

Characterization of microsatellite loci in *Brighamia insignis* and transferability to other genera in the Hawai'ian lobelioid group

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PREMISE: Microsatellite markers were developed to measure genetic diversity and relatedness of ex situ collections of *Brighamia insignis* (Campanulaceae).

METHODS AND RESULTS: Potential microsatellite markers were identified from two sources; 28 were developed for *B. insignis* and an additional 12 markers from a previously published study of *Lobelia villosa*. Primer pairs were tested on 30 individuals of *B. insignis* and 24 individuals of *B. rockii* to provide measures of genetic diversity and inbreeding. We assessed cross-species amplification in an additional 13 taxa that represented all six genera within the Hawai'ian lobelioid group to determine the broader applicability of the markers.

CONCLUSIONS: Results indicate that these primers will provide useful estimates of genetic diversity and relatedness of ex situ collections of both *Brighamia* species. In addition, we have also demonstrated the widespread applicability of these markers for use in population genetic studies of several species within the Hawai'ian lobelioid group.

KEY WORDS *Brighamia*; Campanulaceae; cross-amplification; Hawai'ian lobelioids; *Lobelia*; microsatellites.

With over 126 species, the Hawai'ian lobelioids represent one of the best examples of adaptive radiation (Givnish et al., 2009). Unfortunately, for many species in this group, wild populations are so reduced that they are now of high conservation concern. For example, the genus *Brighamia* A. Gray is composed of two species, *B. insignis* A. Gray and *B. rockii* H. St. John, which were once found on multiple Hawai'ian islands (Gemmill et al., 1998) but are currently reduced to only one individual of *B. insignis* and fewer than 100 individuals of *B. rockii* (Walsh, 2016). Fortunately, the National Tropical Botanical Garden began making collections in the 1970s and has distributed germplasm to botanic gardens around the world (Hannon and Perlman, 2002). According to the global plant collections database PlantSearch (https://tools.bgci.org/plant_search.php), at least 56 other botanic gardens maintain collections of this species. This conservation effort helped *B. insignis* escape extinction and could ultimately provide potential in situ restoration material. However, recent genetic analysis of the ex situ populations suggests a loss of genetic diversity and fitness declines associated with inbreeding depression (Walsh, 2015).

This loss of genetic diversity could be mitigated by developing a robust breeding program that incorporates genetic data into breeding decisions and ex situ management (Fant et al., 2016). An important first step in this process is to track the origins of all material

in collections; however, the majority of individuals have uncertain parentage. Neutral molecular markers can be used to estimate relatedness of individuals with unknown parentage. Unfortunately, previous genetic markers used for *Brighamia* studies have revealed only a limited amount of genetic diversity (Gemmill et al., 1998; Walsh, 2015). Here we report 28 microsatellite markers developed for *B. insignis* and 12 published primers from *Lobelia villosa* (Rock) H. St. John & Hosaka (Tran et al., 2015) that were tested on *B. insignis* and *B. rockii*, as well as on an additional 13 taxa that represented all six genera in the Hawai'ian lobelioid group.

METHODS AND RESULTS

Genomic DNA was extracted using the modified 2× cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). We tested all primers on 15 taxa that represented the six genera within the Hawai'ian lobelioid group; for each taxon, one to two samples were tested. These included both species of *Brighamia* (*B. insignis* and *B. rockii*), two species of *Clermontia* Gaudich. (*C. fauriei* H. Lévl. and *C. samuelii* C. N. Forbes subsp. *hanaensis* (H. St. John) Lammers), six species of *Cyanea* Gaudich. (*C. fissa* Hillebr.,

TABLE 1. Characteristics of 30 microsatellite loci used for screening in this study, including 18 newly developed markers for *Brighamia insignis* and 12 previously published markers for *Lobelia villosa*.

Species and locus	Primer sequences (5'–3')	Repeat motif	Allele size range (bp)	Fluorescent label	GenBank accession no. ^a
<i>Brighamia insignis</i>					
BRIN01	F: CTTGTTGCAGGATGGGAGTT R: GGGTATCCACCCTTTCTTC	(GA) ₁₇	202–222	Green [D3-PA]	MK387344
BRIN05	F: GAATGGTTTTCACTTTCCCAAC R: ATCTCTTACCCCGGAAGCAC	(GT) ₁₇	181–207	Blue [D4-PA]	MK387346
BRIN07	F: TTCAGCACAGATCCCTTTTG R: TCCAGATTCAGCTCCTCCAG	(TG) ₁₆ (AG) ₇	167	Green [D3-PA]	MK387347
BRIN08	F: AGACGGCTCGGGGTATAACT R: GTACCATTCTCGTATTACCCA	(TG) ₁₂	146–154	Blue [D4-PA]	MK387348
BRIN10	F: CAGCTTTACCGTCTTCTGC R: TTTCTAAAGTTACAAATCAAGG	(CT) ₁₁ (CA) ₁₄	148–186	Blue [D4-PA]	MK387349
BRIN41	F: CAACGCTGATGATGATGATTG R: ACCCTTCGTTTCCAAAGATCC	(TG) ₁₄	111–125	Black [D2-PA]	MK387352
BRIN43	F: GGACCAACAATTGGAGAAACA R: CTGATGCTGAACATCTGTAAACAA	(CT) ₂₇	174–248	Blue [D4-PA]	MK387354
BRIN44	F: ACTGGATTGAAAGCCTTACTTTG R: CTAATGCACATTTTGGATTGCT	(AG) ₁₄	142–162	Black [D2-PA]	MK387355
BRIN46	F: GGAAACTGATGTGCGTTGA R: TTGCTTCATGACTTGAGCTTG	(TC) ₁₃ (AC) ₁₀	232–254	Blue [D4-PA]	MK387357
BRIN47	F: CCCCTCCATTTCAGGTTCT R: CCTCAGCAGGGGAAAAGTAA	(GT) ₁₂	152–188	Green [D3-PA]	MK387358
BRIN51	F: GACAAGGGACATGGCTGTTT R: TGAGAGTTTGATCTGCACAAAA	(CT) ₁₆ (CA) ₁₂	170–208	Green [D3-PA]	MK387362
BRIN53	F: GCTTGTGACACAACATGAAA R: TGATAGTCACAGTTTGGCTGA	(AG) ₁₂	165–177	Green [D3-PA]	MK387363
BRIN54	F: TGTGAATGGCTGGTTGGTAA R: GCCTAAAGGCAAAGAGTTTGAA	(GT) ₉	168–176	Green [D3-PA]	MK387364
BRIN56	F: CGCGAAGTCCAGAAAGAAAAC R: TTTTGTTCCTCTTCGTCCA	(CA) ₁₁	104	Black [D2-PA]	MK387366
BRIN57	F: TGGGAGAAAATTGAAAGCAAA R: GTACAAAGAAATCCACTCACTCGC	(GT) ₇ (GA) ₂₂	223–233	Blue [D4-PA]	MK387367
BRIN59	F: ACCAGGGATTGTTTCGCTAAG R: GGCTCTGTGGCATTCAAGT	(GA) ₂₉	—	Green [D3-PA]	MK387351
BRIN61	F: GTGAGCTGGGTGGTTGTTTT R: AGGAGACCCCTCAACAATC	(AG) ₁₄	183–191	Green [D3-PA]	MK387369
BRIN66	F: GTACTGCATGCCCTGTGTT R: GATGCGCTCAAACGAGAGTT	(GT) ₁₀ (GA) ₁₀	157–181	Blue [D4-PA]	MK387370
<i>Lobelia villosa</i>					
LOVI4	F: ACGTCTAGGGGCACTGCCAAGCCAG R: TCCAAATGGGAGACTACTGCAGAAAGG	(AC) ₁₂	248–268	M13F-green [D3-PA]	
LOVI23	F: TCTTTTGTCCATGCCAGCGTG R: AGGTACTCGGTCTGAGCGTTTCG	(AC) ₁₁	401–437	M13F-blue [D4-PA]	
LOVI33	F: ACAGGGGGCAAACTGGTCACC R: TGCAAGGATGACGAAGGGGCTC	(GT) ₈ (GA) ₉	372–378	M13F-black [D2-PA]	
LOVI34	F: ACAGGCGCTATGGCGTCCCT R: GTTGATGATCATGAGACCGTC	(CA) ₁₀	155	M13F-blue [D4-PA]	
LOVI35	F: GCTTACAACAAATTGCCTC R: AGCCTCGAAATCATCCGGCCCA	(CA) ₁₇	230–256	M13F-black [D2-PA]	
LOVI37	F: GGATCACTCAAGGATGAACGCAAGG R: TGTGTAATGGACCTTGGGCTGTCTC	(GA) ₁₄	470–478	M13F-green [D3-PA]	
LOVI48	F: TCACCGAAGATTATCGAACCA R: ACGAGTGAGAGACTTTGGGTGATCA	(AC) ₇ A(AC) ₅	400–420	M13F-green [D3-PA]	
LOVI52	F: CGAATGATCTATTATCCAGCC R: AGACACTTCCTCGAGTTGGTG	(AC) ₁₁	—	M13F-blue [D4-PA]	
LOVI56	F: AGCATCAGCAAGACACTTGC R: AGTAAGGGATAAAGCAGACCTGG	(CT) ₉ (CA) ₁₄	536–640	M13F-black [D2-PA]	
LOVI73	F: TCACAAATGCTCCATCGCGAG R: TGTAGCGAAAGTACCGGATCCA	(CA) ₉	468–478	M13F-black [D2-PA]	
LOVI90	F: ACCGGCTGTAATCACGCGTTGG R: CTACTGTGTGAAGTCGGAACCC	(CA) ₁₂	200–206	M13F-blue [D4-PA]	
LOVI93	F: TCTAGCAGAAGCCTCACCCTCGGA R: CACCAGAACTCAAGCAAGGCGAC	(GT) ₁₀	287–397	M13F-green [D3-PA]	

Note: — = no amplified product.

^aAccession numbers are provided for loci developed for *Brighamia insignis*. Loci for *Lobelia villosa* were previously published in Tran et al. (2015).

TABLE 2. Allele size ranges for the 18 newly developed markers for *Brighamia insignis* and 12 previously published markers for *Lobelia villosa*, tested on 15 taxa from the Hawaiian lobeloid complex.^a

Locus	<i>Brighamia insignis</i> (n = 30)	<i>Brighamia rockii</i> (n = 24)	<i>Clermontia fauriei</i> (n = 2)	<i>Clermontia samuelii</i> (n = 1)	<i>Cyanea fissia</i> (n = 2)	<i>Cyanea hardyi</i> (n = 2)	<i>Cyanea leptostegia</i> (n = 2)	<i>Cyanea hirtella</i> (n = 2)	<i>Cyanea pseudofauriei</i> (n = 2)	<i>Cyanea salicina</i> (n = 1)	<i>Delissea kauaiensis</i> (n = 2)	<i>Delissea rhytidosperra</i> (n = 2)	<i>Delissea walanaensis</i> (n = 1)	<i>Lobelia niihaensis</i> (n = 2)	<i>Trematolobelia kaulensis</i> (n = 2)
BRIN01	202–208	192–206	134–144	144	132–142	138	138	142	138	140–142	140–158	—	140–142	154	142
BRIN02	218–222	214–218	172–194	194	179–197	186	186	156–672	154–162	162	145–149	181	181	180	195–200
BRIN05	181–207	181–205	187–194	194	189–195	186	186	190–196	186	191	181	175–187	181	165–179	144–152
BRIN07	167	149–173	153–160	165	156–163	185	—	158–169	—	—	—	—	181	165–179	195–197
BRIN08	146–154	146–148	111–131	123	123–146	152–154	159–161	146	155–163	146	146	141–143	146	152	152
BRIN10	148–170	150–152	146–154	154	149–165	160	267	149	160	149	175	—	154–171	149–151	143
BRIN41	174–186	168–178	—	—	—	—	—	165–171	—	165	—	—	—	169	163–171
BRIN41	111–125	115–125	—	—	—	—	—	—	—	100	103–106	—	122	110	100
BRIN43	174–190	188–218	198–201	198	201–208	193	183	191	189–193	185–199	190–198	197–205	209–219	183	187–192
BRIN43	228–234	228–234	215–232	206–225	209	209	203–209	201–205	201–215	—	210–213	210–213	—	152	152
BRIN44	142–148	126–138	215–217	182	182	160	223–230	182	211	182	197–215	195	224–228	205–215	185–220
BRIN44	152–162	224	224	—	—	—	—	—	—	—	238–240	207–224	—	—	—
BRIN46	232–254	234–252	244–248	240	263	209–218	240–260	228–238	209	245–249	250–266	250	268	241–258	228–230
BRIN47	152–178	150–164	167–170	169	—	—	—	—	—	172	170–180	167–183	167–180	—	160
BRIN47	182–188	174–188	181–184	182	160–172	168	172	172	153–165	172	156	156	156	170–182	170
BRIN51	170–174	164–176	172–176	173	160–172	168	198	188	184	182	180–182	172–182	184–196	188–198	182
BRIN51	180–208	182–186	184–198	190–210	—	—	—	161	158–162	160	188	185–190	188	145–161	194–228
BRIN53	165–177	171–177	—	173	—	161	165	168–172	163	168	166	163–166	167	156–158	146–161
BRIN54	168–176	158–180	—	160	168–172	163	165	168–172	163	168	166	163–166	167	156–158	166
BRIN54	104	114–116	92–108	104	101–107	98–105	97–103	109–112	98–112	107–113	97–109	106	92–114	85–91	96–115
BRIN56	223–233	219–248	204–206	202	160	218–222	210–222	161–167	218–228	138–161	218	—	222	205	241–247
BRIN59	—	—	178–204	182–195	168–192	—	—	172–190	—	—	—	—	—	—	—
BRIN61	183–191	181–193	—	193	181–190	—	193–208	181–193	191–193	181	181–201	—	181–205	181	—
BRIN66	157–181	157–177	—	161	—	157–175	—	158–174	156–173	173–175	157–159	159	293	205–215	182
LOV04	248	248	253–259	253–259	241–287	254	250–289	242–282	255	252–260	252	253–261	—	174–176	207–209
LOV04	268	397	401	402	400	290–294	400–403	—	393–403	400	417	419	269–276	249–259	148–150
LOV04	417–437	415–417	419	423	421–423	412	—	—	—	424	430–445	—	—	269	255
LOV133	372–378	368–382	355	257	350	348–359	356–360	350–352	358	350–354	368	384	362	403–412	403
LOV134	155	151–165	—	—	156–160	156	155	156–160	156	156–160	—	154–165	154–161	—	422–434
LOV135	230–236	236–242	—	—	261–275	260–271	254	260–265	254–264	261	—	234–255	231	—	346
LOV135	256	256	—	—	—	—	—	—	—	—	—	—	—	—	—
LOV137	470–478	476–484	—	429–433	471–497	—	—	460–494	481	490–502	409–420	480–490	472	428–448	425
LOV148	400–420	376–404	—	452	402–425	410–430	424–434	413	412–418	—	—	—	401	403–408	406
LOV152	—	157–173	—	171	208–222	—	172	172	170–199	172–199	—	—	—	—	—
LOV152	194–204	194–204	—	210	208–222	—	208–218	208–218	208–218	224–242	—	—	—	—	—
LOV156	536–558	520–546	—	552	544–550	548–554	548–550	546	548	546	541	545–562	535	573–581	564–572
LOV156	618–640	618–620	—	620	618–623	622	622	622	622	623	620	620	620	452–474	459
LOV173	468–478	468–478	467	465	—	467	467	467	—	467	466–475	473	477	452–474	459
LOV190	200–206	200	201–211	203	—	200	203–215	204–206	—	205–209	180	200	200	184–204	200–212
LOV193	287	287	382–390	394–402	—	365	375	340	—	310	342	377	288	356–376	—
LOV193	391–397	393	—	—	—	—	—	—	—	—	—	—	—	—	—

Note: — = no data available; n = number of individuals tested.

^aFor primers amplifying more than one locus (>2 bands), the predicted allele ranges are listed separately for each locus. Ranges were determined based on allele sizes in a homozygous state (only two bands produced—one from each loci), presence of a large break in allele size ranges (suggesting no overlap), and cross referencing with other species (assuming common origins of loci).

TABLE 3. Genetic characterization of 18 newly developed markers for *Brighamia insignis* and 12 previously published markers for *Lobelia villosa* across 30 *B. insignis* individuals and 24 *B. rockii* individuals.

Locus	<i>B. insignis</i> (n = 30)						<i>B. rockii</i> (n = 24)					
	Range (bp)	A	A _e	H _o	H _e	F	Range (bp)	A	A _e	H _o	H _e	F
BRIN01	202–208	2	1.1	0.00	0.07	1.00	192–206	4	2.3	0.27	0.56	0.51
	218–222	3	1.5	0.15	0.31	0.53	214–218	2	1.0	0.05	0.05	–0.02
BRIN05	181–207	7	2.5	0.33	0.61	0.45	181–205	9	5.9	0.39	0.83	0.53
BRIN07	167	1	1.0	—	—	—	149–173	7	3.0	0.43	0.67	0.36
BRIN08	146–154	4	2.0	0.18	0.50	0.64	146–148	2	1.8	0.11	0.43	0.76
BRIN10	148–170	6	1.4	0.07	0.30	0.75	150–152	2	2.0	0.09	0.50	0.83
	174–186	5	1.5	0.07	0.32	0.77	168–178	3	2.4	0.33	0.58	0.43
BRIN41	117–125	4	1.9	0.11	0.47	0.77	115–125	2	1.9	0.05	0.48	0.89
BRIN43	—	0	—	—	—	—	188–218	9	2.1	0.50	0.53	0.05
	228–248	5	3.6	0.09	0.72	0.87	228–234	3	2.9	0.11	0.65	0.84
BRIN44	142–148	4	1.9	0.14	0.48	0.70	126–138	5	2.1	0.71	0.52	–0.36
	152–162	4	1.7	0.10	0.42	0.77	—	0	—	—	—	—
BRIN46	232–254	4	2.1	0.17	0.53	0.69	234–254	5	2.7	0.14	0.63	0.77
BRIN47	152–178	6	2.4	0.18	0.58	0.69	150–164	5	2.3	0.77	0.57	–0.36
	182–188	3	1.1	0.04	0.12	0.65	174–188	5	4.0	0.50	0.75	0.33
BRIN51	170–174	2	1.4	0.00	0.28	1.00	164–176	4	2.1	0.20	0.52	0.61
	180–208	4	1.5	0.04	0.32	0.87	182–186	2	1.1	0.10	0.10	–0.05
BRIN53	165–177	4	2.1	0.12	0.51	0.77	171–177	3	1.7	0.00	0.41	1.00
BRIN54	168–176	3	1.9	0.07	0.46	0.85	158–180	3	1.4	0.18	0.31	0.41
BRIN56	104	1	1.0	—	—	—	114–116	2	1.1	0.00	0.10	1.00
BRIN57	223–233	4	3.0	0.25	0.67	0.63	219–248	9	4.2	0.36	0.76	0.52
BRIN61	183–191	3	1.3	0.13	0.23	0.44	181–193	5	2.9	1.00	0.65	–0.53
BRIN66	157–181	8	2.7	0.07	0.63	0.89	157–177	7	2.0	0.32	0.51	0.38
LOVI04	248	1	1.0	—	—	—	248	1	1.0	—	—	—
	268	1	1.0	—	—	—	—	0	—	—	—	—
LOVI23	401–411	4	1.8	0.50	0.45	–0.11	397	1	1.0	—	—	—
	417–437	4	3.7	0.25	0.73	0.66	415–417	2	1.2	0.05	0.15	0.64
LOVI33	372–378	2	1.5	0.14	0.34	0.58	368–382	3	1.9	0.07	0.47	0.85
LOVI34	155	1	1.0	—	—	—	151–165	2	1.5	0.39	0.31	–0.24
LOVI35	230–236	2	1.2	0.14	0.13	–0.08	236–242	4	2.5	0.07	0.59	0.88
	256	1	1.0	—	—	—	256	1	1.0	—	—	—
LOVI37	470–478	3	1.8	0.21	0.44	0.53	476–484	3	2.3	0.23	0.56	0.59
LOVI48	400–420	2	1.1	0.00	0.08	1.00	376–404	3	2.1	0.00	0.51	1.00
LOVI52	—	0	—	—	—	—	157–173	5	3.8	0.57	0.73	0.22
	—	0	—	—	—	—	194–204	2	1.1	0.00	0.12	1.00
LOVI56	536–558	5	1.7	0.00	0.42	1.00	520–546	6	4.9	0.20	0.80	0.75
	618–640	4	1.4	0.00	0.27	1.00	618–620	2	1.2	0.00	0.15	1.00
LOVI73	468–478	2	1.2	0.00	0.17	1.00	468–478	c2	1.6	0.05	0.36	0.85
LOVI90	200–206	3	1.5	0.04	0.35	0.88	200	1	1.0	—	—	—
LOVI93	287	1	1.0	—	—	—	287	1	1.0	—	—	—
	391–397	3	2.2	0.26	0.54	0.52	393	1	1.0	—	—	—

Note: — = no data available; A = number of alleles; A_e = number of effective alleles; F = inbreeding coefficient; H_e = expected heterozygosity; H_o = observed heterozygosity; n = number of individuals tested.

C. hardyi Rock, *C. hirtella* Hilldebr., *C. leptostegia* A. Gray, *C. pseudo-fauriei* Lammers, and *C. salicina* H. Lév.), three species of *Delissea* Gaudich. (*D. kauaiensis* (Lammers) Lammers, *D. rhytidosperra* H. Mann, *D. waianaensis* Lammers), as well as *Lobelia niihauensis* H. St. John and *Trematolobelia kauaiensis* Skottsb. The *Brighamia* sources were identified using the PlantSearch database, and the other Hawaiian lobelioids were obtained from the National Tropical Botanic Garden living collections and DNA Library (Appendix 1).

Genomic DNA from two *B. insignis* individuals was sent to the microsatellite-development company Genetic Marker Services (Brighton, United Kingdom). Libraries were constructed by adapter-ligation of digested genomic DNA, which was then screened with the following filter-bonded synthetic repeat motifs: (AG)₁₇, (AC)₁₇, (AAC)₁₀, (CCG)₁₀, (CTG)₁₀, and (AAT)₁₀. These were transformed into *E. coli*, plated onto agar/ampicillin plates, and screened for

motif-positive clones, which were then isolated, cultured, and sequenced. The online primer design software Primer3 (Rozen and Skaletsky, 1999) was used to select primers that would minimize multi-loading overlap ambiguities during sequencer genotyping. A total of 28 primer pairs were designed from the libraries, and these amplified products ranging from 100–250 bp. These, and an additional 12 published primers for *L. villosa* (Tran et al., 2015), were tested on all samples. To visualize and quantify allele sizes, the forward primer derived from *B. insignis* libraries was modified with WellRedBlack (D2), Green (D3), or Blue (D4) fluorescent dye (Sigma-Aldrich, St. Louis, Missouri, USA). Primers derived from *L. villosa* were modified at the 5' end (5'-CACGACGTTGTAAAACGAC-3') so they could be labeled separately (Schuelke, 2000). The PCR consisted of 10–50 ng of template DNA, 25 μM of modified forward and reverse primer, and Bioline PCR MasterMix 2× (Bioline USA,

Taunton, Massachusetts, USA) and was performed at 94°C for 3 min; followed by 35 cycles of 94°C for 40 s, 57°C for 40 s, and 72°C for 90 s; with a final extension of 72°C for 10 min. For the *L. villosa* primers, the PCR reactions were stopped after 13 cycles to add 5 µL of labeled primer (CACGACGTTGTAAAACGAC [25 µM]) and returned to the thermocycler for the remaining 27 cycles. All products were analyzed and scored using a CEQ 8000 Genetic Analysis System V9.0 (Beckman Coulter, Brea, California, USA).

Of the 28 primers derived from the *B. insignis* libraries, 10 did not amplify in any species tested (GenBank accession no.: MK387345, MK387350, MK387353, MK387356, MK387359, MK387360, MK387361, MK387365, MK387368, MK387371); the remaining 18 primers produced bands in some species tested (Tables 1, 2). All 12 primers from *L. villosa* amplified in at least one species. Of the combined 30 primer pairs that amplified a product, 12 (seven from *B. insignis* and five from *L. villosa*) produced a maximum of two bands, consistent with amplifying a single locus, whereas the remaining 18 primer pairs (11 from *B. insignis* and seven from *L. villosa*) produced more than two bands in some species. This was expected because many of the Campanulaceae species are paleotetraploids (Lammers, 1988). Once we accounted for stutter, A-tails, and spurious, non-reproducible peaks, we assumed alleles clustered within a narrow range and separated from other alleles by a large range (20–30 bp) to be derived from a single locus. Although we could not do this reliably for every primer pair, we were able to cross-reference our putative loci ranges by comparing them to species that amplified a single locus.

Once primer pairs had been characterized across the 15 taxa, we tested them on a larger subset of *B. insignis* ($n = 30$) and *B. rockii* ($n = 24$) samples. Of the 30 primer pairs tested, one did not amplify reliably in either species (locus BRIN59, GenBank accession no. MK387351) and one did not amplify in *B. insignis* (LOVI52; Tran et al., 2015). With the remaining primer pairs, one (LOVI4) was monomorphic in both species, three were monomorphic only in *B. insignis* (BRIN07, BRIN56, and LOVI34), and two were monomorphic only in *B. rockii* (LOVI90 and LOVI93). Of the primer pairs that were polymorphic in one species, 18 produced a maximum of two peaks consistent with amplifying a single locus, and 12 produced multiple bands. Of the 12 primer pairs producing multiple bands, nine produced more than two peaks in both species (BRIN01, BRIN10, BRIN43, BRIN47, BRIN51, LOVI23, LOVI35, LOVI56, and LOVI93), two (BRIN44, LOVI04) produced more than two bands only in *B. insignis*, and one (LOVI52) produced more than two peaks only in *B. rockii*. For all 30 loci, we report the following descriptive parameters: range, number of alleles, effective number of alleles, observed and expected heterozygosity, and inbreeding coefficient (Table 3; calculated in GenAlEx [Peakall and Smouse, 2006]). As most of the individuals within ex situ collections are related, they violate the expectation of random mating required to detect deviation from Hardy–Weinberg equilibrium, null alleles, and linkage disequilibrium.

CONCLUSIONS

Eighteen microsatellite loci developed in *B. insignis* and 12 derived from *L. villosa* (Tran et al., 2015) were polymorphic and amplified reliably in at least one species. These loci reveal low levels of diversity and high inbreeding in both species, although less so in *B. rockii*. Nonetheless, in combination, these 30 loci can provide insights into the relatedness of individuals in the captive population. In addition, these loci also cross-amplified in taxa that span the Hawaiian

lobelioid group, revealing polymorphism in most species, despite low sample sizes. These loci will be useful for assessing patterns of gene flow, genetic diversity, and structure within and among populations of all species in the lobelioid complex.

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DATA AVAILABILITY

The primer sequence information for the 28 newly developed primers was uploaded to the National Center for Biotechnology Information's GenBank database (accession no. MK387344–MK387371).

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APPENDIX 1. List of institutions and accessions for *Brighamia* germplasm used to generate data for Table 3.

Species	Institution	Sample size	Accession no.
<i>Brighamia insignis</i>		30	
	Bishop Museum	2	842, 1475
	Heidelberg University Botanical Garden	1	107707
	National Tropical Botanical Garden	22	020036, 050681, 050682, 060024, 090445 (×2), 100231, 100651, 100652, 1000714, 120043, 920440, 950232, 990833, 990836, 990836, 990839, 990840, 990942, 990945, 20150354 (+1 no record)
	National Tropical Botanical Garden Herbarium	2	538, 10438
	Sukkulanten-Sammlung Zürich	1	991001
	University of California Botanical Garden at Berkeley	2	NA
		24	
<i>Brighamia rockii</i>	Bishop Museum	1	444245
	Ganna Walska Lotusland	1	2005-192
	Maui Nui Botanical Garden	2	Bri Roc-Mo-EHU-A
	Puu O Hoku Ranch	7	F2 from Huelo islet stock
	National Tropical Botanical Garden	5	110072, 1102283, 950402 (×2), 950422
	National Tropical Botanical Garden Herbarium	8	8031, 8033, 8034, 14025, 14027, 14794, 14025b, 14027b

Note: NA = not available.