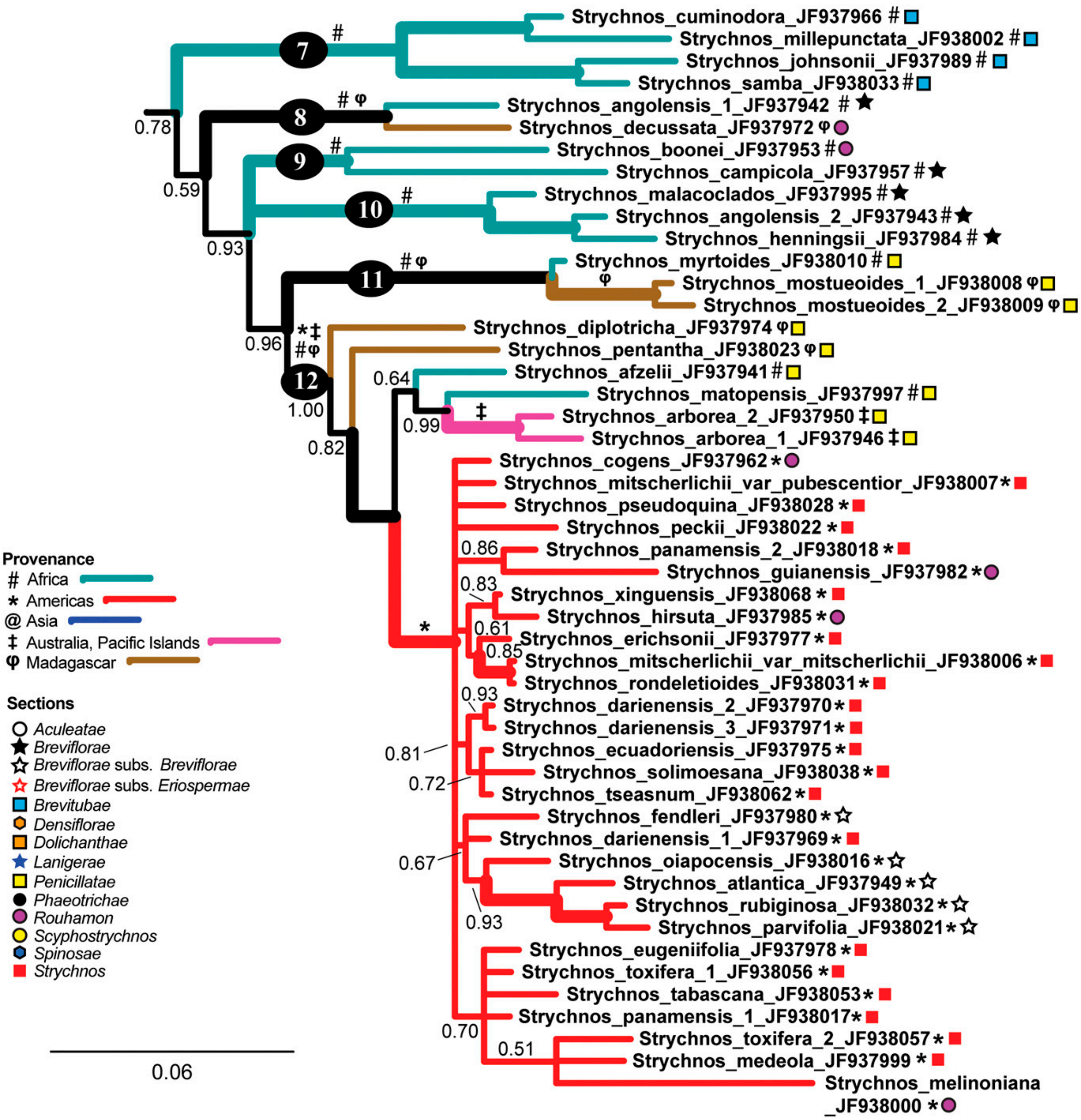

FIG. 3. Partial results from a phylogenetic reconstruction of *Strychnos* and other Loganiaceae genera from a 50% majority rule consensus tree based on ITS sequences from Bayesian analysis (see Figs. 1–3 for complete tree). Posterior probabilities (PP) above or equal to 0.50 are indicated at each branch; thickest branches have 1.00 PP. Symbols above branches indicate area mapping based on distribution of species included in the clade (note, this is not strict character mapping, just an aid in understanding general distributions of clades). Symbols after species names and branch colors indicate provenance of DNA samples and the most recent sectional classification (see legend for symbol explanations). Clade names within *Strychnos* are indicated in black ovals on branches.

intermediary sequences to help with the alignment. In the *Strychnos* work of Frasier (2008), manual coding of ITS secondary structures further enhanced phylogenetic reconstruction in the genus, and this time-consuming but important method was improved upon here by using newly available alignment software that takes secondary structure into account through an automated, reproducible process (see Methods for details).

Although the utility of the ITS region in phylogenetic analyses is well recognized, caution should be exercised when drawing conclusions based solely on this marker. While there are examples where concerted evolution has come to completion (Wendel et al. 1995), Álvarez and Wendel (2003) warned of complications that arise when this has not happened. The persistence of pseudogenes and the presence of more than one rDNA array all contribute to the possibility of inferring incorrect species relationships, a possibility that is increased by polyploidization (Álvarez and Wendel 2003), which is a known phenomenon in *Strychnos* (Gadella 1980). Though our results may be prone to inaccuracies due to the inherent shortcomings of using the ITS region for estimating phylogenies, we present this work as the first taxonomically broad, preliminary hypothesis of phylogenetic relationships in *Strychnos*.

***Tribe Strychneae*—**The placement of *Strychnos* within Loganiaceae is not in question. This is a Gentianales family that has over the last 30 yr shrunk drastically to a core of four tribes and sixteen genera (see Struwe et al. 2018 for a review). However, the placement of the genera within and monophyly of the tribe Strychneae has been consistently unclear in studies over the last few decades and were not well resolved by our results. Tribe Strychneae includes *Strychnos* and two small, mostly Asian genera, *Gardneria* Wall. (five species) and *Neuburgia* Blume (12 species), but the tribe seems to be a non-monophyletic grouping. The position of *Spigelia* L. (mostly Neotropical), the sole member of tribe Spigelieae, has also varied between being nested within Strychneae (Backlund et al. 2000; Gibbons et al. 2012; Yang et al. 2016) or within Loganieae (Oxelman and Bremer 2000; Popovkin et al. 2011), suggesting that at least one of those tribes is paraphyletic. Popovkin et al. (2011) and our analysis resolved the position of *Gardneria* as being not especially close to *Strychnos* and *Neuburgia*, increasing the uncertainty about the proper circumscription of the tribe. The only reported morphological synapomorphy for Strychneae is an indehiscent fruit (Struwe and Albert 1997), but the fruit type varies from berry to drupe (Struwe et al. 2018). The repeated conflict in the placements of *Gardneria*, *Neuburgia*, and *Strychnos* in relation to other Loganiaceae genera calls attention to the need for further study of this group, including additional DNA loci and morphological and chemical data. In all cited studies so far, *Strychnos* has been supported as monophyletic, although sampling of species has been limited when compared to our assessment or to the study of Adebowale et al. (2016b).

***Strychnos Sections and Phylogeny*—**This is the largest study of *Strychnos* to date and suggests that a new sectional system of *Strychnos* is needed to deal with rampant issues of paraphyletic and polyphyletic sections in the current classification (Leeuwenberg 1969; Krukoff and Barneby 1969). This is not a surprise; previous botanists have pointed out that groupings within the genus have been largely based on practical morphological characters, not necessarily evolutionarily informative traits, that helped split this large genus into smaller, more manageable units (see introduction for sectional history).

Of the sections sampled in this phylogeny, only the African *S.* sect. *Spinosae* (Clade 6C) and the American *S.* sect. *Breviflorae* subsect. *Eriospermae* (Clade 1B) were supported as monophyletic. Two of the three monotypic sections, *S.* sect. *Aculeatae* (Clade 3) and *S.* sect. *Phaeotrichae* (Clade 6D), had relatively isolated placements, and therefore might be retained as infrageneric taxa. That is not the case for *S.* sect. *Scyphostrychnos*, with its single species *S. camptoneura*, nested inside the otherwise monophyletic *S.* sect. *Dolichanthae* (Clade 2). Our recovery of a monophyletic *S.* sect. *Spinosae* and nearly monophyletic *S.* sect. *Dolichanthae* are compatible with the results of Adebowale et al. (2016b). The monophyly of *S.* sect. *Spinosae* was also well supported in the analysis of their southern African combined dataset, while *S.* sect. *Dolichanthae* was strongly supported in the analysis of their whole-Africa ITS-only dataset (but *S. camptoneura* was not sampled).

In contrast, *Strychnos* section *Breviflorae* was highly polyphyletic, with the African members located across almost all parts of the tree (Clades 4, 5, 6A, 8, 9, and 10) and with the Neotropical species of *S.* subsect. *Breviflorae* in Clades 1 and 12. Other polyphyletic sections are *S.* sects. *Brevitubae* (Clades 5, 6F, and 7), *Penicillatae* (Clades 6F, 11 and 12), *Rouhamon* (Clades 4, 5, 6E, 8, 9, and 12), and *Strychnos* (Clades 4, 6E, and 12). A similar result was obtained by Adebowale et al. (2016b) from analyses of both the plastid and/or nuclear datasets.

Possibly paraphyletic sections include *S.* sects. *Densiflorae* and *Lanigerae*. In the study of Adebowale et al. (2016b), the monophyly of *S.* sect. *Densiflorae* was well supported in the analysis of the southern African dataset, but when *S. staudtii* was included in the analysis of the ITS2 African dataset, the section was resolved as paraphyletic due to the segregated placement of *S. staudtii*, a similar result with our analysis. The monophyly of the African *S.* section *Lanigerae* was also strongly supported in the analysis of the ITS2 African dataset, but the Asian species that rendered the section paraphyletic in our phylogeny (*S. axillaris* from *S.* sect. *Penicillatae*, and *S. umbellata* and *S. vitiensis* from *S.* sect. *Brevitubae;* Clade 6F) were not included in the Adebowale et al. (2016b) study.

Conversely, some clades recovered by our analysis correspond to some degree with sections recognized by Duvigneaud (1947, 1952), perhaps arguing for their reinstatement and expansion. Examples include Clade 5 with *S. floribunda, S. dale*, and *S. usambarensis* belonging to *S.* sect. *Floribundae* (which might be expanded to include also *S. cuniculina, S. malchairii*, and *S. elaeocarpa*)*;* Clade 6A with *S. mitis* of monotypic sect. *Mites* (which might include also *S. staudtii*); Clade 7 with *S. samba*, type species of *S.* sect. *Sambae* (which might include also *S. cuminodora*, *S. johnsonii*, and *S. millepunctata*); Clade 9 containing *S. boonei*, type species of *S.* sect. *Booneae* (which might include also *S. campicola*); and Clade 10 with *S. angolensis* and *S. malacoclados* belonging to *S.* sect. *Malacocladae* (which might include also *S. henningsii*). Several of Duvigneaud's sectional characters (see further discussion) may be important synapomorphies for these clades within *Strychnos*, but further coding and investigation of morphology are needed for many species.

***Diagnosing the Clades of Strychnos*—**Clade 1 is a strictly Neotropical group of species that are usually forest shrubs or climbing shrubs (except *S. mattogrossensis* that grows as a large liana and *S. brasiliensis* that is a liana, shrub, or tree), with branches and branchlets often quadrangular and conspicuously lenticellate (except *S. nicaraguensis*), bearing mostly solitary or 1 pair of tendrils (1–3 pairs in *S. brachistantha*, absent in *S. brasiliensis* and *S. schultesiana,* unknown in *S. nicaraguensis*) and spines (absent in *S. schultesiana*), and with leaves that usually blacken after drying (Krukoff 1972; Huft 1988). Reproductive characters include terminal inflorescences bearing flowers with proportionally short (with respect to the lobes) corolla tubes that are usually glabrous outside, anthers that are barbate at the base (except *S. mattogrossensis* and *S. schultesiana* with glabrous anthers but the latter with spinule-like appendages at the bases of the theca), glabrous or glabrescent pistils, and small to large-sized, soft to hard-walled, 1- to many-seeded fruits that usually mature yellow or orange (Krukoff 1972; Huft 1988). Besides sharing large fruits, *S. brachistantha* and *S. schultesiana* also present edible fruit pulp with pleasant odor and taste (Krukoff 1972).

Members of Clade 1 are also united in the possession of either a fibrous or cartilaginous testa. Krukoff and Barneby (1969) based *S.* sect. *Breviflorae* subsect. *Eriospermae* on the possession of a fibrous testa (Clade 1B). Adebowale et al. (2016b) found no evidence of the occurrence of a fibrous testa among the African members of *S.* sect. *Breviflorae*, and indeed the African members of the section were not grouped into subsections by Leeuwenberg and Leenhouts (1980). Also, there is no reference for the presence of a cartilaginous testa among African species (Leeuwenberg 1969). Future phylogenetic analyses should expand sampling of American species to test relationships between *S. brasiliensis* ("Clade 1A") and *S. grayi* (both with cartilaginous testa) and *S.* subsect. *Eriospermae* (with fibrous testa), and to evaluate testa characters as possible synapomorphies.

All of the species in the African Clade 2 belong to *S.* sect. *Dolichanthae*, except for *S. camptoneura* from *S.* sect. *Scyphostrychnos.* All species are forest lianas and/or climbing shrubs occupying mostly moist or riverine habitats, with branches and branchlets often not lenticellate, bearing 1–3 pairs of tendrils, and lacking spines (Leeuwenberg 1969). Other characters include congested and few-flowered axillary inflorescences (occasionally also lax and terminal in *S. camptoneura*), glabrous anthers and pistils, and flattened seeds (Leeuwenberg 1969).

While the fruits of species of *S.* sect. *Dolichanthae* are mostly small, soft-walled, and 1–10-seeded, *S. camptoneura* presents a large, hard-walled, and many-seeded fruit used for fish poison in Africa (Leeuwenberg 1969). Other differences include 4-merous flowers (vs. 5-merous in *S. camptoneura*) and well exserted stamens (vs. included). *Strychnos camptoneura* is also the only species in Clade 2 with a 5-lobed constricted corona at the corolla mouth and seeds with a narrow irregular wing. Therefore, while the inclusion of *S. camptoneura* in a recircumscribed *S.* sect. *Dolichanthae* is supported by molecular data, its inclusion would drastically alter the morphological circumscription of the section.

The two accessions of *S. aculeata*, the only species of *S.* sect. *Aculeatae*, clustered together (as "Clade 3") and were placed sister to Clades 4–12. This species is a large forest liana of West and Central Africa bearing 1–3(–4)-paired tendrils, axillary inflorescences, 5-merous flowers, short corolla tubes, glabrous pistils, and large, hard-walled, 1- to many-seeded fruits with flattened seeds, a suite of characters more or less similar to those found in the species of Clade 2 (Leeuwenberg 1969). However, *S. aculeata* is readily distinguished by the presence of prickles on the trunk and stems, and wood with thin medullary rays (Duvigneaud et al. 1952; Leeuwenberg 1969). Its bowl-like fruits are also used for fish poison in Africa, presenting a creamy pulp containing saponin and producing much foam (Leeuwenberg 1969).

Clade 4 unites as sisters an Asian-Australian group and an African group. General habit varies from lianas to small or medium-sized trees with tendrils absent or arranged solitary and usually without spines (present in *S. angustiflora* and *S. lucida*). The species occupy forests and open woodlands (Leenhouts 1962; Leeuwenberg 1969; Conn et al. 1996; Ping-tao and Leeuwenberg 1996). Other shared characters include axillary inflorescences (terminal in *S. angustiflora* and *S. lucida*), exserted anthers, a glabrous pistil, and seeds with sericeous hairs (Leenhouts 1962; Leeuwenberg 1969; Conn et al. 1996; Ping-tao and Leeuwenberg 1996). However, in the Asian-Australian branch, flowers are 5-merous with proportionally long (with respect to the lobes) corolla tubes (except *S. angustiflora*), and small to large, soft to hard, 1–15-seeded fruits that mature orange (Leenhouts 1962; Tirel-Roudet 1972; Conn et al. 1996; Ping-tao and Leeuwenberg 1996). In the African branch, flowers are 4-merous in *S. ignatii* and (4–)5-merous in *S. potatorum* with comparatively short corolla tubes, and small, soft-walled, 1-seeded fruits that mature dark-yellow to black-blue (Leeuwenberg 1969). Note that *S. potatorum* and *S. lucida* are the only species of *Strychnos* with disjunct distributions between continents (Africa and Asia and Asia and Australia, respectively; Leeuwenberg 1969; Conn et al. 1996).

Phytochemical characters may also support Clade 4. *Strychnos nux-vomica, S. ignatii, S. icaja*, together with *S. wallichiana* of Clade 6F, are the main sources for strychnine (Bisset 1980). Strychnine was also detected, but mostly in small amounts, in the extracts of *S. nux-blanda*, *S. lucida* (also from Clade 4), and in the American *S. panamensis* (Clade 12; Marini-Bettòlo et al. 1972; Bisset and Phillipson 1976). *Strychnos icaja* is so far the only African species of *Strychnos* confirmed to synthesize strychnine and a rare derived tertiary alkaloid, 4-hydroxystrychnine, also found in Asian *S. ignatii* (Sandberg et al. 1969a, 1969b; Bisset and Phillipson 1976). In contrast, *S. potatorum* presents mostly curare-type alkaloids and weak strychnine-like activity, a rather common condition among African species (Bisset and Phillipson 1971). Neither strychnine nor its derivatives were detected in *S. angustiflora* (Clade 4), which instead harbors yellow angustine-type bases, a set of alkaloids present mostly in African species (Bisset and Phillipson 1971, 1976). Further investigation is obviously needed to define with more detail the chemical differences between *Strychnos* species and clades, with the need for standardization of extract screening methods for proper comparison among different taxa.

Clade 5 includes large lianas to climbing shrubs or small trees, with unarmed branches bearing solitary to paired tendrils (absent in *S. elaeocarpa*). The species mostly occupy rain forests of West and Central Africa (Leeuwenberg 1969). Inflorescences are few-flowered and axillary (occasionally also terminal in *S. dale, S. malchairii*, and *S. floribunda*), contain 4–5-merous flowers with mostly deeply cordate anthers that are glabrous at the base, and glabrous pistils (Leeuwenberg 1969). An outstanding and apparently invariable character associated with this clade is a small, soft-walled and 1-seeded fruit that usually matures orange and bears seeds with a thin testa that sticks to the yellow fruit pulp.

Within Clade 6A, *S. staudtii* and *S. mitis* are perennial, medium to large-sized trees growing in rain forests of Africa and presenting branchlets devoid of lenticels, spines, and tendrils (Leeuwenberg 1969). Other shared characters include axillary inflorescences (also terminal in *S. mitis*), 4–5-merous flowers, sepals with colleters at the base inside, deeply cordate anthers bearded at the base, glabrous pistils, and small, soft-walled, 1- to few-seeded fruits (Leeuwenberg 1969).

The bulk of *Strychnos* sect. *Densiflorae* species grouped inside Clade 6B (except *S. staudtii*). These species occupy savannas of tropical Africa and Madagascar and are mostly deciduous, small trees or shrubs (except *S. lucens* that grows as a forest liana or climbing shrub), with branches and branchlets usually lenticellate and without tendrils and spines (except *S. lucens* with paired tendrils) (Leeuwenberg

1969). Reproductive characters include axillary or ramiflorous inflorescences (the latter state absent in *S. lucens*), 4–5-merous flowers, sepals without colleters at the base inside, deeply cordate anthers which are glabrous at the base, pilose styles, an often disk-like base of the ovary, and usually large, hard-walled, many-seeded fruits, with granular skin and a slimy edible pulp bearing asperous seeds with very short, erect, and thick hairs (Leeuwenberg 1969).

Interestingly, while the majority of *Strychnos* sect. *Densiflorae* members present a single brush-like ring of lanate hairs inside the mouth of the corolla tube, *S. staudtii* contains two rings of hairs, one positioned in the middle of the corolla tube and the other in a narrow corona at the throat (Leeuwenberg 1969). The monophyly of *S.* sect. *Densiflorae* was supported in the analysis of the southern African dataset of Adebowale et al. (2016b) by the occurrence of a 104 bp indel in the *trnS-trnG* intergenic spacer, but *S. staudtii* was not included in the regional analysis. In the analysis of their ITS2 African dataset, *S. staudtii* was resolved outside of the *S.* sect. *Densiflorae* clade, being placed sister to *S. congolana* and *S. cocculoides*, both from *S.* sect. *Spinosae* (Adebowale et al. 2016b). Morphology and molecular evidence obtained both by Adebowale et al. (2016b) and our analysis supports the exclusion of *S. staudtii* from *S.* sect. *Densiflorae*.

Clade 6C comprises all four species of the African *Strychnos* sect. *Spinosae* consisting of deciduous shrubs or small to medium-sized trees (except *S. congolana* that is a forest liana). These are some of the most dominant *Strychnos* species in the southern African region, occupying preferentially open, dry savanna woodlands (Leeuwenberg 1969; Adebowale et al. 2016b). Vegetative characters include a corky bark, hard wood, branches and branchlets usually not lenticellate and without tendrils (except *S. congolana*), but presenting spines (except *S. ternata*) (Leeuwenberg 1969). The inflorescences are terminal and many-flowered, bearing 5-merous flowers with a narrow white-penicillate corona inside the mouth of the tube and stamens included within the tube. Filaments are inserted near the base of the corolla tube, and the anthers are deeply cordate and ciliate all around the base. Pistils are mostly pubescent, and fruits are large, hard-walled, usually with an edible pulp containing many ochraceous and flattened seeds that have a thick testa when mature. Other remarkable features that support Clade 6C are the presence of deciduous stipules and the absence of interxylary phloem (Leeuwenberg 1969; Mennega 1980).

Strychnos phaeotricha is an African rain forest liana with paired tendrils and unarmed stems that was placed on the isolated branch 6D. This species is unique in the genus by having pubescent glands on the filaments and inside the corolla lobes, the defining character for the monotypic *S.* sect. *Phaeotrichae* (Duvigneaud 1952). Other notable characters of *S. phaeotricha* include large colleters at the base of the petioles, bracts, and calyx, terminal inflorescences, 5-merous flowers, a very short corolla tube, free corolla lobes about three-fourths of the length of the tube, long, well-exserted filaments, glabrous or sparsely puberulent pistils, medium-sized, soft--walled, 3–7-seeded fruits that mature orange, and seeds with a very thin testa (Duvigneaud 1952; Leeuwenberg 1969).

Leeuwenberg (1969) suggested a close relationship between *Strychnos* sects. *Phaeotrichae* and *Spinosae* (Clade 6C) due to their sharing of terminal inflorescences. Indeed, Adebowale et al. (2016b) resolved *S. phaeotricha* as nested inside a well-supported clade including the four species of *S.* sect. *Spinosae* plus *S. staudtii* in the analysis of their ITS2 African dataset, but the last species presents axillary inflorescences. In our phylogeny, *S. phaeotricha* is more closely allied to American Clade 6E and Paleotropical Clade 6F (mostly sect. *Lanigerae*). The relationship of this broader clade was not well resolved with regard to the other branches of Clade 6, reinforcing that more data are needed to clarify these relationships.

Sister to *S. phaeotricha* was Clade 6E containing large forest lianas from Central and South America, especially of the Amazon basin, with branchlets bearing solitary or paired tendrils and devoid of spines (Krukoff 1972). Defining characters include mostly terminal inflorescences (axillary in *S. jobertiana* and *S. panurensis*), a glabrous corolla tube and lobes both within and without (except *S. panurensis* with the corolla tube densely lanose within), very short filaments, glabrous pistils, and discoid seeds with a crustaceous testa (Krukoff 1972; Brant and Davidse 2009). Of the five species, four (*S. chlorantha, S. colombiensis, S. ramentifera,* and *S. jobertiana*) share conspicuously oblong-oblanceolate to linear anthers (either bearded or glabrous at the base). This notable character was used by Krukoff and Monachino (1942) and Krukoff and Barneby (1965) to place these species together in their "*jobertiana*-group." However, according to Brant and Davidse (2009), *S. panurensis* has glabrous and rounded anthers. The *jobertiana*-group species also differ from *S. panurensis* in having the corolla tube longer than the lobes (vs. equal) and large, hard-walled and usually many-seeded fruits (vs. small, soft-walled and 1–2-seeded; Krukoff 1972). As such, our result placing *S. panurensis* among the "*jobertiana*" species should be tested with additional data.

Clade 6F is a large and heterogenous clade composed of 19 species mostly of *S.* sect. *Lanigerae*. The defining character of the section is a medium-sized corolla that is usually glabrous outside, but with a zone of pilosity in the throat, in the tube, and/or inside on the base of the lobes (Leeuwenberg 1969). Of the four species of Clade 6F from other sections, *S. axillaris* and *S. umbellata* present a pilosity in the base of the corolla lobes and/or at the mouth of corolla tube, *S. vitiensis* has very few hairs on the inner surface of the tube, and *S. wallichiana* possesses a vilosity in the base of the tube (Hill 1911; Leenhouts 1962; Tirel-Roudet 1972; Ping-tao and Leeuwenberg 1996).

The predominant habit among all species of Clade 6F is lianas or climbing shrubs occupying tropical rain forests, with branchlets often bearing paired tendrils (absent only in *S. umbellata*, solitary in *S. axillaris*) but usually lacking spines (present only in *S. axillaris*) (Hill 1911, 1917; Leenhouts 1962; Leeuwenberg 1969; Tirel-Roudet 1972; Conn et al. 1996; Ping-tao and Leeuwenberg 1996). Other notable features include usually axillary inflorescences that can in some species also be terminal on the same plant (but exclusively terminal in *S. fallax* and *S. wallichiana*), the anthers more or less bearded at the base (seemingly entirely glabrous in *S. splendens* and *S. wallichiana*), a more or less pilose pistil (entirely glabrous in *S. axillaris, S. vitiensis*, and *S. wallichiana*), and flattened rough-surfaced seeds mostly with very short thick hairs and a rather thick testa (Hill 1911, 1917; Leenhouts 1962; Leeuwenberg 1969; Tirel-Roudet 1972; Conn et al. 1996; Ping-tao and Leeuwenberg 1996). This suite of characters was used by Leeuwenberg (1969) to define *S.* sect. *Lanigerae* and was also used by Khánh (1987) to propose the transfer of S. *umbellata* from *S.* sect. *Brevitubae* to *S.* sect. *Lanigerae*, which is in agreement with our molecular results.

Despite weak support for many of the internal branches within Clade 6F, at least four subordinate clades were strongly supported. The first comprises the African species *S. ngouniensis*, *S. soubrensis*, and *S. splendens*, which have globose fruits that are ca. 3 cm in diam and orange when mature (color unknown in *S. ngouniensis*); the second the Malayan species *S. borneensis* and *S. polytrichantha*, which have lenticellate branchlets; the third the poorly known Indian and Pacific Ocean species *S. umbellata*, *S. lanata*, *S. vitiensis*, and one accession of *S. minor* (from the Solomon Islands); the fourth the Asian species *S. axillaris*, *S. villosa*, *S. wallichiana*, and the three other accessions of *S. mino*r (from the Philippines), which mostly have glabrous pistils (Hill 1911, 1917; Leenhouts 1962; Leeuwenberg 1969; Tirel-Roudet 1972; Conn et al. 1996; Ping-tao and Leeuwenberg 1996). Nevertheless, the positioning of *S. axillaris*, the type species of *S.* sect. *Penicillatae*, within Clade 6F and not clustered among other species of the section (which are in Clades 11 and 12), suggests that the defining character of *S.* sect. *Penicillatae*, a line of erect bristle-like hairs either at the base or along the middle of the inner face of the corolla lobes (Hill 1917), had multiple origins within *Strychnos*.

Clade 7 comprises *S. cuminodora*, *S. johnsonii*, *S. millepunctata*, and *S. samba,* a group of liana species occupying rain forests of tropical Africa. Diagnostic characters of this clade include usually quadrangular (except *S. millepunctata*) branches that lack lenticels and/or branchlets without spines (tendrils unknown), axillary inflorescences (also terminal in *S. samba*), 4–5-merous flowers with the corolla tubes short in proportion to the lobes, mostly glabrous anthers and pistils, soft-walled, 1-seeded fruits, and a thin testa (Duvigneaud 1952; Leeuwenberg 1969).

Clade 8 groups two species, *S. decussata*, a shrub or small tree without tendrils that occurs in the savannas of east and southern Africa and Madagascar; and one of the two accessions of *S. angolensis*, a climbing shrub, small tree, or liana bearing solitary tendrils from tropical rain forests of Africa. Shared characters include unarmed branches, axillary and occasionally also terminal inflorescences (or even ramiflorous in *S. decussata*) with colleters in the bracts, white or yellowish flowers with proportionally (to the lobes) very short corolla tubes that are glabrous or minutely papillose-pubescent outside, densely pilose bases of the lobes inside, glabrous anthers and pistils, and small, soft-walled, 1(–2)-seeded orange-maturing fruits (Leeuwenberg 1969).

Clade 9 is also a small grouping of two species, *S. boonei* and *S. campicola*, which are rain forest lianas of tropical Africa with unarmed branches and branchlets that lack lenticels and bear solitary tendrils. Reproductive characters include axillary inflorescences (also terminal in *S. boonei*), 5-merous flowers with proportionally (to the lobes) short corolla tubes, pilosity on the inner surface of the corolla lobes, and small, soft-walled, 1-seeded fruits (Duvigneaud 1952; Leeuwenberg 1969).

Clade 10 is another small group of three species including the second accession of *S. angolensis*, *S. malacoclados*, and *S. henningsii*. While the first two species are lianas or climbing shrubs from tropical rain forests of Africa bearing solitary tendrils, *S. henningsii* is a shrub or small tree without tendrils from the savannas of east and southern Africa and Madagascar. Shared characters include unarmed branches lacking lenticels, axillary (sometimes also terminal), lax, few-flowered inflorescences (congested and sometimes many-flowered in *S. henningsii*) with colleters in the bracts, 4–5-merous flowers with proportionally (to the lobes) very short corolla tubes, densely pilose bases of the lobes internally, mostly glabrous anthers and pistils, and small, soft-walled, 1-seeded, orange-maturing fruits (Bruce 1955; Leeuwenberg 1969). While *S. angolensis* and *S. malacoclados* share dark brown bean-shaped seeds, *S. henningsii* has pale brown coffee-shaped seeds (Bruce 1955; Leeuwenberg 1969).

Still noteworthy is the distant placements of the two samples of *S. angolensis* in Clades 8 and 10. Adebowale et al. (2016b) suggested that the samples of *S. angolensis* (JF937942) and *S. henningsii* (JF937984) could be possibly misidentified based on their molecular results, but we checked the identifications of the vouchers and found no support for this assertion in the morphologies of the specimens. However, both *S. angolensis* and *S. henningsii* have a wide geographic distribution and each display extensive morphological variation (Bruce 1955; Leeuwenberg 1969), suggesting possible complex diversification histories still unknown.

In Clade 11, *S. mostueoides* and *S. myrtoides* are shrubs or small trees from open and/or low forests or savannas of southern Africa and Madagascar, which are often dichotomously branched and devoid of tendrils and spines. The inflorescences are usually terminal (occasionally axillary in *S. myrtoides*) and the flowers are white and mostly 5-merous (Leeuwenberg 1969).

Within Clade 12, a weakly supported branch includes the African species *S. afzelli, S. matopensis,* and *S. arborea*, all of *S.* sect. *Penicillatae.* Shared characters of these species include solitary tendrils (absent in *S. arborea*), absence of spines (present in young plants of *S. arborea*), axillary inflorescences (terminal and occasionally also axillary in *S. matopensis*), 5-merous flowers with a mostly glabrous corolla but with a line of erect bristle-like hairs either across the base or across the middle of the lobes, included stamens with very short filaments, glabrous pistils, and small, soft-walled, 1(–2)-seeded fruits (Leeuwenberg 1969). These characters were used by Leeuwenberg to define *S.* sect. *Penicillatae*, but members of the section were spread through Clades 6F, 11 and 12 (see previous discussion). The weak support associated with the isolated branches containing *S. diplotricha* and *S. pentantha* (also *S.* sect. *Penicillatae*) suggests that more molecular data are needed to fully resolve the relationships of these species and to determine whether or not to maintain *S.* sect. *Penicillatae*.

All of the species of the American clade within Clade 12 possess a crustaceous seed testa, which may represent a synapomorphy for the group (Krukoff 1972). The ratio of the length of the corolla tube versus the lobes used by Progel (1868) to separate the American species into three sections is obviously highly homoplastic, as demonstrated in our phylogeny. The American species are also highly variable in an assortment of characters, such as habit, ecological preferences, the presence (or absence) of tendrils and spines, inflorescence position, anthers and pistil pubescence, seed number, and fruit size (Krukoff 1972).

Evolutionary Trends in Strychnos—The liana or climbing shrub habit is the predominant growth form in *Strychnos* (although many species can take multiple growth forms depending on habitat). It prevails especially among species grouped within the basally diverging Clades 1, 2, and 3, whereas a tree or shrub-like habit is more common in species placed within the relatively more nested Clades 4–12, suggesting that a non-climbing habit is a derived condition in the genus. Adebowale et al. (2016b) suggested that a tree/shrub-like habit may have become more common as African species dispersed from rain forests into forest edges or open savanna woodlands. A corresponding pattern is apparent among American *Strychnos*, as species of Clade 12 with tree or shrub-like habit occur in both open savannas (e.g. *S. pseudoquina, S. rubiginosa, S. parvifolia*) and in low forests of sandy coastal areas (*S. atlantica*). In Asian and Australian *Strychnos*, a tree/shrub-like habit is also associated with woodlands or open forests (e.g. *S. ignatii, S. nux-blanda, S. lucida*, Clade 4). However, some *Strychnos* species are small to large trees growing in dry forests or rain forests, but these species were not well sampled in our study.

The strongly supported clade grouping Clades 4–12 also seems to be characterized by the absence of tendrils arranged in 2–3 pairs of successive nodes. Duvigneaud (1952) suggested that the evolutionary sequence for the arrangement of the tendrils in *Strychnos* was the reduction from three pairs to one, then a loss of one of the two or both of the remaining tendrils in some lineages. Khánh (1987) argued that the occurrence of tendrils in multiple pairs confers an adaptive advantage for lianas and climbing shrubs that grow in forest habitats. Indeed, our phylogeny suggests that the arrangement of the tendrils into multiple pairs is the ancestral condition of the genus, while 1-paired or solitary tendrils (or the tendrils absent) are derived conditions.

Spines are a rather common feature among American species of *S.* sect. *Breviflorae* (Clades 1 and 12), being present in seven of the eight species placed in Clade 1 (absent in the poorly known *S. schunkei*). In contrast, spines are apparently less common among the Asian-Australian species, being reported in *S. angustiflora*, *S. lucida* (both from Clade 4), *S. axillaris* (Clade 6F), and *S. arborea* (Clade 12). In the African species, spines occur only among members of *S.* sect. *Spinosae* (Clade 6C; except *S. ternata*) and in *S. afzelli* (Clade 12; Leenhouts 1962; Leeuwenberg 1969; Conn et al. 1996). The presence of spines in the African and Asian-Australian *Strychnos* is clearly associated with arid habitats, although the African rain forest *S. aculeata* is the only species in the genus that presents prickles on the trunk and the stems (Clade 3). In the Americas, armed species occur in both open savannas and in tropical rain forests. The Brazilian *S. atlantica* is the only species of the genus with an armed, spine-bearing trunk (Clade 12).

Interxylary phloem has been recorded in most of the more than 70 species of *Strychnos* whose wood anatomy has been investigated (including species of Clades 1–10 and 12). Among the studied species, it appears to be absent only in the African species of *S.* sect. *Spinosae* (Clade 6C) and *S. henningsii* of Clade 10 (Mennega 1980). Thus, interxylary phloem was probably present in the ancestor of the genus and seems to play a key role in providing higher flexibility to the stem of climbers and increased water transport (see Angyalossy et al. 2015 for a review). Its loss among members of *S.* sect. *Spinosae* may be related to the predominant tree or shrub-like habit of that section. In this sense, another remarkable feature of *S.* sect. *Spinosae* members is the presence of interpetiolar stipules (vs. interpetiolar lines in the rest of the genus), a character that may also reflect adaptation to open and dry areas, possibly providing bud protection when exposed to periodic fires in the savanna habitat.

Inflorescence position is an extremely variable, and, based on our phylogeny, a highly homoplastic character within *Strychnos*, with both terminal and axillary inflorescence types occurring simultaneously in both basally branching and more nested clades. Indeed, a number of individual species possess both inflorescence types. Duvigneaud (1952) argued that terminal inflorescences are ancestral for the genus, but based on our results it is not possible to infer the polarity of this character, although exclusively terminal or exclusively axillary inflorescences serve to diagnose some clades of the genus (see previous discussion). Ramiflorous inflorescences are a much rarer condition in *Strychnos*, occurring only in the African savanna species of Clade 6B and in *S. decussata* of Clade 8; thus, they appear to be apomorphies for those groups.

Although the ratio of lengths of the corolla tube to the lobes is a highly homoplastic character in the genus as a whole, a proportionally long corolla tube is imperfectly associated with the clade grouping Clades 4–12. Members of the Asian-Australian branch in Clade 4 are represented exclusively by species of *S.* sect. *Strychnos*, characterized by proportionately long corolla tubes, while species of Clades 1, 2 and 3, as well as the African sister branch within Clade 4 (*S. potatorum* and *S. icaja*), share proportionally short corolla tubes or have intermediate ratios. Therefore, the elongation of the corolla tube relative to lobe length seems to be a derived condition in *Strychnos*, which may be driven by co-evolution with pollinators.

Adebowale et al. (2016b) argued that increases in fruit size and fruit wall thickness among African *Strychnos* are derived adaptations to fire and water shortage, which are both prevalent in savannas and open woodland habitats. Our results, however, do not fully corroborate this hypothesis, as species with large, hard-walled and many-seeded fruits occupy both open and forested habitats and reside in various positions in our phylogeny from Clades 1 to 12. Many large-fruited Amazon species grow in seasonally flooded forests with the fruits being dispersed considerable distances by water and presumably germinating during the dry season (Ducke 1945). In the upland "terra-firme" forest of the Amazon, other species bear large fruits, which, after falling, may remain on the ground for 1–2 yr before breaking up, or remain attached to the plant, where they are consumed by toucans (Krukoff and Monachino 1942; Ducke 1945).

Several other morphological structures are significantly variable across *Strychnos* and have figured prominently in the taxonomy of the genus, but are as yet incompletely described (or described with ambiguous terminology) in many species, thus preventing inference about their evolution. For example, the anthers and pistil supply several taxonomically important characters, especially the length of the pistil and its indumentum, which enables identification of some species after the corolla has fallen. Duvigneaud (1952) called attention to the length, inclusion and level of insertion of the stamens in the corolla tube as important taxonomic characters. However, these characters are unknown in many species. The indumentum of the corolla (especially within the tube and lobes) is another important, although incompletely sampled, taxonomic character, that has been used to distinguish sections by some authors (e.g. *S.* sects. *Dolichanthae*, *Lanigerae*, and *Penicillatae*).

Seed and seed coat characters have also been used to delimit species and define sections. For example, members of the American *S.* sect. *Breviflorae* subsects. *Breviflorae*, *Rouhamon,* and *Strychnos* include 30 species of Clades 6E and 12 with crustaceous testa, while the species of Clade 1 present fibrous (*S.* sect. *Breviflorae* subsect. *Eriospermae*) or cartilaginous testas (*S. brasiliensis*). Among African species, members of Clade 2 share flattened seeds, members of Clade 5 a thin testa that sticks to the fruit pulp, and members of Clade 6B asperous seeds with very short, erect, and thick hairs. However, the descriptions of these seed characters in other taxa of *Strychnos* lack uniformity and consistent definitions of terms. Moreover, very little is known about these characters in the Asian-Australian members of the genus.

Despite the large number of chemical studies already conducted on *Strychnos*, especially related to the chemically active species of the strychnine-like and curare-like groups, the correlation of phytochemical characters and their evolutionary origin still requires in-depth approaches. We found that most of the species already documented that produce strychnine were positioned predominantly among the members of Clade 4. In relation to the 21 species cited as a source of curare by Krukoff (1972), 13 were sampled in our study, two belonging to the Clade 6E (*S. panurensis* and *S. jobertiana*), while the other species were grouped in the Neotropical group within Clade 12.

This work, the first well-sampled, global phylogenetic analysis of *Strychnos*, although based on a single molecular marker, provides many important insights into the phylogeny of this ethnobotanically and ecologically important genus. It also provides critical evolutionary context for an improved understanding of the biogeography and diversification history of *Strychnos* (to appear as a separate study). For example, the Neotropical clades (Clades 6E and 12) have the shortest branches and the most unresolved topologies in the whole phylogeny, indicating relatively recent radiations. The phylogeny also provides a critical cladistic framework for revising the infrageneric classification of *Strychnos*. Future work on the genus should be undertaken to increase sampling of species, in both molecular and morphological datasets (especially Asian species of *S.* sects. *Brevitubae*, *Lanigerae*, and *Penicillatae* and species of several unresolved Neotropical complexes), and should also strive to better integrate molecular data with anatomic, chemical, and morphological evidence.

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AUTHOR CONTRIBUTIONS

RBS provided data regarding the taxonomical and phylogenetic evaluations, table summarizing the history of sectional classification systems in *Strychnos* and was the primary author of the manuscript. CLF provided data regarding taxonomic and phylogenetic evaluations, most of the molecular sequencing used for the phylogeny estimation, made previous phylogenetic analyses and contributed equally as the primary author of the manuscript. JM provided data regarding updated datasets, did the phylogenetic analyses, and contributed to parts of the manuscript. BMT, RCF, and LS provided data regarding taxonomic and phylogenetic evaluations and contributed to the writing of the manuscript. LS also provided the figures and their captions.

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APPENDIX 1. Vouchers used in the phylogenetic analyses of this study. Order of data is: species, voucher, herbarium (abbreviation according to Index Herbariorum), GenBank number for ITS sequence, country, herbarium barcode (when the collection had extra sheets, all the checked material is cited), partial sequence (only when full sequence was not obtained). All *Strychnos* and part of the outgroup sequences are original to this study; other outgroup sequences are from *Gelsemium* and *Logania* (Robbrecht and Manen 2006), *Geniostoma* (Wright et al. 2006), and *Mitreola* and *Spigelia* (Gould and Jansen 1999); see those publications for detailed voucher information.

Outgroup: *Gelsemium sempervirens* (L.) J.St.-Hil., *Longwood 860395*, BG, DQ358881, country not listed in original publication, —. *Antonia ovata* Pohl, *S. Mori 24159*, NY, JF937925, French Guiana, —, ITS1 only. *Bonyunia aquatica* Ducke, *P. Berry et al. 5771*, MO, JF937926, Venezuela, MO-1817430. *Bonyunia minor* N.E.Br., *P. Berry and L. Brako 5522*, NY, JF937927, Venezuela, —. *Gardneria multiflora* Makino, *T. Ceming 9611186*, MO, JF937929, China, —. *Gardneria ovata* Wall., *J. Klackenberg & R. Lundin 214*, NY, JF937930, Sri Lanka, —. *Geniostoma kaalae* (C.N.Forbes) K.L.Gibbons, B.J.Conn & Henwood (=*Labordia kaalae* C.N.Forbes), *T. Motley 1203*, BISH, JF937931, Hawaii, USA, —. *Geniostoma rupestre* J.R.Forst. & G.Forst., (1) source not listed in original publication, DQ499095, Papua New Guinea, —; (2) source not listed in original publication, DQ499096, New Zealand, —. *Logania albiflora* Druce, *Hubbard 4198*, G, DQ358879, country not listed in original publication, —. *Mitrasacme oasena* Dunlop, *P.I. Forste et al. PIF24800*, NY, JF937932, Australia, —. *Mitrasacme polymorpha* R.Br., *Anonymous 20495*, NY, JF937933, Australia, —. *Mitreola petiolata* (Gmel.) Torr. & A.Gray, *Gould 150*, TEX/LL, AF054635, USA, —. *Neuburgia neocaledonica* (Gilg & Gilg-Ben.) J.Molina & Struwe, *L. Struwe 1301*, NY, JF937935, Hawaii (cultivated), USA, —. *Norrisia malaccensis* Gardner, *B.C. Stone 14107*, GH, JF937936, Malaysia, —. *Spigelia coelostylioides* K.Gould, *Gould 139*, TEX/LL, AF177992, Mexico, —. *Spigelia hedyotidea* K.Gould, *Gould 103*, TEX/LL, AF178008, USA, —. *Spigelia loganioides* (Torr. & A.Gray ex Endl. & Fenzl) A.DC., *Goldman 433*, TEX/LL, AF178000, USA, —. *Spigelia marilandica* (L.) L., *Gould 163*, TEX/LL, AF177991, USA, —. *Spigelia texana* (Torr. & A.Gray) A.DC., *Gould 135*, TEX/LL, AF178006, USA, —.

Ingroup: *S. aculeata* Soler., (1) *D.J. Harris & J.M. Fay 109*, MO, JF937939, Central African Republic, —, ITS2 only; (2) *M. Merello 1338*, MO, JF937940, Ghana, MO-041572. *S. afzelii* Gilg, *F.N. Hepper & J. Maley 7962*, WAG, JF937941, Ivory Coast, WAG.1061836. *S. angolensis* Gilg, (1) *F.J. Breteler 14002*, WAG, JF937942, Gabon, WAG.1062138, WAG.1062139; (2) *J.M. Reitsma & B. Reitsma 1300*, WAG, JF937943, Gabon, WAG.1062137. *S. angustiflora* Benth., (1) *Hu & But 22389*, MO, JF937944, Hong Kong, —; (2) *S.Y. Hu 8346*, MO, JF937945, Hong Kong, —. *S. arborea* A.W.Hill, (1) *G.N. Batianoff 11377*, L, JF937946, Australia, L.2688932, ITS1 only; (2), *K.A. Williams s.n.*, US, JF937950, Australia, US03161921. *S. asterantha* Leeuwenb., (1) *A.J.M. Leeuwenberg 8048*, WAG, JF937947, Ivory Coast, WAG.1062229, WAG.1062230, WAG.1062231; (2) *D.K. Harder et al. 2976*, WAG, JF937948, Ghana, WAG.1062235. *S. atlantica* Krukoff & Barneby, *R.P. Belem 3722*, US, JF937949, Brazil, US03160930. *S. axillaris* Colebr., (1) *J.E. Vidal 5984*, US, JF938043, Laos, US03161887, ITS2 only; (2) *J. Munzinger & F. Engelman 237*, MO, JF937951, Laos, —, ITS2 only. *S. barteri* Soler., (1) *C.C.H. Jongkind & J. Assi-Yapo 5002*, WAG, JF937952, Ivory Coast, WAG.1062357, WAG.1062358; (2) *C.C.H. Jongkind et al. 1427*, WAG, JF938040, Ghana, WAG.1062296, WAG.1062297, ITS2 only. *S. boonei* De Wild., *A.J.M. Leeuwenberg 6593*, MO, JF937953, Cameroon, —. *S. borneensis* Leenh., *M.J.E. Coode et al. 7856*, GH, JF937954, Brunei, —. *S. brachistantha* Standl., *E. Lott & S.H. Bullock 1443*, NY, JF937955, Mexico, NY04092342. *S. brasiliensis* (Spreng.) Mart., *C. Medri et al. 446*, NY, JF937956, Brazil, NY00590374. *S. campicola* Gilg ex Leeuwenb., *J. Nemba et al. 615*, MO, JF937957, Cameroon, —. *S. camptoneura* Gilg & Busse, *F.J. Breteler et al. 13093*, WAG, JF937958, Gabon, WAG.1062676, WAG.1062677, WAG.1062678. *S. chlorantha* Progel, *C. Frasier 16A*, CHRB, JF937959, Costa Rica, —, ITS2 only. *S. chrysophylla* Gilg, *Carvalho 6197*, MO, JF937960, Equatorial Guinea, —. *S. cocculoides* Baker, *C.N. Nkhoma et al. 122*, WAG, JF937961, Zambia, WAG.1062901. *S. cogens* Benth., *S. Mori 24607*, NY, JF937962, Guiana, —. *S. colombiensis* Krukoff & Barneby, *D. Penneys 402*, MO, JF937963, Costa Rica, MO-1045179, MO-2193529, ITS2 only. *S. congolana* Gilg, *L. White LJTW0872*, MO, JF937965, Gabon, —. *S. cuminodora* Leeuwenb., *C.C.H. Jongkind et al. 1525*, MO, JF937966, Ghana, —. *S. cuniculina* Leeuwenb., *G. McPherson 16307*, MO, JF937967, Gabon, —. *S. dale* De Wild., *A.J.M. Leeuwenberg 13569*, MO, JF937968, Gabon. *S. darienensis* Seem., (1) *A. Gentry 6386*, NY, JF937969, Panama, —, ITS1 only; (2) *F. Encarnacion C. 974*, MO, JF937970, Peru, MO-2216868, ITS2 only; (3) *S. McDaniel et al. 32823*, US, JF937971, Peru, US03161072, ITS2 only. *S. decussata* (Pappe) Gilg, *R.D. Noyes et al. 1031*, MO, JF937972, Madagascar, —. *S. dinklagei* Gilg, *A.J.M. Leeuwenberg 7932*, WAG, JF937973, Ivory Coast, WAG.1063486, WAG.1063487. *S. diplotricha* Leeuwenb., *G. McPherson & N. Dumetz 14639*, MO, JF937974, Madagascar, —. *S. ecuadoriensis* Krukoff & Barneby, *D. Neill 10443*, MO, JF937975, Ecuador, —. *S. elaeocarpa* Gilg ex Leeuwenb., *A.J.M. Leeuwenberg 5671*, US, JF937976, Cameroon, US03161979, US03161980. *S. erichsonii* M.R.Schomb. ex Progel, *M.-F. Prevost & D. Sabatier 3791*, NY, JF937977, French Guiana. *S. eugeniifolia* Monach., *J.M. Pires et al. 50901*, US, JF937978, Brazil, US03161197. *S. fallax* Leeuwenb., *A.M. Louis 1832*, WAG, JF937979, Gabon, WAG.1063745, WAG.1063746. *S. fendleri* Sprague & Sandwith, *A. Groger 1194*, MO, JF937980, Venezuela, MO-2238359. *S. floribunda* Gilg, *J.M. Reitsma & B. Reitsma 2020*, WAG, JF937981, Gabon, WAG.1063779. *S. guianensis* (Aubl.) Mart., *M. Fleury 1393*, NY, JF937982, French Guiana, —, ITS1 only. *S. henningsii* Gilg, *P. Herman 770*, WAG, JF937984, South Africa, WAG.1064020. *S. hirsuta* Spruce ex Benth., *D.G. Campbell et al. P22104*, MO, JF937985, Brazil, MO-2238335. *S. icaja* Baill., *J.J. Wieringa 2012*, WAG, JF938044, Cameroon, WAG.1063994, WAG.1063996, WAG.1063997. *S. ignatii* P.J.Bergius, (1) *A.C. Church et al. 882*, GH, JF937986, Indonesia, —; (2) *J.E. Vidal 5845*, US, JF938045, Laos, —. *S. innocua* Delile, *A.P.M. de Kruif 541*, WAG, JF937987, Ivory Coast, WAG.1064323. *S. jobertiana* Baill., *R. Vasquez & N. Jaramillo 13899*, MO, JF937988, Peru, MO-1912958. *S. johnsonii* Hutch. & M.B. Moss, *Carvalho 4213*, MO, JF937989, Guinea, —. *S. lanata* A.W.Hill, *A. Boonkongchart 60*, GH, JF937990, Thailand, —. *S. lucens* Baker, *H. Schmidt et al. 1221*, MO, JF937991, Tanzania, —, ITS2 only. *S. lucida* R.Br., (1) *A. Kanis 1978*, US, JF937992, Australia, US03161917, ITS2 only; (2) *D.D. Soejarto et al. 5846*, MO, JF937993, Thailand, —, ITS2 only. *S. madagascariensis* Poir., *G. McPherson 14555*, MO, JF937994, Madagascar, —. *S. malacoclados* C.H.Wright, *L. White 0712*, MO, JF937995, Gabon, —. *S. malchairii* De Wild., *G. McPherson 16724*, MO, JF937996, Gabon, —. *S. matopensis* S.Moore, *M. Merello et al. 970*, MO, JF937997, Zambia, —. *S. mattogrossensis* S.Moore, *T.B. Croat 19648A*, MO, JF937998, Peru, MO-2265559. *S. medeola* Sagot ex Progel, *M. Fleury 1096*, NY, JF937999, French Guiana, —, ITS1 only. *S. melinoniana* Baill., *A. Henderson 305*, NY, JF938000, Brazil, NY02145766. *S. memecyloides* S.Moore, *A.J.M. Leeuwenberg 6324*, MO, JF938001, Cameroon, —, ITS1 only. *S. millepunctata* Leeuwenb., *A.J.M. Leeuwenberg 11040*, MO, JF938002, Ivory Coast, —. *S. minor* Dennst., (1) *Dennis' Collectors 4601*, US, JF938003, Solomon Islands, US03161900; (2) *Ridsdale et al. ISU49*, GH, JF938004, Philippines, —; (3) *J. Molina 7*, CHRB, JF938042, Philippines, —; (4) *J. Molina 23*, CHRB, JF938041, Philippines, —. *S. mitis* S.Moore, *ATBP 668*, MO, JF938005, Uganda, ITS1 only. *S. mitscherlichii* M.R.Schomb. var. *mitscherlichii*, *R. Vasquez et al. 19831*, MO, JF938006, Peru, MO-2265553. *S. mitscherlichii* var. *pubescentior* Sandwith, *M.J. Macía & R. Montufar 1423*, MO, JF938007, Ecuador, MO-2341059. *S. mostueoides* Leeuwenb., (1) *S. Wolhauser SW60262*, MO, JF938008, Madagascar, —; (2) *L. Gautier & C. Chatelain LG2322*, WAG, JF938009, Madagascar, WAG.1065383. *S. myrtoides* Gilg & Busse, *D.W. Thomas 3829*, MO, JF938010, Tanzania, —, ITS1 only. *S. ngouniensis* Pellegr., *A.J.M. Leeuwenberg 7885*, WAG, JF938011, Cameroon, WAG.1065415, WAG.1065416, WAG.1065419. *S. nicaraguensis* Huft, *T.B. Croat 12403*, NY, JF938012, Panama, NY04092349. *S. nigricans* Progel, *M. Nee 38048*, MO, JF938013, Bolivia, MO-2245185. *S. nux-blanda* A.W.Hill, W. *Nanakom & H.T. Beck 880200*, MO, JF938014, Thailand, —. *S. nux-vomica* L., *J.F. Maxwell 90-622*, MO, JF938015, Thailand, —. *S. oiapocensis* Fróes, *Grenand 1956*, WAG, JF938016, French Guiana, WAG.1065599. *S. panamensis* Seem., (1) *J.A. Duke 8604*, US, JF938017, Panama, US0316084, partial ITS2 missing; (2) *M.J. Macía & H. Romero 3000*, MO, JF938018, Ecuador, MO-2341062. *S. panganensis* Gilg, *P. Antilahimena 85*, MO, JF938019, Madagascar, —. *S. panurensis* Sprague & Sandwith, *F. Ayala 1556*, MO, JF938020, Peru, MO-2216869. *S. parvifolia* A.DC., *M.J.N. Rodal et al. 502*, MO, JF938021, Brazil, MO-2238356, partial ITS2 sequence. *S. peckii* B.L.Rob., *R. Ortiz 204*, MO, JF938022, Ecuador, MO-2341052. *S. pentantha* Leeuwenb., *A.J.M. Leeuwenberg* 13920, MO, JF938023, Madagascar, —. *S. phaeotricha* Gilg, *F.J. Breteler 6481*, MO, JF938024, Gabon, —. *S. poeppigii* Progel, *S. McDaniel 15267*, MO, JF938025, Peru, MO-2216701. *S. polytrichantha* Gilg, *J.S. Burley & B. Lee 268*, GH, JF938026, Sarawak, —. *S. potatorum* L.f., *E.A. Banda et al. 3770*, MO, JF938027, Malawi, —. *S. pseudoquina* A. St.-Hil., *H.S. Irwin et al. 13660*, MO, JF938028, Brazil, MO-2238343. *S. pungens* Soler., *R.R.J. van Vuuren 1842*, MO, JF938029, South Africa, —, ITS2 only. *S. ramentifera* Ducke, *M.J. Macía et al. 568*, MO, JF938030, Ecuador, MO-2341061. *S. rondeletioides* Spruce ex Benth., *R. Vasquez 5824*, MO, JF938031, Peru, MO-2265550. *S. rubiginosa* A.DC., *J.R. Abbot & L. Isaacs 16406*, MO, JF938032, Bolivia, MO-2245183. *S. samba* P.A.Duvign., *A.J.M. Leeuwenberg 5675*, NY, JF938033, Cameroon, —. *S. scheffleri* Gilg, *G. McPherson 16226*, MO, JF938034, Gabon, —. *S. schultesiana* Krukoff, (1) *R. Liesner & A. Gonzalez 9170*, MO, JF938036, Venezuela, MO-2238357; (2) *M.F. Quigley 769*, MO, JF938037, Venezuela, MO-2238325, ITS2 only. *S. schunkei* Krukoff & Barneby, *J. Schunke 9175*, MO, JF938035, Peru, MO-2265543. *S. solimoesana* Krukoff, *A. Gentry & S. Estensoro 70699*, MO, JF938038, Bolivia, MO-2245184. *S. soubrensis* Hutch. & Dalziel, *M. Merello et al. 1265*, MO, JF938039, Ghana, —. *S. spinosa* Lam., (1) *L. Struwe 1027*, NY, JF938047, Tanzania, —, ITS2 only; (2) *J. Zarucchi et al. 7614*, NY, JF938048, Madagascar, —, partial ITS2; (3) *T. Motley s.n.*, NY, JF938049, Florida (cultivated), USA, —. *S. splendens* Gilg, *J. Ampunsahi 1987*, MO, JF938050, Ghana, —. *S. staudtii* Gilg (1), *M. Cheek et al. 7056*, WAG, JF938051, Cameroon, WAG.1066830; (2) *M. Cheek 6032*, WAG, JF938052, Cameroon, WAG.1066831. *S. tabascana* Sprague & Sandwith, *C. Frasier 20*, CHRB, JF938053, Costa Rica, —, ITS2 only. *S. talbotiae* S.Moore, *P.P.C. van Meer 1296*, MO, JF938054, Nigeria, —. *S. ternata* Gilg ex Leeuwenb., *A.J.M. Leeuwenberg 6231*, MO, JF938055, Cameroon, —. *S. toxifera* R.H.Schomb. ex Benth., (1) *J.S. Bissett 275*, CHRB, JF938056, Peru, —; (2) *K. Thomsen 1489*, NY, JF938057, Costa Rica. *S. tricalysioides* Hutch. & M.B.Moss, (1) *G. McPherson 16975*, MO, JF938058, Gabon, —; (2) *R.M.A.P. Haegens* & *F.J. Breteler 54*, WAG, JF938059, Gabon, WAG.1067046, WAG.10670, part of ITS2 missing; (3) *X.M. van der Burgt 567*, WAG, JF938060, Cameroon, WAG.1067017. *S. tseasnum* Krukoff & Barneby, *D.K. Evans & L. Najamdai 4408*, MO, JF938062, Ecuador, MO-2341053, part of ITS2 missing. *S. umbellata* Merr., *Hu & But 22313*, MO, JF938063, China, —. *S. usambarensis* Gilg ex Engl., *H. Schmidt 2206*, MO, JF938064, Ghana, MO-047202. *S. villosa* A.W.Hill, *J.V. LaFrankie 2387*, GH, JF938065, Malaysia, —, part of ITS2 missing. *S. vitiensis* A.W.Hill, *A.C. Smith 6198*, NY, JF938066, Fiji, —. *S. wallichiana* Steud. ex A.DC., *A. Gentry & J. Tagi 33792*, MO, JF937964, Malaysia, —. *S. xantha* Leeuwenb., *F. Malaisse 13311*, WAG, JF938067, Congo, WAG.1067397. *S. xinguensis* Krukoff, *J.C. Lindeman et al. 234*, MO, JF938068, Surinam, MO-2238334.