Giant ragweed (Ambrosia trifida L.) belongs to the Asteraceae family (tribe Heliantheae, subtribe Ambrosiinae) together with 40–50 other ragweed (Ambrosia L.) species (Payne, 1964). The genus Ambrosia is native to North America with a center of diversity located in the Sonoran Desert (Martin et al., 2018). Giant ragweed is known as an invasive plant on the European continent together with common ragweed (Ambrosia artemisiifolia L.). Both species are noxious weeds and interfere with the growth development and establishment of various crops (Kong et al., 2007). The primary habitat of A. trifida is flood plains and ditch banks, but in the recent past it has spread to the Corn Belt in the United States, causing great economic losses. In addition to its competition with crops, Ambrosia pollen is responsible for allergic reactions in late summer and autumn (Ghosh et al., 1991) and constitutes a major health problem for ragweed (Ambrosia artemisiifolia L.). Both species are noxiously invasive ragweeds, and we have successfully validated these markers in other giant ragweed populations and related species of Ambrosia.

Ambrosia research is mostly concentrated on common ragweed, and therefore the population structure of other ragweed species is mostly unknown. For A. artemisiifolia, microsatellite markers were developed to determine the origins of invading populations in Europe (Gaudeul et al., 2011), and the history of the invasion was also investigated using herbarium specimens (Martin et al., 2014). Genomic resources are also under development to facilitate further research in ragweed genetics, e.g., the plastid genome of A. trifida (Sablok et al., 2019) and A. artemisiifolia (Amiryousefi et al., 2017; Nagy et al., 2017) have been sequenced. To the best of our knowledge, no reports have been published on chloroplast microsatellite markers for A. trifida, and the cross-species transferability of microsatellite markers has not been investigated.

In the current study, we identified 15 novel chloroplast microsatellite markers, which will enrich the existing genomic resources for ragweeds, and we have successfully validated these markers in relevant giant ragweed populations and related species of Ambrosia.

METHODS AND RESULTS

We searched the complete chloroplast genome of A. trifida (Sablok et al., 2019; GenBank accession number NC036810) for microsatellite...
loci. Simple sequence repeats (SSRs) were identified using MISA software (Thiel et al., 2003). Mononucleotide repeats were excluded from our search, and we applied a threshold based on minimum length criteria (unit size/minimum repeat time): six for di-, four for tri-, and three for tetra-, penta-, and hexanucleotide repeats, respectively. MISA allowed the identification and localization of perfect as well as compound microsatellites. Compound SSRs were considered repeats if: (1) primer length ranging from 18 to 23 bp; (2) PCR product size range of 100–300 bp; (3) melting temperature between 50°C and 70°C, with 55°C as the optimum annealing temperature; and (4) a GC content of 40–70%, with an optimum of 50% (Table 1).

After primer design, amplification efficiency and polymorphism were evaluated using 29 A. trifida DNA samples from two different historical populations collected in Europe and North America. Cross-amplification in the genus Ambrosia was assessed in five individuals of each of A. artemisifolia, A. maritima, A. psilostachya DC., and A. tenuifolia Spreng. DNA samples were taken from the herbarium collection of the Finnish Museum of Natural History (Appendix 1). Leaf samples were rinsed with deionized water and 70% ethanol, and total genomic DNA was isolated using the E.Z.N.A. Plant Kit (Omega Bio-tek, Norcross, Georgia, USA). Laboratory work was carried out in a separate DNA laboratory at the University of Helsinki. Blank samples were processed together with herbarium material during DNA extractions. Final concentrations were measured with a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). PCR amplification was carried out in a 20-µL volume containing 20 ng of genomic DNA, 0.2 mM dNTPs (Thermo Fisher Scientific), 2 µL 1× PCR buffer, 20 pM of each primer, and 0.25 units DyNAzyme DNA polymerase (Thermo Fisher Scientific). All reactions were performed in a MasterCycler ep96 (Eppendorf, Hamburg, Germany) with the following settings: 2 min of initial denaturation at 94°C; 35 cycles of denaturation for 30 s at 94°C, annealing for 1 min at 55°C, and extension for 2 min at 72°C; followed by a final extension for 5 min at 72°C. Amplification products were separated on 1.5% agarose gels (GE Healthcare, Chicago, Illinois, USA) using a GeneScan 500 LIZ Size Standard (Applied Biosystems). The scoring of electropherograms was carried out with Geneious Prime (Biomatters Ltd., Auckland, New Zealand). All primers amplified successfully across the test individuals and 10 proved to be polymorphic, whereas five gave monomorphic results.

**TABLE 1.** The characteristics of 10 polymorphic and five monomorphic chloroplast microsatellite loci developed for *Ambrosia trifida.*

| Locus* | Primer sequences (5′–3′) | Position | Repeat motif | Allele size range (bp) | Fluorescent label | GenBank accession no. |
|--------|--------------------------|----------|--------------|------------------------|------------------|----------------------|
| AART_MS2 | F: GCAGTCTATTAGTGTCCCTTT | psbC gene | (TTT),3 | 142–148 | HEX | MN385584 |
| AART_MS3 | F: GTTCAAAATACGATCAATTC | rbcL gene | (GGATA),3 | 136–148 | 6-FAM | MN385586 |
| ATRI_M9 | F: GGAGCTTTCTGGCTTCATCTT | ycf4-cema IGS | (ATT),3 | 138–144 | TAMRA | MN385585 |
| AART_MS5 | F: CTTGTCACATATTCCTAGCC | petA-psbJ IGS | (TTT),3 | 146–154 | HEX | MN385587 |
| AART_MS9 | F: CAGAATCCAGTTGCTTAAGG | ndhA intron | (TAT),3 | 140–160 | 6-FAM | MN385588 |
| ATRI_M6 | F: TACGTGTAGCAGGATATCG | ycf1 gene | (AGA),3 | 156–162 | TAMRA | MN385593 |
| ATRI_MS8 | F: GTATGGGCAATTTGAAATGATA | ndhD gene | (TAT),3 | 145–161 | HEX | MN385592 |
| AART_MS11 | F: CCAAAATTTGAGCAGAAAAG | trnL-intron | (TTT),3 | 159–167 | 6-FAM | MN385591 |
| AART_MP6 | F: TCTTACAGGAGAAAGGAAA | rpL3-rps18 IGS | (TTT),3 | 137–153 | TAMRA | MN385589 |
| AART_MP1 | F: GCAGTTGTAGATCTACAGCTA | trnK-intron | (ATT),3 | 157–163 | HEX | MN385590 |
| ATRI_M21 | F: GGTAATCACTATATTAAGAAGGAG | trnK-intron | (ATT),3 | 200 | — | — |
| ATRI_M22 | F: AGTGGACCTGCACCTTAAAG | trnT-psbD IGS | (TTT),3 | 130 | — | — |
| ATRI_M23 | F: GATCTCCTGGTGTAGTGTC | trnS-psbZ IGS | (ATC),3 | 103 | — | — |
| ATRI_M24 | F: TGACCGAATAGGAACTTGG | ycfB intron II | (TTT),3 | 163 | — | — |
| ATRI_M25 | F: TGGCCAAATTGGTAGCACCT | trnL intron | (AAC),3 | 198 | — | — |

Note: IGS = intergenic spacer.
*The annealing temperature for all primers is 55°C except for AART_MP6 for which it is 52°C.
patterns (Table 1). The polymorphic markers were used to evaluate the genetic diversity of 49 *Ambrosia* samples.

The number of alleles, effective number of alleles, and Shannon’s information index were calculated using iMEC (Amiryousefi et al., 2018). The number of alleles per locus ranged from two to six, the effective number of alleles ranged from 1.198 to 2.324, Shannon’s information index ranged from 0.305 and 1.467, and the expected heterozygosity ranged from 0.178 to 0.645, while the polymorphism information content ranged from 0.211 to 0.675 (Table 2). The 10 polymorphic loci were also successfully amplified in 20 individuals (five individuals each) in the following four related species: *A. artemisiifolia*, *A. maritima*, *A. psilostachya*, and *A. tenuifolia* (Table 3, Appendix 1).

### CONCLUSIONS

We used the recently sequenced plastid genome of *A. trifida* (Sablok et al., 2019) to develop and characterize 15 chloroplast microsatellite markers; these were then used to identify high genetic diversity among the analyzed giant ragweed samples. Overall population genetic variation was similar to that detected in common ragweed (*A. artemisiifolia*) in North America and in the Rhône-Alpes region (Genton et al., 2005). Given the high level of polymorphism detected with the developed cpSSR primer set in the *A. trifida* population, the markers developed here should be suitable for further studies investigating the origin of invasive populations in Europe and studying the dynamics of invasion and modes of dispersal. Due to the high rate of cross-amplification, the developed polymorphic cpSSR primers will likely be useful in intra- or interspecific genetic studies of the genus *Ambrosia*.

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### DATA AVAILABILITY

All sequence information was deposited in the National Center for Biotechnology Information (NCBI) GenBank database (accession numbers MN385584–MN385593 and NC_036810.2; Table 1).

### LITERATURE CITED

Amiryousefi, A., J. Hyvönen, and P. Poczaí. 2017. The plastid genome sequence of the invasive plant common ragweed (*Ambrosia artemisiifolia*, Asteraceae). *Mitochondrial DNA Part B* 2(2): 753–754.

Amiryousefi, A., J. Hyvönen, and P. Poczaí. 2018. iMEC. Online marker efficiency calculator. *Applications in Plant Sciences* 6(6): e01159.

Gaudeul, M., T. Giraud, L. Kiss, and J. A. Shykoff. 2011. Nuclear and chloroplast microsatellites show multiple introductions in the worldwide invasion history of common ragweed, *Ambrosia artemisiifolia*. *PLoS ONE* 6(3): e17658.

Genton, B. J., J. A. Shykoff, and T. Giraud. 2005. High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple sources of introduction. *Molecular Ecology* 14(14): 4275–4285.

Ghosh, B., M. P. Perry, and D. G. Marsh. 1991. Cloning the cDNA encoding the AmbtV allergen from giant ragweed (*Ambrosia trifida*) pollen. *Gene* 101(2): 231–238.

Kong, C. H., P. Wang, and X. H. Xu. 2007. Allelopathic interference of *Ambrosia trifida* with wheat (*Triticum aestivum*). *Agriculture, Ecosystems & Environment* 119(3–4): 416–420.

Martin, M. D., E. A. Zimmer, M. T. Olsen, A. D. Foote, M. T. P. Gilbert, and G. S. Brush. 2014. Herbarium specimens reveal a historical shift in phylogeographic structure of common ragweed during native range disturbance. *Molecular Ecology* 23(7): 1701–1716.

Martin, M. D., E. Quiroz-Ciaros, G. S. Brush, and E. A. Zimmer. 2018. Herbarium collection-based phylogenetics of the ragweeds (*Ambrosia*, Asteraceae). *Molecular Phylogenetics and Evolution* 120: 335–341.

Montagnani, C., R. Gentili, M. Smith, M. F. Guarino, and S. Citterio. 2017. The worldwide spread, success, and impact of ragweed (*Ambrosia spp.*). *Critical Reviews in Plant Sciences* 36(3): 139–178.

Nagy, E., G. Hegedűs, J. Táller, B. Kutasy, and E. Virág. 2017. Illumina sequencing of the chloroplast genome of common ragweed (*Ambrosia artemisiifolia* L.). *Data in Brief* 15: 606–611.

Payne, W. W. 1964. A re-evaluation of the genus *Ambrosia* (Compositae). *Journal of the Arnold Arboretum* 45(4): 401–438.

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### TABLE 2. Characteristics of 10 polymorphic chloroplast microsatellite markers in 29 individuals of giant ragweed (*Ambrosia trifida*).

| Locus       | *Ambrosia trifida* (N = 29) |
|-------------|-----------------------------|
|             | A | \(A_e\) | I | \(H_e\) | PIC |
| AART_MS2    | 3 | 1.624 | 0.675 | 0.413 | 0.478 |
| AART_MS3    | 3 | 2.324 | 1.467 | 0.645 | 0.675 |
| ATRI_M5     | 3 | 2.112 | 0.808 | 0.573 | 0.597 |
| AART_MS5    | 2 | 1.271 | 0.369 | 0.203 | 0.267 |
| AART_MS9    | 6 | 1.654 | 0.911 | 0.426 | 0.501 |
| ATRI_M56    | 3 | 1.233 | 0.334 | 0.178 | 0.211 |
| ATRI_M58    | 4 | 1.198 | 0.305 | 0.446 | 0.523 |
| AART_MS11   | 2 | 1.424 | 0.414 | 0.288 | 0.312 |
| AART_MP6    | 4 | 1.806 | 0.897 | 0.446 | 0.489 |
| AART_MP1    | 2 | 1.251 | 0.329 | 0.218 | 0.225 |
| Mean        | 3.200 | 1.424 | 0.651 | 0.384 | 0.428 |

Note: \(A\) = number of alleles; \(A_e\) = effective number of alleles; \(H_e\) = expected heterozygosity; \(I\) = Shannon’s information index; N = number of individuals sampled; PIC = polymorphism information content.

*Note: Distinctity and voucher information are provided in Appendix 1.*

### TABLE 3. Results of cross-amplification of 10 polymorphic chloroplast microsatellite markers developed for *Ambrosia trifida* in four related ragweed species.

| Locus       | *A. artemisiifolia* (N = 5) | *A. psilostachya* (N = 5) | *A. tenuifolia* (N = 5) | *A. maritima* (N = 5) |
|-------------|-----------------------------|---------------------------|-------------------------|-----------------------|
| AART_MS2    | 142–148                     | 145–148                   | 148–154                 | 142–148               |
| AART_MS3    | 142–148                     | 148–154                   | 142–154                 | 142–148               |
| ATRI_M5     | 141–144                     | 144–147                   | 147–153                 | 141–144               |
| AART_MS5    | 146–154                     | 150–158                   | 154–162                 | 150–154               |
| AART_MS9    | 144–160                     | 152–160                   | 156–164                 | 148–160               |
| ATRI_M56    | 159–162                     | 156–162                   | 159–168                 | 159–162               |
| ATRI_M58    | 153–165                     | 153–157                   | 153–161                 | 149–161               |
| AART_MS11   | 159–171                     | 163–175                   | 163–175                 | 159–171               |
| AART_MP6    | 141–149                     | 153–157                   | 149–157                 | 141–153               |
| AART_MP1    | 161–163                     | 163–171                   | 159–167                 | 157–163               |

Note: \(N\) = number of individuals sampled.

*Numbers shown represent the size in base pairs (bp) of the amplified fragments.*

*Locality and voucher information are provided in Appendix 1.*
APPENDIX 1. Sampling information for ragweed specimens used in this study.

| Species               | Voucher no. | Collection year | Collection locality            |
|-----------------------|-------------|-----------------|--------------------------------|
| *Ambrosia trifida* L. | H1651188    | 1936            | Viipuri, Maaskola              |
|                       | H1651189    | 1936            | Vipuri, Maasola                |
|                       | H1645552    | 1938            | Helsinki, botanical garden     |
|                       | H1645551    | ca. 1930–1935   | Helsinki, botanical garden     |
|                       | H1273891    | 1977            | Helsinki, botanical garden     |
|                       | H1725761    | 2001            | Missouri, Washington State Park|
|                       | H1591544    | 1957            | Arlington, Virginia, Anderson Hospital|
|                       | H1566112    | 1984            | Cameron, Louisiana             |
|                       | H1076406    | 1966            | Middleton, Dane County, Wisconsin|
|                       | H1076407    | 1966            | Middleton, Dane County, Wisconsin|
|                       | H1018948    | 1970            | Towson, Baltimore County, Maryland|
|                       | H1139339    | 1975            | Florence, Hampshire County, Massachusetts|
|                       | H1137652    | 1959            | The Pas, Manitoba, Canada      |
|                       | H1141347    | 1972            | Lexington, Davidson County, North Carolina|
|                       | H1150975    | 1976            | Saint-Fulgence, Québec         |
|                       | H1208063    | 1976            | Saint-Fulgence, Québec         |
|                       | H1555799    | 1980            | Saint Albans, Franklin County, Vermont|
|                       | H1645543    | 1939            | Arnaud, Manitoba               |
|                       | H1645544    | 1968            | Saint-Gédéon, Québec           |
|                       | H1645548    | 1935            | Rigaud, Québec                 |
|                       | H1645546    | 1961            | Sioux Lookout, Ontario         |
|                       | H1645545    | 1867            | Middleton, Dane County, Wisconsin|
|                       | H1645547    | 1958            | Saint-Fulgence, Québec         |
|                       | H1645550    | 1892            | Wisconsin                      |
|                       | H1282191    | 1979            | City of Thunder Bay, Ontario    |
|                       | H1017127    | 1967            | Mount Horeb, Dane County, Wisconsin|
|                       | H1589855    | 1988            | Luhansk (then Voroshilovgrad), Ukraine|
|                       | H1581440    | 1972            | Litomerice, Czech Republic     |
|                       | H1645540    | 1902            | Schleswig-Holstein, Germany    |
| *Ambrosia artemisiifolia* L. | H1070900 | 1968            | Gyor, Hungary                   |
|                       | H1216521    | 1976            | Graz, Austria                  |
|                       | H1224407    | 1974            | Solosnica, Slovakia            |
|                       | H1679632    | 1992            | Torino, Piemonte, Italy        |
|                       | H1673036    | 1990            | Anvers (Antwerp), Belgium      |
| *Ambrosia maritima* L. | H1645521    | 1964            | Massa, Tuscany, Italy          |
|                       | H1645522    | 1964            | Massa, Tuscany, Italy          |
|                       | H10922472   | 1973            | Berre-l’Étang, France          |
|                       | H1477643    | 1958            | Punta Sabbioni, Venice, Italy   |
|                       | H1475197    | 1981            | Sanlúcar de Barrameda, Cadiz, Spain|
| *Ambrosia psilostachya* DC. | H1155299  | 1947            | Noordwijk, the Netherlands      |
|                       | H1247694    | 1979            | Berlin, (West) Germany         |
|                       | H1116344    | 1975            | Anvers (Antwerp), Belgium      |
|                       | H1594014    | NA              | NA                             |
|                       | H1467250    | NA              | NA                             |
| *Ambrosia tenuifolia* Spreng. | H1645534  | 1908            | Chapelle St. Laurent Beaumarche, France|
|                       | H1487927    | 1967            | Buenos Aires, Rio de Plata     |
|                       | H1491053    | 1974            | Buenos Aires, Florida, Calle General Roca|
|                       | H1486933    | 1967            | Buenos Aires, Magdalena, Arroyo Juan Blanco|
|                       | H1486934    | 1967            | Buenos Aires, Magdalena, Arroyo Juan Blanco|

Note: NA = not available.

*All vouchered specimens are deposited at the herbarium of the Finnish Museum of Natural History (H), University of Helsinki, Finland.*