A Genomic Approach for Isolating Chloroplast Microsatellite Markers for Pachyptera kerere (Bignoniaceae)

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Primer Note

A genomic approach for isolating chloroplast microsatellite markers for *Pachyptera kerere* (Bignoniaceae)[1]

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- *Premise of the study:* In this study, we developed chloroplast microsatellite markers (cpSSRs) for *Pachyptera kerere* (Bignoniaceae) to investigate the population structure and genetic diversity of this species.
- *Methods and Results:* We used Illumina HiSeq data to reconstruct the chloroplast genome of *P. kerere* by a combination of de novo and reference-guided assembly. We then used the chloroplast genome to develop a set of cpSSRs from intergenic regions. Overall, 24 primer pairs were designed, 21 of which amplified successfully and were polymorphic, presenting three to nine alleles per locus. The unbiased haploid diversity per locus varied from 0.207 (Pac28) to 0.817 (Pac04). All but one locus amplified for all other taxa of *Pachyptera*.
- *Conclusions:* The markers reported here will serve as a basis for studies to assess the genetic structure and phylogeographic history of *Pachyptera*.

Key words: Bignoniaceae; Bignonieae; chloroplast genome; microsatellite; *Pachyptera kerere*; transferability.

Pachyptera kerere (Aubl.) Sandwith (Bignoniaceae) is a Neotropical liana that is widely distributed from Belize to central Amazon in Brazil (Lohmann and Taylor, 2014). This species occurs in humid and often flooded forest vegetation almost entirely along stream banks and rivers, where it is found in low densities. The flowers of *P. kerere* are white and infundibuliform and bloom throughout the year, providing a constant nectar source for different species of *Euglossa*, which are the most likely pollinators (Gentry, 1974, 1976). This species falls within the *Anemopaegma* flower type and steady-state phenology proposed by Gentry (1974). Specialized secretory glands are concentrated near the calyx margin and on the upper portion of the corolla tube. In addition, glands are also present at the interpetiolar region and the petiole apex, and play an important role in ant–plant interactions (Lohmann and Taylor, 2014). The seeds of *P. kerere* are corky and most likely water dispersed (Gentry, 1979). The broad distribution of *P. kerere*, combined with its habitat specificity and morphology, make it an interesting model to study the biological processes that determine the patterns of intra- and interpopulation variation of plant species in the Amazon.

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Microsatellites (simple sequence repeats [SSRs]) constitute an important genomic resource for botanical studies (Ellegren, 2004) and have been widely used to study the ecological and evolutionary processes that shape plant populations (Ebert and Peakall, 2009). Next-generation sequencing (NGS) technologies now allow us to easily isolate and develop SSR markers from nuclear and plastid genomes (Egan et al., 2012). In this study, we reconstructed the chloroplast genome of *P. kerere* and used this genome to develop a set of chloroplast microsatellite markers (cpSSRs) for population genetic studies of *P. kerere*. We also tested the transferability of these markers to *P. kerere* var. *incarnata* (Aubl.) A. H. Gentry and the three other recognized species of *Pachyptera* DC. ex Meisn. (Lohmann and Taylor, 2014): *P. aromatica* (Barb. Rodr.) L. G. Lohmann, *P. erythraea* (Dugand) A. H. Gentry, and *P. ventricosa* (A. H. Gentry) L. G. Lohmann.

METHODS AND RESULTS

Whole genomic DNA was extracted from silica-dried leaf tissue of one individual of *P. kerere* (collection *A. Nogueira 162*) using a mini-scale cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). An aliquot of 5 µg of total DNA was fragmented using a Covaris S-series sonicator (Covaris, Woburn, Massachusetts, USA) and used to construct short-insert libraries (300 bp) using the NEBNext DNA Library Prep Master Mix Set and the NEBNext Multiplex Oligos for Illumina (New England BioLabs, Ipswich, Massachusetts, USA) following the manufacturer's instructions. The *P. kerere* library was diluted to a concentration of 10 mM, indexed by tags, and sequenced on an Illumina HiSeq 2000 system (Illumina, San Diego, California, USA) at the Universidade de São Paulo (Escola Superior de Agricultura Luiz de Queiroz [ESALQ], Piracicaba, Brazil). Clean reads (100-bp single-end) were filtered for quality using a Perl script that trimmed reads from the ends until there were three consecutive bases with a Phred quality score of 20 or more. Reads with more than three uncalled bases or fewer than 40 bp in length were removed from the data set. The chloroplast genome of *P. kerere* was reconstructed using a combination of de novo and reference-guided assembly following Nazareno et al. (2015).

The chloroplast genome for *P. kerere* was annotated using the software Geneious version 4.7.5 (Biomatters Ltd., Auckland, New Zealand). Start and stop codons were inspected and adjusted manually.

We used the Imperfect Microsatellite Extractor (IMEx) interface (Mudunuri and Nagarajaram, 2007) to detect perfect and imperfect microsatellites, with minimum thresholds of four repeat units for tri-, tetra-, penta-, and hexa-; six for di-; and 10 for mononucleotide repeats, respectively. Chloroplast microsatellite–flanking primers for cpSSRs found only on intergenic regions were designed using the software Primer3 (Rozen and Skaletsky, 1999) and the following settings: (i) length ranging from 20 to 23 nucleotides, (ii) annealing temperature from 50°C to 62°C, and (iii) minimum GC content of 50%.

In total, 24 primer pairs were designed. To validate those primer pairs, PCR amplifications were performed in 8.5-µL reactions containing 10 ng of template DNA, 0.5 µL 10 mM of each primer with forward primers labeled with 6-FAM or JOE fluorescent dyes (Macrogen, Seoul, South Korea), 5 µL 1× of Kapa2G Fast ReadyMix (Kapa Biosystems, Wilmington, Massachusetts, USA), and 0.6 µL 25 mM $MgCl_2$ (Promega Corporation, Madison, Wisconsin, USA). PCR conditions were as follows: 94°C for 3 min; 20 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, 72°C for 1 min; and a final elongation step at 72°C for 5 min. Initial screens were performed with three *P. kerere* individuals, and their amplicons were visualized on an agarose gel (0.8%) with a 100-bp ladder (Promega Corporation).

Twenty-one of the 24 primer pairs produced a single band with strong amplification and were selected for polymorphism assessment in 65 *P. kerere* samples. These samples were grouped in three populations (11–39 individuals per population; Appendix 1). For these samples, genomic DNA was extracted from silica-dried leaves using an Invisorb Plant Mini Kit (Invitek, Berlin, Germany) following the manufacturer's protocol. Fluorescently labeled amplicons were resolved to genotype on an automated sequencer (ABI 3730XL) with GeneScan 500 ROX Size Standard (Applied Biosystems, Foster City, California, USA). Chloroplast microsatellite profiles were analyzed with GeneMarker (Holland and Parson, 2011). Each cpSSR was considered a locus at a specific site and the length variants were considered alleles. For each polymorphic locus, we obtained the number of alleles (*A*) and unbiased haploid diversity index (*h*) using the program GenAlEx version 6.41 (Peakall and Smouse, 2006). Transferability of polymorphic cpSSRs was tested in five individuals of each of the following taxa: *P. aromatica*, *P. erythraea*, *P. ventricosa*, and *P. kerere* var. *incarnata*. The PCR amplification profile followed the same conditions described above.

We obtained a partial chloroplast genome (149,076 bp) and used it to develop a set of 21 polymorphic chloroplast microsatellite markers (Table 1). Considering all samples ($n = 65$), *A* ranged from three to nine and *h* ranged from 0.207 (Pac28) to 0.817 (Pac04) (Table 2). Most of the polymorphic primers (96%) successfully amplified for *P. kerere* var. *incarnata* and for all species of *Pachyptera* (Table 3).

TABLE 1. Characteristics of 21 intergenic chloroplast microsatellite primers developed for *Pachyptera kerere*.[a]

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	Fluorescent dye	Position	GenBank accession no.
Pac03	F: TCGTTCTAGACCATCGGATT R: GGAACTTCCGTCTAATCAAATG	$(A)_6(G)_{13}$	179–190	JOE	*trnkUUU/rps16*	KP867116
Pac04	F: GGATTCGACGTAAACAATGA R: GGAACTTCCGTCTAATCAAA	$(C)_{11}$	164–174	6-FAM	*trnkUUU/rps16*	KP867117
Pac05	F: TCTAATGATCCGGGGCGTAA R: CCCTCTCTTTCCCTTTCCGT	$(A)_{14}$	166–173	6-FAM	*psbK/psbI*	KP867118
Pac06	F: ACTCCTGCCTTCATCATCTCT R: ACGGTAGAAGAGAAGGTTCCA	$(T)_{10}C(A)_{10}$	145–153	JOE	*rps2/rpoC2*	KP867119
Pac08	F: GTTTGATAAAGATGAGGCCGGT R: ACTAGTAAAGGGTGTCGGGG	$(A)_{10}$	173–180	JOE	*psbM/trnD-GU*	KP867120
Pac09	F: CGCCTCTTGAATCACCAAAGAT R: TGGGTCAGGTCCACTTACTTT	$(A)_{10}$	91–96	JOE	*trnlGRU/psbD*	KP867121
Pac11	F: GCGCGGTTGGTTTCTAAGAT R: ACTTCAGCAAACTTCGCTTCA	$(T)_{13}$	220–230	JOE	*trnSGGA/rps4*	KP867122
Pac12	F: CAAGATTGTTTAGATCTGAGGGG R: CCCATAGATCATTTTCTGCAGG	$(T)_{11}$	157–176	JOE	*accD/psaI*	KP867123
Pac13	F: GGAAATCCTTCTGTGAGATT R: GGAATTAGACCTAACACGAT	$(T)_{10}$	184–199	JOE	*psbE/petL*	KP867124
Pac15	F: GTGACGCTGAATTGGACTCC R: CACGTACAGCATTCCCTCAC	$(A)_{10}$	228–241	6-FAM	*rps12/psi-psbT*	KP867125
Pac16	F: AGATGGTTCTACTTCGTCGGA R: TCCCTGAGTAAGAACCATTGGA	$(A)_{11}$	207–220	JOE	*psbH/petB*	KP867126
Pac17	F: AGACAACTCACCCTCTTTCT R: CTTCTCGAGGTATAATGACAGAC	$(T)_{11}$	144–151	JOE	*rpl36/infA*	KP867127
Pac18	F: GTAGATGCTATGCGAACAAC R: GTGTCTCACGCATATACCT	$(T)_{11}$	187–199	6-FAM	*rps8/rpl14*	KP867128
Pac19	F: GTCCTTTATCCAAGTTTACC R: ATTCACTAATCGGGATGG	$(A)_{11}$	155–162	6-FAM	*rpl16/rp53*	KP867129
Pac20	F: TGACTGCTTCTTTAGATCCAGA R: TTGCTATGCTTAGTGTGTGAC	$(A)_{10}$	119–124	JOE	*rpl16/rp53*	KP867130
Pac21	F: CTGGGTTCTTCTACTTCATT R: CAATGGTCAAATTCTACAGG	$(T)_{10}$	104–110	JOE	*rps12_end/trnV-GAC*	KP867131
Pac23	F: AGGAACCCGCAAATATTGGC R: ACTCGCAGTATGGGTCTAGC	$(A)_{10}$	199–215	JOE	*ndhD/psaC*	KP867132
Pac24	F: TCCTTTGTGTATCTTGGTCTTCC R: TCGAGACTGTTTACCCCAAGA	$(T)_{11}$	161–171	6-FAM	*ndhA/orf188*	KP867133
Pac25	F: TTCCTGTCTTGTTTTCCACA R: TCTTAGCGAGTAGTTCCGAA	$(TA)_7$	185–193	JOE	*trnP-GGG/psaJ*	KP867134
Pac27	F: CCCCTTGTCCCTTTAATTCACA R: CAGGAACCAGGAACCAGACT	$(TAA)_4$	146–155	JOE	*trnL-UAA/trnF-GAA*	KP867136
Pac28	F: AGGTCTTCTGAACCGCTTCC R: TTGACCTACGCCTGTTTGAAC	$(GGA)_4$	181–187	6-FAM	*rbcL/psal*	KU867864

[a]The annealing temperature for all loci was 58°C.

TABLE 2. Characteristics of 21 polymorphic chloroplast microsatellite loci in three populations of *Pachyptera kerere*.[a]

	Amazon (n = 15)		Caracaraí (n = 39)		Rorainópolis (n = 11)		All (n = 65)	
Locus	*A*	*h*	*A*	*h*	*A*	*h*	*A*	*h*
Pac03	5	0.725	4	0.693	3	0.678	7	0.784
Pac04	5	0.755	6	0.737	5	0.854	9	0.817
Pac05	3	0.533	3	0.234	2	0.555	4	0.369
Pac06	4	0.782	4	0.596	3	0.654	5	0.687
Pac08	3	0.560	2	0.229	2	0.545	3	0.377
Pac09	3	0.604	4	0.310	4	0.818	4	0.535
Pac11	4	0.782	5	0.253	3	0.714	6	0.531
Pac12	4	0.525	4	0.279	4	0.694	6	0.395
Pac13	5	0.787	3	0.374	3	0.638	6	0.523
Pac15	5	0.757	4	0.331	7	0.909	9	0.628
Pac16	4	0.712	5	0.477	5	0.818	7	0.615
Pac17	3	0.530	3	0.237	3	0.709	4	0.410
Pac18	4	0.679	4	0.211	5	0.833	5	0.462
Pac19	3	0.703	3	0.316	5	0.892	5	0.599
Pac20	4	0.714	4	0.571	3	0.666	4	0.693
Pac21	2	0.527	3	0.243	4	0.709	5	0.429
Pac23	6	0.802	7	0.369	7	0.890	9	0.597
Pac24	4	0.638	2	0.051	3	0.644	6	0.493
Pac25	4	0.756	4	0.252	5	0.866	6	0.538
Pac27	4	0.742	4	0.475	4	0.777	6	0.629
Pac28	3	0.500	2	0.057	2	0.333	4	0.207
Mean	3.9	0.672	3.8	0.347	3.9	0.724	5.7	0.539

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

TABLE 3. Transferability of 21 microsatellite markers developed for *Pachyptera kerere* across four different taxa of *Pachyptera*.

Locus	Repeat motif	*P. aromatica*	*P. erythraea*	*P. ventricosa*	*P. kerere* var. *incarnata*
Pac03	$(A)_6(G)_{13}$	+	+	+	+
Pac04	$(C)_{11}$	+	+	+	+
Pac05	$(A)_{14}$	+	+	+	+
Pac06	$(T)_{10}C(A)_{10}$	+	+	+	+
Pac08	$(A)_{10}$	+	+	+	+
Pac09	$(A)_{10}$	+	+	+	+
Pac11	$(T)_{13}$	+	+	+	+
Pac12	$(T)_{11}$	+	+	+	+
Pac13	$(T)_{10}$	+	+	+	+
Pac15	$(A)_{10}$	+	+	+	+
Pac16	$(A)_{11}$	+	+	+	+
Pac17	$(T)_{11}$	+	+	+	+
Pac18	$(T)_{11}$	+	+	+	+
Pac19	$(A)_{11}$	+	+	+	+
Pac20	$(A)_{10}$	—	+	+	+
Pac21	$(T)_{10}$	+	+	+	+
Pac23	$(A)_{10}$	+	+	+	+
Pac24	$(T)_{11}$	+	+	+	+
Pac25	$(TA)_7$	+	+	+	+
Pac27	$(TAA)_4$	+	+	+	+
Pac28	$(GGA)_4$	+	+	+	+

Note: + = successful amplification as evidenced by the occurrence of distinct single bands on sequencing gels; — = no amplification.

CONCLUSIONS

We developed and amplified a set of polymorphic chloroplast microsatellite markers for *P. kerere*. These markers will be useful for evolutionary and phylogeographic studies. The applicability of these microsatellite loci in *Pachyptera* congeneric species was confirmed by successful transferability. We plan to use these markers to assess patterns of genetic structure of *Pachyptera* species in the Amazon rainforest.

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Appendix 1. Voucher and locality information for the individuals of *Pachyptera* sampled.

Species	Population code	Locality	Geographic coordinates	Voucher no.
Pachyptera kerere (Aubl.) Sandwith	AM	Brazil, Amazonas, Novo Airão	1°54′21.0″S, 61°20′08.9″W	Beyer 324
	AM	Brazil, Amazonas, Novo Airão	1°54′21.0″S, 61°20′08.9″W	Beyer 324
	AM	Brazil, Amazonas, Novo Airão	2°43′12.2″S, 60°45′16.7″W	Francisco 28
	AM	Brazil, Amazonas, Novo Airão	2°43′12.7″S, 60°45′16.7″W	Francisco 29
	AM	Brazil, Amazonas, Novo Airão	2°43′12.9″S, 60°45′16.6″W	Francisco 29
	AM	Brazil, Amazonas, Novo Airão	2°43′11.9″S, 60°45′16.6″W	Francisco 29
	AM	Brazil, Amazonas, Novo Airão	2°43′11.7″S, 60°45′16.6″W	Francisco 29
	AM	Brazil, Amazonas, Novo Airão	2°43′11.4″S, 60°45′16.8″W	Francisco 29
	AM	Brazil, Amazonas, Novo Airão	2°43′11.8″S, 60°45′17.4″W	Francisco 29
	AM	Brazil, Amazonas, Novo Airão	2°43′12.9″S, 60°45′17″W	Francisco 29
	AM	Brazil, Amazonas, Novo Airão	2°43′12.4″S, 60°45′16.8″W	Francisco 30
	AM	Brazil, Amazonas, Novo Airão	2°43′12.3″S, 60°45′16.4″W	Francisco 31
	AM	Brazil, Amazonas, Novo Airão	2°32′09″S, 60°50′20″W	Lohmann 805
	AM	Brazil, Amazonas, Novo Airão	2°32′09″S, 60°50′49″W	Lohmann 836
	AM	Brazil, Amazonas, Manaus	2°57′42″S, 59°55′40″W	Nogueira 162
	CA	Brazil, Roraima, Caracaraí	1°29′26.1″N, 61°0′13.3″W	Francisco 29
	CA	Brazil, Roraima, Caracaraí	1°29′26.3″N, 61°0′16.8″W	Francisco 36
	CA	Brazil, Roraima, Caracaraí	1°29′10.9″N, 61°0′41.3″W	Francisco 37
	CA	Brazil, Roraima, Caracaraí	1°29′12.6″N, 61°0′39″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°29′11.9″N, 61°0′39″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°29′11.1″N, 61°0′39″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°29′10.9″N, 61°0′39″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°29′10.4″N, 61°0′42.1″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°29′8.4″N, 61°0′42.1″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°29′5.4″N, 61°0′42.1″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°29′0.4″N, 61°0′41.9″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°28′36.9″N, 61°0′54.5″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°28′38″N, 61°0′57.6″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°17′1.5″N, 61°18′50.7″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°17′1.3″N, 61°18′50.7″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°17′1″N, 61°18′50.5″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°29′10.9″N, 61°0′41.3″W	Francisco 38
	CA	Brazil, Roraima, Caracaraí	1°29′24.9″N, 61°0′11.4″W	Francisco 39
	CA	Brazil, Roraima, Caracaraí	1°29′23.3″N, 61°0′09.1″W	Francisco 40
	CA	Brazil, Roraima, Caracaraí	1°29′23.3″N, 61°0′09.1″W	Francisco 40
	CA	Brazil, Roraima, Caracaraí	1°40′29.0″N, 61°11′24.6″W	Francisco 41
	CA	Brazil, Roraima, Caracaraí	1°40′29.0″N, 61°11′24.6″W	Francisco 41
	CA	Brazil, Roraima, Caracaraí	1°33′11.9″N, 61°13′58.3″W	Francisco 43
	CA	Brazil, Roraima, Caracaraí	1°39′45.2″N, 61°11′43.6″W	Francisco 43
	CA	Brazil, Roraima, Caracaraí	1°39′45.3″N, 61°11′43.7″W	Francisco 43
	CA	Brazil, Roraima, Caracaraí	1°34′16.3″N, 61°13′45.6″W	Francisco 43
	CA	Brazil, Roraima, Caracaraí	1°34′10.9″N, 61°13′36.4″W	Francisco 43
	CA	Brazil, Roraima, Caracaraí	1°34′7.1″N, 61°13′24.5″W	Francisco 43
	CA	Brazil, Roraima, Caracaraí	1°31′16.9″N, 61°14′25.8″W	Francisco 43
	CA	Brazil, Roraima, Caracaraí	1°29′24.2″N, 61°0′3.5″W	Francisco 47
	CA	Brazil, Roraima, Caracaraí	1°29′24.1″N, 61°0′2.1″W	Francisco 47
	CA	Brazil, Roraima, Caracaraí	1°29′18″N, 60°59′56.8″W	Francisco 47
	CA	Brazil, Roraima, Caracaraí	1°29′15″N, 60°59′51.6″W	Francisco 47
	CA	Brazil, Roraima, Caracaraí	1°29′24.6″N, 61°0′11.4″W	Francisco 47
	CA	Brazil, Roraima, Caracaraí	1°29′23.4″N, 61°0′8.8″W	Francisco 47
	CA	Brazil, Roraima, Caracaraí	1°25′21.8″N, 60°50′34.2″W	Francisco 47
	CA	Brazil, Roraima, Caracaraí	1°25′21.3″N, 60°50′38.3″W	Francisco 47
	CA	Brazil, Roraima, Caracaraí	1°25′20″N, 60°50′42.1″W	Francisco 57
	CA	Brazil, Roraima, Caracaraí	1°5′46.5″N, 61°52′53″W	Gomes 659
	RR	Brazil, Roraima, Rorainópolis	1°33′14.2″S, 61°30′27.8″W	Beyer 337
	RR	Brazil, Roraima, Rorainópolis	1°33′14.2″S, 61°30′27.8″W	Beyer 337
	RR	Brazil, Roraima, Rorainópolis	1°22′5.2″S, 61°45′55.3″W	Gomes 639
	RR	Brazil, Roraima, Rorainópolis	1°22′5.2″S, 61°45′55.3″W	Gomes 639
	RR	Brazil, Roraima, Rorainópolis	1°22′5.2″S, 61°45′55.3″W	Gomes 639
	RR	Brazil, Roraima, Rorainópolis	1°22′5.2″S, 61°45′55.3″W	Gomes 639
	RR	Brazil, Roraima, Rorainópolis	1°22′5.2″S, 61°45′55.3″W	Gomes 640
	RR	Brazil, Roraima, Rorainópolis	1°23′0.2″S, 61°51′6″W	Gomes 648
	RR	Brazil, Roraima, Rorainópolis	1°12′12.7″S, 61°50′37.3″W	Gomes 651
	RR	Brazil, Roraima, Rorainópolis	1°23′42.0″S, 61°41′45.0″W	Lohmann 336
	RR	Brazil, Roraima, Rorainópolis	0°43′46″S, 61°51′24″W	Thode 424

Appendix 1. Continued.

Species	Population code	Locality	Geographic coordinates	Voucher no.
Pachyptera aromatica (Barb. Rodr.) L. G. Lohmann	Individual	Brazil, Amazonas, Novo Airão	2°32′08″S, 60°50′49″W	Lohmann 794
Pachyptera erythraea (Dugand) A. H. Gentry	Individual	Colombia, Santander	7°09′19″N, 73°50′28″W	Gentry 15372*
Pachyptera kerere var. *incarnata* (Aubl.) A. H. Gentry	Individual	Brazil, Pará, Óbidos	1°52′38.2″S, 55°35′27.4″W	Francisco 122
Pachyptera ventricosa (A. H. Gentry) L. G. Lohmann	Individual	Brazil, Pará, Belterra	2°55′50.2″S, 55°0′44.6″W	Francisco 84

Note: All specimens are deposited at the University of São Paulo Herbarium (SPF), São Paulo, Brazil, except one sample (*) which is deposited at the Missouri Botanical Garden (MO), St. Louis, Missouri, USA.