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PRIMER NOTE

Microsatellite markers for the endangered *Puya raimondii* in Peru

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PREMISE: Microsatellite primers were developed for *Puya raimondii* (Bromeliaceae), an endangered species distributed in the Andean Mountains of Bolivia and Peru.

METHODS AND RESULTS: Genome skimming of *P. raimondii*, *P. macrura*, and *P. hutchisonii* resulted in the selection of 46 pairs of cross-species microsatellite markers. Of these, 12 microsatellite primer pairs produced clear and polymorphic bands in *P. raimondii*. These primer sets were then used for the detection of potential polymorphisms in 84 *P. raimondii* individuals collected from four populations in Peru. The number of alleles per locus ranged from one to six, and the observed and expected levels of heterozygosity ranged from 0.000 to 0.8929 and from 0.000 to 0.7662, respectively.

CONCLUSIONS: The microsatellite markers developed in this study will be useful for future population genetic analyses and breeding system studies in *P. raimondii*.

KEY WORDS Bromeliaceae; codominant markers; genetic variability; genome skimming; next-generation sequencing; *Puya raimondii*.

Puya raimondii Harms (Bromeliaceae), also known as queen of the Andes or, locally, as titanka, grows between 3600 and 4400 m in high-elevation grasslands and along rocky slopes. It is mostly found in scattered populations along the Andes of Peru and Bolivia, where it plays an important role, serving as a critical refuge, food source, and nesting place for a number of bird species (Salinas et al., 2007). *Puya raimondii* is the largest species in the Bromeliaceae, producing tens of thousands of flowers per inflorescence. Its stem can reach 5 m tall, on top of a rosette of hundreds of thorny leaves. Being monocarpic, the inflorescence is produced at the end of its life cycle (~100 years), reaching up to 8 m tall. With an estimated 800,000 individuals in Peru, and 30,000–35,000 individuals in Bolivia, the species is considered endangered (Lambe, 2009). The main threats to its survival are anthropogenic fire disturbance, climate change, and declining genetic diversity.

To date, accurate and comprehensive studies on the genetic structure of remaining *P. raimondii* populations are lacking. Although Sgorbati et al. (2004) found high levels of genetic similarity among eight populations of *P. raimondii* in Peru based on a combination of amplified fragment length polymorphism (AFLP), cpSSR, and random-amplified polymorphic DNA (RAPD) analyses, a high ratio of polymorphic AFLP markers has also been reported for populations from the Huascarán National Park and neighboring areas (Hornung-Leoni et al., 2013). In addition, Vadillo (2011) found significant morphological variation for the number of spines on the leaf apices of plants sampled from 15 populations located in the central and southern part of Peru. Collectively, these studies can provide some insight into the genetic structure of *P. raimondii* populations. However, some of the methods used (i.e., analyses based on morphological traits and dominant genetic markers) are not useful for assessing ecological or evolutionary processes that are critical to development of conservation strategies for the species, such as mating system investigations or parentage analysis.

Thus, there is an urgent need to develop codominant genetic markers that can be used to better assess the genetic and ecological impacts of small population size associated with the potential endangerment of *P. raimondii*. Next-generation sequencing technology is now widely used in many areas of conservation biology, including for the development of microsatellite markers to assess the genetic structure of populations. In this study, we used next-generation sequencing (i.e., genome skimming techniques) to develop a set of microsatellite markers for *P. raimondii*.

METHODS AND RESULTS

To design primers for microsatellite markers in *P. raimondii*, one individual each of *P. raimondii*, *P. macrura* Mez, and *P. hutchisonii* L. B. Sm. was sampled for genome skimming. The latter two species and *P. macropoda* L. B. Sm. were used to conduct cross-species

screening of microsatellite markers in *P. raimondii*. *Puya raimondii* is closely related to *P. macrura* (Jabaily and Sytsma, 2010), whereas the phylogenetic relationship to *P. hutchisonii* and *P. macropoda* remains unknown. All four species are distributed in arid regions of the high Andes and are morphologically similar at the juvenile stage. For this study, plant material was collected from Peru: *P. raimondii* was collected from Chupaca, Lampa, and Bolognesi provinces; *P. hutchisonii* was collected from Huaylas Province; *P. macrura* was collected from Huari Province; and *P. macropoda* was collected from Yungay Province. Voucher specimens for each species were deposited in the Herbarium of the Museo de Historia Natural of Universidad Nacional Mayor de San Marcos (USM), Lima, Peru (Appendix 1).

Total genomic DNA was extracted from silica-dried leaves using a modified cetyltrimethylammonium bromide (CTAB) procedure (Doyle and Doyle, 1987; a higher concentration [3%] of beta-mercaptoethanol was used in the extraction buffer) and sent to the Beijing Genomics Institute (BGI; Shenzhen, China) for library construction and sequencing. The genomic libraries were sequenced on an Illumina X Ten platform (Illumina, San Diego, California, USA) with a 150-bp paired-end strategy; approximately 10 million raw reads and 95,000 assembled contigs (longer than 590 bp) were generated for each species. The raw reads were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (BioProject number: PRJNA562459, PRJNA562611; SRA number: SRR10023784, SRR10023783, SRR10028124; Appendix 1). The library reads of each of the three species were assembled using SPAdes 3.13.0 (Bankevich et al., 2012). Plastome contigs were identified by queries to GenBank based on BLASTX analysis and subsequently excluded in the assembled genomes. Microsatellite regions were screened in the assembled genome of *P. raimondii* by using the microsatellite search tool SciRoKo 3.4 (Kofler et al., 2007). PCR primer pairs for microsatellites were designed using Primer3web version 4.1.0 (Untergasser et al., 2012) with the default parameter settings. In total, 220 microsatellite loci from *P. raimondii* were identified. They belonged to di-, tri-, tetra-, penta-, and hexanucleotide repeats (50%, 22.7%, 11.3%, 9%, and 7%, respectively). Each locus was checked for homology in the assembled *P. macrura* and *P. hutchisonii* genomes using BioEdit version 7.0.9.0 (Hall, 1999). In total, 70 cross-species microsatellite loci were selected for primer design and synthesis (Majorbio Company, Shanghai, China).

PCR amplification was performed with three primers: a sequence-specific forward primer with an M13(−21) tail at its 5′ end, a sequence-specific reverse primer, and the universal fluorescent-labeled M13(−21) primer (FAM, HEX, or TAMRA; Invitrogen, Guangzhou, China) (Schuelke, 2000). Amplification was performed in 10-μL reactions that include: 2 μL 5× buffer mix (TaKaRa Biotechnology Co., Dalian, China), 0.8 μL of dNTP, 0.1 μL of *Taq* (PrimeSTAR, TaKaRa Biotechnology Co.), 1 μL 0.2 mM aqueous solution for each of three primers (3 μL in total), 30–50 ng of template DNA in 1 μL of aqueous solution, and 3.1 μL of ddH_2O. PCR conditions include: 3 min at 94°C, followed by 35 cycles of denaturation at 94°C for 3 min, denaturation of 94°C for 30 s, annealing of 60°C for 30 s, and DNA extension at 72°C for 5 min. The PCR products were scanned by an ABI PRISM 3100 Genetic Analyzer using GeneScan 500 LIZ internal size standard (Applied Biosystems, Waltham, Massachusetts, USA). The size of the alleles at each locus was scored by GeneMarker version 1.5 (SoftGenetics, State College, Pennsylvania, USA). Preliminary PCR screening resulted in the successful amplification of 46 of the 70 primer pairs; one clear band was generated for each of the 46 primer pairs in *P. raimondii*. These primer pairs (Table 1, Appendix 2) were then screened for polymorphisms across nine individuals selected from four different *P. raimondii* populations (Cachi, Huascar, Pachapaqui,

TABLE 1. Characteristics of 12 polymorphic microsatellite loci identified in *Puya raimondii*.

Locus[a]	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	Fluorescent dye	GenBank accession no.
Puya-002	F: CTCTCTGCGCCATCACATTA R: TCGTGATCGGGTTGATCTT	$(GGT)_8$…$(GGTGGA)_6$	199–216	FAM	MN218732
Puya-009	F: TATGTACCCGATCCGAACC R: TACCCGACCCGACCAAATA	$(ATTTT)_6$…$(TTCGGG)_4$	207–222	FAM	MN218735
Puya-012	F: CTTTCGTATGGGAAGGTGA R: CGAGCCAAGAAAGATGAAGG	$(TAAAA)_4$…$(CT)_6$	246–263	HEX	MN218737
Puya-016	F: GTCCTCGACATCTTCCCAGA R: TGCGGAACGAAAAATAGATG	$(AAAG)_5$	180–194	TAMRA	MN218740
Puya-037	F: GCTTTGGGTTCAACGGTCTA R: GCGGAGACTAAGAGGACGAA	$(TTC)_5$…$(GA)_8$	240–248	HEX	MN218753
Puya-039	F: GCCCATGTATGTGCGTGTAT R: CCCTCCTCCACTGCTTCC	$(GA)_7$	190–202	FAM	MN218754
Puya-042	F: AAGGAATTATGAGCGCATGG R: TGTGAACCCACAGAATCAGC	$(AG)_{19}$	180–194	HEX	MN218755
Puya-046	F: AGGGCTCCTTCTCTCTCCTG R: GGCCAGAGGTAAAGGGGTAG	$(CT)_{12}$	200–213	HEX	MN218756
Puya-049	F: GCAAAATACACGAAGGAAGC R: GGGATGGTGAAGAAATGGTG	$(TC)_6$	210–222	HEX	MN218757
Puya-052	F: TGCGGAAACAGAGAAGAACC R: CTGCTGCAGCTCCTCTTAGG	$(CT)_{13}$	202–210	TAMRA	MN218760
Puya-065	F: TTGGGACTTCCAGGTCACTC R: GAGAGAAGGAGCCCTCATCA	$(CT)_7$…$(CT)_7$	272–284	FAM	MN218769
Puya-069	F: AGGGGAGCTCTCTTGGAGAC R: AAACAGAAACCAACCGCAAC	$(TA)_7$	187–212	TAMRA	MN218772

[a]Annealing temperature for all loci was 60°C.

and Choconchaca; Appendix 1). Twelve primer pairs (Table 1) producing clear and polymorphic bands were then used to screen 84 *P. raimondii* individuals collected from four populations in Peru (Table 2, Appendix 1).

GenAlEx 6.51b2 (Peakall and Smouse, 2012) was used to calculate the number of alleles and the observed and expected levels of heterozygosity. The fixation index (*F*) was calculated using GENEPOP 4.3 (Rousset, 2008). The deviation from Hardy–Weinberg equilibrium and genotypic linkage disequilibrium among all pairs of loci within populations were estimated using GENEPOP 4.3 based on default parameter settings. We found no consistent deviation from Hardy–Weinberg equilibrium or linkage disequilibrium for any loci within the populations. The levels of observed heterozygosity and expected heterozygosity of the *P. raimondii* populations varied from 0.000 to 0.8929 and from 0.000 to 0.7662, respectively (Table 2). For the 12 polymorphic loci, the number of alleles per locus ranged from one to six (Table 2), with loci Puya-002 and Puya-049 having the highest number of alleles.

Cross-species amplification success rates in *P. hutchisonii*, *P. macropoda*, and *P. macrura* indicate that 14–18 of the 46 microsatellite loci developed in *P. raimondii* could also be successfully amplified in this set of taxa (Table 3). Among these successfully cross-amplified loci, six, 14, and 13 loci are polymorphic and 11, four, and two are monomorphic for *P. hutchisonii*, *P. macropoda*,

TABLE 2. Genetic diversity of 12 microsatellite loci in four populations of *Puya raimondii*.[a]

	Cachi (*N* = 15)				Huascar (*N* = 14)				Pachapaqui (*N* = 28)				Choconchaca (*N* = 27)			
Locus	***A***	H_o	H_e	***f***	***A***	H_o	H_e	***f***	***A***	H_o	H_e	***f***	***A***	H_o	H_e	***f***
Puya-002	2	0.0667	0.0667	—	3	0.2857	0.3148	0.0957	6	0.3214	0.4773	0.3306[b]	2	0.0370	0.0370	—
Puya-009	2	0.0667	0.0667	—	3	0.2500	0.2355	−0.0645	3	0.1071	0.2305	0.5398[b]	3	0.0741	0.0734	−0.0097
Puya-012	4	0.2000	0.3057	0.3538	2	0.0714	0.0714	—	3	0.1481	0.2048	0.2803	4	0.1111	0.1433	0.2277
Puya-016	1	0.0000	0.0000	—	1	0.0000	0.0000	—	3	0.1481	0.2621	0.4394[b]	3	0.1111	0.1740	0.3659
Puya-037	1	0.0000	0.0000	—	2	0.0714	0.1984	0.6486	4	0.1786	0.2019	0.1176	3	0.1111	0.1754	0.3710
Puya-039	3	0.0667	0.1908	0.6585[b]	4	0.2500	0.3080	0.1951	5	0.4583	0.5488	0.1678	2	0.0741	0.0727	−0.0196
Puya-042	1	0.0000	0.0000	—	1	0.0000	0.0000	—	3	0.4286	0.5162	0.1724	4	0.1481	0.1433	−0.0348
Puya-046	4	0.2667	0.2506	−0.0667	5	0.3571	0.6138	0.4273[b]	5	0.3571	0.4656	0.2362[b]	1	0.0000	0.0000	—
Puya-049	2	0.1333	0.1287	−0.0370	3	0.2143	0.2619	0.1875	6	0.8929	0.7662	−0.1688	3	0.0741	0.0734	−0.0097
Puya-052	2	0.1333	0.1287	−0.0370	2	0.1667	0.1594	−0.0476	3	0.4444	0.4354	−0.0213	3	0.1538	0.2119	0.2780
Puya-065	2	0.0667	0.0667	—	3	0.0714	0.2619	0.7347[b]	5	0.0714	0.2630	0.7320[b]	1	0.0000	0.0000	—
Puya-069	3	0.1538	0.1508	−0.0213	3	0.1818	0.2554	0.2982	2	0.0800	0.1502	0.4725	2	0.0370	0.0370	—
Overall		0.0961	0.1129	0.1558		0.1599	0.2234	0.3010		0.303	0.3768	0.1985		0.0776	0.0951	0.1820

Note: — = not applicable; *A* = number of alleles; *f* = inbreeding coefficient; H_e = unbiased expected heterozygosity; H_o = observed heterozygosity; *N* = number of individuals.
[a]See Appendix 1 for locality and voucher information.
[b]Deviation from Hardy–Weinberg equilibrium after Bonferroni correction ($P < 0.05$).

TABLE 3. Cross-species amplification success of microsatellites developed in *Puya raimondii* in three related *Puya* species.[a]

	Puya macrura (*N* = 5)				*Puya macropoda* (*N* = 4)				*Puya hutchisonii* (*N* = 2)			
Locus	***A***	H_o	H_e	**Allele size range (bp)**	***A***	H_o	H_e	**Allele size range (bp)**	***A***	H_o	H_e	**Allele size range (bp)**
Puya-002	6	1.0000	0.8889	209–224	2	0.5000	0.4286	210–213	1	—	—	215
Puya-004	7	0.8000	0.9111	197–227	3	0.5000	0.7143	209–215	2	1.0000	0.6667	211–213
Puya-008	—	—	—	—	—	—	—	—	2	1.0000	0.6667	244–248
Puya-014	3	0.2000	0.6000	263–271	2	1.0000	0.5714	263–267	—	—	—	—
Puya-015	—	—	—	—	1	—	—	214	1	—	—	210
Puya-016	2	0.2000	0.2000	242–246	2	1.0000	0.5714	242–246	2	1.0000	0.6667	242–246
Puya-017	2	0.5000	0.5000	244–248	1	0.0000	0.0000	244	1	—	—	246
Puya-022	3	0.2000	0.3778	163–193	6	0.7500	0.9286	169–202	2	1.0000	0.6667	182–188
Puya-028	3	0.6667	0.6000	208–226	3	0.0000	0.7143	220–226	1	—	—	221
Puya-030	5	0.8000	0.8444	243–250	5	0.7500	0.8571	240–254	1	—	—	246
Puya-031	4	0.6000	0.7778	260–269	2	0.3333	0.3333	260–263	1	—	—	259
Puya-033	1	—	—	306	1	—	—	306	1	—	—	305
Puya-034	1	—	—	243	1	—	—	243	—	—	—	—
Puya-037	—	—	—	—	—	—	—	—	1	—	—	245
Puya-042	—	—	—	—	—	—	—	—	2	1.0000	0.6667	177–181
Puya-049	—	—	—	—	2	0.5000	0.4286	213–215	1	—	—	215
Puya-052	—	—	—	—	2	0.0000	0.5714	240–242	2	1.0000	0.6667	227–241
Puya-053	2	0.0000	0.6667	266–268	3	0.5000	0.6786	264–270	–	—	—	—
Puya-054	4	0.4000	0.5333	198–208	3	0.2500	0.7500	206–209	1	—	—	207
Puya-055	4	0.4000	0.8000	172–184	3	0.5000	0.6071	175–184	—	—	—	—
Puya-067	5	0.8000	0.7556	254–266	2	0.2500	0.2500	254–258	1	—	—	257
Overall	—	0.3127	0.4026	—	—	0.3254	0.4002	—	—	0.2857	0.1905	—

Note: — = not applicable; *A* = number of alleles; H_e = unbiased expected heterozygosity; H_o = observed heterozygosity; *N* = sample size.
[a]See Appendix 1 for locality and voucher information.

and *P. macrura*, respectively. These results demonstrate that these primer pairs may be of broad utility throughout the genus *Puya*.

CONCLUSIONS

The design of microsatellite primers for *P. raimondii* will greatly assist future efforts to assess the ecological and genetic ramifications of small population size in this species. This study not only contributes directly to the development of future conservation strategies for *P. raimondii* but also may benefit similar efforts in closely related taxa.

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AUTHOR CONTRIBUTIONS

X.J.G. and M.L.S. designed the experiment, L.T. and Y.Q.Z. conducted genetic work, and Z.F.W. and K.S.B. conducted genetic analyses. All authors assisted with manuscript preparation and approved the final manuscript.

DATA AVAILABILITY

All primer sequences developed for this study have been deposited to the National Center for Biotechnology Information (NCBI) GenBank database; accession numbers are listed in Table 1 (polymorphic loci) and Appendix 2 (monomorphic loci). The raw reads were deposited to the NCBI Sequence Read Archive (BioProject number: PRJNA562459, PRJNA562611; SRA number: SRR10023784, SRR10023783, SRR10028124).

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APPENDIX 1. Locality information of the four *Puya* species used for microsatellite primer design.

Species	Population name	Location	*N*	Geographic coordinates	Elevation (m)	Voucher (Herbarium)[a]	BioProject no.[b]
Puya hutchisonii L. B. Sm.*	—	Prov. Huaylas	2	77.811W, 9.046S	4250	Xue-Jun Ge et al. 221 (USM)	SRR10028124/ PRJNA562611
Puya macropoda L. B. Sm.	—	Prov. Yungay	4	77.64W, 9.07S	3850	Xue-Jun Ge et al. 32 (USM)	—
Puya macrura Mez*	—	Prov. Huari	5	77.183W, 9.319S	3450	Xue-Jun Ge et al. 165 (USM)	SRR10023783/ PRJNA562459
Puya raimondii Harms	Cachi	Prov. Chupaca, Yanacancha	15	75.475W, 12.247S	4124	G. Prado et al. s.n. (USM-315310)	—
Puya raimondii	Huascar	Prov. Chupaca, Yanacancha	14	75.440W, 12.236S	4170	G. Prado et al. s.n. (USM-315311)	—
*Puya raimondii**	Pachapaqui	Prov. Bolognesi, Aquia	28	77.088W, 9.958S	3800	M. Suni et al. s.n. (USM-315307)	SRR10023784/ PRJNA562459
Puya raimondii	Choconchaca	Prov. Lampa, Lampa	27	70.088W, 15.258S	3962	L. Tumi et al. s.n. (USM-315308)	—

Note: N = number of individuals.
[a]Vouchers are deposited at the Herbarium of the Museo de Historia Natural of Universidad Nacional Mayor de San Marcos (USM), Lima, Peru.
[b]NCBI Sequence Read Archive (SRA)/BioProject no. for genome skimming data.
*Species used for genome skimming.

APPENDIX 2. Characteristics of 34 monomorphic microsatellite loci identified in *Puya raimondii*.

Locus[a]	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	Fluorescent dye	GenBank accession no.
Puya-004	F: GTCCACGCAAAAAGGATCA R: GAGGGGAATTGGAAACCCTA	$(TTCCCG)_6…(CT)_{12}$	261	TAMRA	MN218733
Puya-008	F: AGAGGGTTCACCGTAGAGCA R: CGCAGGTAGGAGAAGAGCTG	$(TATGTG)_4$	229	FAM	MN218734
Puya-010	F: AGAAAATTCCCAAGGCTGTG R: GGAATAGCCAGCCAAGGTAG	$(TCCTAT)_7$	237	FAM	MN218736
Puya-014	F: TGAAGATGCTGTGTGCTGTG R: TTTGCCCTTTGGACTCATCT	$(GCAA)_4$	244	FAM	MN218738
Puya-015	F: ACGCTTCAGAACTCAAGAATC R: CGACCGTAGGAGGAAGAGAA	$(TAAT)_4$	193	FAM	MN218739
Puya-017	F: TCCCCTCCTTTTGCTAGAAC R: TCGGTGAAGCCCATATGAA	$(TTTC)_4$	228	HEX	MN218741
Puya-018	F: CGCAACTCTGCGAACTGTAG R: GAAGGTTCTCCACCACCAAA	$(AGAA)_5$	227	FAM	MN218742
Puya-019	F: CGGCAACCAGAAAGAAGAAG R: TTCTCTCCCTTCTCTCGGCT	$(TTC)_{13}$	230	FAM	MN218743
Puya-021	F: ATGAGGAAGCAGCTCAAGGAGA R: TATTTTGAACCGATCCGAGG	$(TCG)_5$	240	FAM	MN218744
Puya-022	F: ACTTGCACCTCGTCAGCAC R: GGCGAAGCTTGATGAGAGAA	$(CTC)_7$	156	FAM	MN218745
Puya-023	F: AAAACGATACCAAAATCCATGT R: GGTGGTGCAATTAATTTGGTG	$(TCA)_6$	229	FAM	MN218746
Puya-025	F: TTCATGTTGCATTGTGCTGA R: TGAACCCATGCAGAACAAAC	$(TTG)_7$	152	FAM	MN218747
Puya-028	F: TGATCAGCCGAATACATTGC R: GCCAATGCAATTCCCTTCTA	$(TTC)_{10}$	205	FAM	MN218748
Puya-030	F: AATTCGATTCCCCAAAGTCC R: GACTCGTCGTTGAGGAGCAC	$(GTC)_8$	232	TAMRA	MN218749
Puya-031	F: ATTCGGCTGAAGGTGCAGTA R: ATGCGAGCTTGTAAGGAAGC	$(CTT)_{12}$	235	TAMRA	MN218750
Puya-033	F: CCGAATTTGCCACAAATCTT R: AAAGGGTTCAGGCGATGTTA	$(AGA)_5$	291	TAMRA	MN218751
Puya-034	F: ATAGAGGCGACCATTTGTCA R: TTGCTTGTGGTGCTATTTGC	$(GAT)_7$	226	FAM	MN218752
Puya-040	F: AAGGAATTATGAGCGCATGG R: TGTGAACCCACAGAATCAGC	$(AG)_{19}$	182	FAM	MN218755
Puya-044	F: AGGGCTCCTTCTCTCTCCTG R: GGCCAGAGGTAAAGGGGTAG	$(CT)_{12}$	205	FAM	MN218756
Puya-048	F: TGCAAAATACACGAAGGAAGC R: GGGATGGTGAAGAAATGGTG	$(TC)_6$	216	FAM	MN218757
Puya-050	F: TGTATTATCCCTTCAGAACTTGC R: TCGCATACATAGGACGAGTCA	$(CT)_7$	181	FAM	MN218758
Puya-051	F: AACACCGAAGGTGGTTCTTG R: GCCTAGTTGCTTCGCATTTC	$(TG)_{12}$	199	FAM	MN218759
Puya-053	F: GTTTTCGATGCCGATTGATT R: GTCTTTGTGGCTGAGCGATT	$(AT)_9$	246	TAMRA	MN218761
Puya-054	F: TCTTTACGTCCACACCTCCA R: TCTCTTCATCAGCGGGATCT	$(CA)_7$	190	FAM	MN218762
Puya-055	F: AGCTCGGAGGAGGGTCTTAG R: CGAGATGAGCCTCAGAATCC	$(CTC)_8$	160	FAM	MN218763
Puya-057	F: ACGGCAGCTCTATCCTCGTA R: GAGGACGTGAAGGTGTGGAT	$(TCG)_8$	181	TAMRA	MN218764
Puya-059	F: ATCCGTTGTCGTCGGAATAG R: CTCCCTCTCTCTGTGGTTCG	$(GCC)_5$	234	FAM	MN218765
Puya-060	F: CTACCGTTGATTCCCTGGAC R: CTCCGCCTACGAACAAAAAC	$(TTC)_8$	228	FAM	MN218766
Puya-062	F: CCTTCCAACTCCTCAGCTTG R: CAATCACTCTGGCTCACGAC	$(TTG)_9$	246	FAM	MN218767
Puya-064	F: GGTGTGTGGTGTTGTCAAGG R: GCTTCAAGATTTGTGCAGATG	$(AGG)_{11}$	226	FAM	MN218768

(Continues)

APPENDIX 2. (Continued)

Locus[a]	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	Fluorescent dye	GenBank accession no.
Puya-066	F: TTGGGACTTCCAGGTCACTC R: GAGAGAAGGAGCCCTCATCA	$(CT)_7…(CT)_7$	272	FAM	MN218769
Puya-067	F: TCAGCGTTTGCTTATCGTTG R: TTTCCAGTGATTTGGGGTGT	$(AG)_6$	236	TAMRA	MN218770
Puya-068	F: GGAAATGAGGTGTCGGTTGT R: GCTTGCTTTGTTCTTTGGCT	$(AT)_{11}$	170	FAM	MN218771
Puya-070	F: ATCCTGCAACCAAACAGGAC	$(TA)_{12}$	205	FAM	MN218773

[a]Annealing temperature for all loci was 60°C.