Primer Note

Using genomic data to develop chloroplast DNA SSRs for the Neotropical liana Stizophyllum riparium (Bignoniaceae, Bignoniaceae)

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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 (Bignoniaceae, 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 Bignoniaceae, 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...
## 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<sub>a</sub> (°C) | Fluorescent dye<sup>a</sup> | Position | GenBank accession no. |
|-------|--------------------------|--------------|------------------------|----------------|------------------------------|----------|----------------------|
| Stiz2 | F: CTTTGAGGTTGTTGTTGATTCC | (A)₉(G)₁₀ | 211–216 | 49.9 | JOE | trnK-UUU/rps16 | KP863512 |
| Stiz2 | R: GGGTGAGGCTTGTGTTGATTCC | (T)₁₀ | 170–176 | 54.0 | JOE | rps16/rps16 | KP863513 |
| Stiz4 | F: ATCTGATAGTTGAGGAGG | (A)₁₀ | 160–169 | 55.8 | 6-FAM | psbH/psbA | KP863514 |
| Stiz5 | R: T CTCCCTCCCGGTCTGAGCTT | (A)₁₁ | 176–181 | 57.4 | JOE | atpF/atpF | KP863515 |
| Stiz6 | R: AGAACATCATCATGTGGGGC | (A)₁₂ | 143–150 | 57.4 | JOE | atpH/atpF | KP863516 |
| Stiz7 | F: C TCTTGTATGTTGCTGCCAACAA | (A)₁₀ | 164–171 | 57.4 | JOE | rps2/rpsC2 | KP863517 |
| Stiz10 | R: AGAAGAGAAGGTCTCATGCGCA | (T)₁₀ | 165–173 | 57.4 | 6-FAM | petN/petM | KP863519 |
| Stiz11 | R: CTGTCATGCTTGGGTGAC | (A)₁₀ | 190–199 | 54.0 | JOE | psbMs/rpsD-GUC | KP863520 |
| Stiz12 | F: GATTTGTACGAGGAGCCTCC | (A)₁₁ | 167–180 | 56.9 | 6-FAM | psbMs/rpsD-GUC | KP863521 |
| Stiz13 | F: CTGTCATGCTTGGGTGAC | (T)₁₀ | 201–210 | 54.0 | JOE | psbChmS-UGA | KP863522 |
| Stiz14 | F: GCAACATCATCATGTGGGGC | (A)₁₃ | 136–149 | 54.0 | JOE | psaB/ycf3 | KP863523 |
| Stiz15 | F: C TCTTGTATGTTGCTGCCAACAA | (A)₁₀ | 90–96 | 51.7 | 6-FAM | trnT-UGU/trnL-UAA | KP863525 |
| Stiz16 | F: TCTCTTGTATGTTGCTGCCAACAA | (T)₁₀ | 179–184 | 54.7 | JOE | ycf4/ycfA | KP863527 |
| Stiz20 | F: TTTGAGGAGTTGCTTGTCC | (T)₁₂ | 183–191 | 56.9 | 6-FAM | petA/petB | KP863528 |
| Stiz21 | F: TCTGTACATCTACATCTATGCT | (T)₁₀ | 186–197 | 48.6 | 6-FAM | petL/petG | KP863529 |
| Stiz22 | F: ACCACCTATACATTAGAACGTT | (T)₁₀ | 161–169 | 56.9 | JOE | rpl20/rpl15 | KP863531 |
| Stiz23 | F: GATCAGATCACAGATGCGAGG | (A)₁₀ | 153–162 | 56.9 | 6-FAM | ClpP/psi_psbT | KP863532 |
| Stiz24 | F: AGTGGAGATCACAGATGCGAGG | (A)₁₁ | 179–183 | 61.4 | 6-FAM | psbH/psbB | KP863534 |
| Stiz25 | R: AGAACATCATCATGTGGGGC | (T)₁₁ | 168–175 | 61.4 | JOE | infA/rps8 | KP863535 |
| Stiz26 | F: TCTCTGACTGTCGAATTGGA | (T)₁₀ | 168–170 | 61.4 | 6-FAM | rps8/rpl14 | KP863536 |
| Stiz27 | F: TCTCTGACTGTCGAATTGGA | (T)₁₀ | 243–248 | 53.2 | JOE | rpl16/rps3 | KP863537 |
| Stiz28 | F: TCTTTGCTTACATTACAGG | (A)₁₀ | 168–169 | 55.8 | JOE | rpl16/rps3 | KP863538 |
| Stiz33 | F: GCTGATCTCATCTGATTAGAAC | (T)₁₃ | 230–238 | 49.9 | 6-FAM | ycf1/ycf1 | KP863539 |
| Stiz34 | F: AGACTGAGGAGATCACAGATGCGAGG | (A)₁₁ | 179–183 | 61.4 | 6-FAM | psbH/psbB | KP863534 |
| Stiz36 | R: C TCTTGTATGTTGCTGCCAACAA | (T)₁₀ | 182–206 | 56.9 | JOE | ycf1/ycf1 | KP863540 |
| Stiz39 | F: C TCTTGTATGTTGCTGCCAACAA | (T)₁₀ | 168–170 | 56.9 | 6-FAM | trnP-UGG/psaI | KP863530 |
| Stiz40 | F: GATCAGATCACAGATGCGAGG | (A)₁₀ | 130–132 | 53.2 | JOE | trnL-UAATrnl-GAA | KP863526 |
| Stiz45 | F: GAAATCCATATTAGGCCAACCA | (TCTCCTT)₁₀ | 156–171 | 53.2 | JOE | ClpP/psi_psbT | KP863533 |

<sup>a</sup>Fluorescent label used for the forward primer sequence.

**Note:** T<sub>a</sub> = annealing temperature.

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 Bio-Labs, Ipswich, Massachusetts, USA), following the manufacturer’s protocol. The library concentration was diluted to 10 mM and sequenced (single end) on an Illumina 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
SAM (the number of alleles \( A \)) was used to detect perfect microsatellites, with a threshold of 10 repeat units for mononucleotide and six repeats for dinucleotide, trinucleotide, tetranucleotide, penta-, and hexanucleotides. The annotated chloroplast genome was used to select 36 primer pairs designed. The PCR amplifications were performed in a final volume of 10 \( \mu \)L containing 15 ng of genomic DNA, 0.5 \( \mu \)L (10 mM) of each primer with forward primers labeled with 6-FAM or JOE fluorophore (Promega Corporation, Madison, Wisconsin, USA), and 5 \( \mu \)L 1x of KAPA2G Fast ReadyMix (Kapa Biosystems, 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 40 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).

| Locus   | CAM (n = 19) | SAM (n = 22) | EAM (n = 18) | All (n = 59) |
|---------|--------------|--------------|--------------|-------------|
| 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       | 2            | 0.712 | 6       | 0.760 |
| Stiz13  | 7            | 0.419        | 6            | 0.766       | 3            | 0.365 | 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  | 5            | 0.156        | 5            | 0.743       | 0.601        | 0.317 |
| 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            | 0.662        | 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.

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

## 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.

## LITERATURE CITED

Beyer, A., and E. P. S. R. P. A. 2009. Chloroplast simple sequence repeats (cpSSRs): Technical resources and recommendations for expanding cpSSR discovery and applications to a wide array of plant species. *Molecular Ecology Resources* 9: 673–690.

Francisco, J. N., C. A. G. N. A. R. Z. and L. G. L. O. H. M. 2016. A genomic approach for isolating chloroplast microsatellite markers for *Pachyptera kerere* (Bignoniaceae). *Applications in Plant Sciences* 4: 1600055.
APPENDIX 1. Voucher and locality information for all individuals of Stizophyllum sampled.

| Species                          | Population code | Locality               | Geographic coordinates | Voucher no. |
|----------------------------------|-----------------|------------------------|------------------------|-------------|
| Stizophyllum riparium (Kunth) Sandwich | CAZ             | Brazil, Amazonas, Manaus | 3°00′14″N, 59°55′07″W | A. Nogueira 170° |
|                                  | CAM             | Mexico, Campeche, Calakmul | 18°48′39″N, 89°18′28″W | D. Álvarez 5027° |
|                                  | CAM             | Mexico, Quintana Roo, Playa del Carmen | 20°36′58″N, 87°03′58″W | E. Cabrera 6450° |
|                                  | CAM             | Mexico, Yucatan, Buctozot | 21°12′60″N, 88°45′60″W | E. Cabrera 13716° |
|                                  | CAM             | Mexico, Quintana Roo, Adolfo de la Huerta | 19°34′45″N, 89°03′31″W | D. Álvarez 9469° |
|                                  | CAM             | Mexico, Campache, Adolfo de la Huerta | 18°32′27″N, 89°54′53″W | D. Álvarez 5168° |
|                                  | CAM             | Belize, Toledo, Maya Mts. | 16°24′25″N, 89°06′07″W | B. K. Holtz 4319° |
|                                  | CAM             | Belize, Coyo | 17°21′05″N, 88°55′12″W | D. E. Ata 1040° |
|                                  | CAM             | Mexico, Chias, Ocosingo | 16°54′18″N, 92°02′17″W | E. Martinez 12499° |
|                                  | CAM             | Mexico, Chias, Ocosingo | 17°01′39″N, 91°16′31″W | J. P. Abascal 79° |
|                                  | CAM             | Mexico, Chias, Ocosingo | 17°01′30″N, 91°18′22″W | G. Aguilera 1688° |
|                                  | CAM             | Mexico, Veracruz, Los Tuxtla | 18°34′60″N, 95°05′60″W | C. S. Sinaca 1561° |
|                                  | CAM             | Mexico, Veracruz, San Andres Tuxtla | 18°26′60″N, 95°12′58″W | C. S. Sinaca 339° |
|                                  | CAM             | Guatemala, Peten, San Lorenzo | 17°01′39″N, 89°52′60″W | B. Wallhofer 5907° |
|                                  | CAM             | Costa Rica, Puntarenas, Peninsula de Osa | 8°24′01″N, 83°17′30″W | R. Aguilar 1960° |
|                                  | CAM             | Costa Rica, Guanacaste, Nandayure | 9°50′57″N, 85°20′26″W | A. Estrada 87° |
|                                  | CAM             | Costa Rica, Puntarenas, Palmar Norte | 8°58′60″N, 83°27′60″W | A. H. Gentry 78803° |
|                                  | CAM             | Costa Rica, San José, Tarcoles | 9°47′29″N, 84°31′37″W | J. F. Morales 4012° |
|                                  | CAM             | Costa Rica, Puntarenas, Golfito | 8°33′03″N, 83°20′60″W | A. Azofeifa 719° |
|                                  | CAM             | Costa Rica, Puntarenas, Bueno Aires | 9°02′04″N, 83°25′49″W | L. González 897° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°10′12″S, 50°21′02″W | M. Beyer 295° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°09′46″S, 50°20′52″W | M. Beyer 295° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°10′13″S, 50°21′04″W | M. Beyer 295° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°09′45″S, 50°20′51″W | M. Beyer 295° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°09′48″S, 50°20′52″W | M. Beyer 295° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°10′08″S, 50°21′01″W | M. Beyer 295° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°10′04″S, 50°21′02″W | M. Beyer 295° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°10′04″S, 50°21′02″W | M. Beyer 295° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°10′04″S, 50°21′02″W | M. Beyer 295° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°10′04″S, 50°21′02″W | M. Beyer 295° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°09′46″S, 50°20′51″W | M. Beyer 295° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°09′48″S, 50°20′52″W | M. Beyer 295° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°09′36″S, 50°25′21″W | M. Beyer 302° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°04′10″S, 50°14′46″W | M. Beyer 303° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 06°03′55″S, 50°12′57″W | M. Beyer 303° |
|                                  | EAM             | Brazil, Pará, Paraíapebas | 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° |
|                                  | 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. *
|---------------------------------|-----------------|---------------------------------|---------------------------|-----------------|
| Stizophyllum perforatum (Cham.) Miers | Brazil, Mato Grosso, Paraná | 9°35′28″S, 56°30′49″W | M. Beyer 353 |
| —                               | Brazil, Mato Grosso, Paraná | 9°35′28″S, 56°30′49″W | M. Beyer 353 |
| —                               | Brazil, Mato Grosso, Paraná | 9°23′13″S, 56°30′07″W | M. Beyer 354 |
| —                               | Brazil, Mato Grosso, Paraná | 9°23′13″S, 56°30′07″W | M. Beyer 354 |
| —                               | Brazil, Mato Grosso, Paraná | 9°23′13″S, 56°30′07″W | M. Beyer 354 |
| —                               | Brazil, Mato Grosso, Paraná | 9°23′13″S, 56°30′07″W | M. Beyer 354 |
| —                               | Brazil, Mato Grosso, Paraná | 9°23′13″S, 56°30′07″W | M. Beyer 354 |
| —                               | Brazil, Mato Grosso, Paraná | 9°23′13″S, 56°30′07″W | M. Beyer 354 |
| —                               | Brazil, Mato Grosso, Paraná | 9°23′13″S, 56°30′07″W | M. Beyer 354 |
| —                               | Brazil, Mato Grosso, Paraná | 9°23′13″S, 56°30′07″W | M. Beyer 354 |
| —                               | Brazil, Mato Grosso, Paraná | 9°31′48″S, 9°31′48″W | M. Beyer 356 |
| —                               | Brazil, Mato Grosso, Paraná | 9°31′48″S, 9°31′48″W | M. Beyer 356 |
| —                               | Brazil, Mato Grosso, Paraná | 9°31′48″S, 9°31′48″W | M. Beyer 356 |
| —                               | Brazil, Mato Grosso, Paraná | 9°31′48″S, 9°31′48″W | M. Beyer 356 |
| —                               | Brazil, Mato Grosso, Paraná | 9°31′48″S, 9°31′48″W | M. Beyer 356 |
| —                               | Brazil, Mato Grosso, Paraná | 9°31′48″S, 9°31′48″W | M. Beyer 356 |
| —                               | Brazil, Mato Grosso, Paraná | 9°31′48″S, 9°31′48″W | M. Beyer 356 |
| —                               | Brazil, São Paulo, Candido Mota | 22°45′27″S, 50°22′06″W | J. P. Souza 9703 |

| Species                          | Localities                                                   | Geographic coordinates     | Voucher no. *
|---------------------------------|--------------------------------------------------------------|---------------------------|-----------------|
| Stizophyllum inaequilaterum      | 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 105 |
| —                               | Brazil, Mina Gerais, Belo Horizonte                         | 11°54′S, 71°22″W | J. Lombardi 2431 |
| —                               | 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. |
| —                               | 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                                  | 0°09′02″S, 75°17′20″W | J. Schunke-Vigo 15997 |
| —                               | French Guiana, Gaul                                       | 03°37′22″N, 53°12′34″W | S. A. Mori 24242 |
| —                               | Peru, San Martin, Tocache                                  | 08°11′22″S, 76°30′57″W | J. Schunke-Vigo 14609 |
| —                               | Brazil, Amazonas, Leticia                                  | 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.

*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 (1) that is deposited at the New York Botanical Garden Herbarium (NY), Bronx, New York, USA; and one sample (1) that is deposited at the Herbarium of the Federal University of Ceará (EAC), Fortaleza, Ceará, Brazil.

*Sample used for DNA extraction.