Microsatellite Markers for the Yam Bean Pachyrhizus (Fabaceae)

Authors: Delêtre, Marc, Soengas, Beatriz, Utge, José, Lambourdière, Josie, and Sørensen, Marten

Source: Applications in Plant Sciences, 1(7)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1200551

Applications in Plant Sciences

Applications in Plant Sciences 2013 1(7): 1200551

Primer Note

Microsatellite markers for the yam bean *Pachyrhizus* (Fabaceae)[1]

Marc Delêtre[2,5], Beatriz Soengas[2], José Utge[3], Josie Lambourdière[3], and Marten Sørensen[4]

[2]UMR 7206 Eco-anthropologie et ethnobiologie, Muséum National d'Histoire Naturelle, Département Hommes Natures Sociétés, CP 135, 57 rue Cuvier, 75231 Paris CEDEX 05, France; [3]SSM-UMS 2700 MNHN-CNRS, Muséum National d'Histoire Naturelle, Département Systématique et Evolution, CP 26, 57 rue Cuvier, 75231 Paris CEDEX 05, France; and [4]Plant Systematic Group, Department of Plant and Environmental Sciences, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 21, DK-1958 Frederiksberg C, Denmark

- *Premise of the study:* Microsatellite loci were developed for the understudied root crop yam bean (*Pachyrhizus* spp.) to investigate intraspecific diversity and interspecific relationships within the genus *Pachyrhizus*.
- *Methods and Results:* Seventeen nuclear simple sequence repeat (SSR) markers with perfect di- and trinucleotide repeats were developed from 454 pyrosequencing of SSR-enriched genomic libraries. Loci were characterized in *P. ahipa* and wild and cultivated populations of four closely related species. All loci successfully cross-amplified and showed high levels of polymorphism, with number of alleles ranging from three to 12 and expected heterozygosity ranging from 0.095 to 0.831 across the genus.
- *Conclusions:* By enabling rapid assessment of genetic diversity in three native neotropical crops, *P. ahipa*, *P. erosus*, and *P. tuberosus*, and two wild relatives, *P. ferrugineus* and *P. panamensis*, these markers will allow exploration of the genetic diversity and evolutionary history of the genus *Pachyrhizus*.

Key words: cross-species amplification; Fabaceae; microsatellites; *Pachyrhizus*; pyrosequencing; yam bean.

Yam beans (*Pachyrhizus* Rich. ex DC., Fabaceae) are little-studied plants with edible tuberous roots native to South and Central America. The genus comprises five species, two wild (*P. panamensis* R. T. Clausen and *P. ferrugineus* (Piper) M. Sørensen) and three cultivated (*P. ahipa* (Wedd.) Parodi, *P. erosus* (L.) Urb., and *P. tuberosus* (Lam.) Spreng.). Yam beans are grown for their starchy root but are propagated exclusively through seeds. To stimulate root growth, farmers prune flower buds but leave either one pod on each plant or select a few plants dedicated to seed production. To set conservation strategies, it is necessary to understand how these different methods influence the crop's dynamics of genetic diversity, but this requires molecular tools that yield information on important parameters such as heterozygosity and allelic frequencies needed for the computation of most population genetic statistics. There are to date no available genetic markers for *Pachyrhizus* species. Socially and culturally important but economically marginalized, yam beans are "orphans" to crop science, and few resources have been invested in evaluating the current status of genetic diversity in these minor yet promising crops. The lack of molecular tools has probably stymied efforts to document these largely untapped genetic resources.

In this paper, we report the isolation and characterization of 17 polymorphic simple sequence repeat nuclear markers for *P. ahipa* and their successful cross-amplification in other *Pachyrhizus* species. Phylogenetic relationships among *Pachyrhizus* species remain largely unresolved. This new set of molecular markers will permit investigation of the phylogeography of the *Pachyrhizus* complex.

METHODS AND RESULTS

Total genomic DNA was extracted from herbarium specimens from 20 mg of lyophilized leaf tissue using NucleoSpin 96 Plant kits (Macherey-Nagel, Hoerdt, France) following the manufacturer's instructions. Purified DNA was eluted in a final volume of 200 µL, and final concentration was checked using a Nanodrop ND-1000 spectrophotometer (Labtech, Palaiseau, France). A sample of 3 µg total DNA at 60 ng/µL final concentration, representing a pool of 12 *P. ahipa* accessions spanning the whole distribution range of the species in Bolivia, was sent to Genoscreen (Lille, France) for production of enriched DNA libraries and 454 GS-FLX Titanium (Roche Applied Science, Meylan, France) pyrosequencing (Malausa et al., 2011). A total of 3454 sequences containing potential microsatellite motifs were produced. Following sequence cleaning and removal of duplicates, 252 primer pairs (only perfect repeats with at least five repeats) were designed using the QDD bioinformatics pipeline (Meglécz et al., 2010).

We selected a set of markers that would cover a wide range of amplification product sizes and could be used in multiplex reactions (i.e., that minimized differences in annealing temperatures and complementarity among primer pairs), targeting in priority loci with the longest di- and trinucleotide repeats (six repeats or more). A cost-efficient approach to selecting markers is to prescreen microsatellites for polymorphism using in silico DNA sequences (Hoffman and Nichols,

[1]Manuscript received 17 October 2012; revision accepted 3 January 2013.

The authors thank Gérard Aymonin and Myriam Gaudeul (Muséum National d'Histoire Naturelle, Paris, France) for providing access to *Pachyrhizus* spp. exsiccatae from the National Herbarium of Paris (P).

[5]Author for correspondence: deletre@mnhn.fr

doi:10.3732/apps.1200551

2011), but very little sequence information is available for the understudied genus *Pachyrhizus*. Blasting primer sequences against sequences available at GenBank for the closest Fabaceae species, we obtained the best results with the model crop *Glycine max* (L.) Merr. (subtribe Glycininae), with a mean query coverage (±SE) of 88% (±23) and 93% (±8) identity between *G. max* and *P. ahipa* homologous sequences. Targeting conserved flanking regions among distantly related species can also be a potent way to enhance cross-species utility of microsatellite markers (Dawson et al., 2010). Using microsatellite variability in *G. max* as a proxy to infer variability among putative microsatellites in *Pachyrhizus* spp., we targeted loci most likely to be polymorphic. Thirty-six primer pairs were tested in separate PCRs. Nine pairs failed to produce clear amplicons. A second test was carried out on the 27 primer pairs that amplified using a sample of 144 accessions (wild and cultivated) from herbarium specimens representing varietal, morphological, and potential genetic variation across the natural distribution area of the genus (Appendix 1). Multiplex PCR were carried out on an Eppendorf Mastercycler ep gradient thermocycler (Eppendorf, Hamburg, Germany) using phosphoramidite-labeled oligonucleotides (Applied Biosystems, Warrington, United Kingdom) in a final volume of 12.5 µL. Along with 1 µL of nondiluted DNA template, each well contained 6.25 µL of QIAGEN Type-it Master Mix (QIAGEN, Hilden, Germany), 1.25 µL of 10× primer mix (with primers at 2 µM), and 4 µL of RNase-free water. An initial activation step at 95°C for 30 s preceded 20 cycles of amplification, each starting with an annealing step of 90 s at 56°C and continuing with an extension at 72°C for 30 s. Amplification ended with a final extension at 60°C for 30 min. To ensure unambiguous peak assignment, primer pairs were pooled in two different sets (M1 and M2) as indicated in Table 1. Multiplex Manager 1.2 software (Holleley and Geerts, 2009) was used to optimize primer combinations.

Genotyping was performed on an ABI PRISM 3130 Genetic Analyzer (Perkin Elmer/Applied Biosystems, Foster City, California, USA). Each sample was prepared from 1 µL of PCR template to which 8.8 µL formamide and 0.2 µL GeneScan 500 LIZ Size Standard (Applied Biosystems) were added. Genotypes were extracted and analyzed using GeneMapper 4.0 software (Applied Biosystems). To reduce the risk of typing errors, allele peaks were checked by eye. Cross-species amplification tests succeeded for all loci across the genus. Six loci were strictly monomorphic across all species and were discarded. At the species level, 15 out of the 17 remaining loci were monomorphic in *P. ahipa*, six in the cultivated *P. tuberosus*, and four in the cultivated *P. erosus* (Table 2). Only two and three loci were monomorphic in the wild *P. tuberosus* and wild *P. erosus*, respectively. Number of alleles, observed and expected heterozygosities, and tests for deviation from Hardy–Weinberg equilibrium (HWE) were estimated using GenAlEx version 6.41 (Peakall and Smouse, 2006). Results for each locus and species are summarized in Table 2. The number of alleles ranged from three to 12, with a mean value of (±SE) 6.4 ± 3.0 alleles across loci and species. Expected heterozygosity ranged from 0.095 (AIP9) to 0.831 (AIP30). All loci showed significant deviation from HWE in the three cultivated species ($P < 0.001$). Linkage disequilibrium was checked using GENEPOP 4.1.4 (Rousset, 2008). Two pairs of loci showed significant linkage disequilibrium in the cultivated *P. erosus* after Bonferroni correction for multiple comparisons ($P < 0.0004$). Yam beans are predominantly self-pollinating species with outcrossing rates typically ranging between 2% and 4% (Sørensen, 1996), and physical linkage of loci cannot be distinguished from disequilibrium due to nonrandom mating.

CONCLUSIONS

Conservation of crop genetic resources hinges on the availability of efficient molecular tools to characterize population genetic structure and decipher the dynamics of crop genetic diversity. The case of *Pachyrhizus* illustrates the spillover benefits

Table 1. Characteristics of the 17 microsatellite loci developed for *Pachyrhizus* spp.

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	T_a (°C)	Primer set	5′ dye	GenBank accession no.
AIP1	F: CAGTAGCACCTCCACCGTTT	$(CT)_9$	86–92	56	M1	6-FAM	JX846809
	R: GTAGAGATCTCCGGTGCCAG						
AIP5	F: GTCGCCTTGTCCTCACTTTC	$(GAA)_7$	97–109	56	M1	NED	JX846810
	R: CAACGCACTGTTCTTCCAAC						
AIP9	F: GTGATCTGTGGTTCTCACGG	$(AC)_{10}$	121–127	56	M2	PET	JX846811
	R: TGCAATACAACCCTTTGGTTC						
AIP10	F: TAATCCAAAATGGGCTTGGA	$(GAA)_7$	122–148	56	M1	6-FAM	JX846812
	R: GGAACATATTCACTGCTTCTTCTTC						
AIP15	F: AATCCCGATCCTATTCCACC	$(CAA)_{14}$	146–167	56	M2	6-FAM	JX846813
	R: TTGGAAGGCTGATCATAGGG						
AIP16	F: TGGTTAAAGCCTCTGAATTTGC	$(TC)_7$	172–186	62	M1	6-FAM	JX846814
	R: AGTCAGCACCAAGTCTCCGT						
AIP17	F: TCAGCTGCATAAGTTGAAGACTC	$(TTC)_{15}$	157–211	60	M2	NED	JX846815
	R: TGCAGGTGATCTTCTGAACTC						
AIP19	F: AGTGACATGATCACCCCATTC	$(AG)_9$	201–205	56	M1	PET	JX846816
	R: TCGAATCCAGAGATTTATGATGG						
AIP21	F: ATGTAACAGTGCGGTTTGGC	$(TC)_8$	227–237	56	M1	NED	JX846817
	R: GAGGCAGTGAATTACACTAAGAAATC						
AIP22	F: CCTCTTGTCACTCTTTCATCTCC	$(TTC)_{10}$	227–263	56	M2	VIC	JX846818
	R: CTCTGCAATTCCCTTCCTGA						
AIP23	F: CAAATCTGACCCTTTAGGCG	$(TCT)_9$	231–252	56	M2	PET	JX846819
	R: AAGCAGGCATAACCTTGTTGA						
AIP27	F: AGCAACTTCCTTCATCTTCCA	$(AAC)_6$	295–301	62	M1	VIC	JX846820
	R: CAAGGGAGAATTTGAGCAGC						
AIP28	F: GTAGCCATTGCTATGCCATT	$(TC)_{10}$	85–107	56	M1	PET	JX846821
	R: CGACTGCGTGATAGACTCTG						
AIP30	F: TCCATCGTTGTCTACAACACC	$(CTT)_{17}$	281–329	56	M2	6-FAM	JX846822
	R: TGAGGAGGAAGAAAGTCAGAGTG						
AIP31	F: CCACTAACTTCGTCCATTGC	$(CT)_{10}$	162–198	56	M1	PET	JX846823
	R: CCAAAGGGATATGGAAACGA						
AIP34	F: ACGATGGATAACTGTTGTACGTG	$(CT)_9$	86–90	56	M2	6-FAM	JX846824
	R: AAATGAGGGAGAAGATTGGTTG						
AIP36	F: CCCAAACAACTATAATGAACTTGAA	$(AG)_{11}$	188–198	56	M2	6-FAM	JX846825
	R: TGTTCCCTATGAGATGTCTGCTAT						

Note: F = forward primer sequence; R = reverse primer sequence; T_a = optimal annealing temperature.

TABLE 2. Results of initial primer screening in *Pachyrhizus ahipa*, *P. erosus*, and *P. tuberosus* (wild and cultivated) for the 17 polymorphic loci. Cross-amplification tests were also carried in two wild species, *P. ferrugineus* and *P. panamensis*.

	P. ahipa (cultivated)				*P. erosus* (cultivated)				*P. erosus* (wild)				*P. ferrugineus*				*P. panamensis*				*P. tuberosus* (cultivated)				*P. tuberosus* (wild)			
Locus	*n*	*A*	H_o	H_e	*n*	*A*	H_o	H_e	*n*	*A*	H_o	H_e	*n*	*A*	H_o	H_e	*n*	*A*	H_o	H_e	*n*	*A*	H_o	H_e	*n*	*A*	H_o	H_e
AIP1	46	1	—	—	19	1	—	—	14	3	0.071	0.554	4	1	—	—	2	2	0.500	0.375	50	3	0.000	0.340	8	3	0.125	0.477
AIP5	46	1	—	—	19	3	0.000	0.460	14	2	0.000	0.490	4	1	—	—	2	1	—	—	50	1	—	—	8	1	—	—
AIP9	46	1	—	—	18	1	—	—	14	2	0.000	0.459	4	3	0.500	0.594	2	1	—	—	50	1	—	—	8	1	—	—
AIP10	46	1	—	—	19	3	0.105	0.517	14	5	0.143	0.758	4	4	0.500	0.719	2	2	0.000	0.500	50	2	0.000	0.113	8	5	0.250	0.773
AIP15	46	1	—	—	19	4	0.000	0.681	14	5	0.000	0.673	4	2	0.000	0.375	2	3	0.500	0.625	50	2	0.000	0.241	8	5	0.250	0.719
AIP16	46	1	—	—	19	1	—	—	14	1	—	—	4	2	0.250	0.219	2	2	0.000	0.500	50	2	0.000	0.077	8	3	0.125	0.461
AIP17	46	2	0.000	0.043	19	5	0.000	0.637	14	7	0.143	0.668	4	1	—	—	2	2	0.000	0.500	50	2	0.000	0.113	8	5	0.250	0.727
AIP19	46	1	—	—	17	2	0.000	0.208	14	2	0.071	0.497	4	2	0.000	0.375	2	1	—	—	50	2	0.000	0.039	8	2	0.000	0.219
AIP21	46	1	—	—	16	3	0.063	0.643	14	3	0.071	0.538	4	3	0.500	0.531	2	2	0.000	0.500	50	1	—	—	8	3	0.250	0.586
AIP22	45	1	—	—	17	3	0.000	0.637	14	5	0.071	0.543	4	2	0.000	0.500	2	3	0.500	0.625	50	2	0.000	0.113	8	5	0.250	0.750
AIP23	46	1	—	—	11	2	0.000	0.397	8	3	0.000	0.594	4	2	0.000	0.375	2	2	0.000	0.500	50	2	0.000	0.365	7	3	0.143	0.622
AIP27	46	1	—	—	14	2	0.000	0.337	14	1	—	—	4	2	0.250	0.469	2	1	—	—	50	1	—	—	8	2	0.000	0.219
AIP28	46	1	—	—	20	2	0.000	0.480	14	3	0.071	0.554	4	3	0.500	0.594	2	2	0.000	0.500	50	1	—	—	8	4	0.125	0.680
AIP30	45	2	0.000	0.411	18	3	0.000	0.475	14	4	0.000	0.612	4	3	0.250	0.531	2	2	0.000	0.500	49	6	0.000	0.493	8	6	0.250	0.734
AIP31	45	1	—	—	15	4	0.000	0.436	11	2	0.091	0.236	1	1	—	—	1	1	—	—	48	3	0.000	0.322	8	5	0.250	0.625
AIP34	46	1	—	—	19	2	0.000	0.188	14	2	0.214	0.436	4	2	0.000	0.375	2	1	—	—	50	1	—	—	8	2	0.125	0.117
AIP36	45	1	—	—	19	1	—	—	14	1	—	—	4	1	—	—	2	2	0.500	0.375	50	2	0.000	0.343	8	4	0.500	0.695

Note: — = H_e and H_o could not be calculated because the locus is monomorphic in this species; A = number of alleles detected; H_e = expected heterozygosity; H_o = observed heterozygosity; n = number of samples genotyped.

to be reaped from next-generation sequencing and research on model plants for the study of minor crops (Varshney et al., 2010). The markers we developed showed high levels of polymorphism and enough discriminant power for distinguishing among varietal groups within species. They will be available for a wide range of applications, from breeding to population genetic studies. Markers also revealed a surprisingly low level of genetic variability in the Bolivian root crop, *P. ahipa*. While the wild parent of the crop has yet to be identified, we will use the new markers to investigate the origin of *P. ahipa*. Results should shed new light on the evolutionary history of the *Pachyrhizus* genus.

LITERATURE CITED

DAWSON, D. A., G. J. HORSBURGH, C. KÜPPER, I. R. K. STEWART, A. D. BALL, K. L. DURRANT, B. HANSSON, ET AL. 2010. New methods to identify conserved microsatellite loci and develop primer sets of high cross-species utility—As demonstrated for birds. *Molecular Ecology Resources* 10: 475–494.

HOFFMAN, J. I., AND H. J. NICHOLS. 2011. A novel approach for mining polymorphic microsatellite markers *in silico*. *PLoS ONE* 6: e23283.

HOLLELEY, C., AND P. GEERTS. 2009. Multiplex Manager 1.0: A cross-platform computer program that plans and optimizes multiplex PCR. *BioTechniques* 46: 511–517.

MALAUSA, T., A. GILLES, E. MEGLÉCZ, H. BLANQUART, S. DUTHOY, C. COSTEDOAT, V. DUBUT, ET AL. 2011. High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. *Molecular Ecology Resources* 11: 638–644.

MEGLÉCZ, E., C. COSTEDOAT, V. DUBUT, A. GILLES, T. MALAUSA, N. PECH, AND J. F. MARTIN. 2010. QDD: A user-friendly program to select microsatellite markers and design primers from large sequencing projects. *Bioinformatics (Oxford, England)* 26: 403–404.

PEAKALL, R., AND P. E. SMOUSE. 2006. GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

ROUSSET, F. 2008. GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.

SØRENSEN, M. 1996. Yam bean (*Pachyrhizus* DC.). Promoting the conservation and use of underutilized and neglected crops, vol. 2. Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany, and International Plant Genetic Resources Institute, Rome, Italy.

VARSHNEY, R. K., J.-C. GLASZMANN, H. LEUNG, AND J.-M. RIBAUT. 2010. More genomic resources for less-studied crops. *Trends in Biotechnology* 28: 452–460.

Appendix 1. List of exsiccatae used in cross-species amplification tests. Wild and cultivated specimens are indicated as well as varietal types (when available).

Species	Voucher specimen	Herbarium	Status	Varietal type	Geographic origin	Geographic coordinates		*n*
P. ahipa	AC102	CP	Cult.		Bolivia	−21.516667	−64.75	7
	AC201	CP	Cult.		Bolivia	−16.991785	−67.65667	3
	AC202	CP	Cult.		Bolivia	−16.991785	−67.65667	3
	AC203	CP	Cult.		Bolivia	−17.003605	−67.632637	3
	AC204	CP	Cult.		Bolivia	−16.991785	−67.65667	4
	AC205	CP	Cult.		Bolivia	−17.578248	−65.908356	3
	AC206	CP	Cult.		Bolivia	−17.578248	−65.908356	2
	AC207	CP	Cult.		Bolivia	−17.578248	−65.908356	2
	AC208	CP	Cult.		Bolivia	−17.115358	−66.866082	2
	AC209	CP	Cult.		Bolivia	−16.702337	−67.928724	2
	AC213	CP	Cult.		Bolivia	−16.565948	−67.450075	5
	AC214	CP	Cult.		Bolivia	−16.816619	−67.58327	5
	AC521	CP	Cult.		Bolivia	−17.386354	−66.166935	2
	AC526	CP	Cult.		Bolivia	−22.191736	−64.679739	3
P. erosus	EC004	CP	Cult.		Mexico	21.036201	−104.371755	1
	EC006	CP	Cult.		Mexico	17.084025	−96.750269	1
	EC033	CP	Cult.		Mexico	20.694622	−88.805437	1
	EC040	CP	Cult.		Guatemala	14.183014	−90.022237	1
	EC042	CP	Cult.		Guatemala	14.198991	−90.051012	1
	EC043	CP	Cult.	Jícama	Guatemala	13.850747	−90.107489	1
	EC104	CP	Cult.		Mexico	20.172634	−89.018154	1
	EC116	CP	Cult.		Guatemala	14.272535	−90.038137	1
	EC204	CP	Cult.		Mexico	19.453644	−96.958523	1
	EC205	CP	Cult.	Agua Dulce	Mexico	20.574095	−100.748026	1
	EC214	CP	Cult.		Guatemala	16.968801	−89.912224	1
	EC216	CP	Cult.		Guatemala	16.792709	−89.93351	1
	EC219	CP	Cult.	Jícama	Guatemala	16.514523	−89.415679	1
	EC250	CP	Cult.		Guatemala	16.968801	−89.912224	1
	EC352	CP	Cult.		Honduras	14.89834	−88.721695	1
	EC353	CP	Cult.		Honduras	14.398769	−89.197369	1
	EC502	CP	Cult.	Cristalina	Mexico	17.224758	−93.603516	1
	EC510	CP	Cult.		Mexico	19.848102	−90.522079	1
	EC559	CP	Cult.	Tipo Nayarit	Mexico	21.813775	−105.207667	1
	EC560	CP	Cult.	Agua Dulce	Mexico	21.054305	−104.484372	1
	EW048	CP	Wild		Costa Rica	10.495914	−85.358734	1
	EW049	CP	Wild		Costa Rica	10.495914	−85.358734	1
	EW050	CP	Wild		Costa Rica	10.495914	−85.358734	1
	EW051	CP	Wild		Costa Rica	10.495914	−85.358734	1
	EW053	CP	Wild		Costa Rica	10.51883	−85.25425	1
	EW054	CP	Wild		Costa Rica	10.522919	−85.254135	1
	EW115	CP	Wild		Costa Rica	15.801297	−91.755159	1
	EW203	CP	Wild		Mexico	19.489088	−96.950426	1
	EW212	CP	Wild		Guatemala	15.078426	−89.436391	1
	EW222	CP	Wild		Costa Rica	10.578947	−85.404396	1
	EW223	CP	Wild		Costa Rica	10.547559	−85.681744	1
	EW229	CP	Wild		Costa Rica	18.457018	−70.121276	1
	EW230	CP	Wild		Dominican Republic	18.755268	−70.017257	1
	EW522	CP	Wild		Mauritius	−20.233892	57.497052	1
P. ferrugineus	FW044	CP	Wild		Guatemala	15.2835	−89.0653	1
	FW220	CP	Wild		Costa Rica	10.041001	−83.545998	1
	FW237	CP	Wild		Martinique	14.74463	−61.172655	1
	1713	FHO	Wild		Honduras	15.28333333	−87.65	1
P. panamensis	PW055	CP	Wild		Panama	9.211261	−79.616092	1
	PW056	CP	Wild		Panama	−2.235923	−80.0773	1
P. tuberosus	TC063	CP	Cult.	Ashipa	Bolivia	−17.402899	−63.769538	1
	TC210	CP	Cult.	Ashipa	Bolivia	−16.313055	−67.604899	1
	TC239	CP	Cult.	Jíquima	Ecuador	−0.78052	−80.259619	1
	TC303	CP	Cult.	Iwa	Ecuador	−1.516623	−77.983546	1
	TC306	CP	Cult.	Iwa	Ecuador	−1.034976	−77.665193	1
	TC307	CP	Cult.	Capamu	Ecuador	−1.197423	−77.394104	1
	TC308	CP	Cult.	Capamu	Ecuador	−1.197423	−77.394104	1
	TC309	CP	Cult.	Namaou	Ecuador	−1.931854	−77.867203	1
	TC311	CP	Cult.	Jíquima	Ecuador	−1.350635	−80.579531	1
	TC313	CP	Cult.	Jíquima	Ecuador	−1.04433	−80.65846	1
	TC314	CP	Cult.	Jíquima	Ecuador	−1.049994	−80.516596	1
	TC350	CP	Cult.	Chuin morado	Peru	−4.913096	−73.683014	1
	TC351	CP	Cult.	Ashipa	Peru	−3.784781	−73.343725	1
	TC352	CP	Cult.	Chuin morado	Peru	−5.816514	−74.399128	1

Appendix 1. Continued.

Species	Voucher specimen	Herbarium	Status	Varietal type	Geographic origin	Geographic coordinates		*n*
	TC353	CP	Cult.	Chuin amarillo	Peru	−4.995186	−73.982391	1
	TC354	CP	Cult.	Chuin blanco	Peru	−9.462608	−74.191132	1
	TC355	CP	Cult.	Chuin morado	Peru	−9.462608	−74.191132	1
	TC356	CP	Cult.	Ashipa	Peru	−4.981505	−73.820343	1
	TC357	CP	Cult.	Ashipa maron	Peru	−3.783925	−73.344755	1
	TC358	CP	Cult.	Ashipa maron	Peru	−3.783925	−73.344755	1
	TC359	CP	Cult.	Ashipa	Peru	−6.914839	−75.171905	1
	TC361	CP	Cult.	Chuin morado	Peru	−9.462608	−74.191132	1
	TC362	CP	Cult.	Chuin morado	Peru	−9.462608	−74.191132	1
	TC374	CP	Cult.	Ashipa	Peru	−8.538923	−74.876347	1
	TC375	CP	Cult.	Ashipa	Peru	−8.393583	−74.42399	1
	TC376	CP	Cult.	Yushpe	Peru	−8.688282	−74.432602	1
	TC532	CP	Cult.	Ajipa	Bolivia	−15.166667	−67.066667	1
	TC533	CP	Cult.	Ajipa	Bolivia	−14.349548	−67.950125	1
	TC534	CP	Cult.	Ashipa	Peru	−6.027214	−76.966839	1
	TC537	CP	Cult.	Ashipa	Peru	−12.982437	−71.284111	1
	TC538	CP	Cult.	Ashipa	Peru	−13.896077	−71.501198	1
	TC544	CP	Cult.	Chuin morado	Peru	−4.554522	−73.620987	1
	TC547	CP	Cult.	Chuin morado	Peru	−4.570265	−73.685417	1
	TC548	CP	Cult.	Chuin morado	Peru	−4.570265	−73.685417	1
	TC549	CP	Cult.	Chuin morado	Peru	−4.625704	−73.752708	1
	TC550	CP	Cult.	Jíquima	Ecuador	−0.78052	−80.259619	1
	TC551	CP	Cult.	Jíquima	Ecuador	−0.78052	−80.259619	1
	TC552	CP	Cult.	Jíquima	Ecuador	−0.922554	−80.446064	1
	TC553	CP	Cult.	Jíquima	Ecuador	−1.206948	−80.369039	1
	TC554	CP	Cult.	Jíquima	Ecuador	−0.92267	−80.445679	1
	TC555	CP	Cult.	Jíquima	Ecuador	−0.92267	−80.445679	1
	TC556	CP	Cult.	Iwa	Ecuador	−1.516623	−77.983546	1
	TC557	CP	Cult.	Iwa	Ecuador	−1.482921	−78.002413	1
	TC564	CP	Cult.	Cocotichuin	Peru	−3.708167	−73.200167	1
	TC565	CP	Cult.	Cocotichuin	Peru	−8.735792	−74.540977	1
	TC566	CP	Cult.	Chuin blanco	Peru	−8.764296	−74.529991	1
	TC568	CP	Cult.	Ashipa	Peru	−8.692863	−74.414377	1
	TC575	CP	Cult.	Chuin morado	Peru	−3.708041	−73.200045	1
	TC577	CP	Cult.	Cocotichuin	Peru	−9.354223	−74.306488	1
	TC578	CP	Cult.	Chuin blanco	Peru	−8.764296	−74.529991	1
	TW378	CP	Wild		Ecuador	−0.91659	−77.750037	1
	TW379	CP	Wild		Ecuador	−2.299945	−78.100054	1
	TW380	CP	Wild		Ecuador	−3.406414	−78.572431	1
	TW381	CP	Wild		Ecuador	−3.88318	−78.783488	1
	TW558	CP	Wild		Ecuador	−1.066685	−79.466693	1
	TW559	CP	Wild		Ecuador	−1.066642	−79.466693	1
	TW560	CP	Wild		Ecuador	−1.066642	−79.466693	1
	TW561	CP	Wild		Ecuador	−0.016136	−79.383488	1

Note: CP = Royal Veterinary and Agricultural University Herbarium, Copenhagen, Denmark; cult. = cultivated; FHO = University of Oxford, Daubeny Herbarium, Oxford, United Kingdom; *n* = number of individuals per accession.