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Source: Applications in Plant Sciences, 4(1)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1500084

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Applications in Plant Sciences 2016 4(1): 1500084

Primer Note

Microsatellites from *Fosterella christophii* (Bromeliaceae) by de novo transcriptome sequencing on the Pacific Biosciences RS platform[1]

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- *Premise of the study:* Microsatellite markers were developed in *Fosterella christophii* (Bromeliaceae) to investigate the genetic diversity and population structure within the *F. micrantha* group, comprising *F. christophii*, *F. micrantha*, and *F. villosula*.
- *Methods and Results:* Full-length cDNAs were isolated from *F. christophii* and sequenced on a Pacific Biosciences RS platform. A total of 1590 high-quality consensus isoforms were assembled into 971 unigenes containing 421 perfect microsatellites. Thirty primer sets were designed, of which 13 revealed a high level of polymorphism in three populations of *F. christophii*, with four to nine alleles per locus. Each of these 13 loci cross-amplified in the closely related species *F. micrantha* and *F. villosula*, with one to six and one to 11 alleles per locus, respectively.
- *Conclusions:* The new markers are promising tools to study the population genetics of *F. christophii* and to discover species boundaries within the *F. micrantha* group.

Key words: Bromeliaceae; *Fosterella christophii*; microsatellites; Pacific Biosciences; single-molecule real-time (SMRT) sequencing; transcriptome.

Fosterella christophii Ibisch, R. Vásquez & J. Peters, *F. micrantha* (Lindl.) L. B. Sm., and *F. villosula* (Harms) L. B. Sm. form a well-circumscribed species group within the genus *Fosterella* L. B. Sm., known as the *F. micrantha* group (Pitcairnioideae; Bromeliaceae) (Wagner et al., 2013). The three species are morphologically very similar terrestrial rosette plants with small, whitish, insect-pollinated flowers (Peters, 2009). Such high levels of similarity are surprising, given that *F. micrantha* is endemic to Central America, whereas the other two species reside in the Bolivian Andes. Controlled pollination experiments indicated that all three species are self-compatible but also form viable hybrids (Wagner et al., 2015). To investigate the genetic diversity and differentiation in this closely related species complex, we used Pacific Biosciences' single-molecule real-time (SMRT) technology (Eid et al., 2009) to develop a set of genic microsatellite markers in *F. christophii.*

[1]Manuscript received 18 July 2015; revision accepted 24 September 2015.

The authors thank the Botanical Gardens of Hannover and Heidelberg, Dr. Jule Peters (University of Kassel, Kassel, Germany), Dr. Nicole Schütz (Stuttgart State Museum of Natural History, Stuttgart, Germany), and Dr. Ivón Ramírez-Morillo (Centro de Investigación Científica de Yucatán, Mérida, Yucatán, Mexico) for kindly providing plant material and Sascha Herwig for assistance in the laboratory.

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doi:10.3732/apps.1500084

METHODS AND RESULTS

Total RNA was isolated from fresh leaves of one *F. christophii* plant (NW09.030-11) using the RNeasy Plus Micro Kit (QIAGEN, Venlo, The Netherlands). RNA quality and quantity were assessed by capillary electrophoresis on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, California, USA). Polyadenylated RNA was isolated with the NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs, Ipswich, Massachusetts, USA), followed by an integrity check via capillary electrophoresis. An aliquot of 1 ng of poly(A) RNA was selected as an input for cDNA synthesis with a SMARTer PCR cDNA Synthesis Kit (Clontech Laboratories, Mountain View, California, USA). A SMRTbell library was prepared as recommended by Pacific Biosciences (PacBio, Menlo Park, California, USA). The amplified cDNA was size-fractionated on agarose gels, and fragments with insert sizes >1.5 kb were recovered. SMRTbell templates were bound to polymerases using the PacBio DNA/polymerase binding kit P4 and v2 primers. Polymerase-template complexes were bound to magnetic beads using a MagBead Kit (PacBio, part #100-133-600). Sequencing was carried out on the PacBio RS II sequencer using C2 sequencing reagents with a movie length of 180 min. Full-length cDNAs were identified with the PacBio SMRT analysis software (version 2.2.0). High-quality sequences were achieved by running the protocol with a filter for a minimum of three full passes of a cDNA and discarding all non–full length cDNAs and chimeric products. The read output was further trimmed and assembled into unigenes using CAP3 (Huang and Madan, 1999).

A total of 1590 high-quality consensus isoforms with an average size of 1322 bp were assembled into 971 unigenes. BatchPrimer3 (You et al., 2008) was applied to detect perfect microsatellites, accepting minimum thresholds of seven repeat units for di-, six for tri-, five for tetra-, and four for penta- and hexanucleotide repeats, respectively. A total of 421 microsatellites were present in 275 unigenes. Motif types are compiled in Appendix S1. Flanking sequences of appropriate quality and length were present at 335 microsatellite loci.

Microsatellite-flanking primers were designed using the BatchPrimer3 interface (You et al., 2008), applying the following criteria: length ranging from 18 to 23 nucleotides, product size ranging from 100 to 300 bp, annealing

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Table 1. Characteristics of 22 microsatellite loci and flanking primer pairs developed for *Fosterella christophii*.

Locus[a,b]	Primer sequences (5′–3′)	Repeat motif	Allele size (bp)	T_a (°C)	GenBank accession no.	BLASTX description	%[c]	SSR location
Foc_01	F: CCTCACTATCGCTCACTACAT R: GTCACGCACACCAACTTC	$(AG)_{15}$	146	55.2	KT036677	PREDICTED: cinnamoyl-CoA reductase 1-like isoform X1 [*Phoenix dactylifera*]	80	5′UTR
Foc_02	F: GAGGCATTGGGTTTTTCT R: AGATCTGCGGCTACATCTC	$(TC)_{17}$	147	55.3	KT036678	No match found	—	—
Foc_03	F: CCTTATTCCCCAAATCATAAA R: CTACCTCCTCTTCCTCTTCCT	$(AG)_{17}$	148	55.8	KT036679	PREDICTED: tetraspanin-3-like [*Musa acuminata* subsp. *malaccensis*]	80	5′UTR
Foc_04	F: GCCATTGAGTTCACCAAGT R: ACAACCCAAGCAATAATAACA	$(AG)_{20}$	145	55.5	KT036680	PREDICTED: tricetin 3′,4′,5′-*O*-trimethyltransferase [*Phoenix dactylifera*]	77	3′UTR
Foc_05	F: CTTCTCCTTCTCCTCCATCT R: AATAGGTCAGAGGATTTGAGC	$(TCG)_7$	136	55.4	KT036681	PREDICTED: ribonuclease 2 [*Musa acuminata* subsp. *malaccensis*]	78	5′UTR
Foc_06	F: CGTCAATCTCAATCCCTTC R: ACCTGCACTACTCAGAGGAA	$(CCT)_7$	138	55.3	KT036682	PREDICTED: secretory carrier-associated membrane protein 2-like isoform X1 [*Elaeis guineensis*]	88	5′UTR
Foc_07	F: TTCATCGCCTCTCGTTTAT R: CTTCGCCGTACCTCCAGTAG	$(CTC)_7$	130	57.9	KT036683	PREDICTED: uncharacterized protein LOC103707719 isoform X2 [*Phoenix dactylifera*]	86	5′UTR
Foc_09*	F: TAAAGGGAGAGAGAGGAAGAA R: GATGAGCTGCTGCTTCTG	$(AGA)_8$	168	55.3	KT581625	PREDICTED: phosphoribulokinase, chloroplastic [*Pyrus ×bretschneideri*]	93	5′UTR
Foc_10*	F: CTCCTTTTTCCTTTTCCTTTA R: CCGTGTTCTTCTTGTTGTACT	$(AGG)_8$	140	54.6	KT581626	Ubiquitin-conjugating enzyme 32 [*Theobroma cacao*]	87	5′UTR
Foc_11*	F: GAGGGTAAATTTCTCTGCTTC R: TACGATGTACAGCTAGGGATG	$(GAA)_9$	204	55.2	KT581627	Best match <75% sequence similarity	—	—
Foc_12	F: CACAAATGTGCTCTTCTGG R: CGTGGGATCTCTATCGTG	$(TCT)_{14}$	153	55.0	KT036684	Best match <75% sequence similarity	—	—
Foc_15*	F: GAGGACTTCGGTGTAATTTGT R: CCAACGGAAGAGTTCATAATA	$(ATTTT)_4$	185	55.2	KT581628	Best match <75% sequence similarity	—	—
Foc_16**	F: CTCAGCTGAACAATTCTGAG R: ACTTGGAGATGGAAGATAAGG	$(GAAGA)_5$	194	54.3	KT581629	PREDICTED: sec-independent protein translocase protein TATC, chloroplastic-like [*Oryza brachyantha*]	90	5′UTR
Foc_17*	F: GCCATTGTCCAGAAGTCC R: TAATAATTAGGGGATGAGCAG	$(TCCTC)_4$	153	55.1	KT581630	PREDICTED: carboxyvinyl-carboxyphosphonate phosphorylmutase, chloroplastic [*Amborella trichopoda*]	82	CDS
Foc_18	F: CATCGTCCTCTACCTCTACG R: GCCCTCCTTGTAGTCCTC	$(CCGCTC)_4$	193	54.8	KT036685	Best match <75% sequence similarity	—	—
Foc_19*	F: GAAAGAGGAAGAAACCGTAGA R: ATCAAAAGATGGAGGAGGAG	$(TCTCCT)_5$	156	55.6	KT581631	PREDICTED: PTI1-like tyrosine-protein kinase 1 isoform X1 [*Elaeis guineensis*]	91	5′UTR
Foc_20**	F: GAGGAAGAGAGAGGAAGAGAG R: AGGAGTAGAGGCGTCTCAG	$(TCTTCC)_5$	134	54.4	KT581632	RING/FYVE/PHD zinc finger superfamily protein [*Theobroma cacao*]	80	5′UTR
Foc_21**	F: CTCCAAAACGAACCCAAC R: CGAATCTAGGGCTGTATTTTT	$(GA)_{12}$	134	55.9	KT581633	PREDICTED: cation transport regulator-like protein 2 [*Musa acuminata* subsp. *malaccensis*]	76	5′UTR
Foc_25	F: CTCTCTCCCCATCGACAC R: GCTTGCAGTAGTAGACGAAGA	$(AG)_{12}$	159	55.8	KT036686	PREDICTED: UPF0235 protein At5g63440 isoform X1 [*Elaeis guineensis*]	95	5′UTR
Foc_27	F: TACTCACTTCCAAGCACTCTC R: CTTCAGCGTCTCCCACAT	$(AG)_{16}$	122	56.0	KT036687	Membrane steroid-binding protein 1, partial [*Oryza sativa* Indica group]	84	5′UTR
Foc_28	F: AAAGGGAAGTACAGAATCAGG R: GGGACAAGTCATTATCAAGTG	$(GCA)_{10}$	147	54.9	KT036688	Best match <75% sequence similarity	—	—
Foc_30	F: CATTTCCATTTTAACGAAGC R: TCATTCTCTTCTTCCTCTTCC	$(CT)_{14}$	150	55.2	KT036689	PREDICTED: uncharacterized protein LOC102721803 [*Oryza brachyantha*]	79	5′UTR

Note: 5′UTR = 5′ untranslated region; 3′UTR = 3′ untranslated region; CDS = coding region; T_a = annealing temperature.
[a] All loci were amplified using a standard touchdown PCR.
[b] Loci that were monomorphic (*) among the seven initially tested individuals of *F. christophii*, *F. villosula*, and *F. micrantha*; loci that were monomorphic (**) within each of the three tested species but showed some potential to differentiate between species.
[c] Sequence similarities of unigenes with more than 75% identity (%) to known genes obtained using BLASTX (Altschul et al., 1990).

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Table 2. Results of primer screening for 13 polymorphic loci developed for *Fosterella christophii*.[a]

Locus	NW09.005 (N = 11)			NW09.030 (N = 9)			NW09.034 (N = 9)			*F. christophii* (N = 29)	*F. micrantha* (N = 31)	*F. villosula* (N = 21)	Total (N = 81)
	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	A	A	A_{total}
Foc_01	4	0.09*	0.59	5	0.44	0.72	4	0.78	0.78	7	9	4	13
Foc_02	2	0.00	0.42	5	0.78	0.75	5	1.00	0.83	8	4	6	13
Foc_03	3	0.09	0.50	4	0.56	0.60	4	0.22	0.47	9	8	2	16
Foc_04	1	—	—	2	0.22	0.52	6	0.89	0.80	9	1	1	9
Foc_05	2	0.00*	0.52	5	0.78	0.71	3	0.11	0.57	7	2	2	9
Foc_06	1	—	—	4	0.89	0.66	3	0.56	0.57	6	4	2	8
Foc_07	2	0.00	0.42	2	0.22	0.21	4	0.67	0.75	4	2	2	4
Foc_12	2	0.00*	0.52	4	0.56	0.61	4	0.67	0.70	7	11	3	15
Foc_18	2	0.00	0.17	5	0.44	0.66	4	0.00*	0.65	7	4	2	11
Foc_25	2	0.09	0.25	1	—	—	3	0.89	0.69	4	3	2	6
Foc_27	3	0.09	0.18	4	0.78	0.71	4	0.67	0.75	7	5	4	8
Foc_28	1	—	—	3	0.56	0.60	2	0.33	0.29	4	4	2	6
Foc_30	2	0.00	0.51	2	0.67	0.52	4	0.22	0.78	6	4	5	9
Mean	2.08	0.04	0.41	3.54	0.57	0.61	3.85	0.54	0.66	6.54	4.69	2.85	9.77

Note: A = number of alleles; A_{total} = number of alleles across all tested accessions; H_e = expected heterozygosity; H_o = observed heterozygosity; N = number of individuals sampled.

[a] See Appendix 1 for locality and voucher information.

* Highly significant deviation from Hardy–Weinberg equilibrium ($P \leq 0.001$).

temperature from 50°C to 70°C, and GC content from 30% to 70%. Based on optimal primer characteristics, 30 loci representing all repeat types (12 di-, 10 tri-, two tetra-, three penta-, and three hexanucleotide repeats) were selected for further analysis. Primer functionality was validated by genotyping 29 *F. christophii* plants from three natural populations, with nine to 11 individuals each (Appendix 1). DNA was extracted from dried leaves according to Tel-zur et al. (1999). PCR amplifications were conducted in 12.5-μL final volumes in a T-Gradient thermocycler (Biometra, Göttingen, Germany), following the touchdown protocol previously described (Wöhrmann et al., 2012).

For the initial screens, PCR products from three *F. christophii* individuals (including NW09.030-11 as a positive control) and two plants each of *F. micrantha* and *F. villosula* were electrophoresed on an automated sequencer (LI-COR 4300 IR^2; LI-COR Biosciences, Lincoln, Nebraska, USA). Fragment sizes were scored manually as described by Wöhrmann et al. (2012). Twenty-two of the 30 primer pairs yielded one or two distinct bands of the expected size range in each tested individual, depending on the homo- or heterozygous state of the respective amplicon. Locus characteristics, primer sequences, GenBank accession numbers, and the results of a BLASTX similarity search in GenBank of these 22 loci are summarized in Table 1. Eight primer pairs failed to amplify in any of the specimens and were not considered further.

Thirteen markers proved to be polymorphic among the three tested *F. christophii* specimens and were used to genotype the full set of samples listed in Appendix 1. Allele numbers and observed and expected heterozygosity values were calculated with Arlequin 3.5.1.2 (Excoffier et al., 2005). GENEPOP 1.2 (Raymond and Rousset, 1995) was used to perform exact tests of Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium. All 13 loci were polymorphic, exhibiting four to nine alleles per locus among the 29 analyzed *F. christophii* plants (Table 2). Observed and expected heterozygosity values ranged from zero to 1 and from 0.17 to 0.83, respectively. Significant deviations from HWE in terms of heterozygote deficiency were detected at three loci (Foc_01, Foc_05, Foc_12) in population NW09.005 and at one locus (Foc_18) in population NW09.034, which is possibly explained by the potential of *Fosterella* species to self-pollinate (Wagner et al., 2015). With one exception (Foc_04), all 13 loci were also polymorphic in *F. micrantha* (two to 11 alleles) and in *F. villosula* (two to six alleles) (Table 2). Significant linkage disequilibrium was only found between two loci (Foc_12 and Foc_18).

NTSYSpc version 2.1 (Rohlf, 2000) was used to perform principal coordinate analyses (PCoA) based on a square-root transformed distance matrix calculated with the index of Bray and Curtis (1957). Distinct groups were formed by PCoA (Appendix S2), illustrating the potential of the markers to differentiate between the three closely related species of the *F. micrantha* group. Four individuals with identical multilocus genotypes were found in *F. villosula* and three in *F. christophii*, indicating clonal growth within populations.

Cross-amplification in more distant Bromeliaceae was analyzed with one individual each of *F. rusbyi* (Mez) L. B. Sm., *Deuterocohnia longipetala* (Baker) Mez, *Dyckia marnier-lapostollei* L. B. Sm. var. *estevesii* Rauh, *Encholirium* spp. Mart. ex Schult. f. (all Pitcairnioideae), *Ananas comosus* (L.) Merr. (Bromelioideae), *Catopsis morreniana* Mez (Tillandsioideae), and *Puya mirabilis* (Mez) L. B. Sm. (Puyoideae) (Appendices 1 and 2). Although physically linked to expressed genes, only three of the markers were particularly well conserved and showed consistent amplification of one or two distinct bands in the expected size range in four or more of the seven species included in the test panel (Appendix 2).

CONCLUSIONS

So far, PacBio's SMRT technology has only rarely been applied to microsatellite marker development (e.g., Grohme et al., 2013; Wei et al., 2014). To our knowledge, the present report is the first using cDNAs as source material for this purpose. The increasing popularity of the PacBio RS II system compared with earlier sequencing technologies is primarily attributed to its high sequence accuracy obtained by circular consensus sequencing and the extraordinarily long reads of up to 20 kb. The analysis of full-length cDNAs is appealing not only for detecting genic microsatellite markers but also for many other applications such as gene mapping or gene expression profiling. The cDNA-based microsatellite markers developed for *F. christophii* represent promising tools for population genetic analyses and species delimitation within the *F. micrantha* group and presumably other species complexes of the Pitcairnioideae.

LITERATURE CITED

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.

Bray, J. R., and J. T. Curtis. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs* 27: 325–349.

Eid, J., A. Fehr, J. Gray, K. Luong, J. Lyle, G. Otto, P. Peluso, et al. 2009. Real-time DNA sequencing from single polymerase molecules. *Science* 323: 133–138.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47–50.

Grohme, M. A., R. F. Soler, M. Wink, and M. Frohme. 2013. Microsatellite marker discovery using single-molecule real-time circular

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consensus sequencing on the Pacific Biosciences RS. *BioTechniques* 55: 253–256.

Huang, X., and A. Madan. 1999. CAP3: A DNA sequence assembly program. *Genome Research* 9: 868–877.

Peters, J. 2009. Revision of the genus *Fosterella* (Bromeliaceae). PhD Thesis, University of Kassel, Kassel, Germany.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248–249.

Rohlf, F. J. 2000. NTSYSpc: Numerical taxonomy and multivariate analysis system, ver. 2.1. Applied Biostatistics Inc., Port Jefferson, New York, USA.

Tel-Zur, N., S. Abbo, D. Myslabodski, and Y. Mizrahi. 1999. Modified CTAB procedure for DNA isolation from epiphytic cacti of the genera *Hylocereus* and *Selenicereus* (Cactaceae). *Plant Molecular Biology Reporter* 17: 249–254.

Wagner, N., D. Silvestro, D. Brie, P. L. Ibisch, G. Zizka, K. Weising, and K. Schulte. 2013. Spatio-temporal evolution of *Fosterella* (Bromeliaceae) in the Central Andean biodiversity hotspot. *Journal of Biogeography* 40: 869–880.

Wagner, N. D., T. Wöhrmann, V. Öder, A. Burmeister, and K. Weising. 2015. Reproduction biology and chloroplast inheritance in Bromeliaceae: A case study in *Fosterella* (Pitcairnioideae). *Plant Systematics and Evolution* 301: 2231–2246.

Wei, N., J. B. Bemmels, and C. W. Dick. 2014. The effects of read-length, quality and quantity on microsatellite discovery and primer development: From Illumina to PacBio. *Molecular Ecology Resources* 14: 953–965.

Wöhrmann, T., N. Wagner, F. Krapp, B. Huettel, and K. Weising. 2012. Development of microsatellite markers in *Fosterella rusbyi* (Bromeliaceae) using 454-pyrosequencing. *American Journal of Botany* 99: e160–e163.

You, F. M., N. Huo, Y. Q. Gu, M.-C. Luo, Y. Ma, D. Hane, G. R. Lazo, et al. 2008. BatchPrimer3: A high throughput web application for PCR and sequencing primer design. *BMC Bioinformatics* 9: 253.

Appendix 1. Plant material analyzed in this study. Representative samples of *F. christophii*, *F. villosula*, and *F. micrantha* populations were collected with the largest possible distances from each other (between 20 cm and 3–4 m, depending on the total size of the patch).

N	Species	Location	Plant ID/Voucher (Herbarium)[a]	GPS coordinates	
				Latitude	Longitude
11	*Fosterella christophii* Ibisch, R. Vásquez & J. Peters	Florida, Santa Cruz (BOL)	NW09.005 (LPB)	−18.09952	−63.60210
9	*Fosterella christophii*	Larecaja, La Paz (BOL)	NW09.030 (LPB)	−15.66240	−67.71320
9	*Fosterella christophii*	Larecaja, La Paz (BOL)	NW09.034 (LPB)	−15.46787	−67.97005
5	*Fosterella villosula* (Harms) L. B. Sm.	Caranavi, La Paz (BOL)	NW09.012 (LPB)	−16.03293	−67.63168
3	*Fosterella villosula*	Sud Yungas, La Paz (BOL)	NW09.019 (LPB)	−15.45160	−67.17057
7	*Fosterella villosula*	José Ballivián, Beni (BOL)	NW09.024 (LPB)	−14.54075	−67.49963
6	*Fosterella villosula*	Larecaja, La Paz (BOL)	NW09.035 (LPB)	−15.30580	−67.36843
5	*Fosterella micrantha* (Lindl.) L. B. Sm.	Veracruz (MEX)	Schütz 11.04 (KAS)	18.41262	−95.09468
10	*Fosterella micrantha*	Oaxaca (MEX)	Schütz 11.05 (KAS)	17.85203	−96.21658
6	*Fosterella micrantha*	Oaxaca (MEX)	Schütz 11.06 (KAS)	17.73767	−96.32755
4	*Fosterella micrantha*	Oaxaca (MEX)	Schütz 11.17 (KAS)	16.79033	−95.35933
6	*Fosterella micrantha*	Oaxaca (MEX)	Schütz 11.19 (KAS)	15.86467	−96.47219
1	*Fosterella rusbyi* (Mez) L. B. Sm.	South Yungas, La Paz (BOL)	JP 06.0078 (LPB)	−16.36167	−67.46333
1	*Dyckia marnier-lapostollei* L. B. Sm. var. *estevesii* Rauh	Goias (BRA)	BGHD 130151	−16.66667	−49.26667
1	*Deuterocohnia longipetala* (Baker) Mez	Tarija (BOL)	Schütz 06.068 (KAS)	−22.57043	−64.41242
1	*Encholirium* spp. Mart. ex Schult. f.	NA (BRA)	BGHD 125585	NA	NA
1	*Ananas comosus* (L.) Merr.	NA (NA)	HERRH 0000-G-33	NA	NA
1	*Catopsis morreniana* Mez	Veracruz (MEX)	BGHD 131731	NA	NA
1	*Puya mirabilis* (Mez) L. B. Sm.	Salta (ARG)	BGHD 130040	NA	NA

Note: ARG = Argentina; BGHD = Botanical Garden Heidelberg; BOL = Bolivia; HERRH = Botanical Garden Hannover; MEX = Mexico; *N* = number of individuals per sampling location; NA = not available; PE = Peru.

[a] Herbarium abbrevations are according to Index Herbariorum (http://sweetgum.nybg.org/science/ih/).

Appendix 2. Cross-species amplification of 13 microsatellite markers developed for *Fosterella christophii* in seven heterologous bromeliad species.

Species	Foc_01	Foc_02	Foc_03	Foc_04	Foc_05	Foc_06	Foc_07	Foc_12	Foc_18	Foc_25	Foc_27	Foc_28	Foc_30
Fosterella rusbyi	+	—	+	+	+	+	—	—	—	+	+	+	—
Dyckia marnier-lapostollei var. *estevesii*	+	—	+	+	+	—	—	—	—	+	—	+	—
Deuterocohnia longipetala	—	—	—	—	+	—	—	—	—	+	—	+	—
Encholirium spp.	—	—	+	—	+	—	—	—	—	+	—	+	—
Ananas comosus	—	—	—	+	+	+	—	—	—	—	—	+	—
Catopsis morreniana	—	—	—	—	—	—	—	—	—	—	—	—	—
Puya mirabilis	+	—	—	—	+	—	—	—	—	—	—	+	—

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

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