Using Genomic Data to Develop Chloroplast DNA SSRs for the Neotropical Liana Stizophyllum riparium (Bignonieae, Bignoniaceae)

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Source: Applications in Plant Sciences, 5(10)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1700061

Applications in Plant Sciences

Applications in Plant Sciences 2017 5(10): 1700061

Primer Note

Using genomic data to develop chloroplast DNA SSRs for the Neotropical liana *Stizophyllum riparium* (Bignonieae, Bignoniaceae)[1]

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- *Premise of the study:* We developed chloroplast microsatellite markers (cpSSRs) to be used to study the patterns of genetic structure and genetic diversity of populations of *Stizophyllum riparium* (Bignonieae, Bignoniaceae).
- *Methods and Results:* We used genomic data obtained through an Illumina HiSeq sequencing platform to develop a set of cpSSRs for *S. riparium*. A total of 36 primer pairs were developed, of which 28 displayed polymorphisms across 59 individuals from three populations. Two to 12 alleles were recorded, and the unbiased haploid diversity per locus ranged from 0.037 to 0.905. All 28 cpSSRs presented transferability to two closely related species, *S. inaequilaterum* and *S. perforatum*.
- *Conclusions:* We report a set of 28 cpSSRs for *S. riparium*. All markers were shown to be variable in *S. riparium*, indicating that these markers will be valuable for population genetic studies across *S. riparium* and congeneric species.

Key words: Bignoniaceae; chloroplast microsatellites; cross-amplification; Neotropical flora; *Stizophyllum inaequilaterum*; *Stizophyllum perforatum.*

Stizophyllum Miers (Bignoniaceae) is a small genus of Bignonieae, the largest tribe in the Bignoniaceae (Lohmann and Taylor, 2014). The genus is clearly monophyletic (Lohmann, 2006) and includes three species, i.e., *S. inaequilaterum* Bureau & K. Schum., *S. perforatum* (Cham.) Miers, and *S. riparium* (Kunth) Sandwith (Lohmann and Taylor, 2014). All species of *Stizophyllum* are lianas with trumpet-shaped flowers that are pollinated by medium-sized bees (Gentry, 1974). Their winged seeds are dispersed by wind (Gentry, 1974). Members of *Stizophyllum* are easily recognized by their hollow stems, pellucid-punctate leaflets, urceolate calyces, and linear fruits (Lohmann and Taylor, 2014). The genus as a whole is broadly distributed, occurring from southern Mexico to the Atlantic Forest in southern Brazil (Lohmann and Taylor, 2014). Although the generic circumscription is clear, the three species of *Stizophyllum* currently recognized are morphologically similar and can occur sympatrically in Amazonia.

Nuclear DNA polymorphisms based on microsatellites (simple sequence repeats [SSRs]) are powerful sources for population genetic studies (Kalia et al., 2011). These molecular markers, present also in the genomes of organelles (e.g., chloroplast), allow us to access genetic information that facilitates genotype identification, mainly due to their multiallelic nature (Masi et al., 2003). The chloroplast microsatellite and nuclear microsatellite markers (cpSSR and nSSR, respectively) have been widely used to study phylogenetic and genetic diversity in plants (Kalia et al., 2011). Unlike nSSRs, which are highly polymorphic, codominant, and biparentally inherited, cpSSRs are a nonrecombinant molecule and uniparentally inherited, allowing us to trace the history of populations through their haplotype diversity (Ebert and Peakall, 2009). Despite these differences, the two markers are complements for understanding the genetic structure in natural populations (Ebert and Peakall, 2009; Kalia et al., 2011).

Furthermore, the advent of high-throughput sequencing technologies (Metzker, 2010) has allowed the development of SSR markers for multiple taxonomic groups (Zalapa et al., 2012; Francisco et al., 2016). Here, we used chloroplast genome sequence data obtained by de novo and reference-guided assembly to develop and characterize chloroplast microsatellite markers for *S. riparium*. Cross-amplification of the cpSSR markers developed for *S. riparium* was tested in all congeneric species to evaluate the utility of those markers for population genetic studies in *Stizophyllum* as a whole.

METHODS AND RESULTS

We first obtained the chloroplast genome sequence of *S. riparium* using an Illumina platform. For that, we extracted the genomic DNA from fresh leaf material dried in silica gel from a single individual of *S. riparium* (voucher: *Nogueira*

[1]Manuscript received 29 May 2017; revision accepted 21 July 2017.

The authors thank Comissão de Aperfeiçoamento de Pessoal do Nível Superior (CAPES) for a scholarship to M.B., Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for a Pq-1C grant to L.G.L. (307781/2013-5), and Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) for a scholarship to A.G.N. (2013/12633-8) and a regular research grant (2011/508559-2) and a collaborative FAPESP-NSF-NASA Dimensions of Biodiversity Grant (2012/50260-6) to L.G.L.

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doi:10.3732/apps.1700061

TABLE 1. Characteristics of 28 intergenic chloroplast microsatellite markers developed for *Stizophyllum riparium*.

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	T_a (°C)	Fluorescent dye[a]	Position	GenBank accession no.
Stiz2	F: CCTTGTTGGTTAGTTGAGTTCC	$(A)_6(G)_{10}$	211–216	49.9	JOE	*trnK-UUU/rps16*	KP863512
	R: GGGGTAGCGACTTGTATATAACT						
Stiz3	F: AGGAAGTTTTCTCCTCGTAC	$(T)_{10}$	170–176	54.0	JOE	*rps16/rps16*	KP863513
	R: CTTGAGTTATGAGTACGAATGG						
Stiz4	F: ATCTAATGATCCGGGGCG	$(A)_{10}$	160–169	55.8	6-FAM	*psbI/trns-GCU*	KP863514
	R: TTCCCTTTCCGTTGATGACT						
Stiz5	F: CCCTTCCCGAACCAAACATG	$(A)_{11}$	176–181	57.4	JOE	*atpF/atpF*	KP863515
	R: TTGTCAGAAGAGTCCTCCAAGT						
Stiz6	F: GCCACACATACATTGCTTTGC	$(A)_{12}$	143–150	57.4	JOE	*atpH/atpI*	KP863516
	R: TCCCTGTCATGTTCCTTGGA						
Stiz7	F: TCCTAGTATGCTGGCCAACA	$(A)_{10}$	164–171	57.4	JOE	*rps2/rpsC2*	KP863517
	R: AGAAGAGAAGGTTCCATCGGA						
Stiz10	F: CCAACCTAAAATTCTTCCGA	$(T)_{10}$	165–173	57.4	6-FAM	*petN/psbM*	KP863519
	R: CTTATAGAAAGTGCGGTGC						
Stiz11	F: CAGATTGCCTTTCCACTTCGA	$(A)_{10}$	190–199	54.0	JOE	*psbM/trnD-GUC*	KP863520
	R: TCGAAGAAATCCCCAACCCT						
Stiz12	F: GGATTTCTTCGATGGGCCCT	$(A)_{11}$	167–180	56.9	6-FAM	*psbM/trnD-GUC*	KP863521
	R: GCCAGTCCCGACAGATCC						
Stiz13	F: CCTGTTCTTTCCATGACCCC	$(T)_{10}$	201–210	54.0	JOE	*psbC/trnS-UGA*	KP863522
	R: AAAGAAAGGGGAATGGCTCG						
Stiz14	F: GCATAGCTAGCAATCCATTCT	$(A)_{13}$	136–149	54.0	JOE	*psaA/ycf3*	KP863523
	R: GGAACAATTGGAATAGAATGCC						
Stiz15	F: CATTCCTCGGCTTTCATTCGT	$(A)_{10}$	90–96	51.7	6-FAM	*trnT-UGU/trnL-UAA*	KP863525
	R: TCTTCCTGCCATTTCTCCCA						
Stiz18	F: CATTCGTTCTTCAGCCTCA	$(T)_{10}$	179–184	54.7	JOE	*ycf4/cemA*	KP863527
	R: TTGAGTTGCATGGATTTGGA						
Stiz20	F: TTCTAGGAGGCTTGTCTTCC	$(T)_{12}$	183–191	56.9	6-FAM	*petA/psbJ*	KP863528
	R: TGTCCACTACTTTACTGTACGT						
Stiz21	F: TACTGCGTCAATTGCCAATT	$(T)_{10}$	186–197	48.6	6-FAM	*petL/petG*	KP863529
	R: TCACCACGCCTGTATTGTAA						
Stiz22	F: ACCCACCTATACAGTAACGGT	$(T)_{10}$	161–169	56.9	JOE	*rpl20/rpl1s*	KP863531
	R: TCTCCATTGGTAGCAAATGGT						
Stiz23	F: GATTCTCTATCTGCTGTGTG	$(A)_{10}$	153–162	56.9	6-FAM	*ClpP/psi_psbT*	KP863532
	R: TCTTGAATGGAAAGTAAGGG						
Stiz24	F: AGTCGAGTATCTGAAACACGA	$(A)_{11}$	179–183	61.4	6-FAM	*psbH/petB*	KP863534
	R: AGAACCATTGGATCATCACGT						
Stiz25	F: TGGAGGAGAAACGATATTAG	$(T)_{11}$	168–175	61.4	JOE	*infA/rps8*	KP863535
	R: CTTCTCGAGGTATAATGACA						
Stiz26	F: TCTAGCCATATCAGCATTTCGT	$(T)_{10}$	168–170	61.4	6-FAM	*rps8/rpl14*	KP863536
	R: GTGTCTCACGGCATATACCT						
Stiz27	F: TCTTCTCATCCAGCTCCTCG	$(T)_{10}$	243–248	53.2	JOE	*rpl16/rps3*	KP863537
	R: CGAATAAGCGCTACGACTGA						
Stiz28	F: GTCCTTTATCCAAGTTTACC	$(A)_{10}$	168–169	55.8	JOE	*rpl16/rps3*	KP863538
	R: TCTTACTGATTCACTAGTCG						
Stiz33	F: TGGATCTCTCAGTCTAAGCAGG	$(T)_{13}$	230–238	49.9	6-FAM	*ycf1/ycf1*	KP863539
	R: CCACAAGCCGAGGAGATCTT						
Stiz34	F: GACACGCAGGATAATTCATA	$(T)_{10}$	182–206	56.9	JOE	*ycf1/ycf1*	KP863540
	R: TCCCAGAATGAATACAGAAC						
Stiz36	F: ACATCCCTATTTCCTCCATTG	$(TA)_6$	168–170	56.9	6-FAM	*trnP-UGG/psaJ*	KP863530
	R: TCTTAGCGAGTAGTTCCGA						
Stiz39	F: CTCTAACCTCTGAGCTAAGC	$(ATA)_4$	175–176	58.0	JOE	*trnT-UGU/trnL-UAA*	KP863524
	R: CGAAATTCTATATCGCTGCA						
Stiz40	F: GATCCAAGAAATTACAGGAC	$(TAA)_4$	130–132	53.2	JOE	*trnL-UAA/trnF-GAA*	KP863526
	R: TGAGCTATCCTGACCATT						
Stiz45	F: GAAATCCCATATGACCCA	$(TCTCTT)_7$	156–171	53.2	JOE	*ClpP/psi_psbT*	KP863533
	R: CCCCAAAGAAAGAGAAAG						

Note: T_a = annealing temperature.
[a]Fluorescent label used for the forward primer sequence.

170; Appendix 1) collected in Manaus (Amazonas State, Brazil) using the Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany) and following the manufacturer's instructions. Approximately 5 µg of total DNA was fragmented using a Covaris S-Series Focused-ultrasonicator (Covaris, Woburn, Massachusetts, USA) and a short-insert (300 bp) library was constructed with NEBNext DNA Library Prep Master Mix Set and NEBNext Multiplex oligos for Illumina (New England BioLabs, Ipswich, Massachusetts, USA), following the manufacturer's protocol. The library concentration was diluted to 10 mM and sequenced (single end) on an Ilumina HiSeq 2000 system (Illumina, San Diego, California, USA) at the University of São Paulo (Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo [ESALq]) in Piracicaba, Brazil. A Perl script was used to filter for quality using a Phred score of 20 or more for the cleaned reads, with the exclusion of reads with more than three uncalled bases, or shorter than 40 bp. We used a combination of reference-guided and de novo assembly to construct the chloroplast genome of *S. riparium* following Nazareno et al. (2015). The chloroplast genome for *S. riparium* was annotated using DOGMA (Dual Organellar

TABLE 2. Characteristics of 28 polymorphic chloroplast microsatellite markers in three populations of *Stizophyllum riparium*.[a]

	CAM (n = 19)		SAM (n = 22)		EAM (n = 18)		All (n = 59)	
Locus	*A*	*h*	*A*	*h*	*A*	*h*	*A*	*h*
Stiz2	3	0.368	4	0.614	4	0.654	6	0.660
Stiz3	4	0.551	2	0.524	4	0.575	6	0.671
Stiz4	5	0.719	3	0.626	3	0.451	9	0.773
Stiz5	3	0.550	2	0.095	2	0.125	4	0.533
Stiz6	3	0.433	4	0.726	2	0.209	6	0.668
Stiz7	4	0.618	3	0.511	2	0.529	6	0.719
Stiz10	5	0.693	2	0.505	2	0.118	6	0.633
Stiz11	2	0.529	3	0.426	4	0.802	5	0.653
Stiz12	5	0.683	4	0.671	4	0.712	6	0.760
Stiz13	4	0.419	6	0.766	3	0.385	7	0.584
Stiz14	7	0.850	8	0.884	4	0.600	10	0.893
Stiz15	4	0.575	2	0.467	4	0.582	6	0.627
Stiz18	3	0.608	4	0.633	3	0.228	6	0.588
Stiz20	3	0.342	8	0.833	2	0.294	9	0.780
Stiz21	7	0.596	5	0.745	3	0.601	8	0.717
Stiz22	1	0.000	2	0.416	4	0.467	5	0.703
Stiz23	4	0.642	4	0.676	3	0.582	6	0.698
Stiz24	2	0.441	3	0.706	2	0.111	4	0.665
Stiz25	2	0.525	3	0.552	2	0.523	4	0.732
Stiz26	3	0.632	2	0.395	1	0.000	3	0.420
Stiz27	4	0.608	3	0.386	3	0.600	4	0.625
Stiz28	2	0.125	2	0.233	1	0.000	2	0.458
Stiz33	4	0.419	5	0.737	2	0.309	9	0.796
Stiz34	2	0.485	3	0.338	2	0.476	5	0.725
Stiz36	2	0.264	3	0.451	2	0.111	3	0.516
Stiz39	3	0.542	2	0.521	1	0.000	3	0.493
Stiz40	1	0.000	2	0.100	1	0.000	2	0.037
Stiz45	5	0.788	9	0.900	3	0.621	12	0.905
Mean	3.4	0.500	3.6	0.551	2.6	0.381	5.7	0.644

Note: *A* = number of alleles; *h* = unbiased haploid diversity.
[a]Voucher and locality information are provided in Appendix 1.

TABLE 3. Transferability of 28 chloroplast microsatellite markers developed for *Stizophyllum riparium* across two related *Stizophyllum* species.[a]

Locus	Repeat motif	*S. perforatum*	*S. inaequilaterum*
Stiz2	$(A)_6(G)_{10}$	+	+
Stiz3	$(T)_{10}$	+	+
Stiz4	$(A)_{10}$	+	+
Stiz5	$(A)_{11}$	+	+
Stiz6	$(A)_{12}$	+	+
Stiz7	$(A)_{10}$	+	+
Stiz10	$(T)_{10}$	+	+
Stiz11	$(A)_{10}$	+	+
Stiz12	$(A)_{11}$	+	+
Stiz13	$(T)_{10}$	+	+
Stiz14	$(A)_{13}$	+	+
Stiz15	$(A)_{10}$	+	+
Stiz18	$(T)_{10}$	+	+
Stiz20	$(T)_{12}$	+	+
Stiz21	$(T)_{10}$	+	+
Stiz22	$(T)_{10}$	+	+
Stiz23	$(A)_{10}$	+	+
Stiz24	$(A)_{11}$	+	+
Stiz25	$(T)_{11}$	+	+
Stiz26	$(T)_{10}$	+	+
Stiz28	$(T)_{10}$	+	+
Stiz27	$(A)_{10}$	+	+
Stiz33	$(T)_{13}$	+	+
Stiz34	$(T)_{10}$	+	+
Stiz36	$(TA)_6$	+	+
Stiz39	$(ATA)_4$	+	+
Stiz40	$(TAA)_4$	+	+
Stiz45	$(TCTCTT)_7$	+	+

[a]Successful amplification (+) evidenced by the occurrence of a distinct single band on the sequencing gel.

GenoMe Annotator; Wyman et al., 2004; http://dogma.ccbb.utexas.edu/). Start and stop codons were inspected and adjusted manually.

The Imperfect Microsatellite Extractor (IMEx) interface (Mudunuri and Nagarajaram, 2007) was used to detect perfect microsatellites, with a threshold of 10 repeat units for mononucleotide and six repeats for di-, tri-, tetra-, penta-, and hexanucleotides. The annotated chloroplast genome was used to select markers located in noncoding regions exclusively. Primer pairs were designed from microsatellite sequence sites using Primer3web 4.0 (Rozen and Skaletsky, 1999), with the following parameters: primer size of 18–23 bp, temperature of 50–62°C with maximum difference between forward and reverse primers of 1°C, and GC content of 40–80%.

In total, 36 primer pairs were designed. The PCR amplifications were performed in a final volume of 10 µL and contained 15 ng of genomic DNA, 0.5 µL (10 mM) of each primer with forward primers labeled with 6-FAM or JOE fluorescent dyes (Macrogen, Seoul, South Korea; Table 1), 0.6 µL (25 mM) $MgCl_2$ (Promega Corporation, Madison, Wisconsin, USA), and 5 µL 1× of KAPA2G Fast ReadyMix (Kapa Biosytems, Wilmington, Massachusetts, USA). The cycling conditions were as follows: an initial denaturation step of 3 min at 94°C; followed by 30 cycles of 30 s at 94°C for denaturation, 30 s at the specific annealing temperature for each primer pair (Table 1), and 72°C for 60 s; and a final extension of 5 min at 72°C. To test the utility of the individual primers, PCR products were detected using a 1.0% agarose gel electrophoresis with a 100-bp range DNA ladder (Promega Corporation).

Of the 36 primer pairs tested, 28 successfully amplified (Table 1) and were checked for polymorphism in 59 individuals from three *S. riparium* populations (ranging from 18 to 22 individuals; Appendix 1). All samples tested were also extracted using fresh leaf material dried in silica gel or from herbarium specimens using the Invisorb Spin Plant Mini Kit, following the manufacturer's instructions. The amplicons with fluorescent labels were resolved to genotype on an ABI 3500 XL automated DNA sequencer with GeneScan 500 ROX Size Standard (Applied Biosystems, Foster City, California, USA). The microsatellite marker profiles were analyzed using GeneMarker (Holland and Parson, 2011). For each cpSSR, the number of alleles (*A*) and unbiased haploid diversity index (*h*) were obtained using GenAlEx 6.41 (Peakall and Smouse, 2006). In addition, we calculated chloroplast haplotype variation within populations by estimating the total number of haplotypes and the unbiased haplotype diversity as $H_e = [n/(n-1)](1-\sum pi^2)$, where n is the number of individuals analyzed in each population and p is the frequency of the i^{th} haplotype in the respective population (Nei, 1978). Cross-amplification of polymorphic cpSSRs was tested in six individuals of *S. inaequilaterum* and *S. perforatum* using the same PCR conditions described above.

A partial chloroplast genome (128,239 bp) was obtained and used to develop a set of 28 polymorphic cpSSRs (Table 2). The number of alleles ranged from two to 12, and unbiased haplotype diversity varied from 0.037 (Stiz40) to 0.905 (Stiz45) (Table 2). When alleles at each of the 28 loci were jointly analyzed, we observed 18, 19, and 22 haplotypes for EAM (i.e., east of Amazonia), CAM (i.e., Central America), and SAM (i.e., south of Amazonia) populations, respectively. Furthermore, the maximum unbiased haplotype diversity (H_e = 1.00) was observed for all *S. riparium* populations. All polymorphic cpSSR markers successfully amplified for *S. inaequilaterum* and *S. perforatum* (Table 3).

CONCLUSIONS

We developed and characterized 28 chloroplast microsatellite markers for *S. riparium*. Due to the high rate of cross-amplification (100%), these chloroplast microsatellite markers will be useful for genetic studies involving *Stizophyllum* as a whole.

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Appendix 1. Voucher and locality information for all individuals of *Stizophyllum* sampled.

Species	Population code	Locality	Geographic coordinates	Voucher no.[a]
Stizophyllum riparium (Kunth) Sandwith	CAZ	Brazil, Amazonas, Manaus	3°00′14″N, 59°55′07″W	A. Nogueira 170*
	CAM	Mexico, Campache, Calakmul	18°48′39″N, 89°18′28″W	D. Álvarez 5027[1]
	CAM	Mexico, Quintana Roo, Playa del Carmen	20°36′58″N, 87°03′58″W	E. Cabrera 6450[1]
	CAM	Mexico, Yucatan, Buctzotz	21°12′60″N, 88°45′60″W	E. Cabrera 13716[1]
	CAM	Mexico, Quintana Roo, Adolfo de la Huerta	19°34′45″N, 89°03′31″W	D. Álvarez 9469[1]
	CAM	Mexico, Campache, Calakmul	18°32′27″N, 89°54′53″W	D. Álvarez 5168[1]
	CAM	Belize, Toledo, Maya Mts.	16°24′25″N, 89°06′07″W	B. K. Holst 4319[1]
	CAM	Belize, Cayo	17°21′05″N, 88°55′12″W	D. E. Atha 1040[1]
	CAM	Mexico, Chipas, Ocosingo	16°54′18″N, 92°02′17″W	E. Martinez 12499[1]
	CAM	Mexico, Chipas, Ocosingo	17°01′39″N, 91°16′31″W	J. P. Abascal 79[1]
	CAM	Mexico, Chipas, Ocosingo	17°01′30″N, 91°18′22″W	G. Aguilar 1688[1]
	CAM	Mexico, Veracruz, Los Tuxtla	18°34′60″N, 95°05′60″W	C. S. Sinaca 1561[1]
	CAM	Mexico, Veracruz, San Andres Tuxtla	18°26′60″N, 95°12′58″W	C. S. Sinaca 339[1]
	CAM	Guatemala, Peten, San Jose	17°01′39″N, 89°52′60″W	B. Wallnöfer 5907[1]
	CAM	Costa Rica, Puntarenas, Peninsula de Osla	8°24′01″N, 83°17′30″W	R. Aguilar 1960[1]
	CAM	Costa Rica, Guanacaste, Nandayure	9°50′57″N, 85°20′26″W	A. Estrada 87[1]
	CAM	Costa Rica, Puntareans, Palmar Norte	8°58′60″N, 83°27′60″W	A. H. Gentry 78803[1]
	CAM	Costa Rica, San José, Tircoles	9°47′29″N, 84°31′37″W	J. F. Morales 4012[1]
	CAM	Costa Rica, Puntarenas, Golfito	8°33′03″N, 83°20′60″W	A. Azofeifa 719[1]
	CAM	Costa Rica, Puntarenas, Bueno Aires	9°02′04″N, 83°25′49″W	L. González 897[1]
	EAM	Brazil, Pará, Parauapebas	06°10′12″S, 50°21′02″W	M. Beyer 295
	EAM	Brazil, Pará, Parauapebas	06°09′46″S, 50°20′52″W	M. Beyer 295
	EAM	Brazil, Pará, Parauapebas	06°10′13″S, 50°21′04″W	M. Beyer 295
	EAM	Brazil, Pará, Parauapebas	06°09′45″S, 50°20′51″W	M. Beyer 295
	EAM	Brazil, Pará, Parauapebas	06°09′48″S, 50°20′52″W	M. Beyer 295
	EAM	Brazil, Pará, Parauapebas	06°10′08″S, 50°21′01″W	M. Beyer 295
	EAM	Brazil, Pará, Parauapebas	06°10′04″S, 50°21′02″W	M. Beyer 295
	EAM	Brazil, Pará, Parauapebas	06°10′04″S, 50°21′02″W	M. Beyer 295
	EAM	Brazil, Pará, Parauapebas	06°10′02″S, 50°21′02″W	M. Beyer 295
	EAM	Brazil, Pará, Parauapebas	06°09′46″S, 50°20′51″W	M. Beyer 295
	EAM	Brazil, Pará, Parauapebas	06°09′48″S, 50°20′53″W	M. Beyer 301
	EAM	Brazil, Pará, Parauapebas	06°09′36″S, 50°25′21″W	M. Beyer 302
	EAM	Brazil, Pará, Parauapebas	06°04′10″S, 50°14′46″W	M. Beyer 303
	EAM	Brazil, Pará, Parauapebas	06°03′55″S, 50°12′57″W	M. Beyer 303
	EAM	Brazil, Pará, Parauapebas	06°04′14″S, 50°14′44″W	M. Beyer 303
	EAM	Brazil, Pará, Novo Repartimento	04°19′57″S, 49°56′57″W	M. Beyer 315
	EAM	Brazil, Pará, Altamira	03°12′20″S, 51°14′56″W	M. Beyer 317
	EAM	Brazil, Pará, Moju	03°50′25″S, 46°06′40″W	M. Beyer 318
	SAM	Brazil, Mato Grosso, Paranaíta	9°35′28″S, 56°30′49″W	M. Beyer 353
	SAM	Brazil, Mato Grosso, Paranaíta	9°35′28″S, 56°30′49″W	M. Beyer 353
	SAM	Brazil, Mato Grosso, Paranaíta	9°35′28″S, 56°30′49″W	M. Beyer 353
	SAM	Brazil, Mato Grosso, Paranaíta	9°35′28″S, 56°30′49″W	M. Beyer 353
	SAM	Brazil, Mato Grosso, Paranaíta	9°35′28″S, 56°30′49″W	M. Beyer 353

APPENDIX 1. Continued.

Species	Population code	Locality	Geographic coordinates	Voucher no.[a]
	SAM	Brazil, Mato Grosso, Paranaíta	9°35′28″S, 56°30′49″W	M. Beyer 353
	SAM	Brazil, Mato Grosso, Paranaíta	9°35′28″S, 56°30′49″W	M. Beyer 353
	SAM	Brazil, Mato Grosso, Paranaíta	9°23′13″S, 56°30′07″W	M. Beyer 354
	SAM	Brazil, Mato Grosso, Paranaíta	9°23′13″S, 56°30′07″W	M. Beyer 354
	SAM	Brazil, Mato Grosso, Paranaíta	9°23′13″S, 56°30′07″W	M. Beyer 354
	SAM	Brazil, Mato Grosso, Paranaíta	9°23′13″S, 56°30′07″W	M. Beyer 354
	SAM	Brazil, Mato Grosso, Paranaíta	9°23′13″S, 56°30′07″W	M. Beyer 354
	SAM	Brazil, Mato Grosso, Paranaíta	9°23′13″S, 56°30′07″W	M. Beyer 354
	SAM	Brazil, Mato Grosso, Paranaíta	9°23′13″S, 56°30′07″W	M. Beyer 354
	SAM	Brazil, Mato Grosso, Paranaíta	9°23′13″S, 56°30′07″W	M. Beyer 354
	SAM	Brazil, Mato Grosso, Paranaíta	9°23′13″S, 56°30′07″W	M. Beyer 354
	SAM	Brazil, Mato Grosso, Paranaíta	9°31′48″S, 9°31′48″W	M. Beyer 356
	SAM	Brazil, Mato Grosso, Paranaíta	9°31′48″S, 9°31′48″W	M. Beyer 356
	SAM	Brazil, Mato Grosso, Paranaíta	9°31′48″S, 9°31′48″W	M. Beyer 356
	SAM	Brazil, Mato Grosso, Paranaíta	9°31′48″S, 9°31′48″W	M. Beyer 356
	SAM	Brazil, Mato Grosso, Paranaíta	9°31′48″S, 9°31′48″W	M. Beyer 356
	SAM	Brazil, Mato Grosso, Paranaíta	9°31′48″S, 9°31′48″W	M. Beyer 356
Stizophyllum perforatum (Cham.) Miers	—	Brazil, São Paulo, Candido Mota	22°45′27″S, 50°22′06″W	J. P. Souza 9703
	—	Brazil, Paraná, Londrina	23°34′12″S, 50°57′42″W	L. H. M. Fonseca 105
	—	Brazil, Paraná, Londrina	23°34′12″S, 50°57′42″W	L. H. M. Fonseca 103
	—	Brazil, Minas Gerais, Belo Horizonte	11°54′S, 71°22′W	J. Lombardi 2431[1]
	—	Brazil, Rio de Janeiro, São Pedro de Aldeia	22°50′10″S, 42°06′13″W	J. A. Kallunki s.n.
	—	Brazil, Piauí, Eliseu Martins	08°05′27″S, 43°39′42″W	P. Martins & E. Nunes s.n.[4]
Stizophyllum inaequilaterum Bureau & K. Schum.	—	Brazil, Acre, Marechal Taumaturgo	08°56′29″S, 72°47′33″W	L. G. Lohmann 454
	—	Equador, Amazonia, Orellana	0°40′59″S, 76°24′W	H. Romero-Saltos 2831
	—	Peru, Ucayali, Pedro Abad	09°09′02″S, 75°47′20″W	J. Schunke-Vigo 15997
	—	French Guiana, Saul	03°37′22″N, 53°12′34″W	S. A. Mori 24242[2]
	—	Peru, San Martin, Tocache	08°11′22″S, 76°30′57″W	J. Schunke-Vigo 14609[3]
	—	Brazil, Amazonas, Letícia	04°12′19″S, 69°55′58″W	A. H. Gentry 18302

Note: CAM = Central America; CAZ = Central Amazonia; EAM = east of Amazonia; SAM = south of Amazonia.

[a] Most specimens are deposited at the Herbarium of the University of São Paulo (SPF), São Paulo, Brazil, except for 20 samples ([1]) that are deposited at the Missouri Botanical Garden Herbarium (MO), St. Louis, Missouri, USA; one sample ([2]) that is deposited at the Herbarium of the Muséum National d'Histoire Naturelle (P), Paris, France; one sample ([3]) that is deposited at the New York Botanical Garden Herbarium (NY), Bronx, New York, USA; and one sample ([4]) that is deposited at the Herbarium of the Federal University of Ceará (EAC), Fortaleza, Ceará, Brazil.

* Sample used for DNA extraction.