Microsatellite markers for *Saussurea polylepis* (Asteraceae), a vulnerable continental island species endemic to Korea

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**PREMISE:** Nuclear microsatellite markers were developed for *Saussurea polylepis* (Asteraceae), a vulnerable species with very limited distribution in a few southwestern continental islands of the Korean peninsula, in order to facilitate future population genetic studies.

**METHODS AND RESULTS:** Based on the Illumina sequence data, a total of 21 microsatellite primer pairs were designed and tested for their suitability. Nineteen of these primers, with two to 11 alleles per locus, were polymorphic in three natural populations of *S. polylepis*. The levels of expected and observed heterozygosity ranged from 0.000 to 0.842 and 0.000 to 0.933, respectively. Sixteen of these sequence repeat (SSR) markers were successfully cross-amplified in five congeneric species, namely *S. gracilis*, *S. grandifolia*, and *S. tanakae* for all 21 loci, and *S. maximowiczii* and *S. pulchella* for 18 loci.

**CONCLUSIONS:** The SSR markers developed here will be useful for future population genetic studies on *S. polylepis* and related species.

**KEY WORDS** Asteraceae; conservation; continental islands; microsatellite markers; *Saussurea polylepis*.

*Saussurea* DC. (ca. 400 species) is one of the largest genera in the tribe Cardueae (Asteraceae) and is also one of the most speciose groups in the flora of Korea (Lipschitz, 1979; Im, 2007). Of the approximately 40 species of *Saussurea* in Korea, nearly 40% (15 species) are endemic, mostly occurring in small-sized populations in isolated regions. Of these endemic species, *S. polylepis* Nakai, a perennial herb, is highly restricted to small continental islands (less than 10 locations) in the southwestern corner of the Korean peninsula. Its population size is extremely small, normally less than 30 individuals per population, and it is currently categorized as a vulnerable species (VU B2ab (iii, iv)) in the Korean Red List (National Institute of Biological Resources, 2014). Although the overall phylogenetic framework among the species of *Saussurea* in Korea is lacking, *S. polylepis* can be morphologically distinguished from its congeneric species by its glossy and reniform middle cauline leaves, hairs on adaxial and abaxial leaf surfaces, petioles without wings, and irregularly dentate leaf margins (Im, 2007).

*Saussurea* includes numerous ecologically and ethnomedicinally important species. For example, some species, such as *S. lappa* (Decne.) Sch. Bip., *S. gnaphalodes* (Royle ex DC.) Sch. Bip., *S. costus* (Falc.) Lipsch., and *S. ceratocarpa* Decne., contain many important chemical compounds that have become important drugs in the international market (Pandey et al., 2007; Zeng et al., 2012). In addition, some species occur at higher elevations than any vascular plant (e.g., *S. gnaphalodes* in the vicinity of Mount Everest at 6400 m; Yoshida, 2002; Yang et al., 2008). Because of their distribution in the subalpine/alpine regions and overexploitation for different medicinal and commercial purposes, many of these species are sensitive to climatic change and are subject to extinction. Several endemic species of *Saussurea* in the backbone mountain range (i.e., Baekdudaegan) in the Korean peninsula are not an exception to this situation (e.g., *S. grandicapitula* W. Lee & Im, *S. chabyoungsanica* Im, and *S. calcicola* Nakai). Despite the ecological and economic importance of the genus *Saussurea*, very few studies on population genetics and molecular marker development have been conducted (Jeong et al., 2012; Zeng et al., 2012). In this study, we selected one very rare species, *S. polylepis*, occurring in several continental islands in Korea, and developed polymorphic microsatellite markers for future population genetic studies. In addition, we tested whether some of these markers could be cross-amplified to five closely related species of *Saussurea*, namely *S. gracilis* Maxim., *S. grandifolia* Maxim., *S. maximowiczii* Herder, *S. pulchella* (Fisch.) Fisch. ex Colla, and *S. tanakae* Franch. & Sav. ex Maxim., which are commonly found in mountain regions of Korea.
METHODS AND RESULTS

Total genomic DNA was extracted from a fresh leaf sample collected in Hong-do, Korea (Appendix 1), and deposited in the National Center for Biotechnology Information (NCBI) BioSample database (BioSample accession number SAMN10362162) using a DNeasy Plant Mini Kit (QIAGEN, Carlsbad, California, USA), following the manufacturer instructions. An Illumina paired-end genomic library was constructed using a TruSeq DNA LT Sample Prep Kit (Illumina, San Diego, California, USA), and the library sequencing was conducted with the Illumina MiSeq platform at the Macrogen Company (Seoul, Korea). A total of 15,210,742 paired-end reads (4.5 Gbp, NCBI Sequence Read Archive BioProject ID PRJNA503488) were obtained. The raw reads were trimmed to remove low-quality bases using Sickle (Joshi and Fass, 2011) and adapters using Scythe (v0.994 BETA) (Buffalo, 2014). The paired-end reads were then assembled using SPAdes version 2.4 (Bankevich et al., 2012), and a total of 814,626 contigs were obtained. MIcroSAtellite identification software (MISA) with default settings (Thiel et al., 2003) was used to detect simple sequence repeat (SSR) motifs with a repeat unit ranging from two to six nucleotides. The minimum number of nucleotide repeats was set to six for dinucleotide repeats and five for trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide repeats, and a total of 40,773 regions were found. Primer3 version 2.3.6 software (Koressaar and Remm, 2007; Untergasser et al., 2012) was used to design SSR primers with the following settings: optimal conditions of length of 20 bp (18–27 bp), annealing temperature of 60°C (57–63°C), and product size range of 100–300 bp. Of the 40,773 regions, 35 candidate primer

| Locus | Primer sequences (5′–3′) | Repeat motif | Allele size range (bp) | T<sub>a</sub> (°C) | Fluorescent dye | GenBank accession no. |
|-------|-------------------------|--------------|------------------------|-------------------|----------------|----------------------|
| SP01  | F: TCACAAGCCCATTCAGCCAT | (CT)<sub>10</sub> | 237–249 | 59 | FAM | MK285611 |
|       | R: TACAGGGAACCAGAAGTCG  |             |                       |                   |                |                      |
| SP02  | F: TACAGGCGAGGTTTGTCGA  | (TC)<sub>11</sub> | 269–273 | 59*, 60 | HEX | MK285612 |
|       | R: TCAGGGTCACACCGTTTGA |             |                       |                   |                |                      |
| SP03  | F: CTGCCGAGTCGCTCCTCTAG | (AAT)<sub>10</sub> | 234–297 | 59 | FAM | MK285613 |
|       | R: TTTGGTCTCACACCCCTTGG |             |                       |                   |                |                      |
| SP04  | F: CGGAGGACACACATACGACCC | (AGT)<sub>5</sub> | 225–234 | 60 | HEX | MK285614 |
|       | R: TTCAGGAGATGTAACATGACG |             |                       |                   |                |                      |
| SP05  | F: TCAAGGATGGGACAAAAACCA | (AC)<sub>10</sub> | 233–247 | 59 | FAM | MK285615 |
|       | R: AAAAGGGTCGGGCTCTCAAC  |             |                       |                   |                |                      |
| SP07  | F: CTCTGCAAGGATCGCTCAA | (GA)<sub>11</sub> | 233–287 | 60 | FAM | MK285616 |
|       | R: CAATAGCCGGCTAAACCAGAA |             |                       |                   |                |                      |
| SP10  | F: GTTTTCGGCGCTACCAAAAC | (GGAG)<sub>2</sub> | 222–231 | 60 | FAM | MK285617 |
|       | R: AACAATCCGGCTACCGCTC |             |                       |                   |                |                      |
| SP11* | F: CAGCGAGTTGGAGCAAGAG | (TCA)<sub>6</sub> | 191, 200 | 59*, 60 | HEX | MK285618 |
|       | R: CTAGGGAGGCAACTCGAGA |             |                       |                   |                |                      |
| SP12  | F: AGCGATTCTGATGTGCTCTCT| (TTG)<sub>9</sub> | 268–295 | 58*, 59 | FAM | MK285619 |
|       | R: ACAACATGTTTGTCTTCTGAGG |             |                       |                   |                |                      |
| SP13  | F: GCATACCGGCGAGTGAAGTG | (TCT)<sub>10</sub> | 222–246 | 59 | FAM | MK285620 |
|       | R: CCATCAGTGGCGTGGCAGAT |             |                       |                   |                |                      |
| SP19<sup>b</sup> | F: CTGCTAGGATGTGACTAAGAG | (GAT)<sub>6</sub> | 249 | 58 | FAM | MK285621 |
|       | R: CGACGATGTTGATGTGCAAA |             |                       |                   |                |                      |
| SP20  | F: GCCTTCTCTACCTGCTGCCACA | (TAG)<sub>3</sub> | 223–250 | 59 | FAM | MK285622 |
|       | R: GTTTCGGCCGTCAACCTTG |             |                       |                   |                |                      |
| SP21  | F: TGCTTCAACCTGATGATCAGC | (TCT)<sub>10</sub> | 253–256 | 59 | FAM | MK285623 |
|       | R: TGACCAACCTTGGTTCACGAA |             |                       |                   |                |                      |
| SP22  | F: TCACAACCCGGAGCAACAT | (TGGT)<sub>12</sub> | 273–285 | 60 | HEX | MK285624 |
|       | R: CCTCTCTATTGGGCAACCATGG |             |                       |                   |                |                      |
| SP23  | F: ACCAGATTGTGAGCAACCTCA | (ATAC)<sub>6</sub> | 272–348 | 58*, 59 | HEX | MK285625 |
|       | R: CGCACAATTGGAGATACCGG |             |                       |                   |                |                      |
| SP25  | F: GACACACAGTTGGTTTCCGC | (ATAC)<sub>6</sub> | 253–265 | 59 | FAM | MK285626 |
|       | R: GCCCTAGTCTCCTCATGCTTA |             |                       |                   |                |                      |
| SP26  | F: GCCCTAGGCTCTTTTGAGTGA | (GC)<sub>6</sub> | 237–239 | 59 | FAM | MK285627 |
|       | R: CGACTGGCGCTACATAGGGTT |             |                       |                   |                |                      |
| SP29  | F: TGTCGCGCAGGTCTCTATCT | (GGTT)<sub>5</sub> | 267–276 | 59*, 60 | HEX | MK285628 |
|       | R: CAATACGGACTGACTGGCT |             |                       |                   |                |                      |
| SP30  | F: ACTGAACTGCTGTCTTGGCTA | (ATAG)<sub>2</sub> | 225–253 | 59*, 60 | HEX | MK285629 |
|       | R: TGCCATCATCTCTGCTCTACTCA |             |                       |                   |                |                      |
| SP31  | F: TGCTAGCACAACACAGCTTGT | (TAA)<sub>5</sub> | 213–281 | 59*, 60 | HEX | MK285630 |
|       | R: GATGAGTGGCACTGACATGACG |             |                       |                   |                |                      |
| SP32  | F: TGTTGGAAAGGCGATGATGGA | (CATATA)<sub>10</sub> | 202–242 | 59 | FAM | MK285631 |
|       | R: TCCATGGATGTTTGTCATGGT |             |                       |                   |                |                      |

Note: T<sub>a</sub> = annealing temperature.
<sup>a</sup>Fixed heterozygous locus.
<sup>b</sup>Monomorphic locus.
<sup>*</sup>Although most of the samples were amplified based on a higher T<sub>a</sub> setting, some samples failed to amplify and thus lowered annealing temperatures were used for PCR.
Significant deviation from Hardy–Weinberg equilibrium (Table 2). The SNP34 locus showed polymorphisms across all eight individuals. These 19 primer pairs were subsequently scored using 48 individuals of *S. polylepis* sampled from the same three island populations and five individuals from each of the other species, namely *S. gracilis*, *S. grandifolia*, *S. maximowiczii*, *S. pulchella*, and *S. tanakae* (Appendix 1), for congeneric cross-transferability of the markers. The PCR thermocycling conditions and genotyping method were the same as described above.

Population genetic diversity parameters, including number of alleles, expected heterozygosity, and observed heterozygosity, were calculated using GenAlEx v6.502 (Peakall and Smouse, 2006). Deviations from Hardy–Weinberg equilibrium were calculated for each locus with GENEPOP 4.2 (Rousset, 2008). Of the 21 SSRs developed in this study, 19 were polymorphic. The expected and observed heterozygosity values ranged from 0.000 to 0.842 and from 0.000 to 0.933, respectively (Table 2). Of the 19 polymorphic loci, five in the Hong-do population, five in the Heuksan-do population, and three in the Ui-do population deviated from Hardy–Weinberg equilibrium (Table 2).

### TABLE 3. Cross-amplification of 21 microsatellite loci developed for *Saussurea polylepis* in five related congeneric species.

| Locus | *S. grandifolia (N = 5)* | *S. maximowiczii (N = 5)* | *S. pulchella (N = 5)* | *S. tanakae (N = 5)* | *S. gracilis (N = 5)* |
|-------|--------------------------|---------------------------|-----------------------|----------------------|-----------------------|
|       | Allele size range (bp)   | Allele size range (bp)    | Allele size range (bp)| Allele size range (bp)| Allele size range (bp) |
| SP01  | 7 231–245                | 3 281–285                 | 1 197                 | 2 237–239            | 3 237–241            |
| SP02  | 5 263–285                | 3 281–309                 | 4 283–293             | 3 271–275            | 4 261–279            |
| SP03  | 5 258–315                | 1 258                     | 1 258                 | 5 258–297            | 5 258–297            |
| SP04  | 5 201–252                | 0 —                      | 0 —                   | 1 225                | 4 222–237            |
| SP06  | 7 231–255                | 4 239–251                 | 5 235–251             | 6 235–249            | 6 217–251            |
| SP07  | 6 225–267                | 3 223–235                 | 0 —                   | 7 223–253            | 8 227–265            |
| SP10  | 3 222–231                | 3 222–231                 | 2 222–225             | 3 216–225            | 4 222–231            |
| SP11  | 2 191–200                | 0 —                      | 6 167–197             | 2 191–200            | 2 191–200            |
| SP12  | 5 265–283                | 3 175–271                 | 3 214–229             | 3 271–277            | 5 268–280            |
| SP13  | 4 202–274                | 1 226                     | 1 226                 | 3 218–242            | 4 194–242            |
| SP19  | 1 249                    | 2 246–249                 | 1 249                 | 1 249                | 2 249–255            |
| SP20  | 5 217–232                | 2 220–223                 | 2 211–223             | 4 220–232            | 4 217–229            |
| SP21  | 3 256–280                | 2 304–307                 | 1 292                 | 4 262–271            | 2 259–265            |
| SP22  | 5 265–285                | 1 237                     | 2 245–269             | 2 273–281            | 2 265–273            |
| SP23  | 7 264–352                | 4 272–352                 | 4 272–329             | 4 272–372            | 7 264–372            |
| SP25  | 3 233–253                | 2 249–253                 | 2 249–253             | 3 253–261            | 6 253–277            |
| SP26  | 3 233–237                | 2 235–237                 | 2 235–237             | 4 233–239            | 2 235–237            |
| SP29  | 4 264–279                | 2 270–276                 | 0 —                   | 1 270                | 4 267–276            |
| SP31  | 5 221–241                | 2 229–237                 | 3 217–265             | 5 225–249            | 5 221–245            |
| SP34  | 2 221–281                | 1 221                     | 1 221                 | 2 221–281            | 1 221                |
| SP35  | 4 202–218                | 0 —                      | 2 130–174             | 2 202–210            | 6 202–240            |

Note: — unsuccessful amplification; A = number of alleles; N = number of individuals sampled.

*Locality and voucher information provided in Appendix 1.

For *S. polylepis*, applications in plant sciences 2019 7(6): e11270 Yun and Kim—amplification of 21 microsatellite loci developed for *Saussurea polylepis*.
Cross-amplification of the 21 primer pairs was conducted in five widely distributed congeneric species of *Saussurea* on the Korean peninsula. Three species (*S. gracilis*, *S. grandiflora*, and *S. tanakae*) were all successfully amplified and polymorphic for the 21 loci, while two species (*S. maximowiczii* and *S. pulchella*) were successfully amplified and polymorphic for 18 loci.

**CONCLUSIONS**

The results of the present study represent the second set of SSR markers developed for the genus *Saussurea*. The 21 SSR markers developed in this study will be useful for studying genetic diversity, gene flow, and population structure in *S. polylepis*. In addition, the resulting population genetic studies will help to develop appropriate conservation strategies and management plans of the endangered *S. polylepis* on continental islands in Korea. Lastly, the successful cross-amplification of these markers among commonly found *Saussurea* species in Korea will provide a novel population genetic tool in one of the most speciose groups in Korea.

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**AUTHOR CONTRIBUTIONS**

S.A.Y. and S.-C.K. designed the project. S.A.Y. collected samples in the field, conducted the experiments, analyzed the data, and wrote the draft of the manuscript. S.-C.K. supervised the study and revised the manuscript. All authors approved the final version of the manuscript.

**DATA ACCESSIBILITY**

All sequence information was deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (BioProject ID PRJNA503488), and genomic DNA was deposited in the NCBI BioSample database (accession number SAMN10362162).

**APPENDIX 1.** Locality and voucher information of three *Saussurea polylepis* populations and five congeneric species used in this study.

| Species                  | Collection locality                  | Geographic coordinates | N   | Voucher specimen  |
|--------------------------|-------------------------------------|------------------------|-----|------------------|
| *Saussurea polylepis* Nakai | Hongdo, Jeolla Province, Korea      | —                      | 13  | SKKU044834       |
|                          | Heuk-san-do, Jeolla Province, Korea | —                      | 20  | SKKU044835       |
|                          | Ui-do, Jeolla Province, Korea       | —                      | 15  | SKKU044836       |
| *Saussurea gracilis* Maxim. | Mt. Gaya, Gyeongsang Province, Korea | 35.825868°N, 128.117077°E | 1   | SKKU044840       |
|                          | Mt. Yongmoon, Gyeonggi Province, Korea | 37.560846°N, 127.543898°E | 1   | SKKU044841       |
|                          | Mt. Naejang, Jeolla Province, Korea | 35.485817°N, 126.81233°E | 1   | SKKU044843       |
|                          | Mt. Doksal, Jeolla Province, Korea  | 34.076889°N, 125.10550°E | 1   | SKKU044847       |
|                          | Jeju island, Korea                 | 33.358472°N, 126.50391°E | 1   | SKKU031085       |
| *Saussurea grandifolia* Maxim. | Mt. Odae, Gangwon Province, Korea | 37.80222°N, 128.54722°E | 1   | SKKU031089       |
|                          | Mt. An, Gangwon Province, Korea     | 38.141981°N, 128.32650°E | 1   | SKKU031079       |

(Continued)
| Species                      | Collection locality                           | Geographic coordinates<sup>a</sup> | N | Voucher specimen<sup>b</sup> |
|------------------------------|-----------------------------------------------|-----------------------------------|---|-------------------------------|
| Saussurea polylepis          | Mt. Daeam, Gangwon Province, Korea             | 38.182150°N, 128.093567°E         | 1 | SKKU031080                    |
| Saussurea polylepis          | Uleung island, Gyeongsang Province, Korea      | 37.513883°N, 130.883817°E         | 1 | SKKU044837                    |
| Saussurea polylepis          | Russia                                        | 43.032722°N, 134.150306°E         | 1 | SKKU044844                    |
| Saussurea maximowiczii Herder| Mt. Chilbo, Gyeonggi Province, Korea           | 37.258249°N, 126.933596°E         | 1 | SKKU044838                    |
| Saussurea maximowiczii Herder| Mt. Bisle, Gyeongsang Province, Korea          | 35.714933°N, 128.525200°E         | 1 | SKKU044846                    |
| Saussurea maximowiczii Herder| Mt. Baekun, Jeolla Province, Korea             | 35.067500°N, 127.646917°E         | 1 | SKKU031088                    |
| Saussurea maximowiczii Herder| Mt. Mudeung, Jeolla Province, Korea            | 35.137000°N, 127.015000°E         | 1 | SKKU031087                    |
| Saussurea maximowiczii Herder| Jeju island, Korea                             | 33.358033°N, 126.462873°E         | 1 | SKKU031078                    |
| Saussurea pulchella (Fisch.) Fisch. ex Colla | Mt. Hambaek, Gangwon Province, Korea | 37.180667°N, 128.893850°E         | 1 | SKKU044839                    |
| Saussurea pulchella (Fisch.) Fisch. ex Colla | Mt. Odae, Gangwon Province, Korea            | 37.792383°N, 128.563083°E         | 1 | SKKU044853                    |
| Saussurea pulchella (Fisch.) Fisch. ex Colla | Mt. Goyang, Gangwon Province, Korea           | 37.414733°N, 128.752617°E         | 1 | SKKU044852                    |
| Saussurea pulchella (Fisch.) Fisch. ex Colla | Bongwha, Gyeongsang Province, Korea           | 37.030693°N, 128.985611°E         | 1 | SKKU044854                    |
| Saussurea pulchella (Fisch.) Fisch. ex Colla | Russia                                        | 43.031028°N, 131.556639°E         | 1 | SKKU044845                    |
| Saussurea tanakae Franch. & Sav. ex Maxim. | Mt. Hwaak, Gyeonggi Province, Korea | 37.990133°N, 127.500517°E         | 1 | SKKU044842                    |
| Saussurea tanakae Franch. & Sav. ex Maxim. | Mt. Hambaek, Gangwon Province, Korea          | 37.148183°N, 128.908917°E         | 1 | SKKU044848                    |
| Saussurea tanakae Franch. & Sav. ex Maxim. | Mt. Seorak, Gangwon Province, Korea           | 38.136000°N, 128.346965°E         | 1 | SKKU044850                    |
| Saussurea tanakae Franch. & Sav. ex Maxim. | Mt. Deokhang, Gangwon Province, Korea         | 37.325683°N, 129.003617°E         | 1 | SKKU044851                    |
| Saussurea tanakae Franch. & Sav. ex Maxim. | Mt. An, Gangwon Province, Korea               | 38.141981°N, 128.326502°E         | 1 | SKKU044849                    |

<sup>Note: N = number of samples.  
<sup>aGeographic coordinates are not provided for *Saussurea polylepis* due to the vulnerable status of the species.  
<sup>bAll voucher specimens are deposited in the Ha Eun Herbarium (SKK; Sungkyunkwan University, Korea).</sup>