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MICROSATELLITE MARKERS FOR THE YAM BEAN _PACHYRHIZUS_ (FABACEAE)\(^1\)

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- **Premise of the study:** Microsatellite loci were developed for the understudied root crop yam bean (_Pachyrhizus_ spp.) to inves-
tigate 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 poly-
morphism, 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 diver-
sity 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 strate-
gies, it is necessary to understand how these different methods in-
fluence 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
454 GS-FLX Titanium (Roche Applied Science, Meylan, France) pyrosequenc-
ing (Malausa et al., 2011). A total of 3454 sequences containing potential micro-
satellite 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 dif-
fences 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 micro-
satellites for polymorphism using in silico DNA sequences (Hoffman and Nichols,
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 vari-
etal, morphological, and potential genetic variation across the natural distribution area of the genus (Appendix 1). Multiplex PCR were carried out on an Eppe-
dorf 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 pre-
ceded 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 assign-
ment, 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
testing 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 GenAIEx version 6.61 (Peakall and Smouse, 2006). Results for each lo-
cus 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 avail-
ability of efficient molecular tools to characterize population genetic structure and decipher the dynamics of crop genetic di-
versity. The case of *Pachyrhizus* illustrates the spillover benefits

| Locus | Primer sequences (5’–3’) | Repeat motif | Allele size range (bp) | T\textsubscript{a} (°C) | Primer set | 5’ dye | GenBank accession no. |
|-------|--------------------------|--------------|------------------------|----------------|------------|-------|---------------------|
| AIP1  | F: CATGAGCCCTCCACCGGTTT | (CT)\textsubscript{6} | 86–92 | 56 | M1 | 6-FAM | JX846809 |
| R: GTAGGAAGCTTCCGCTGCAG | | | | | | |
| AIP5  | F: GTGCGTGTTGCTCCACTCTTC | (GAA)\textsubscript{3} | 97–109 | 56 | M1 | NED | JX846810 |
| R: CAAAGTACCTGTTCTTACAC | | | | | | |
| AIP9  | F: GTATCTGTTGCTTCTCCAGG | (AC)\textsubscript{10} | 121–127 | 56 | M2 | PET | JX846811 |
| R: TGCAATACACCTCTTTCAC | | | | | | |
| AIP10 | F: TAAACAAAGGGCTTGGGA | (GAA)\textsubscript{3} | 122–148 | 56 | M1 | 6-FAM | JX846812 |
| R: GAGAACATTACGTGCTTCTTC | | | | | | |
| AIP15 | F: ATGCCCTGCTTCCACC | (CAA)\textsubscript{14} | 146–167 | 56 | M2 | 6-FAM | JX846813 |
| R: TTGGAGGCGTATGTACG | | | | | | |
| AIP16 | F: TGTTAAAGGCCTGTAATGGC | (TC)\textsubscript{2} | 172–186 | 62 | M1 | 6-FAM | JX846814 |
| R: AGTCAGCCAAAGCTCTCAGT | | | | | | |
| AIP17 | F: TCACGTGCTAAAGTTAGAATC | (TTT)\textsubscript{15} | 157–211 | 60 | M2 | NED | JX846815 |
| R: TGCAAGGTGACTCTGACACTC | | | | | | |
| AIP19 | F: AGTGAACATGACACCCCTTAT | (AG)\textsubscript{9} | 201–205 | 56 | M1 | PET | JX846816 |
| R: TCCGGACTGCAAGATTTATGAGT | | | | | | |
| AIP21 | F: ATGGAAGGCTGCTTGGGC | (TC)\textsubscript{8} | 227–237 | 56 | M1 | NED | JX846817 |
| R: GAGGGCTGTTATCACTACAAATC | | | | | | |
| AIP22 | F: CCGTCTGTGCTCTCTTCCTTCCA | (TTT)\textsubscript{10} | 227–263 | 56 | M2 | VIC | JX846818 |
| R: CTCTGGATTTTCCTTTGCA | | | | | | |
| AIP23 | F: CAAATGCTGGCCTTTAGGGGC | (TCT)\textsubscript{9} | 231–252 | 56 | M2 | PET | JX846819 |
| R: AAGCAAGTTAACCCCTTGTTGA | | | | | | |
| AIP27 | F: AGCAATCTTCTCTTCCACCTCACCA | (AAT)\textsubscript{6} | 295–301 | 62 | M1 | VIC | JX846820 |
| R: CAAAGGGAGATGGTAAAGCGC | | | | | | |
| AIP28 | F: GTCAAGCTTGGCTGCTGATT | (TC)\textsubscript{9} | 85–107 | 56 | M1 | PET | JX846821 |
| R: CGACTCCGTGATAGACTCTCTG | | | | | | |
| AIP30 | F: TCACGTGCTTCTCAACACC | (CTT)\textsubscript{17} | 281–329 | 56 | M2 | 6-FAM | JX846822 |
| R: TGAGGAGGGAGAAAGTACGCTTG | | | | | | |
| AIP31 | F: CCACTAACCTCTGCTCTTGCC | (CT)\textsubscript{10} | 162–198 | 56 | M1 | PET | JX846823 |
| R: CCAAAGGATATGGTAAAGCAG | | | | | | |
| AIP34 | F: AGATGGAATACCTGTGCTGACTG | (CT)\textsubscript{9} | 86–90 | 56 | M2 | 6-FAM | JX846824 |
| R: AATAGGGGAGAGATTGTTTGG | | | | | | |
| AIP36 | F: CACCAACAATGTAATTGAAGCTTAGA | (AG)\textsubscript{11} | 188–198 | 56 | M2 | 6-FAM | JX846825 |
| R: TGGTCCCTCCTGATAATGGTCTGCTCATT | | | | | | |

**Note:** F = forward primer sequence; R = reverse primer sequence; \( T_a \) = optimal annealing temperature.
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* (cultivated) *P. erosus* (cultivated) *P. erosus* (wild) *P. ferrugineus* *P. panamensis* *P. tuberosus* (cultivated) *P. tuberosus* (wild)

| Locus    | n | A | H<sub>o</sub> | H<sub>e</sub> | HE 46 | n | A | H<sub>o</sub> | H<sub>e</sub> | HE 46 | n | A | H<sub>o</sub> | H<sub>e</sub> | HE 46 | n | A | H<sub>o</sub> | H<sub>e</sub> | HE 46 |
|----------|---|---|---------|--------|-----|---|---|---------|--------|-----|---|---|---------|--------|-----|---|---|---------|--------|-----|
| AIP5     | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP9     | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP10    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP15    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP16    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP17    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP19    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP21    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP22    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP23    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP27    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP28    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP30    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP31    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP34    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |
| AIP36    | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   | 46| 1 | 0.000   | 0.000  | 0   |

Note: = He and Ho could not be calculated because the locus is monomorphic in this species; n = number of alleles detected; He = expected heterozygosity; Ho = observed heterozygosity.

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**LITERATURE CITED**

Dawson, D. A., G. J. Horsburgh, C. Kupper, 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.

Hofmann, J. I., and H. J. Nichols. 2011. A novel approach for mining polymorphic microsatellite markers in *silico*. *PLoS ONE* 6: e23283.

Holley, C., and P. Gerhts. 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. Costeodat, V. Dubet, 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. Costeodat, V. Dubet, 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. GenAIEx 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. Liung, 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 | −67.56567 | 3 |
|         | AC202 | CP | Cult. | Bolivia | −16.991785 | −67.56567 | 3 |
|         | AC203 | CP | Cult. | Bolivia | −17.003605 | −67.632637 | 3 |
|         | AC204 | CP | Cult. | Bolivia | −16.991785 | −67.56567 | 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. | Guatemala | 14.183014 | −90.02237 | 1 |
|          | EC040 | CP | Cult. | Guatemala | 14.198991 | −90.051012 | 1 |
|          | EC043 | CP | Cult. | Jícamá | 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 | −96.950075 | 1 |
|          | EC205 | CP | Cult. | Agua Dulce | 20.574095 | −100.748026 | 1 |
|          | EC214 | CP | Cult. | Guatemala | 16.968801 | −89.912224 | 1 |
|          | EC216 | CP | Cult. | Guatemala | 16.792709 | −89.9353 | 1 |
|          | EC219 | CP | Cult. | 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 | 17.224758 | −93.603516 | 1 |
|          | EC510 | CP | Cult. | Mexico | 19.848102 | −90.52079 | 1 |
|          | EC559 | CP | Cult. | Tipo Nayarit | 21.813775 | −105.207667 | 1 |
|          | EC560 | CP | Cult. | Agua Dulce | 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 | −96.950075 | 1 |
|          | EW212 | CP | Wild | Guatemala | 15.078426 | −89.436391 | 1 |
|          | EW222 | CP | Wild | Costa Rica | 10.578947 | −85.403936 | 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 | 47.970852 | 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 | 15.283333 | −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. | Ashiya | −17.402899 | −63.765938 | 1 |
|            | TC210 | CP | Cult. | Ashiya | −16.313055 | −67.048989 | 1 |
|            | TC239 | CP | Cult. | Jíquima | −0.78052 | −80.259619 | 1 |
|            | TC303 | CP | Cult. | Iwa | −1.516623 | −77.98546 | 1 |
|            | TC306 | CP | Cult. | Iwa | −1.034976 | −77.665193 | 1 |
|            | TC307 | CP | Cult. | Capamu | −1.197423 | −77.394104 | 1 |
|            | TC308 | CP | Cult. | Capamu | −1.197423 | −77.394104 | 1 |
|            | TC309 | CP | Cult. | Namaou | −1.931854 | −77.867203 | 1 |
|            | TC311 | CP | Cult. | Jíquima | −1.350635 | −80.579531 | 1 |
|            | TC313 | CP | Cult. | Jíquima | −1.04433 | −80.65846 | 1 |
|            | TC314 | CP | Cult. | Jíquima | −1.049994 | −80.516936 | 1 |
|            | TC350 | CP | Cult. | Chuin morado | −4.913096 | −73.60314 | 1 |
|            | TC351 | CP | Cult. | Chuin morado | −3.784781 | −73.343725 | 1 |
|            | TC352 | CP | Cult. | Chuin morado | −5.816514 | −74.399128 | 1 |

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### 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 |
| TC352   | CP               | Cult.     | Ajipa        | Bolivia       | −15.166667       | −67.066667             | 1 |
| TC353   | CP               | Cult.     | Ajipa        | Bolivia       | −14.349548       | −67.950125             | 1 |
| TC354   | CP               | Cult.     | Ashipa       | Peru          | −6.027214        | −76.966839             | 1 |
| TC357   | CP               | Cult.     | Ashipa       | Peru          | −12.982437       | −71.284111             | 1 |
| TC338   | CP               | Cult.     | Ashipa       | Peru          | −13.896077       | −71.501198             | 1 |
| TC344   | CP               | Cult.     | Chuin morado | Peru          | −4.554522        | −73.620987             | 1 |
| TC347   | CP               | Cult.     | Chuin morado | Peru          | −4.570265        | −73.685417             | 1 |
| TC348   | CP               | Cult.     | Chuin morado | Peru          | −4.570265        | −73.685417             | 1 |
| TC349   | 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      |               |               | −0.91659         | −77.750037             | 1 |
| TW379   | CP               | Wild      |               |               | −2.299945        | −78.100054             | 1 |
| TW380   | CP               | Wild      |               |               | −3.406414        | −78.572431             | 1 |
| TW381   | CP               | Wild      |               |               | −3.883318        | −78.783488             | 1 |
| TW558   | CP               | Wild      |               |               | −1.066685        | −79.466693             | 1 |
| TW559   | CP               | Wild      |               |               | −1.066642        | −79.466693             | 1 |
| TW560   | CP               | Wild      |               |               | −1.066642        | −79.466693             | 1 |
| TW561   | CP               | Wild      |               |               | −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.

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