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**DEVELOPMENT AND CHARACTERIZATION OF EST-SSR MARKERS FOR *BEGONIA LUZHAEIENSIS* (BEGONIACEAE)**

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**Method and Results:** Based on the transcriptome data of *B. luzhaiensis*, 60 primer pairs were selected for initial validation, of which 16 yielded polymorphic microsatellite loci in 57 individuals. The number of alleles observed for these 16 loci ranged from one to nine. The observed and expected heterozygosity ranged from 0.000 to 1.000 and from 0.000 to 0.804 with averages of 0.370 and 0.404, respectively. Five loci could be successfully amplified in *B. leprosa*.

**Conclusions:** The expressed sequence tag–simple sequence repeat markers are the first specifically developed for *B. luzhaiensis* and the first developed in *Begonia* sect. *Coelocentrum*. These markers will be useful for future studies of the genetic structure and phylogeography of *B. luzhaiensis*.

**Key words:** *Begonia leprosa*; *Begonia luzhaiensis*; Begoniaceae; expressed sequence tag–simple sequence repeat (EST-SSR); section *Coelocentrum*; transcriptome.

The immense terrain of limestone karsts stretching from southern China to northern Vietnam (Sino-Vietnamese limestone karsts; SVLK) is a renowned biodiversity hotspot. It is noted for extremely high species diversity and endemicity of narrowly distributed calciphilous plants that are increasingly threatened by habitat destruction caused by rapid economic growth in recent decades (Chung et al., 2014). To design effective conservation strategies, a better understanding of the evolutionary mechanisms underlying the rich and distinct SVLK flora is essential. With ca. 70 species distributed exclusively in the SVLK, *Begonia* L. sect. *Coelocentrum* Irmsch. (Begoniaceae) is one of the most characteristic plant groups (Chung et al., 2014; Peng et al., 2014, 2015; Li et al., 2016), presenting an ideal model system for studying limestone plant speciation. Species of the section exhibit great morphological variation in leaf shape, texture, and variegation; they are usually confined to caves and cave-like microhabitats that occur abundantly in the karst areas of southeastern China (Guangdong, Guangxi, and Yunnan provinces) and northern Vietnam (Chung et al., 2014). Most species of sect. *Coelocentrum* are known from a single or a few localities, with the exception of *B. cavalieri* H. Lév., *B. leprosa* Hance, and *B. luzhaiensis* T. C. Ku (Gu et al., 2007).

Based on phylogenetic analyses of Asian limestone *Begonia* species, Chung et al. (2014) proposed that the excess of moisture brought by the onset of East Asian monsoons since the late Miocene accelerated rates of karstification of Sino-Vietnamese limestone terrains, triggering widespread allopatric speciation in the SVLK. To test the microevolutionary processes underlying the speciation hypothesis of Chung et al. (2014), we investigate the population genetics and phylogeography of *B. luzhaiensis*. In this article, we report microsatellite markers developed for *B. luzhaiensis*. Expressed sequenced tag–simple sequence repeat (EST-SSR) markers are valuable in tests of cross-transferability, facilitating studies of population genetic diversity in many plant species (e.g., Dikshit et al., 2015; Zhou et al., 2016). Here, we used next-generation transcriptome sequencing to develop a set of microsatellite markers for *B. luzhaiensis*. Additionally, we tested the transferability of these markers for *B. leprosa*, another widespread species of sect. *Coelocentrum*.

**METHODS AND RESULTS**

To maximize potential loci, total RNAs were extracted from fresh leaves and male floral buds (from *C.-I Peng 18732*, denoted as Pool AC) and fruit (*C.-I Peng 18735*, Pool B) of *B. luzhaiensis*, respectively. RNA extraction was performed using the PureLink RNA Mini Kit (Invitrogen, Carlsbad, California, USA) according to the manufacturer’s instructions, and quality and quantity were measured by an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Monica, CA).
### Table 1. Characteristics of 16 microsatellite loci developed for *Begonia luzhaiensis*.

| Locus  | Primer sequences (5′–3′) | Repeat motif | Fluorescent label | Allele size range (bp) | Putative function [Organism] | E-value | GenBank accession no. |
|--------|--------------------------|--------------|-------------------|------------------------|-----------------------------|---------|----------------------|
| BLZ01  | F: TCGTGTGAGACGTCTCTTTTT  | (GA)$_{20}$  | FAM               | 188–228                | No hit                      | –       | KY659210             |
|        | R: CCGCAAGATTCGCTCTTTT   |              |                   |                        |                             |         |                      |
| BLZ02  | F: AAGCTCCTCTCTCTCTTCTCTT | (TCT)$_{11}$ | FAM               | 164–200                | No hit                      | –       | KY659211             |
|        | R: CGCTTCTCTCTCTCTCTTCTCT |              |                   |                        |                             |         |                      |
| BLZ03  | F: GAGCAAGAGGAGGTCTGTG   | (CGAAC)$_{5}$| NED               | 205–240                | Cyclin-L1-1 isoform X1 [Lupinus angustifolius] | 3.7     | KY659213             |
|        | R: CGGTCTAGATTACACCGAC   |              |                   |                        |                             |         |                      |
| BLZ04  | F: TGGTTCGAGGAAGACATTG   | (AGGAG)$_{5}$| NED               | 163–217                | Transcription factor MYB114 isoform X2 [Eucalyptus grandis] | 0.004   | KY659214             |
|        | R: CTCTGCTGGTTCTTCTGT    |              |                   |                        |                             |         |                      |
| BLZ05  | F: TGAGCCAGAGCTGAGAGTG   | (GATGGA)$_{5}$| PET              | 209–257                | Mlo-related protein [Corchorus capsiculum] | 8e-08   | KY659215             |
|        | R: ACTGAGACCACCATTTACCG  |              |                   |                        |                             |         |                      |
| BLZ06  | F: AGATCAGAGGAGAAGCTCG   | (TT)$_{12}$  | VIC               | 135–153                | 1,4-dihydroxy-2-naphthoyl-CoA thioesterase 1 [Malus domestica] | 2e-11   | KY659220             |
|        | R: CCTCCATCTCTAATCGATA   |              |                   |                        |                             |         |                      |
| BLZ07  | F: GCAGGAGTTGAGAGGGAGAG  | (CAG)$_{11}$ | VIC               | 200–248                | No hit                      | –       | KY659216             |
|        | R: CCAAACCATGGGACAGCTAG  |              |                   |                        |                             |         |                      |
| BLZ08  | F: TTGTTCGAGAGAAGAGCTTT  | (GAAAG)$_{5}$| NED               | 182–192                | Hypothetical protein [Gossypium raimondii] | 0.17    | KY659217             |
|        | R: AGCCAGAGCACCCATTTTC   |              |                   |                        |                             |         |                      |
| BLZ09  | F: GTAGTCGAGAGGTTGAGAA   | (CCCATT)$_{5}$| PET              | 185–227                | Hypothetical protein [Citrus clementina] | 3e-12   | KY659218             |
|        | R: GTGCTGTACTTTCAGAGATA  |              |                   |                        |                             |         |                      |
| BLZ10  | F: AGATCGAGAAGACGTGAAA   | (GAA)$_{10}$ | VIC               | 150–210                | No hit                      | –       | KY659219             |
|        | R: CCTCGATCATCTCTCGAAA   |              |                   |                        |                             |         |                      |
| BLZ11  | F: AGCTGCGACTCTGGAAACT   | (AAAT)$_{5}$ | FAM               | 226–242                | Uncharacterized protein [Vitis vinifera] | 0.46    | KY659221             |
|        | R: GCCCTTCATCTCTCGAAA    |              |                   |                        |                             |         |                      |
| BLZ12  | F: AATGGAGGAGAAGACGGTTA  | (TTT)$_{5}$  | NED               | 151–199                | Hypothetical protein [UPL1 isoform X3 [Cucumis melo] | 9e-13   | KY659222             |
|        | R: GTATAGCGCGAGCCTCGAAA  |              |                   |                        |                             |         |                      |
| BLZ13  | F: TCTCAGTCTGACTCAGCAA   | (TTCC)$_{5}$ | PET               | 186–222                | 1,4-dihydroxy-2-naphthoyl-CoA thioesterase 1 [Malus domestica] | 2e-11   | KY659220             |
|        | R: GTATAGCGCGAGCCTCGAAA  |              |                   |                        |                             |         |                      |
| BLZ14  | F: ATCTGCTGCTGCTGATAAGT  | (GACCG)$_{5}$| VIC               | 197–227                | No hit                      | –       | KY659223             |
|        | R: GTATAGCGCGAGCCTCGAAA  |              |                   |                        |                             |         |                      |
| BLZ15  | F: AGCCAGAGCAGACTTCACT   | (GAGATG)$_{3}$| FAM               | 210–228                | Hypothetical protein [Gossypium raimondii] | 1e-13   | KY659224             |
|        | R: GCCCTGTACTTTCAGAAA    |              |                   |                        |                             |         |                      |
| BLZ16  | F: GACGTGATGAAAGCCATAAC  | (TCTCAA)$_{5}$| NED              | 223–259                | No hit                      | –       | KY659225             |
|        | R: CCGCTTCTCTCAGGGACGA   |              |                   |                        |                             |         |                      |

*Annealing temperature was 53°C for all loci.*
Heterozygosity;

| Locus | BLZ01 | BLZ02 | BLZ03 | BLZ04 | BLZ05 | BLZ06 | BLZ07 | BLZ08 | BLZ09 | BLZ10 | BLZ11 | BLZ12 | BLZ13 | BLZ14 | BLZ15 | BLZ16 |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| BLZ01 | —     | +     | —     | +     | —     | +     | —     | +     | —     | +     | —     | +     | —     | +     | —     | +     |
| BLZ02 | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| BLZ03 | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     |
| BLZ04 | +     | —     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| BLZ05 | +     | +     | —     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| BLZ06 | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| BLZ07 | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     |
| BLZ08 | —     | +     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     |
| BLZ09 | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     |
| BLZ10 | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     |
| BLZ11 | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     |
| BLZ12 | —     | +     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     |
| BLZ13 | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| BLZ14 | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| BLZ15 | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| BLZ16 | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |

Note: + = successful amplification; — = failed amplification; *= PCR has not been performed.

Localization and voucher information are provided in Appendix 1.

Between any pair of loci (P < 0.001). The putative functions of SSR-associated sequences were determined by BLASTX against the nonredundant GenBank database.

The interspecific transferability of the 16 markers was evaluated in B. leprosa, another widespread species of sect. Coelocentrum (Table 3). Five markers were successfully cross-amplified in B. leprosa.

CONCLUSIONS

The 16 microsatellite markers described here are the first developed for B. luzhaiensis and also the first study in Begonia sect. Coelocentrum. These microsatellites can be applied to the investigation of genetic diversity, population genetic structure, mating system, and gene flow, thus facilitating our understanding of evolutionary mechanisms and species diversification of the limestone flora. Data from such studies will contribute to the conservation and management of B. luzhaiensis that is increasingly threatened by habitat destruction.

LITERATURE CITED

Chung, K.-F., W.-C. Liou, R. R. Rubite, R. Repin, R. Kiew, Y. Liu, and C.-I. Peng. 2014. Phylogenetic analyses of Begonia sect. Coelocentrum and allied limestone species of China shed light on the evolution of Sino-Vietnamese karst flora. Botanical Studies (Taipei, Taiwan) 55:1.

Dikshiti, H. K., A. Singh, D. Singh, M. S. Aski, P. Prakash, N. Jain, S. Meena, et al. 2015. Genetic diversity in Lens species revealed by EST and genomic simple sequence repeat analysis. PLoS ONE 10: e0138101.

Gu, C.-Z., C.-I. Peng, and N. J. Turbaland. 2007. Begoniaceae. In Z.-Y. Wu, P. H. Raven, and D. Y. Hong [eds.], Flora of China, vol. 13, 153–207. Science Press, Beijing, China, and Missouri Botanical Garden Press, St. Louis, Missouri, USA.

Holland, M. M., and W. Parison. 2011. Genemark® HII: A reliable software tool for the analysis of forensic STR data. Journal of Forensic Sciences 56:29–35.

Li, C., L.-H. Yang, D.-K. Tian, Y. Chen, R.-J. Wu, and N.-F. Fu. 2016. Begonia lepingensis (Begoniaceae), a new compound-leaved species with unique petiolule pattern from Guangxi of China. Phytotaxa 244: 45–56.
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24°43'41.0"N, 110°31'44.0"E

C.-I Peng 18732

B. luzhaiensis

collection locality

Notes: n = number of individuals sampled.