**DEVELOPMENT, CHARACTERIZATION, AND CROSS-AMPLIFICATION OF 16 MICROSATELLITE PRIMERS FOR ATRIPLEX TATARICA (AMARANTHACEAE)**

**EVA KONDRYSOVÁ**, **KAROL KRÁK**<sup>2,3</sup>, **AND BOHUMIL MANDÁK**<sup>2,3,4</sup>

<sup>2</sup>Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, CZ-165 21 Czech Republic; and
<sup>3</sup>Institute of Botany of the Czech Academy of Sciences, Zámecká 1, CZ-252 43 Průhonice, Czech Republic

**Methods and Results:** Sixteen novel microsatellite primers were developed for *A. tatarica* based on high-throughput sequencing of enriched libraries. All markers were polymorphic, with the number of alleles per locus ranging from three to 25 and observed and expected heterozygosity ranging from 0.08 to 0.74 and 0.10 to 0.87, respectively. In addition, some of these loci were successfully amplified and showed polymorphisms in four *Atriplex* and seven *Chenopodium* species.

**Conclusions:** The microsatellite markers published here will be useful in assessing genetic diversity, structure, and gene flow within and across populations of *A. tatarica*, as well as in other species of *Atriplex* and the related genus *Chenopodium*.

**Key words:** Amaranthaceae; *Atriplex*; *Chenopodium*; cross-amplification; microsatellites.

The genus *Atriplex* L. (*Amaranthaceae*) numbers about 270 species (McArthur and Sanderson, 1984) distributed mainly in the deserts and semideserts in southwestern North America, in southern Australia, in southern Central Asia, in southwestern South America (Osmond et al., 1980; McArthur and Sanderson, 1984), or in coastal and solonchak regions of the Northern Hemisphere (Osmond et al., 1980). Most previous population studies in *Atriplex* used allozymes (Mandák et al., 2005, 2006a, 2006b), and highly variable microsatellites have been employed only in the study of the Australian species *A. nummularia* Lindl. (Byrne et al., 2008). To date, no nuclear simple sequence repeat markers (SSRs) have been developed specifically for *A. tatarica* L. and successfully cross-amplified to closely related *Atriplex* and *Chenopodium* L. species to enable population-level assessment of various representatives of the genus. Given the number of species in both the genus and the whole family, we expect that these markers will have broad applicability for conservation and population-level analyses.

*Atriplex tatarica* is an annual diploid (2n = 2x = 18) with a mixed mating system (Mandák et al., 2005) and is native to a wide area of Eurasia (Kochánková and Mandák, 2008). Along with 13 other species, it belongs to the section *Sclerocalyx* (Asch.) Asch. & Graebn. The distribution center of this section is located in southern Central Asia (Kochánková and Mandák, 2008). In Europe the species has a continental distribution. The northwestern border of its current continuous European range runs through the Czech Republic (southern Moravia), southern Slovakia, eastern Poland, and central Belarus, and its expansion in these countries has recently been reported (Kochánková and Mandák, 2008). The species possesses remarkable heterocarpy, which is morphologically manifested in the shape and size of bracteoles and in the size and color of fruits. Heterocarpy enables colonizing species such as *A. tatarica* to survive both major disturbances and unfavorable conditions (by ensuring that at least some seeds persist) and to expand during periods of favorable conditions (by ensuring that some seeds effectively spread and germinate) (Doudová et al., 2017). In this paper, we report the development and characterization of 16 novel microsatellite loci for *A. tatarica*. Additionally, we cross-amplified these loci in four and seven species of the genera *Atriplex* and *Chenopodium*, respectively.

**METHODS AND RESULTS**

**Microsatellite development**—Total genomic DNA of *A. tatarica* was extracted from 20–25 mg of silica gel–dried leaf tissue from seven samples of different population origin (Appendix 1) using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). These samples were used by GenoScreen (Lille, France) to develop microsatellite loci following the protocol of Malausa et al. (2011) based on GS FLX Titanium pyrosequencing (454 Life Sciences, a Roche Company, Branford, Connecticut, USA) of microsatellite-enriched DNA libraries. Microsatellite enrichment was carried out using eight microsatellite probes [(AG)10, (AC)10, (AAC)8, (AGG)8, (ACG)8, (AAG)8, (ACAT)6, (ATCT)6]. The sequencing yielded 32,229 reads, and 1956 of these contained microsatellite

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2 Author for correspondence: mandak@fpz.czu.cz

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Multiplex 1

| Locus | Primer sequences (5′−3′) | Repeat motif | Allele size range (bp) | Fluorescent label | Concentration in multiplex reaction (µM) | GenBank accession no. |
|-------|-------------------------|--------------|-----------------------|-------------------|----------------------------------------|----------------------|
| AT22  | F: GTGCAACGAAGAGTGAATGTTT | (TGT)₇ | 114−134 | 6-FAM | 0.1 | MF598744 |
|       | R: TGAGAAGCTGAGAAGAGTTTG | | | | | |
| AT28  | F: CTCGACACTGTTAAGAACTCAA | (AG)₈ | 161−222 | 6-FAM | 0.6 | MF598747 |
|       | R: CGTACCTATAGGGTTCTCTCCC | | | | | |
| AT15  | F: GCTATTGCTGATTTCCCC | (TGT)₉ | 101−134 | VIC | 0.1 | MF598741 |
|       | R: CGAGTTGAGTCCGAATAGAA | | | | | |
| AT20  | F: GGTGGAATTAATGUGGAAAA | (AGA)₇ | 139−150 | NED | 1.0 | MF598743 |
|       | R: CTTGAGCTCGCTTTGGACTC | | | | | |
| AT17  | F: GGTGTTACCTACGCTTTTATTC | (TG)₉ | 100−118 | PET | 0.2 | MF598742 |
|       | R: ATTTGGAAATTCGAGGATCTC | | | | | |
| AT07  | F: GTGCTGGTGAACAACCTCTTC | (TGT)₁₁ | 153−188 | PET | 0.2 | MF598740 |
|       | R: AGTCCAGCCGATCAGTTTTTT | | | | | |
| AT38  | F: ATGGCCCAACCATATTTCTCA | (AC)₈ | 102−104 | 6-FAM | 0.1 | MF598750 |
|       | R: GATCTTGTAGTGTTAAGCAGGG | | | | | |
| AT27  | F: GCACTGAGGACAGAATCCC | (TGC)₆ | 218−233 | VIC | 0.1 | MF598746 |
|       | R: CCAATGCTTTGGATTTTCCTCA | | | | | |
| AT44  | F: TTTGATCTGTCTGCTGATAACC | (TG)₅ | 165−180 | VIC | 0.1 | MF598753 |
|       | R: TCCCTTTGATGACACCCCAA | | | | | |

Multiplex 2

| Locus | Primer sequences (5′−3′) | Repeat motif | Allele size range (bp) | Fluorescent label | Concentration in multiplex reaction (µM) | GenBank accession no. |
|-------|-------------------------|--------------|-----------------------|-------------------|----------------------------------------|----------------------|
| AT04  | F: TGTGAAAGTTGCTCTTTTGGACAC | (TCT)₁₄ | 94−200 | 6-FAM | 0.4 | MF598738 |
|       | R: CGAGTTAGAGGAGATAAAGAGGG | | | | | |
| AT05  | F: TTTCTTGGGCTTTCTTTGGA | (TGT)₁₁ | 86−108 | VIC | 0.1 | MF598739 |
|       | R: TGACCCCTTTTTCCTTTTAGGG | | | | | |
| AT25  | F: TAACGTGCTGGTTCTGGTGAAGG | (AGG)₈ | 266−283 | NED | 0.2 | MF598745 |
|       | R: TAGGTACCTCTGCTGGGTTG | | | | | |
| AT30  | F: GAGGCAGAGCTGAAAGTGGG | (GAA)₉ | 136−150 | PET | 0.1 | MF598748 |
|       | R: TTCGACCTCTCTCTGCTGCTG | | | | | |
| AT31  | F: GAACGCCAGAGGAGGTGGAG | (GT)₈ | 137−161 | NED | 0.2 | MF598749 |
|       | R: CTGCGACAGCAGACTAATTA | | | | | |
| AT41  | F: TTTCGACATAACTCGATTA | (TACA)₆ | 190−230 | PET | 0.2 | MF598751 |
|       | R: AATAAGGCAAAGATCCCCCG | | | | | |
| AT42  | F: GAGGCTCTGACTGAGAAGG | (CAT)₈ | 172−188 | VIC | 0.1 | MF598752 |
|       | R: CTTCCCGGCTCTTATCATAAA | | | | | |

*Annealing temperature was 55°C for both multiplexes.

Biological validation—Forty-seven candidate loci possessing perfect repeat motifs and different expected amplicon lengths within the 100−400-bp interval were selected and tested for amplification from all seven individuals. The PCR reactions were performed in 5-µL reaction volumes containing 1 µL of genomic DNA, 0.1 µM of both primers, and 1× QIAGEN Multiplex PCR Master Mix (QIAGEN). Reactions were performed with the following conditions: an initial denaturation step at 95°C for 15 min; followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min; and a final extension at 72°C for 10 min. The PCR products were checked on 2% agarose gels. Of the markers that amplified successfully from all seven individuals, 16 polymorphic markers with well-scorable peaks were selected and combined into two multiplexes (Table 1). The sequences of the motifs. Primers were designed based on reads of the positive strands using QDD software (Meglécz et al., 2010).

Microsatellite data analysis—Allele size was determined using GeneMarker 2.6.4 (SoftGenetics, State College, Pennsylvania, USA). FSTAT 2.9.3 (Goudet, 1995) was used to calculate summary statistics for SSR loci such as the average number of alleles per locus and Weir and Cockerham’s parameter f (Weir and Cockerham, 1984) as a measure of departure from within-population random mating. Observed and expected heterozygosities were calculated using GENEPOP (Rousset, 2008), and the deviation from Hardy–Weinberg equilibrium was determined based on 10,000 permutations in FSTAT 2.9.3 (Goudet, 1995). MICRO-CHECKER version 2.2.3 (van Oosterhout et al., 2004) was used to test for evidence of stuttering, allele dropout, and the presence of null alleles at each locus. The Brookfield 1 equation (Brookfield, 1996) was used to calculate null allele frequencies.

We identified 143 alleles at 16 microsatellite loci, with an average of 8.9 alleles per locus. The summary statistics for genetic variability across and within populations are presented in Table 3. The deficit of heterozygotes, computed over all populations and loci, was significant, as indicated by a relatively high inbreeding coefficient (f = 0.171). Eight out of 16 loci were not in Hardy–Weinberg equilibrium (Table 2), which might be due to high levels of self-pollination and the strong bottlenecks within populations. No signs of stuttering or large allele dropout were detected. The average null allele frequency for each locus calculated using the Brookfield method detected the presence of null alleles at five loci (Table 2).

Fifteen microsatellite loci were successfully cross-amplified from some of the species tested (Table 3). The cross-amplification was more successful with closely related species of the genus *Atriplex* than of *Chenopodium* (Table 3).
## Table 2. Genetic characterization of 16 newly developed polymorphic microsatellite loci across six populations of *Atriplex tatarica*.

| Locus       | Bulgaria (11A, n = 20) | Poland (25A, n = 20) | Germany (30A, n = 20) | Romania (44A, n = 20) | Hungary (62A, n = 20) | Czech Republic (70A, n = 20) | Overall (n = 120) |
|-------------|------------------------|----------------------|-----------------------|------------------------|------------------------|-------------------------------|------------------|
|             | B A H                  | f                    | A H A H f            | A H A H f              | A H A H f              | A H A H f                     | A H A H f         |
| Multiplex 1 |                        |                      |                      |                        |                        |                               |                  |
| AT38        | 0.124 2 0.10 0.26 0.62* | 1 ND ND ND         | 1 ND ND ND         | 2 0.25 0.22 -0.12     | 1 ND ND ND             | 2 0.10 0.10 -0.03            | 3 0.08 0.10 0.28* |
| AT22        | 0.124 4 0.40 0.61 0.36* | 5 0.75 0.70 -0.07    | 4 0.37 0.58 0.38*    | 6 0.55 0.61 0.10        | 2 0.25 0.30 0.16        | 4 0.70 0.64 -0.10            | 7 0.50 0.61 0.12* |
| AT28        | 0.223† 7 0.41 0.84 0.52* | 6 0.65 0.77 0.16    | 5 0.68 0.75 0.10      | 5 0.50 0.60 0.17        | 4 0.25 0.59 0.59*        | 6 0.55 0.67 0.18            | 12 0.51 0.80 0.28* |
| AT15        | -0.030 7 0.65 0.62 -0.05 | 8 0.75 0.78 0.04    | 8 0.63 0.77 0.18      | 9 0.85 0.82 -0.04       | 4 0.65 0.57 -0.14        | 3 0.40 0.47 0.14            | 11 0.66 0.79 0.02 |
| AT44        | -0.005 2 0.11 0.10 -0.03 | 3 0.15 0.14 -0.04    | 2 0.05 0.05 0.00      | 1 ND ND ND             | 2 0.00 0.12 1.00*         | 2 0.16 0.31 0.50            | 5 0.08 0.13 0.37* |
| AT27        | -0.008 3 0.15 0.14 -0.04 | 4 0.55 0.47 -0.18    | 3 0.37 0.32 -0.17     | 4 0.35 0.38 0.07        | 5 0.60 0.75 0.52*         | 5 0.60 0.74 0.20            | 8 0.53 0.83 0.30* |
| AT20        | 0.227† 5 0.35 0.77 0.55* | 6 0.53 0.73 0.28*    | 7 0.53 0.85 0.39*     | 4 0.85 0.75 -0.14       | 4 0.30 0.62 0.52*         | 5 0.60 0.74 0.20            | 8 0.53 0.83 0.30* |
| AT17        | 0.000 4 0.90 0.71 -0.27 | 4 0.32 0.63 0.51*    | 5 0.63 0.63 0.01      | 4 0.20 0.27 0.27        | 5 0.60 0.57 -0.06         | 4 0.70 0.68 -0.03            | 5 0.56 0.67 0.04 |
| AT07        | 0.197† 4  0.40 0.76 0.48* | 5 0.06 0.69 0.92*    | 5 0.21 0.71 0.71*     | 6 0.50 0.74 0.33*       | 3 0.16 0.23 0.33          | 4 0.25 0.65 0.62*            | 12 0.26 0.71 0.58* |
| Multiplex 2 |                        |                      |                      |                        |                        |                               |                  |
| AT04        | 0.010 5 0.50 0.58 0.05 | 8 0.50 0.82 0.40*    | 12 0.65 0.90 0.29*    | 6 0.20 0.74 0.74*       | 2 0.35 0.46 0.24         | 13 0.50 0.88 0.44*           | 25 0.46 0.87 0.38* |
| AT05        | -0.074 5 0.65 0.55 -0.19 | 5 0.70 0.60 -0.17    | 5 0.40 0.43 0.07      | 7 0.50 0.50 0.00        | 4 0.55 0.45 -0.23        | 6 0.60 0.66 0.09            | 8 0.57 0.68 -0.07 |
| AT42        | -0.096 3 0.60 0.47 -0.28 | 6 0.60 0.71 0.16     | 6 0.89 0.77 -0.16     | 6 0.85 0.73 -0.18       | 2 0.50 0.57 0.12         | 5 0.60 0.57 -0.06           | 8 0.67 0.71 -0.06 |
| AT31        | 0.018 8 0.80 0.86 0.07 | 6 0.42 0.63 0.34     | 9 0.85 0.86 0.01      | 6 0.85 0.81 -0.05       | 3 0.70 0.62 -0.14        | 6 0.80 0.78 -0.03           | 9 0.74 0.84 0.03 |
| AT25        | -0.015 3 0.45 0.44 -0.02 | 5 0.78 0.68 -0.14    | 5 0.68 0.71 0.04      | 6 0.60 0.79 0.25*       | 3 0.05 0.05 0.00         | 5 0.70 0.62 -0.14           | 7 0.54 0.67 0.02 |
| AT30        | -0.014 4 0.50 0.49 -0.02 | 5 0.50 0.68 0.27     | 3 0.63 0.52 -0.22     | 3 0.30 0.27 -0.10       | 2 ND ND ND               | 5 0.40 0.49 0.19           | 6 0.39 0.46 0.05 |
| AT41        | 0.246† 6  0.29 0.74 0.61* | 4 0.40 0.60 0.34    | 2 0.11 0.11 -0.03     | 5 0.06 0.45 0.87*       | 2 0.39 0.47 0.19         | 5 0.35 0.66 0.47*           | 11 0.27 0.61 0.48* |
| Mean        | 4.50 0.46 0.56 0.19* | 5.06 0.51 0.64 0.21* | 5.13 0.51 0.60 0.15* | 5.00 0.49 0.58 0.15*    | 2.81 0.37 0.43 0.16*     | 4.75 0.49 0.59 0.17*        | 8.94 0.44 0.61 0.17* |
| s.d.        | 1.79 0.23 0.23 0.25 | 1.73 0.21 0.16 0.20 | 2.85 0.25 0.27 0.16 | 2.85 0.27 0.21 0.17 | 1.28 0.23 0.19 0.34 | 2.67 0.21 0.19 0.18 | 5.03 0.20 0.25 0.21 |

Note: A = number of alleles; B = null allele frequency averaged over all populations using the Brookfield 1 equation (Brookfield, 1996); f = inbreeding coefficient; $H_e$ = expected heterozygosity; $H_o$ = observed heterozygosity; $n$ = number of individuals sampled; ND = not determined.

* Significant deviation from Hardy–Weinberg equilibrium ($P < 0.05$).

† Loci with null alleles.
Sixteen polymorphic microsatellite loci were developed for *A. tatarica*. These markers will be valuable for investigating the population genetic structure, mating system, and phylogeographic pattern of this species. The cross-species amplification of these markers indicates that they may be widely useful in related Amaranthaceae species. We conclude that the SSRs described here will facilitate ecological and evolutionary studies of *A. tatarica* and related species.

**CONCLUSIONS**

**LITERATURE CITED**

**Brookfield, J.** 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology* 5: 453–455.

**Byrne, M., M. Hankinson, J. F. Sampson, and S. Stankovski.** 2008. Microsatellite markers isolated from a polyploid saltbush, *Atriplex nummularia* Lindl. (Chenopodiaceae). *Molecular Ecology Resources* 8: 1426–1428.

**Doudova, J., J. Douda, and B. Mandak.** 2017. The complexity underlying invasiveness precludes the identification of invasive traits: A comparative study of invasive and non-invasive holarctic *Atriplex* congeners. *Plos ONE* 12: e0176455.

**Goudet, J.** 1995. *FSTAT* (Version 1.2): A computer program to calculate F-statistics. *Journal of Heredity* 86: 485–486.

**Kochanova, J., and B. Mandak.** 2008. Biological flora of Central Europe: *Atriplex tatarica*. *Perspectives in Plant Ecology, Evolution and Systematics* 10: 217–229.

**Malaua, T., A. Gilles, E. Miglécž, H. Blanquart, S. Duthoy, C. Costedoat, V. Dubet, et al.** 2011. High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. *Molecular Ecology Resources* 11: 638–644.

**Mandak, B., K. Bimova, I. Plackova, V. Mahelka, and J. Chirtek.** 2005. Loss of genetic variation in geographically marginal populations of *Atriplex tatarica* (Chenopodiaceae). *Annals of Botany* 96: 901–912.

**Mandak, B., K. Bimova, and I. Plackova.** 2006a. Genetic structure of experimental populations and reproductive fitness in a heterocarpic plant *Atriplex tatarica* (Chenopodiaceae). *American Journal of Botany* 93: 1640–1649.

**Mandak, B., K. Bimova, V. Mahelka, and I. Plackova.** 2006b. How much genetic variation is stored in the seed bank? A study of *Atriplex tatarica* (Chenopodiaceae). *Molecular Ecology* 15: 2653–2663.

**McArthur, E. D., and S. C. Sanderson.** 1984. Distribution, systematics, and evolution of Chenopodiaceae: An overview. In *A. R. Tiedmann, A. D. McArthur, H. C. Stutz, R. Stevens, and K. L. Johnson* [eds.], Proceedings—symposium on the biology of *Atriplex* and related chenopods, 14–24. Intermountain Forest and Range Experiment Station, U.S. Department of Agriculture, Forest Service, Provo, Utah, USA.

**Miglécz, E., C. Costedoat, V. Dubet, A. Gilles, T. Malaua, N. Pech, and J. F. Martin.** 2010. QDD: A user-friendly program to select microsatellite markers and design primers from large sequencing projects. *Bioinformatics* (Oxford, England) 26: 403–404.

**Osmond, C. B., O. Bjorkman, and D. J. Anderson.** 1980. Physiological processes in plant ecology: Towards a synthesis with *Atriplex*. Springer, Heidelberg, Germany.

**Rousset, F.** 2008. GENEPOP007: A complete reimplementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.

**Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley.** 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535–538.

**Weir, B. S., and C. C. Cockermith.** 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358–1370.
### Appendix 1. Geographic information on *Atriplex* and *Chenopodium* populations in this study.

| Species Voucher specimen accession no. | N | Collection locality | Latitude (°N) | Longitude (°E) |
|----------------------------------------|---|---------------------|---------------|---------------|
| **Atriplex tatarica** L.            |   |                     |               |               |
| B. Mandak 1A                      | 1 | Győr, Hungary       | 47.70633      | 17.48802      |
| B. Mandak 3A                      | 1 | Nagyhegyes, Hungary | 47.51795      | 21.35765      |
| B. Mandak 5A                      | 1 | Sizileu, Romania    | 46.44174      | 25.76171      |
| B. Mandak 7A                      | 1 | Constanta, Romania | 44.28178      | 28.55689      |
| B. Mandak 9A                      | 1 | Dragodana, Romania  | 44.72501      | 25.34959      |
| B. Mandak 11A                     | 20| Sofia, Bulgaria     | 42.74245      | 23.25354      |
| B. Mandak 12A                     | 1 | Popovitsa, Bulgaria | 42.13607      | 25.05463      |
| B. Mandak 25A                     | 20| Wielgosz Brzeżiński, Poland | 52.20053 | 21.41598 |
| B. Mandak 30A                     | 20| Berlin, Germany     | 52.30090      | 13.19406      |
| B. Mandak 44A                     | 20| Tečcani, Romania    | 47.03009      | 26.86970      |
| B. Mandak 62A                     | 20| Hegyháthodász, Hungary | 46.93466 | 16.68857 |
| B. Mandak 70A                     | 20| Velké Němčice, Czech Republic | 49.01046 | 16.68755 |

**Species used for cross-amplification**

| Species Voucher specimen accession no. | N | Collection locality | Latitude (°N) | Longitude (°E) |
|----------------------------------------|---|---------------------|---------------|---------------|
| *A. oblongifolia* Waldst. & Kit.      |   |                     |               |               |
| B. Mandak 930                         | 5 | Cluj-Napoca, Apahida, Romania | 46.81814 | 23.74997 |
| B. Mandak 932                         | 5 | Vlădeni, Romania     | 47.41469      | 27.37454      |
| B. Mandak 938                         | 5 | Horoměchle, Czech Republic | 50.13175 | 14.35309 |
| B. Mandak 937                         | 5 | Ladná, Czech Republic | 48.80956      | 16.89302      |
| B. Mandak 933                         | 5 | Vlădeni, Romania     | 47.40971      | 27.37309      |
| B. Mandak 928                         | 5 | Cluj-Napoca, Barai, Romania | 46.85796 | 23.90922 |
| B. Mandak 931                         | 5 | Vlădeni, Romania     | 47.41469      | 27.37454      |

**Chenopodium bonus-henricus** L.

| Species Voucher specimen accession no. | N | Collection locality | Latitude (°N) | Longitude (°E) |
|----------------------------------------|---|---------------------|---------------|---------------|
| B. Mandak 379                         | 3 | Kladrubce, Czech Republic | 49.48618 | 13.68160 |
| B. Mandak 383                         | 3 | Plužná, Czech Republic | 50.47151      | 14.79743      |
| B. Mandak 394                         | 1 | Praha, Czech Republic | 50.11596      | 14.43306      |
| **C. hybridum** L.                    |   |                     |               |               |
| B. Mandak 287                         | 3 | Velké Němčice, Czech Republic | 48.99786 | 16.67289 |
| B. Mandak 295                         | 3 | Kunszentmiklós, Hungary | 46.98325      | 19.25014      |
| B. Mandak 311                         | 2 | Víť, Slovakia       | 47.74325      | 18.32589      |
| B. Mandak 394                         | 2 | Čehnice, Czech Republic | 49.22747 | 14.03011 |
| **C. pumilio** R. Br.                |   |                     |               |               |
| B. Mandak 327                         | 3 | Praha, Czech Republic | 50.08723      | 14.40689      |
| B. Mandak 362                         | 3 | Bachkovo, Bulgaria  | 41.94260      | 24.85230      |
| **C. rubrum** L.                      |   |                     |               |               |
| B. Mandak 281                         | 2 | Rejsice, Czech Republic | 50.31997 | 14.97881 |
| B. Mandak 312                         | 2 | Víť, Slovakia       | 47.74325      | 18.32589      |
| **C. suecicum** Murr                 |   |                     |               |               |
| B. Mandak 264                         | 3 | Rumiškiškes, Lithuania | 54.87940 | 24.19795 |
| B. Mandak 328                         | 3 | Švermlov, Czech Republic | 50.17681 | 14.10547 |
| **C. urbicicum** L.                   |   |                     |               |               |
| B. Mandak 335                         | 4 | Hortobágy, Hungary  | 47.58125      | 21.15842      |

**Note:** N = number of individuals used.

* One voucher was collected from each sampled population. Herbarium vouchers are deposited in the author’s collection at the Czech University of Life Sciences in Prague. Collector name and unique population number in our database are indicated.

* Individual used to create a DNA genomic library.

* Populations used for evaluating the quality of developed microsatellite loci.