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PRIMER NOTE

# 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. Lév. and *C. samuelii* C. N. Forbes subsp. *hanaensis* (H. St. John) Lammers), six species of *Cyanea* Gaudich. (*C. fissa* Hilldebr.,

 

**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] |
|---|---|---|---|---|---|
| *Brighamia insignis* | | | | | |
| BRIN01 | F: CTTGTTGCAGGATGGGAGTT | $(GA)_{17}$ | 202–222 | Green [D3-PA] | MK387344 |
| | R: GGGTATCCACCCTTTCCTTC | | | | |
| BRIN05 | F: GAATGGTTTTCACTTTCCCAAC | $(GT)_{17}$ | 181–207 | Blue [D4-PA] | MK387346 |
| | R: ATCTCTTACCCCGGAAGCAC | | | | |
| BRIN07 | F: TTCAGCACAGATCCCTTTTG | $(TG)_{16}(AG)_{7}$ | 167 | Green [D3-PA] | MK387347 |
| | R: TCCAGATTCAGCTCCTCCAG | | | | |
| BRIN08 | F: AGACGGCTCGGGGTATAACT | $(TG)_{12}$ | 146–154 | Blue [D4-PA] | MK387348 |
| | R: GTACCATTCTCGTATTTACCCA | | | | |
| BRIN10 | F: CAGCTGTTACCGTCTTCTGC | $(CT)_{11}(CA)_{14}$ | 148–186 | Blue [D4-PA] | MK387349 |
| | R: TTTCTAAAGTTACAAAATCAAGG | | | | |
| BRIN41 | F: CAACGCTGATGATGATGATTG | $(TG)_{14}$ | 111–125 | Black [D2-PA] | MK387352 |
| | R: ACCCTTCGTTCCAAAGATCC | | | | |
| BRIN43 | F: GGACCAACAATTGGAGAAACA | $(CT)_{27}$ | 174–248 | Blue [D4-PA] | MK387354 |
| | R: CTGATGCTGAACATCTGTAAACAA | | | | |
| BRIN44 | F: ACTGGATTGAAAGCCTTACTTTG | $(AG)_{14}$ | 142–162 | Black [D2-PA] | MK387355 |
| | R: CTAATGCACATTTTTGGATTGCT | | | | |
| BRIN46 | F: GGAAAACTGATGTGCGTTGA | $(TC)_{13}(AC)_{10}$ | 232–254 | Blue [D4-PA] | MK387357 |
| | R: TTGCTTCATGACTTGAGCTTG | | | | |
| BRIN47 | F: CCCCTCCATTTCAAGGTTCT | $(GT)_{12}$ | 152–188 | Green [D3-PA] | MK387358 |
| | R: CCTCAGCAGGGGAAAAGTAA | | | | |
| BRIN51 | F: GACAAGGGACATGGCTGTTT | $(CT)_{16}(CA)_{12}$ | 170–208 | Green [D3-PA] | MK387362 |
| | R: TGAGAGTTTGATCTGCACAAAA | | | | |
| BRIN53 | F: GCTTGTTGCACAACATGAAA | $(AG)_{12}$ | 165–177 | Green [D3-PA] | MK387363 |
| | R: TGATAGTCACAGTTTGGCTGA | | | | |
| BRIN54 | F: TGTGAATGGCTGGTTGGTAA | $(GT)_{9}$ | 168–176 | Green [D3-PA] | MK387364 |
| | R: GCCTAAAGGCAAAGAGTTTGAA | | | | |
| BRIN56 | F: CGCGAAGTCCAGAAGAAAAC | $(CA)_{11}$ | 104 | Black [D2-PA] | MK387366 |
| | R: TTTTGTTTCACTCTTCGTCCA | | | | |
| BRIN57 | F: TGGGAGAAAATTGAAAGCAAA | $(GT)_{7}(GA)_{22}$ | 223–233 | Blue [D4-PA] | MK387367 |
| | R: GTACAAAGAATCCACTCACTCGC | | | | |
| BRIN59 | F: ACCAGGGATTGTTCGCTAAG | $(GA)_{29}$ | — | Green [D3-PA] | MK387351 |
| | R: GGCTCTGTGGCATTCAAAGT | | | | |
| BRIN61 | F: GTGAGCTGGGTGGTTGTTTT | $(AG)_{14}$ | 183–191 | Green [D3-PA] | MK387369 |
| | R: AGGAGGACCCCTCAACAATC | | | | |
| BRIN66 | F: GTACTGCATGCCCTGTGTT | $(GT)_{10}(GA)_{10}$ | 157–181 | Blue [D4-PA] | MK387370 |
| | R: GATGCGCTCAAACGAGAGTT | | | | |
| *Lobelia villosa* | | | | | |
| LOVI4 | F: ACGTCTAGGGGCACTGCCAAGCCAG | $(AC)_{12}$ | 248–268 | M13F-green [D3-PA] | |
| | R: TCCAAATGGGAGACTACTGCAGAAAGG | | | | |
| LOVI23 | F: TCTTTTGTCCATGCCAGCGTG | $(AC)_{11}$ | 401–437 | M13F-blue [D4-PA] | |
| | R: AGGTACTCGGTCTGAGCGTTTCG | | | | |
| LOVI33 | F: ACAGGGGGCAAAACTGGTCACC | $(GT)_{8}(GA)_{9}$ | 372–378 | M13F-black [D2-PA] | |
| | R: TGCAAGGATGACGAAGGGGCTC | | | | |
| LOVI34 | F: ACAGGCGCTATGGCGTCCCT | $(CA)_{10}$ | 155 | M13F-blue [D4-PA] | |
| | R: GTTGTATGCATCATGAGACCGTC | | | | |
| LOVI35 | F: GCTTACAACAAATTGCCTC | $(CA)_{17}$ | 230–256 | M13F-black [D2-PA] | |
| | R: AGCCTCGAAATCATCCGGCCCA | | | | |
| LOVI37 | F: GGATCACTCAAGGATGAACTCGCAAGG | $(GA)_{14}$ | 470–478 | M13F-green [D3-PA] | |
| | R: TGTGTAATGGACCTTGGGCTGTCTC | | | | |
| LOVI48 | F: TCACCGAACGATTCATCGAACCA | $(AC)_{7}A(AC)_{5}$ | 400–420 | M13F-green [D3-PA] | |
| | R: ACGAGTGAGAGACTTTTGGGTGATCA | | | | |
| LOVI52 | F: CGAATGATCTATTATTCCAGCC | $(AC)_{11}$ | — | M13F-blue [D4-PA] | |
| | R: AGACACTTCCTCGAGTTGGTG | | | | |
| LOVI56 | F: AGCATCAGCAAGACACTTGC | $(CT)_{9}(CA)_{14}$ | 536–640 | M13F-black [D2-PA] | |
| | R: AGTAAGGGATAAAGCAGACCTGG | | | | |
| LOVI73 | F: TCACAAATGCTCCATCGCGAG | $(CA)_{9}$ | 468–478 | M13F-black [D2-PA] | |
| | R: TGTAGCGGAAAGTACCGGATCCA | | | | |
| LOVI90 | F: ACCGGCTGTAATCACGCGTTGG | $(CA)_{12}$ | 200–206 | M13F-blue [D4-PA] | |
| | R: CTACTGTGTGAAGTCGGAAAACC | | | | |
| LOVI93 | F: TCTAGCAGAAGCCTCACCCCGGA | $(GT)_{10}$ | 287–397 | M13F-green [D3-PA] | |
| | R: CACCAGAACTCAAGCAAGGCGAC | | | | |

*Note:* — = no amplified product.
[a]Accession 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 Hawai'ian lobelioid complex.[a]

| Locus | *Brighamia insignis* (*n* = 30) | *Brighamia rockii* (*n* = 24) | *Clermontia fauriei* (*n* = 2) | *Clermontia samuelii* (*n* = 1) | *Cyanea fissa* (*n* = 2) | *Cyanea hardyi* (*n* = 2) | *Cyanea leptostegia* (*n* = 2) | *Cyanea hirtella* (*n* = 2) | *Cyanea pseudofauriei* (*n* = 2) | *Cyanea salicina* (*n* = 1) | *Delissea kauaiensis* (*n* = 2) | *Delissea rhytidosperma* (*n* = 2) | *Delissea waianaeensis* (*n* = 1) | *Lobelia niihauensis* (*n* = 2) | *Trematolobelia kauaiensis* (*n* = 2) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BRIN01 | 202–208<br>218–222 | 192–206<br>214–218 | 134–144<br>172–194 | 144<br>194 | 132–142<br>179–197 | 138 | 138 | 142<br>156–672 | 138 | 140–142<br>162 | 140–158 | — | 140–142 | 154 | 142 |
| BRIN05 | 181–207 | 181–205 | 187–194 | 194 | 189–195 | 186 | 186 | 190–196 | 154–162<br>186 | 191 | 145–149<br>181 | 181 | 181 | 180 | 195–200 |
| BRIN07 | 167 | 149–173 | 153–160<br>187–191 | 165<br>194 | 156–163<br>196 | 185 | — | 158–169 | — | — | — | 175–187 | 181 | 165–179 | 144–152<br>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<br>174–186 | 150–152<br>168–178 | 146–154 | 154 | 149–165 | 160 | 267 | 149<br>165–171 | 160 | 149<br>165 | 175 | — | 154–171 | 149–151<br>169 | 143<br>163–171 |
| BRIN41 | 111–125 | 115–125 | — | — | — | — | — | — | — | 100 | 103–106 | — | 122 | 110 | 100 |
| BRIN43 | 174–190<br>228–248 | 188–218<br>228–234 | 198–201<br>215–232 | 198<br>206–225 | 201–208 | 193<br>209 | 183<br>203–209 | 191<br>201–205 | 189–193<br>201–215 | 185–199 | 190–198 | 197–205<br>210–213 | 209–219 | 183 | 187–192 |
| BRIN44 | 142–148<br>152–162 | 126–138 | 215–217<br>224 | 182 | 182 | 160 | 223–230 | 182 | 211 | 182 | 197–215<br>238–240 | 195<br>207–224 | 224–228 | 205–215 | 185–220 |
| BRIN46 | 232–254 | 234–252 | 244–248 | 240 | 263 | 209–218<br>224–234 | 240–260 | 228–238<br>260 | 209<br>230–240 | 245–249 | 250–266 | 250 | 268 | 241–258 | 228–230 |
| BRIN47 | 152–178<br>182–188 | 150–164<br>174–188 | 167–170<br>181–184 | 169<br>182 | — | — | — | — | — | 172 | 170–180 | 167–183 | 167–180 | — | 160<br>170 |
| BRIN51 | 170–174<br>180–208 | 164–176<br>182–186 | 172–176<br>184–198 | 173<br>190–210 | 160–172 | 168 | 172<br>198 | 172<br>188 | 153–165<br>184 | 172<br>182 | 156<br>180–182 | 156<br>172–182 | 156<br>184–196 | 170–182<br>188–198 | 182<br>194–228 |
| BRIN53 | 165–177 | 171–177 | — | 173 | — | 161 | — | 161 | 158–162 | 160 | 188 | 185–190 | 188 | 145–161 | 146–161 |
| BRIN54 | 168–176 | 158–180 | — | 160 | 168–172 | 163 | 165 | 168–172 | 163 | 168 | 166 | 163–166 | 167 | 156–158 | 166 |
| BRIN56 | 104 | 114–116 | 92–108 | 104 | 101–107 | 98–105 | 97–103 | 109–115 | 98–112 | 107–113 | 97–109 | 106 | 92–114 | 85–91 | 96–115 |
| BRIN57 | 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<br>220–240 | 182–195<br>200–210 | 168–192 | — | — | 172–190 | — | — | — | — | — | — | — |
| BRIN61 | 183–191 | 181–193 | — | 193 | 181–190 | — | 193–208 | 181–193 | 191–193<br>250–268 | 181 | 181–201 | — | 181–205<br>293 | 181<br>205–215 | 182<br>207–209 |
| BRIN66 | 157–181 | 157–177 | — | 161 | — | 157–175 | — | 158–174 | 156–173 | 173–175 | 157–159 | 159 | 173 | 174–176 | 148–150 |
| LOVI04 | 248<br>268 | 248 | 253–259<br>260–266 | 253–259 | 241–287 | 254<br>290–294 | 250–289 | 242–282 | 255 | 252–260<br>277–288 | 252 | 253–261 | — | 249–259<br>269–276 | 255<br>269 |
| LOVI23 | 401–411<br>417–437 | 397<br>415–417 | 401<br>419 | 402<br>423 | 400<br>421–423 | 401–403<br>412 | 400–403 | — | 393–403 | 400<br>424 | 417<br>430–445 | 419 | 415–429 | 403–412 | 403<br>422–434 |
| LOVI33 | 372–378 | 368–382 | 355 | 257 | 350 | 348–359 | 356–360 | 350–352 | 358 | 350–354 | 368 | 384 | 362 | 367–395 | 346 |
| LOVI34 | 155 | 151–165 | — | — | 156–160 | 156 | 155 | 156–160 | 156 | 156–160 | — | 154–165 | 154–161 | — | — |
| LOVI35 | 230–236<br>256 | 236–242<br>256 | — | — | 261–275 | 260–271 | 254 | 260–265 | 254–264 | 261 | — | 234–255 | 231 | — | — |
| LOVI37 | 470–478 | 476–484 | — | 429–433<br>452 | 471–497 | — | — | 460–494 | 481 | 490–502 | 409–420 | 480–490 | 472 | 428–448 | 425 |
| LOVI48 | 400–420 | 376–404 | — | 422–428 | 402–425 | 410–430 | 424–434 | 413 | 412–418 | — | — | — | 401 | 403–408 | 406 |
| LOVI52 | — | 157–173<br>194–204 | — | 171<br>210 | 171–199<br>208–222 | — | 172<br>208–218 | 172 | 170–199<br>208–218 | 172–199<br>224–242 | — | — | — | — | — |
| LOVI56 | 536–558<br>618–640 | 520–546<br>618–620 | — | 552<br>620 | 544–550<br>618–623 | 548–554<br>622 | 548–550<br>622 | 546<br>622 | 548<br>622 | 546<br>623 | 541<br>620 | 545–562<br>620 | 535<br>620 | 573–581 | 564–572 |
| LOVI73 | 468–478 | 468–478 | 467 | 465 | — | 467 | 467 | 467 | — | 467 | 466–475 | 473 | 477 | 452–474 | 459 |
| LOVI90 | 200–206 | 200 | 201–211 | 203 | — | 200 | 203–215 | 204–206 | — | 205–209 | 180 | 200 | 200 | 184–204 | 200–212 |
| LOVI93 | 287<br>391-397 | 287<br>393 | 382–390 | 394–402 | — | 365 | 375 | 340 | — | 310 | 342 | 377 | 288 | 356–376 | — |

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

[a]For 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 breaks 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.

| | ***B. insignis* (*n* = 30)** | | | | | | ***B. rockii* (*n* = 24)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Locus** | **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. pseudofauriei* Lammers, and *C. salicina* H. Lév.), three species of *Delissea* Gaudich. (*D. kauaiensis* (Lammers) Lammers, *D. rhytidosperma* H. Mann, *D. waianaeensis* 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 Hawai'ian 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 WellRed Black (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 Hawaiʻian 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. |
|---|---|---|---|
| *Brighamia insignis* | | 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 |
| | Sukkulenten-Sammlung Zürich | 1 | 991001 |
| | University of California Botanical Garden at Berkeley | 2 | NA |
| *Brighamia rockii* | | 24 | |
| | 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.