# Novel Microsatellite Development and Characterization for Phacelia formosula (Hydrophyllaceae)

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## NOVEL MICROSATELLITE DEVELOPMENT AND CHARACTERIZATION FOR *PHACELIA FORMOSULA* (HYDROPHYLLACEAE)<sup>1</sup>

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- *Premise of the study:* Microsatellite primers were developed to characterize genetic diversity and structuring in the genus *Phacelia* (Hydrophyllaceae) and to further conservation efforts for *P. formosula*.
- Methods and Results: Fifteen novel microsatellite primers were developed for P. formosula. These were characterized for genetic variation in three separate P. formosula populations. Two to nine alleles were found per locus. Overall observed heterozygosity and expected heterozygosity ranged from 0.000 to 0.800 and 0.000 to 0.840, respectively. Additionally, these loci were successfully amplified and showed polymorphism in P. gina-glenneae and a potential new Phacelia species.
- *Conclusions:* These microsatellite markers will be useful in assessing genetic diversity, structuring, and gene flow within and among populations of the rare *P. formosula*, in addition to related *Phacelia* species. These markers will provide important genetic data needed for appropriate conservation and management of these rare plants.

Key words: Colorado; conservation genetics; Hydrophyllaceae; microsatellite; Phacelia; Phacelia formosula.

Phacelia Juss. (Hydrophyllaceae) is a speciose genus with approximately 167 (USDA NRCS, 2017) species in the United States, predominantly in western states. We follow the taxonomy of the Boraginales Working Group (Luebert et al., 2016) in conserving Phacelia within the family Hydrophyllaceae as opposed to in a subfamily of the Boraginaceae (e.g., APG IV, 2016). In addition to the ongoing debate regarding the status of the Hydrophyllaceae, this maintains agreement with the recent Flora of Colorado (Ackerfield, 2015). Given the number of species in both the genus and family, we expect that these markers will have broad applicability for conservation and populationlevel studies. Additionally, there are many rare and locally endemic species in *Phacelia* (34 species with a G1 or G2 rank; NatureServe, 2017). Whereas previous population genetic studies in Phacelia used cpDNA (Levy et al., 1996) or allozymes (Levy and Neal, 1999), we developed the first primers specifically for population-level assessments in the genus.

*Phacelia formosula* Osterh. (North Park phacelia) is a rare endemic found only in the North Park basin in Jackson County, Colorado, USA. Within this area, *P. formosula* is found in scattered small populations restricted to soils derived from the Coalmont Formation (U.S. Fish and Wildlife Service, 2011). An understanding of the genetic diversity and distribution of *P. formosula* would be extremely useful in guiding management

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and conservation actions. Currently, these data are lacking for *P. formosula*, as well as other *Phacelia* species.

Here we report the development and characterization of 15 novel microsatellite loci for *Phacelia*, all of which were tested for polymorphism in *P. formosula*. Additionally, we cross-amplified these loci in a presumably closely related species, *P. gina-glenneae* N. D. Atwood & S. L. Welsh, and in a recently discovered population of uncertain specific status (*Phacelia* sp. in Table 1).

#### METHODS AND RESULTS

Microsatellite development using DNA extracted from silica-dried P. formosula leaf tissue was conducted by Ecogenics GmbH (Balgach, St. Gallen, Switzerland). Microsatellite content of the genomic DNA fragments was enriched via biotin-labeled tetranucleotide (GTAT, GATA, AAAC, and AAAG; Roche 454 platform [Basel, Basel-Stadt, Switzerland] with GS FLX Titanium reagents) and dinucleotide (CT and GT; Illumina MiSeq platform [San Diego, California, USA] using the Nano 2 × 250 version 2 format) repeats using magnetic streptavidin beads. The enrichments were multiplexed with additional species and produced libraries with 4264 and 13,858 reads (respectively), which were assessed for microsatellites using Primer3 (Rozen and Skaletsky, 1999). The tetranucleotide reads averaged 415 bp in length with 151 reads containing a tetra- or trinucleotide microsatellite insert ≥6 repeat units. The dinucleotide reads averaged 402 bp in length, and 1502 reads contained a dinucleotide microsatellite insert ≥10 repeat units. Suitable primer design was possible in 83 of the tetranucleotide reads and 918 of the dinucleotide reads, of which 24 and 16 reads (respectively) were tested for functionality and polymorphism in seven samples using the methods of Schuelke (2000). The assessment resulted in 40 loci, which were then narrowed to 15 by the authors based on multiplex potential. These loci were multiplexed in two panels for data collection and analyses (Table 1).

For locus amplification within populations, total genomic DNA was extracted from silica-dried leaf tissue at Denver Botanic Gardens using the Omega E.Z.N.A. DNA Mini Kit (Omega Bio-tek, Norcross, Georgia, USA; short protocol with both elution steps). Amplification was carried out at the Nevada Genomics Center (Reno, Nevada, USA) using two PCR panels with different

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	Locus	Primer sequences (5'-3')	Repeat motif	$T_{\rm a}$ (°C)	Primer concentration (µM)	Fluorescent label	Allele size range (bp)	GenBank accession no.
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Phafor_00006	F: GAGTTCGCGGACAAAGATGC	$(TATG)_8$	63.8	0.75	6-FAM	189–197	KP281305
R:       AGGAATTCGCATTAGGTCG       ATTACTCGATTAGGTCG       ATTACTCGATTAGGTCG       ATTACTCGAATTAGGTCG       ATTACTCGAATTAGGTCGCATTCACTTG       ATTACTCGAAAACCCACCCATTCACTTG       ATTACTCGAAAACCCACCCATTCACTTG       ATTACTCGAAAACCCACCCATTCACTTG       ATTACTCGAAAACCCACCCATTCACTTG       ATTACTCGAAAACCCACCCATTCACTTG       ATTACTCGAAAACCCACCATTC       ATTACTCGAAAACCCACCATTCACTTG       ATTACTCGAAAACCCATCACC       ATTACTCGAAAACCCATCACC       ATTACTCGAAAACCCATCACC       ATTACTCGAAAAACCATTC       ATTACTCGAAAAACCATTC       ATTACTCAAAAACCAACC       ATTACTCAAAAAAACCATTCATTCACTCACC       ATTACTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	Phafor 00246	R: CTTGTGATCGCGTCCTAAGC F: CCGCCATCTCCTTCTTCCAC	(TACA) <sub>10</sub>	63.8	2	NED	203–222	KP281306
F: MAANCGEACCTCTACTURG       (KTAC) <sub>11</sub> 62       2       VIC       222-260         F: ATTCCGEATCCACTCC       (TGT) <sub>1</sub> 63.8       2       PET       194-222         F: ATTCCGEATCCATACTCTC       (TTC) <sub>18</sub> 63.8       2       PET       194-222         F: AGGAGCGGCATATACTCTC       (TTC) <sub>18</sub> 63.8       2       NED       253-271         F: AGGAGCGGCATATATACTC       (TTC) <sub>18</sub> 63.8       2       PET       194-222         F: GCAAGGAGTATATAGTC       (TTC) <sub>18</sub> 63.8       2       PET       233-271         F: AGGAGCGATATATAGTC       (TTC) <sub>18</sub> 63.8       1.5       VIC       110-133         F: AGGAGCGATACATAGTCCACACC       (AG) <sub>14</sub> 6.2       2       28-240       28-240         F: AGGCGTTAGAAGGAGCACCACC       (ATC) <sub>18</sub> 6.3       1.5       VIC       110-133         F: AGGCGTTAGAAGGAGAACAAAAG       (ATC) <sub>18</sub> 6.3       1.5       VIC       110-133         F: CCCATTAGAAGATCCACCC       (ATC) <sub>18</sub> 6.3       1.5       VIC       110-133         F: TCCCATTAGACACCC       (ATC) <sub>18</sub> 6.3       1.5       VIC       110-133         F: TCCCATTAGACACACCC       (ATC) <sub>18</sub> 6.3 <td< td=""><td></td><td>R: AGCAATTCGCATTAGGTCCG</td><td></td><td></td><td></td><td></td><td></td><td></td></td<>		R: AGCAATTCGCATTAGGTCCG						
R: TATCGGATARAAGGGGATIG       (TGTA),       6.3.8       2       PET       194-222         R: TATCGGATARAAGGGGAATIG       (TTGTA),       6.3.8       2       NED       253-271         R: TARCGGCATGAAGCGGAATIG       (TTGT),       6.3.8       2       NED       253-271         F: GGGGAGGAAGGACGTAAGCGG       (TTGT),       6.3.8       2       6-FAM       83-96         R: GGGGAATGAAGCGGGAATIG       (GA),       62       2       6-FAM       83-96         R: GGGGAATGAAGGGG       (GA),       62       2       PET       228-240         R: AGGCAATGAAGGGGGGAATGGG       (GA),       6.2       2       PET       228-240         R: AGGCAATGAAGGGGGGAATGGGGG       (AG),       6.2       2       PET       228-240         R: AGGCAATGAAGGGGGGAATGGGGG       (AG),       6.2       2       VIC       110-133         R: AGGCAATGAAGGAGGGAACAAAAG       (TVG),       6.3       4       6-FAM       20-249         R: AGGCAATGAAGGGGG       (TVG),       6.3       4       6-FAM       20-249         R: ACGGATAAGGAAGGAGGAAGGGGG       (TVG),       6.3       4       6-FAM       20-249         R: CGGTAAGGAAGGAAGGAGGGGGAAGGGGGGGGGGAAGGGGGG	Phafor_00567	F: AAAACGCACCGTCTCACTTG	(ATAC) <sub>11</sub>	62	2	VIC	222–260	KY442304
F: MACTGAARCOCCUCCATUR(101A)0.382PEI194-222F: MACTGAARCOCCUCCATTARTET(TTCT) $6.38$ 2NBD253-271F: GCARGCAGCGATTARTET(TTCT) $6.38$ 2 $6.FAM$ $83-96$ F: GCARGCATCATTCATCCATCCATC(CAA); $6.2$ 2 $6.FAM$ $83-96$ F: GCARGCATCATTCATCCATCCATCCATCCATCCATCA(GA); $6.2$ 2 $0.FET$ $238-240$ F: TCCCATTTARCCATCCATCCATCCATCCATCCATCCATCC	DI C DOLEO	R: TTCCGCATAAAACGCCATCG		0 07	¢		000 101	EAC LOOG X
F:AGGGCGAACACATACTUC C(TTC) $6.3.8$ 2NBD $253-271$ F:GCAACCAGGCATACTUC CCAATGAACCCC R:(CAA) $6.3.8$ 2 $6.FAM$ $8.3-96$ F:GCGAACCAACCTUC 	Phator_00650	F: ATACTGAAACCGCCGCAATC B: TTACCGCCTTTCAAACCGCAATC	$(IGIA)_7$	63.8	7	L'EL	194-222	KP281307
R:       GCAGACCAGGCTFTTAGTG       (CAA);       62       2       6-FAM       83-96         F:       GCAGACCAGGCTATTGGG       (GA);       62       2       bFT       228-240         R:       AGGCTATTGTGTCCACATTG       (GA);       62       2       PET       228-240         R:       AGGCTATTGTGTCCACATTG       (GA);       63       1.5       VIC       110-133         R:       AGGCTATTACGGGAACAAAAGG       (ATC);       63       1.5       VIC       110-133         R:       AGGCCTATTGCACATTCAACGGG       (ATC);       62       4       6-FAM       208-223         R:       TGCCGTTGGGGGAACAAAAGG       (ATC);       63       4       0       208-223         R:       TGCGATTGGAGAACAAAAGG       (ATC);       63       4       0       208-223         R:       TGCGATTGGAGAACAAAAGG       (TATC);       63       4       0       208-223         R:       CGGATAACTGAACTGCAACTG       (TATC);       63       1.5       0       208-223         R:       CGGATAACTGCAACTGCAACTG       (TATC);       63       1.5       0       208-233         R:       CGGATAACTGCAACTGCAACG       (TATC);       63       1.5       0<	Phafor_00745	F: AGGGTCGACACCATACTTCTC	(TTCT) <sub>s</sub>	63.8	2	NED	253-271	KP281308
$ \begin{array}{llllllllllllllllllllllllllllllllllll$		R: GCAGACCAGGCTGTTTAGTG						
R:       GTCGTATAAAGGGATCATTGG         F:       TGCCAAAAGGGATCATTGG         C:       F:       TGCCAAAAGGGATCACCACC         R:       AGGCGTTATCGACATC       (GA) <sub>14</sub> 62       2         R:       AGGGGTTATCGACATC       (AG) <sub>14</sub> 63.8       1.5       VIC       110-133         R:       GCCGTTGGGAGAACAAAG       (ATC) <sub>8</sub> 63.8       1.5       VIC       110-133         R:       GCCCGTTGGAGAACAAAG       (ATC) <sub>8</sub> 62       4       6-FAM       28-249         R:       TGGGTAACTGGAGGAGAACAAAG       (ATC) <sub>13</sub> 62       4       0-10       20-249         R:       TGGGTAACGGAGGAGGAGGGGG       (ATC) <sub>13</sub> 62       4       0-10       20-249         R:       TGGGTAACGGAGGAGGAGGGGGG       (TATC) <sub>13</sub> 62       4       0-10       21-249         R:       TGGGTAACGATGGGGGG       (TATC) <sub>13</sub> 63       4       0-10       21-249         R:       CGGTTGGATGGTCGAGGGGG       (TATC) <sub>13</sub> 63       4       0-10       21-249         R:       CGGTTGGATGGTCGATGGTG       (TATC) <sub>13</sub> 63       1       5       21-249         R:       CGGTTGGATGGATGGATGGTG       (TATC) <sub>13</sub>	Phafor_01477	F: GCAAGCAATGAACACACTCC	$(CAA)_7$	62	2	6-FAM	83–96	KP281309
$ \begin{array}{llllllllllllllllllllllllllllllllllll$		R: GTCGTATCAAAGGCATCATTGG						
R:AGGCCTATTCFGTCCACATCR:AGGCCTATTCFGTCCCACATCR:CCCGTTGGGAGAACAAGGGF:ATCGGGTTGGGAGAACAAGAGF:CCCGTTGGGAGAACAAGAGR:CCCGTTGGGAGAACAAGAGR:TCCCGTTGGGAGAACAAGAGR:TCCCGTTGGAGAACAAGAGGR:TCCCGTTGGTGGGAGAACAAGGGR:TCCCGTTGGTGGCGATCAGTGCGGGR:TCCCGTTGGTGGCGATCAGTGCGGGR:TCCCGTTGGTGGCGATCAGTGCGGGR:CAAATAGCACCCTAACTCAGGGR:CAAATAGCACCCTAGTGGGGGGGGGGGGGGGGGGGGGGG	Phafor_01499c	F: TGCAAAAGAGATCCATCCACG	$(GA)_{14}$	62	2	PET	228–240	KP281303
$ \begin{array}{llllllllllllllllllllllllllllllllllll$		R: AGGCCTATTCTGTCCCACATC						
R:GCCCGTTGGGAGAACAAAGF:TCCANGTTGGGAACAAAGF:TCCANGTTGGCATTGGCATTGGCATTGGCATTGGCATTGGCATTGGCATCAGGF:TCCCANGTTGGCATCGGGACGAGGF:GGGAACGAGGACGAGGGGF:GATCAGGGAATCGGGGAF:GATCAGGGAATCGGGGAF:GATCAGGGAATCGGGAGF:GATCAGCGAATCGGGGAF:CCGTTGGTGGATGCATCCTCCF:CCGTTGGTGGATGCATGCTCGGF:CCGTTGGTGGATGCATGCTCGGF:CCGTTGGTGGATGGATGGATGF:AACACATCCATGTTGCAGGGGGGF:AACACATCCATGTTGGAGGGF:GGGTCACGGTTGGGGGGGGAAGGGGF:GGGTCACGGTTGGGGGGGGAAGGGGF:GGGTCACGGTTGGGGGGGGAAGGGGF:GGGTCACGGTGGGGGGAAACGGCF:GGGTTAAGGTGGGGGGGAAACGAGCF:F:TTGGAACGTGGGGGGAAAACGAGCF:F:TTGGCACGGGGGAAAACGAGCF:F:TTGGCACGGGGGAAAAAGGAGGGAAAACGAGCF:F:TGGGTCGAAGGGAAAAAAGGACGAAACGAGCCT) <sub>14</sub> 6:F:F:TGGCGCTAAAGAAAAAGGACCT) <sub>14</sub> 6:F:ATTGGAAGGAACAAAAAAAGAAAAAAAAAAAAAAAAAAA	Phafor_01817c	F: ATCGGGTTACAGAGATGGGC	$(AG)_{14}$	63.8	1.5	VIC	110-133	KY442307
F:TCCCATGTTAGCGAGTAGGC(ATC)86246-FAM208-223R:TGCGTAATTCTGACTTCTAACGAG(TATG)12624NED200-249R:CAAATAGCACCAATTCTAACGAG(TATG)12634NED200-249R:CAAATAGCATGCTGATGTCACC(TATC)1863.846-FAM262-288R:CACATGCTGTGGATGTTCACC(TATC)1863.846-FAM262-288R:CACATGCTGGTGGATGTTCACC(TATC)1863.81.5VIC231-239R:TGGGTCAGTTGTCACTTTCTGTCG(TATC)163.81.5VIC231-239R:TGGGTCAGTTGGTGGTGATGG(TATC)163.81.5VIC231-239R:TGGGTCAGTTGGTGAGTGTTCTTGTCGG(TATC)1622NED165-189R:ATGGATTGCAGTGGTGAGGGAAAGGG(GT)126226-FAM155-167R:ATGGAGTCGTGGGGGAGAAGGGG(CT)14621VIC106-140R:ATGGAGTCTGGGGGAAAGGG(CT)14621VIC106-140		R: GCCCGTTGGGAGAACAAAG						
R:TGGTAATCTTGACTTCTAACGAGF:GATCAGCGATCAGCGGTCR:CAATAGCACCGATCAGCGGTR:CAATAGCACCCAACTCCACCF:CCGTTGCTGGTGCACTCACCF:CCGTTGCTGGTGCACGCTCACCF:CCGTTGCTGGTGCACGTTCAGCR:CAACAGCGTGCAGGTGCACGCTCAGCR:CACATGGTGCAGGTGCAGGTCAGGTGCAGGTGCAGGTGCAGGTGCAGGTAGCAGGTGCAGGTTGCAGGTGCAGGTAGGAGGAGGGAG	Phafor_02245	F: TCCCATGTTAGCGAGTAGGC	$(ATC)_8$	62	4	6-FAM	208–223	KP281310
F:GATCAGCGATCAGTGGTG(TATG)624NED220-249R:CAATAGCACCCAACTCACC(TATC)63.846-FAM262-288R:CCGTTGCTGGTGGTGGTGTGTC(TATC)63.81.5VIC231-239R:TGGGTCAAGTTGGTGTGTGTGGT(TATC)63.81.5VIC231-239R:TGGGTCAAGTTGGTGTGTGTGTGTGTGTGGTGGGTGAGGTGAGGTGAGGTGAGGTGAGGTGAGGTGAGGTGAGGTGAGGTGAGGTGAGGTGAGGTGAGGGTGAGGGTGAGGGAGGGGGG		R: TGCGTAATCTTGACTTCTAACGAG						
R: CAATAGCACCCAACTCCACC F: CCGTTGCTGATGTCCTCACC R: CCGTTGCTGATGTTCAG F: CCGTTGCTGATGTTCAG F: CGTTGCTGATGTTCAGG F: CAACATCCATGTTGCAGGTTAGGTCG F: TGGGTCAGGTTAGGTAGG F: ATTGAACTTCCATTGTAGG F: TTTGTCCAGAGGCAAAGGA F: TTTGTCCAGAGGCAAAAGGA F: TTTGTCCAGAGGCAAAAGGA F: TTTGTCCAGAGGCAAAAGGA F: TTGGTCGAGGGAAAAGGA F: TTGGTCAGAGGCAAAAGGA F: TTGGTCAGAGGCAAAAGGA F: TTGGTCAGAGGCAAAAGGA F: TTGGTCAGAGGCAAAAGGA F: TGGTGCTAAGAATATCTGCCG F: TGGTGCTAAGAATAATCTGCCG F: TGGTGCTAAGAATAATCTGCCG F: TGGTGCTAAGAATAATCTGCCG F: TGGTGCTAAGAATAATCTGCCG F: TGGTGCTAAGAATAATCTGCCG F: TGGTGCTAAGAATAATCTGCCG F: TTGGTGCAGAGGCAAAAGGA F: TTGGTGCAGAGGCAAAAGGA F: TTGGTGCAGAGGCAAAAGGA F: TTGGTGCAGAGGCAAAAGGA F: TTGGTGCAGAGGCAAAAGGA F: TTGGTGCAGAGGCAAAAGGA F: TTGGTGCAGAGGCAAAAGGAC F: TTGGTGCAGAGGCAAAAGGA F: TTGGTGCAGAGGCAAAAGGA F: TTGGTGCAGGCAAAAGGAGGAAAAGGA F: TTGGTGCAGAGGCAAAAGGAGGAAAAGGA F: TTGGTGCAGGGAAAAGGA F: TTGGTGCAGGGAAAAGGA F: TTGGTGGAGGGAAAAGGA F: TTGGTGCAGGGAAAAGGAGGAAAAGGA F: TTGGTGCAGGGAAAAGGA F: TTGGTGCAGGGAAAAGGA F: TTGGTGCAGGGAAAAGGAGGAAAAGGA F: TTGGTGCAGGGAAAAGGA F: TTGGTGCAGGGGAAAAGGA F: TTGGTGCAGGGGAAAAGGA F: TTGGTGCAGGGGAAAAGGA F: TTGGTGCAGGGGAAAAGGAGGAGGAGGAGGAGGAGGAGAAAGGA F: TTGGTGCAGGGGAAAAGGAGGAGGAGGAGGAGGAGGGAAAAGGAGG	Phafor_02638	F: GATCAGCGATCAGTGCGTG	(TATG) <sub>12</sub>	62	4	NED	220–249	KP281312
F: CGGTGGCTGATGTTGTC (TATC) <sub>18</sub> 63.8 4 6-FAM 262-288 R: CAACATGGTTGGTTGGGTGGTTGGG F: AACATCATGTTGGTGGCTGGG F: AACATCATGTTGGTGGGTAGG F: TGGGTCAGGTTAGGTAGGTAGG F: GGGTTAAGTATCGTTGTGTGGG F: TTGTCCAGTATGATTGGC F: TTGTCCAGAGGGAAAGG F: TTGTCCAGAGGGAAAGGA F: TTGGTCGGGAGGGAAAGGA F: TTGGTCGGGAGGGAAAGGA F: TTGGTCGGGAGGGAAAGGA F: TTGGTCGGGAGGGAAAGGA F: TTGGTCGGGAGGGAAAGGA F: TTGGTCGGAGGGAAAGGA F: TTGGTCGTGAGGGAAAGGA F: TTGGTCGTGAGGGAAAGGA F: TTGGTCGGAGGGAAAGGA F: TTGGTCGGAGGGAAAGGA F: TTGGTCGGAGGGAAAGGA F: TTGGTCGGAGGGAAAGGA F: TTGGTCGGAGGGAAAGGA F: TTGGTCGGGAGGGAAAGGA F: TTGGTCGGAGGGAAAGGA F: TTGGTCGGGGGAAAGGA F: TTGGTCGGGGGGAAAGGA F: TTGGTCGGGAGGGGAAAGGA F: TTGGTCGGGAGGGAAAGGA F: TTGGTCGTGGGGGAAAGGA F: TTGGTCGGGGGAAAGGA F: TTGGTCGGGGGAAAGGA F: TTGGTCGTGGGGGAAAGGA F: TTGGTCGTGGGGGGAAAGGA F: TTGGTCGTGGGGGGAAGGAGGAGGGGGAAAGGA F: TTGGTCGTGGGGGGAAGGAGGGGAGGGGGAAGGGGGGGGG		R: CAAATAGCACCCCAACTCCACC						
R: CAACATGGTTGCATGCTTCAG F: CAACATGGTTGCATGCTTCAG F: AACACTGCTTGCAGGTTGCAGG R: TGGGTCAAGGTTAGGTGCAGGT F: GGGTTAAGTATGTCATGTAGGTGG F: TTGAAGTATCATGTATGTAGGTTG F: TTGTCAGTAGGAGACAGG F: TTGTCAGGAGGAAAGGA F: TTGGTCAGGAGGAAAAGGA F: TGGTGGAGGAAAAGGA F: TGGTGGTGAGGAAAAGGA F: TGGTGGTGAGGAAAAGGA F: TGGTGGTGAGAGAGGAAAAGGA F: TGGTGGTGAGGAAAAGGA F: TGGTGGTGAGGAAAAGGA F: TGGTGGTGAGAGAGGAAAAGGA F: TGGTGGTGAGAGAGAGGAAAAGGA F: TGGTGGTGAGAGAAGAGGA F: TGGTGGTGAGAGAGAGGAAAAGGA F: TGGTGGTGAGAGAGAGGAAAAGGA F: TGGTGGTGAGAGAGAGAGAGGAGAGGA F: TGGTGGTGAGAGAGAGAGGA F: TGGTGGTGAGAGAGAGAGGAGAGAGGAGAGGAGAGAGGAG	Phafor_02824	F: CCGTTGCTGATGTCCTTGTC	(TATC) <sub>18</sub>	63.8	4	6-FAM	262–288	KY442308
F: AACATCCATGTTGCACCG (TATC), $63.8$ $1.5$ VIC $231-239$ R: TGGGTCACAGTTAGGTGATG F: GGGTCACAGTTAGGTGATG F: ATTGACTTCCACTATTGACTTG R: ATTGACTTCACTATTGACTTG R: ATTGTCCGGAGACGCA F: TTGTCCGGAGAAACG F: TGGTCGTGAGGAAACGG (CT) <sub>14</sub> $62$ $1$ VIC $106-140$ R: ATTGGAGTAGACTAGAACAGC (CT) <sub>14</sub> $62$ $1$ VIC $106-140$		R: CAACATGGTTGCATGCTTCAG						
R: TGGGTCACAGTTAGGTGATG F: GGGTTAACTACTATGTACTACTATGTACTACTATCATCATTGTACTACTATCATCATCATCATCATCATCATCATCATCATC	Phafor_03037	F: AACACATCCATGTTGCACCG	$(TATC)_7$	63.8	1.5	VIC	231–239	KP281311
F: GGGTTAAGTACTGTCATCATGTAGG (TATG), 62 2 NED 165-189 R: ATTGAACTTCCACTATGGACTTG (GT) <sub>12</sub> 62 2 6-FAM 155-167 F: TTGTCCAGAGGAAAGG (CT) <sub>14</sub> 62 1 VIC 106-140 R: ATGGAAGTGATAGAACAGC (CT) <sub>14</sub> 62 1 VIC 106-140		R: TGGGTCACAGTTAGGTGATG						
R: ATTGAACTTCCACTATCGACTTG F: TTTGTCCAGACGAACGAC (GT) <sub>12</sub> 62 2 6-FAM 155-167 R: AAGCAGTCGTGAGGAAAACG (CT) <sub>14</sub> 62 1 VIC 106-140 R: ATTGGAAGTGACTAGAACAAGC (CT) <sub>14</sub> 62 1 VIC 106-140	Phafor_03754	F: GGGTTAAGTACTGTCATCTTGTACG	(TATG) <sub>9</sub>	62	2	NED	165-189	KP281313
F: TTTGTCCAGACGAAAGGAC (GT) <sub>12</sub> 62 2 6-FAM 155-167 R: AAGCAGTCGTGAGGAAAAGG (CT) <sub>14</sub> 62 1 VIC 106-140 R: ATTGGAAGTGACTAGAACAAGC (CT) <sub>14</sub> 62 1 VIC 106-140		R: ATTGAACTTCCACTATCGACTTG						
R: AAGCAGTCGTGAGGAAAACG F: TGGTGCTAAAGAATATCTGCCG (CT) <sub>14</sub> 62 1 VIC 106–140 R: ATTGGAAGTGACTAGAACAAGC	Phafor_05461c	F: TTTGTCCAGACGCAAACGAC	$(GT)_{12}$	62	2	6-FAM	155-167	KP281302
F: TGGTGCTAAAGAATATCTGCCG (CT) <sub>14</sub> 62 1 VIC 106–140 R: ATTGGAAGTGACTAGAACAAGC								
R: ATTGGAAGTGACTAGAACAAGC	Phafor_13597s		$(CT)_{14}$	62	1	VIC	106-140	KP281304
		R: ATTGGAAGTGACTAGAACAAGC						

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*Note*:  $T_{\rm a}$  = annealing temperature.

annealing temperatures to maximize multiplexing effectiveness. Final PCR volume was 11 µL:1 µL aliquot of panel mix (containing: forward primers [labeled with a universal M13 tail: 5'-TGTAAAACGACGGCCAGT-3'], reverse primers [primer concentration varied by loci, see Table 1], a fluorescently labeled [6-FAM, NED, PET, or VIC] 5' tag, and 20 ng of DNA template) and 10 µL of QIAGEN Multiplex PCR Mastermix (QIAGEN, Hilden, Germany). Reaction conditions were as follows: an initial 15-min 95°C denaturing step; followed by 40 amplification cycles of 95°C for 30 s, 62°C (panel 1) or 63.8°C (panel 2) for 45 s, and 72°C for 45 s; followed by a final elongation step at 72°C for 30 min using a GeneAmp 9700 thermocycler (Applied Biosystems, Carlsbad, California, USA). PCR products were diluted to an appropriate concentration determined by PicoGreen dilution tests. One microliter of diluted PCR product was added to 10 µL of HiDi Formamide with the size standard GeneScan 500 LIZ (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and 7 µL of molecular-grade water followed by electrophoresis on an ABI Prism 3730 DNA Analyzer (Applied Biosystems) at the Nevada Genomics Center. Genotype data were visualized and fragment sizes scored at Denver Botanic Gardens using Geneious version 6.0.6 (Kearse et al., 2012).

GenAlEx version 6.3 (Peakall and Smouse, 2006) was used to calculate observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ) and to test for deviation from Hardy–Weinberg equilibrium (HWE). GENEPOP (Raymond and Rousset, 1995; Rousset, 2008) was used to test for linkage disequilibrium for each pair of loci in each population. ML-NullFreq (Kalinowski and Taper, 2006; 10,000 replicates) was used to estimate the frequency of null alleles.

All 15 microsatellite loci were variable and polymorphic in all three of the P. formosula populations. The number of alleles per locus ranged from two to nine (JC1, N = 30), from two to seven (JC2, N = 30), and from two to eight (JC3, N = 30). The  $H_0$  and  $H_c$  at JC1 ranged from 0.133 to 0.800 and 0.124 to 0.840, respectively. At JC2,  $H_0$  and  $H_e$  ranged from 0.033 to 0.700 and 0.064 to 0.707, respectively. At JC3, the  $H_{\rm o}$  ranged from 0.000 to 0.700 and  $H_{\rm e}$  ranged from 0.067 to 0.742 (Table 2). Three loci (Phafor\_00567, Phafor\_02245, and Phafor\_02824) at the JC1 population and four loci at both the JC2 population (Phafor\_00567, Phafor\_01817c, Phafor\_02245, and Phafor\_03754) and the JC3 population (Phafor\_00246, Phafor\_00567, Phafor\_01817c, and Phafor\_02245) showed significant deviation from HWE (Table 2). After Bonferroni correction. no evidence of significant linkage disequilibrium was detected. Heterozygote deficiencies, possibly indicating the presence of null alleles, were detected for five loci at the JC1 population (Phafor\_00006, Phafor\_00567, Phafor\_01817c, Phafor\_02245, and Phafor\_02824), six loci at the JC2 population (Phafor\_00567, Phafor\_1477, Phafor\_01817c, Phafor\_02245, Phafor\_3754, and Phafor\_13597s), and seven loci at the JC3 population (Phafor\_00246, Phafor\_00567, Phafor\_00745, Phafor\_01817c, Phafor\_02245, Phafor\_02824, and Phafor\_05461c). All 15 microsatellite loci were successfully cross-amplified in both P. gina-glenneae (GC, N = 30) and a newly discovered *Phacelia* population (LC, N = 30) of uncertain specific status. All of the amplified loci were polymorphic in the P. gina-glenneae population while two loci (Phafor\_03037 and Phafor\_01817c) were monomorphic in the Phacelia sp. population (LC) (Table 2).

#### CONCLUSIONS

The novel microsatellite markers described here are the first developed not only for Phacelia, but also for the Hydrophyllaceae. These markers will be valuable for investigating population genetic structure in Phacelia and potentially other genera within Hydrophyllaceae. Knowledge of genetic diversity present within and among the scattered populations of the rare P. formosula will be used to better manage the known populations to ensure their future persistence. Additionally, these markers will be useful for assessing genetic diversity in a newly discovered population of *Phacelia* that is morphologically similar to *P*. formosula but occurs in different habitats more than 40 km away. Investigating potential gene flow between this new population and existing *P. formosula* populations will be helpful in inferring its specific status. Cross-amplification in P. gina-glenneae demonstrates the utility of these markers in assessing genetic diversity in other species of Phacelia. Our results indicate the presence of potential null alleles. Several methods (Chapuis and Estoup, 2007) and programs can be used to detect and account for null alleles in population-level analyses (such as Kalinowski and

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$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	JCIJC2JC3JC3LCCC $A$ $H_o$ $H_e$ $HWE^b$ $A$ $H_o$ $H_e$ $HWE^b$ $A$ $H_o$ $H_e$ $H_WE^b$ $A$ $H_o$ $H_e$ $H$							$Phac\epsilon$	Phacelia formosula	sula						$P_{\cdot}$	Phacelia sp.			Phaceli	Phacelia gina-glenneae	nneae
$A$ $H_o$ $H_w$ $HWE^b$ $A$ $H_o$ $H_w$ $HWE^b$ $A$ $H_o$ $H_w$ $HWE^b$ $A$ $H_o$ $H_w$ $HWE^b$ $A$ $H_o$	$A$ $H_o$ $H_c$ $HWE^b$ $A$ $H_o$ $H_e$ $HWE^b$ $A$ $H_o$ $H_e$ $HWE^b$ $A$ $H_o$				JC1				JC2				JC3				LC				GC	
2 0.367 0.486 0.178 3 0.133 0.126 0.985 2 0.467 0.498 0.732 3 0.267 0.500 0.002** 2 0.033 0.033 0.031 (3.91 0.269 0.109) 3 0.200 0.653 0.301 0.301 (3.91 0.266 0.001*** 4 0.095 0.543 0.000*** 2 0.233 0.339 0.087 2 0.267 0.391 (3.91 0.268 0.001*** 4 0.096 2 0.000 0.653 0.429 0.128 0.000*** 3 0.1045 0.206 0.295 4 0.400 0.408 (3.01 0.203 0.333 0.678 0.300 0.653 0.501 0.203 0.303 0.007 11 0.667 0.206 0.235 0.000 0.653 0.533 0.673 0.301 0.523 0.967 0.206 0.523 0.301 0.523 0.429 0.728 0.000*** 2 0.233 0.295 4 0.400 0.408 (4.0 0.733 0.674 0.678 2 0.057 0.301 0.523 0.429 0.731 0.748 0.986 5 0.000 0.523 0.976 4 0.567 0.599 0.331 6 0.833 0.795 0.000*** 2 0.233 0.205 0.000*** 2 0.233 0.205 0.000*** 2 0.233 0.205 0.000*** 2 0.033 0.095 0.000*** 2 0.230 0.255 (5.0 0.333 0.743 0.748 0.106 2 0.033 0.095 0.000*** 4 0.743 0.286 0.006** 3 0.417 0.232 0.664 2.0 0.300 0.472 0.556 0.506 0.006** 3 0.417 0.522 0.030 0.239 0.551 0.500 0.555 0.500 0.556 0.506 0.006** 3 0.000 0.000 5 0.030 0.255 0.500 0.556 0.556 0.506 0.006** 3 0.417 0.52 0.500 0.556 0.55	2 0.367 0.486 0.178 3 0.133 0.126 0.985 2 0.467 0.498 0.732 3 0.267 0.500 0.002** 2 0.033 0.033 0.033 (0.391 2 0.269 0.109 3 0.209 0.240 0.080 2.50 0.000*** 2 0.233 0.339 0.087 2 0.267 0.391 4 0.267 0.306 0.653 0.578 0.968 2 0.1096 0.904 3 0.429 0.152 0.206 0.205 4 0.400 0.653 14 0.733 0.678 0.968 2 0.379 0.094 3 0.429 0.152 2 0.167 0.206 0.295 4 0.400 0.663 0.733 0.741 0.678 0.566 0.293 0.414 0.649 2 0.053 0.439 0.1391 2 0.033 0.095 0.297 11 0.667 0.206 0.255 1 0.206 0.255 0.000*** 2 0.233 0.295 0.77 0.76 0.776 0.776 0.776 0.773 0.744 0.578 0.966 2 0.301 0.553 0.499 0.391 2 0.033 0.095 0.000*** 2 0.233 0.206 0.207 0.700 0.655 0.500 0.256 0.000 0.667 0.748 0.256 0.506 0.256 0.506 0.556 0.506 0.556 0.506 0.556 0.566	Locus	A	$H_{\rm o}$	$H_{\rm e}$	HWE <sup>b</sup>	A	$H_{\rm o}$	$H_{\rm e}$	HWEb	A	$H_{\rm o}$	$H_{\rm e}$	$\mathrm{HWE}^{\mathrm{b}}$	A	$H_{\rm o}$	$H_{ m e}$	HWE <sup>b</sup>	P	$H_{\rm o}$	$H_{\rm e}$	HWE <sup>b</sup>
5         0.200         0.269         0.109         3         0.200         0.240         0.807         2         0.200         0.653         0.877         2         0.267         0.391           4         0.267         0.366         0.001***         4         0.096         0.533         0.067         0.005         0.543         0.000****         3         0.433         0.429         0.733         0.147         0.267         0.549         0.733         0.645         0.203         0.094         3         0.490         0.733         0.167         0.007         4         0.647         0.653         0.649         0.233         0.743         0.733         0.783         0.297         11         0.667         0.672           6         0.733         0.674         0.678         2         0.003         0.533         0.949         0.331         0.293         0.976         4         0.676         0.673         0.672         0.673         0.672         0.673         0.674         0.667         0.673         0.672         0.733         0.783         0.783         0.783         0.786         0.767         0.776         0.776         0.776         0.776         0.776         0.776         0.77	50.2000.2690.10930.2000.2400.08020.0000.0670.000****20.2330.3390.08720.2670.39140.2670.3660.001****40.0950.5430.000****40.0530.2570.0000.65340.6330.1240.6660.011****40.0590.2370.01440.6690.2230.97640.4000.66340.7330.1440.69620.03790.0140.6490.3310.93120.0330.097110.66740.7330.7450.98650.0670.0640.8300.39120.0330.29560.7330.7450.98650.6000.5230.97640.5990.53160.8330.7890.89060.76740.3670.4480.10620.0330.0950.000****30.0250.0000.55720.1330.7440.96650.6000.5230.97640.5660.6460.9670.7670.77640.3670.4480.10620.3330.34710.9000.5360.5660.6660.6560.6670.73750.5330.5440.3060.5330.5450.7100.2360.7420.3680.5660.6460.9650.7670.776 <td< td=""><td>Phafor_00006</td><td>7</td><td>0.367</td><td>0.486</td><td>0.178</td><td>3</td><td>0.133</td><td>0.126</td><td>0.985</td><td>6</td><td>0.467</td><td>0.498</td><td>0.732</td><td>3</td><td>0.267</td><td>0.500</td><td><math>0.002^{**}</math></td><td>2</td><td>0.033</td><td>0.033</td><td>0.926</td></td<>	Phafor_00006	7	0.367	0.486	0.178	3	0.133	0.126	0.985	6	0.467	0.498	0.732	3	0.267	0.500	$0.002^{**}$	2	0.033	0.033	0.926
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	4 $0.267$ $0.366$ $0.001^{****}$ 4 $0.067$ $0.366$ $0.001^{****}$ 4 $0.067$ $0.208$ $0.295$ 4 $0.400$ $0.653$ 2 $0.133$ $0.124$ $0.666$ $0.904$ 3 $0.423$ $0.297$ 11 $0.667$ $0.672$ 2 $0.133$ $0.124$ $0.666$ $2$ $0.067$ $0.672$ 3 $0.297$ 11 $0.667$ $0.672$ 4 $0.733$ $0.745$ $0.986$ 5 $0.067$ $0.672$ $0.093$ $0.999$ $0.331$ $0.297$ $11$ $0.667$ $0.673$ $0.233$ $0.995$ $0.000$ $0.833$ $0.789$ $0.300$ $0.567$ $0.757$ $0.206$ $0.996$ $0.999$ $0.300$ $0.667$ $0.677$ $0.775$ $0.745$ $0.233$ $0.233$ $0.233$ $0.233$ $0.236$ $0.544$ $0.776$ $0.775$ $0.649$ $0.567$ $0.775$ $0.649$ $0.767$ $0.$	Phafor_00246	S	0.200	0.269	0.109	С	0.200	0.240	0.080	0	0.000	0.067	$0.000^{***}$	0	0.233	0.339	0.087	0	0.267	0.391	0.081
4 0.633 0.678 0.968 3 0.100 0.096 0.994 3 0.433 0.429 0.752 2 0.167 0.206 0.295 4 0.400 0.408 2 0.133 0.124 0.696 2 0.379 0.414 0.649 2 0.069 0.128 0.013 3 0.207 11 0.667 0.622 4 0.400 0.408 4 0.733 0.674 0.678 2 0.033 0.098 0.914 0.649 2 0.069 0.391 2 0.033 0.095 0.000*** 2 0.233 0.206 4 0.757 0.778 0.778 0.789 0.890 6 0.767 0.776 0.776 1 0.563 0.341 0.020* 1 0.000 0.000 $-e$ 5 0.500 0.536 0.713 0.231 0.020* 3 0.100 0.239 0.000*** 4 0.400 0.433 0.029* 1 0.000 0.000 $-e$ 5 0.500 0.556 0.767 0.776 0.776 0.776 0.733 0.684 0.274 0.700 0.200 0.000 $-e$ 5 0.500 0.556 0.767 0.776 0.776 0.733 0.584 0.274 1 0.020* 3 0.100 0.239 0.000*** 4 0.700 0.666 0.546 5 0.546 5 0.546 0.556 0.546 0.556 0.546 0.556 0.566 0.546 0.566 0.566 0.546 0.56	4         0.633         0.678         0.968         3         0.100         0.096         0.994         3         0.413         0.124         0.206         0.295         4         0.400         0.408           2         0.133         0.124         0.696         2         0.379         0.414         0.649         2         0.069         0.128         0.095         0.0095         0.0097         0.111         0.667         0.623           4         0.733         0.574         0.966         2         0.633         0.499         0.391         2         0.095         0.0097         0.767         <	Phafor_00567	4	0.267	0.366	$0.001^{***}$	4	0.095	0.543	$0.000^{***}$	4	0.069	0.250	$0.000^{***}$	ю	0.045	0.278	$0.000^{***}$	З	0.000	0.653	$0.003^{**6}$
2 0.133 0.124 0.696 2 0.379 0.414 0.649 2 0.069 0.128 0.013 3 0.200 0.283 0.297 11 0.667 0.622 4 0.733 0.674 0.678 2 0.007 0.642 0.850 3 0.499 0.391 2 0.033 0.095 0.0007*** 2 0.233 0.206 6 0.776 0.776 0.776 0.733 0.748 0.106 2 0.033 0.095 0.0007*** 2 0.233 0.206 0.353 0.333 0.347 0.778 0.778 0.778 0.778 0.778 0.733 0.231 0.207** 3 0.100 0.000 $$ 5 0.333 0.255 0.646 0.356 0.006** 3 0.447 0.352 0.645 2 0.330 0.350 0.357 0.475 0.778 0.776 0.776 0.754 0.556 0.664 0.566 0.066* 3 0.417 0.352 0.645 2 0.330 0.255 0.546 2 0.333 0.347 0.007*** 4 0.700 0.656 0.006** 3 0.417 0.352 0.645 2 0.330 0.256 0.641 0.007*** 6 0.333 0.347 0.007*** 4 0.700 0.656 0.742 0.368 3 0.000 $$ 4 0.567 0.754 0.754 0.754 0.258 0.641 0.007*** 5 0.445 0.611 3 0.413 0.515 0.615 3 0.357 0.445 0.611 0.007*** 4 0.367 0.414 0.458 2 0.112° 3 0.000 0.667 0.112° 3 0.000 0.472 0.368 0.631 0.333 0.333 0.347 0.033 0.528 0.540 0.356 0.006** 3 0.000 0.667 0.112° 3 0.000 0.472 0.368 0.530 0.356 0.641 0.0007*** 4 0.357 0.445 0.671 3 0.403 0.547 0.358 0.540 0.358 0.540 0.556 0.0007 0.566 0.006** 2 0.000 0.667 0.112° 3 0.000 0.472 0.368 0.531 0.333 0.333 0.347 0.043 0.547 0.358 0.540 0.356 0.0007 0.566 0.006** 4 0.357 0.445 0.671 3 0.433 0.515 0.615 3 0.357 0.444 0.458 2 0.100 0.095 0.773 5 0.267 0.566 0.033 0.333 0.380 0.373 0.557 0.547 0.431 0.357 0.445 0.521 0.406 0.295 2 0.300 0.000 4 0.600 0.446 0.573 0.548 0.033 0.524 5 0.500 0.472 0.566 0.0007 4 0.560 0.075 0.572 0.561 0.572 0.571 0.574 0.556 0.0007 0.066 0.295 2 0.300 0.095 0.773 5 0.267 0.572 0.570 0.572 0.561 0.550 0.0000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.071 0.446 0.566 0.561 0.651 0.651 0.651 0.656 0.0007 0.055 0.540 0.0550 0.573 0.550 0.572 0.560 0.0752 0.561 0.572 0.560 0.0752 0.561 0.0551 0.571 0.551 0.550 0.0000 0.000	2 0.133 0.124 0.696 2 0.379 0.414 0.649 2 0.069 0.128 0.013 3 0.207 11 0.667 0.622 4 0.733 0.674 0.678 2 0.067 0.064 0.850 3 0.499 0.391 2 0.033 0.095 0.000**** 2 0.233 0.206 6 0.733 0.745 0.986 5 0.600 0.523 0.976 4 0.567 0.599 0.531 6 0.833 0.789 0.890 6 0.767 0.776 4 0.367 0.448 0.106 2 0.033 0.095 0.000*** 4 0.400 0.433 0.029* 1 0.000 0.000 $-^{\circ}$ 5 0.500 0.536 2 0.133 0.231 0.020* 3 0.100 0.239 0.000*** 4 0.700 0.443 0.065 0.546 5 2 0.300 0.000 $-^{\circ}$ 5 0.500 0.556 7 0.633 0.684 0.274 4 0.367 0.321 0.959 4 0.700 0.665 0.540 5 0.645 2 0.300 0.000 $-^{\circ}$ 5 0.567 0.574 6 0.286 0.641 0.000*** 6 0.333 0.347 1.000 7 0.667 0.742 0.368 3 0.000 0.000 $-^{\circ}$ 5 0.567 0.574 6 0.286 0.641 0.000*** 6 0.333 0.347 1.000 7 0.665 0.540 5 0.546 0.965 0.544 0.955 6 0.567 0.574 6 0.286 0.641 0.000*** 5 0.700 0.496 0.000*** 4 0.367 0.474 0.458 2 0.100 0.000 $-^{\circ}$ 5 0.567 0.577 3 0.337 0.445 0.671 3 0.433 0.515 0.617 0.413 0.458 2 0.300 0.300 0.000 $-^{-1}$ 4 0.600 0.476 5 0.367 0.378 5 0.700 0.637 0.837 0.474 0.458 2 0.300 0.339 0.524 5 0.500 0.575 9 0.800 0.840 0.725 7 0.567 0.577 0.431 8 0.667 0.681 0.831 8 0.767 0.730 0.944 6 0.700 0.773 mmber of alleles per population; $H_a = \expected heterozygosity; H_o = observed heterozygosity; HWE = P values of \chi^2 test of Hardy–Weinberg equilibrium.$	Phafor_00650	4	0.633	0.678	0.968	ŝ	0.100	0.096	0.994	З	0.433	0.429	0.752	0	0.167	0.206	0.295	4	0.400	0.408	0.106
4 0.733 0.674 0.678 2 0.067 0.064 0.850 3 0.637 0.499 0.391 2 0.033 0.095 0.000*** 2 0.233 0.206 0.767 0.776 0.776 0.773 0.748 0.106 2 0.033 0.095 0.000*** 4 0.567 0.599 0.531 6 0.833 0.789 0.890 6 0.767 0.776 0.776 0.733 0.367 0.448 0.106 2 0.033 0.095 0.000*** 3 0.417 0.352 0.645 2 0.300 0.556 0.576 0.595 0.540 3 0.000 $$ 5 0.500 0.556 0.757 0.574 0.753 0.386 0.641 0.000*** 3 0.417 0.352 0.667 0.112° 3 0.300 0.255 0.574 0.472 0.353 0.540 0.356 0.0667 0.146 0.965 0.546 0.956 0.757 0.574 0.472 0.333 0.387 0.445 0.671 0.321 0.000*** 4 0.367 0.472 0.368 0.664 0.965 6 0.567 0.574 0.472 0.353 0.531 0.445 0.671 0.321 0.000*** 4 0.367 0.403 0.255 1 0.000 $$ 4 0.600 0.445 0.656 3 0.333 0.533 0.540 0.550 0.095 0.577 0.574 0.472 0.556 0.656 0.566 0.354 0.373 0.571 0.576 0.576 0.574 0.472 0.556 0.000*** 4 0.367 0.473 0.556 0.006 $$ 4 0.600 0.472 0.556 0.666 0.556 0.570 0.574 0.472 0.556 0.546 0.565 0.570 0.576 0.574 0.472 0.353 0.333 0.338 0.338 0.331 0.347 0.043 0.557 0.473 0.566 0.445 0.566 0.046 0.255 1 0.000 0.00 0.000 $$ 4 0.600 0.472 0.556 0.656 0.656 0.656 0.096 0.000 0.000 0.000 $$ 4 0.560 0.572 0.656 0.656 0.656 0.333 0.339 0.524 5 0.500 0.572 0.557 0.572 0.570 0.572 0.576 0.576 0.576 0.546 0.525 7 0.500 0.572 0.576 0.576 0.576 0.576 0.566 0.546 0.556 0.546 0.556 0.546 0.556 0.546 0.556 0.566 0.566 0.566 0.566 0.556 0.566 0.566 0.566 0.566 0.556 0.566 0.566 0.556 0.566 0.556 0.566 0.556 0.	4 0.733 0.674 0.678 2 0.067 0.064 0.850 3 0.633 0.499 0.391 2 0.033 0.095 0.000*** 2 0.233 0.206 6 0.767 0.776 0.776 0.733 0.748 0.106 2 0.033 0.095 0.000*** 4 0.567 0.599 0.531 6 0.833 0.789 0.890 6 0.767 0.776 0.776 2 0.133 0.231 0.020** 3 0.417 0.352 0.645 2 0.500 0.536 0.536 0.646 0.966 0.060 $$ 5 0.500 0.255 7 0.633 0.206 0.000*** 3 0.417 0.352 0.645 2 0.300 0.255 7 0.533 0.333 0.333 0.333 0.333 0.331 0.333 0.331 0.333 0.331 0.333 0.331 0.333 0.331 0.333 0.331 0.333 0.333 0.333 0.333 0.333 0.333 0.331 0.333 0.331 0.333 0.331 0.333 0.331 0.333 0.333 0.333 0.333 0.357 0.446 0.965 0.540 0.346 0.965 0.567 0.574 0.476 0.472 0.356 0.667 0.112° 3 0.000 0.472 0.355 0.633 0.533 0.533 0.533 0.533 0.533 0.337 0.496 0.906 * 4 0.367 0.442 0.367 0.445 0.667 0.112° 3 0.333 0.330 0.330 0.330 0.330 0.330 0.330 0.347 0.035 0.647 0.357 0.446 0.955 0.647 0.573 0.572 0.633 0.558 0.567 0.574 0.575 0.574 0.557 0.574 0.556 0.563 0.564 0.356 0.566 0.000 0.000 0.000 0.472 0.333 0.528 0.567 0.572 0.337 0.496 0.9000*** 4 0.367 0.443 0.255 1 0.000 0.000 0.000 0.000 0.472 0.333 0.528 0.567 0.773 5 0.267 0.575 0.577 0.555 0.530 0.333 0.528 0.557 0.733 0.528 0.567 0.733 0.558 0.567 0.733 0.558 0.567 0.733 0.558 0.567 0.733 0.557 0.576 0.576 0.576 0.576 0.576 0.576 0.576 0.566 0.663 0.540 0.255 1 0.000 0.000 0.000 0.000 0.073 0.557 0.557 0.576 0.576 0.576 0.556 0.566 0.566 0.566 0.256 0.566 0.000 0.000 0.000 0.000 0.000 0.566 0.566 0.566 0.566 0.255 0.540 0.556 0.567 0.557 0.576 0.576 0.556 0.568 0.566 0.568 0.566 0.566 0.566 0.566 0.566 0.000 0.000 0.000 0.000 0.000 0.566 0.566 0.566 0.566 0.056 0.000 0.557 0.557 0.557 0.557 0.577 0.557 0.576 0.576 0.576 0.576 0.576 0.576 0.556 0.566 0.566 0.566 0.566 0.566 0.000 0.000 0.000 0.000 0.000 0.000 0.566 0.5	Phafor_00745	0	0.133	0.124	0.696	0	0.379	0.414	0.649	0	0.069	0.128	0.013	З	0.200	0.283	0.297	11	0.667	0.622	0.885
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	6 0.733 0.745 0.986 5 0.600 0.523 0.976 4 0.567 0.599 0.531 6 0.833 0.789 0.890 6 0.767 0.776 0.776 4 0.367 0.448 0.106 2 0.033 0.095 0.000*** 4 0.400 0.433 0.029* 1 0.000 0.000 $-e$ 5 0.500 0.536 2 0.133 0.231 0.020* 3 0.100 0.239 0.000*** 4 0.400 0.433 0.029* 1 0.000 0.000 $-e$ 5 0.500 0.536 1 0.633 0.533 0.684 0.274 4 0.367 0.321 0.959 4 0.770 0.665 0.540 5 0.546 5 0.566 0.645 0.579 0.774 1 0.352 0.645 0.333 0.333 0.337 0.445 0.671 3 0.443 0.515 0.615 0.367 0.742 0.368 3 0.000 0.667 0.112° 3 0.000 0.472 3 0.333 0.333 0.538 0.671 3 0.446 0.965 0.540 5 0.540 5 0.540 5 0.540 5 0.570 0.574 0.472 0.333 0.333 0.533 0.533 0.533 0.531 0.000 0.667 0.112° 3 0.000 0.472 3 0.333 0.533 0.533 0.533 0.533 0.531 0.500 0.496 0.000*** 4 0.367 0.444 0.455 2 0.000 0.000 $$ 5 0.500 0.446 0.955 5 0.560 0.005** 3 0.331 0.333 0.339 0.337 0.435 0.571 0.555 0.533 0.588 0.567 0.773 5 0.257 0.572 0.573 0.555 0.533 0.533 0.330 0.325 7 0.537 0.445 0.455 0.500 0.000 $$ 4 0.357 0.445 0.567 0.773 5 0.700 0.057 0.575 0.572 0.573 0.572 0.556 0.000 0.000 $$ 5 0.760 0.773 5 0.757 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.567 0.572 0.566 0.0840 0.0255 2 0.700 0.093 0.572 5 0.700 0.572 0.567 0.572 0.572 0.566 0.0660 0.773 5 0.767 0.572 0.656 0.0660 0.773 5 0.767 0.773 5 0.767 0.773 5 0.767 0.773 5 0.767 0.773 5 0.767 0.773 5 0.767 0.773 5 0.767 0.773 10.000 0.000 $$ 4 0.651 0.811 0.831 0.830 0.372 0.557 0.744 6 0.770 0.773 10.0001 0.094 16 0.773 10.0001 0.094 16 0.773 0.544 5 0.770 0.773 10.0001 0.001 $$ 4 0.661 0.681 0.981 0.973 0.851 0.773 0.924 6 0.700 0.773 0.572 0.765 0.700 0.565 0.766 0.591 0.773 5 0.767 0.773 10.0001 0.073 0.939 0.773 10.0561 0.773 10.0001 0.773 0.924 16 0.770 0.773 0.9561 0.770 0.773 0.9561 0.770 0.773 0.9561 0.773 0.9561 0.770 0.773 0.9561 0.770 0.773 0.9561 0.770 0.755 0.766 0.766 0.770 0.752 0.766 0.770 0.773 0.9761 0.773 0.9761 0.770 0.770 0.9761 0.773 0.9761 0.770 0.770 0.9761 0.773 0.9761 0.770 0.770 0.770 0.770 0.770 0.770 0.770 0.770 0.770 0.770 0.770 0.	Phafor_01477	4	0.733	0.674	0.678	0	0.067	0.064	0.850	З	0.633	0.499	0.391	0	0.033	0.095	$0.000^{***}$	0	0.233	0.206	0.469
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	4 $0.367$ $0.448$ $0.106$ 2 $0.033$ $0.095$ $0.000^{***}$ 4 $0.400$ $0.433$ $0.029^{*}$ 1 $0.000$ $0.000$ $-^{c}$ 5 $0.500$ $0.536$ $0.255$ 2 $0.133$ $0.231$ $0.020^{*}$ 3 $0.100$ $0.239$ $0.000^{***}$ 3 $0.217$ $0.532$ $0.645$ 2 $0.300$ $0.255$ 7 $0.633$ $0.264$ $0.965$ $0.540$ $0.25$ $0.574$ $0.574$ $0.255$ $0.586$ $0.641$ $0.2065$ $0.574$ $0.574$ $0.255$ $0.570$ $0.574$ $0.574$ $0.357$ $0.647$ $0.112^{c}$ $3$ $0.000$ $0.472$ $0.573$ $0.635$ $0.536$ $0.646$ $0.965$ $0.540$ $0.955$ $6$ $0.567$ $0.574$ $0.574$ $0.573$ $0.533$ $0.633$ $0.671$ $3$ $0.475$ $0.647$ $0.112^{c}$ $3$ $0.000$ $0.472$ $0.533$ $0.533$ $0.525$ $3$ $0.500$ $0.006^{***}$ $4$ $0.367$ $0.446$ $0.955$ $6$ $0.567$ $0.746$ $0.955$ $0.540$ $0.572$ $0.533$ $0.533$ $0.523$ $0.533$ $0.523$ $0.500$ $0.446$ $0.955$ $0.540$ $0.515$ $0.646$ $0.956$ $0.540$ $0.572$ $0.572$ $0.533$ $0.533$ $0.528$ $0.530$ $0.373$ $0.533$ $0.529$ $0.773$ $5$ $0.257$ $0.555$ $0.530$ $0.572$ $0.530$ $0.572$ $0.550$ $0.572$ $0.560$ $0.773$ $5$ $0.550$ $0.572$ $0.570$ $0.773$ $0.560$ $0.773$ $0.572$ $0.570$ $0.773$ $0.570$ $0.773$ $0.560$ $0.773$ $0.570$ $0.773$ $0.560$ $0.773$ $0.560$ $0.770$ $0.572$ $0.500$ $0.773$ $0.560$ $0.773$ $0.560$ $0.773$ $0.572$ $0.560$ $0.770$ $0.572$ $0.560$ $0.770$ $0.572$ $0.560$ $0.770$ $0.572$ $0.560$ $0.770$ $0.572$ $0.560$ $0.773$ $0.560$ $0.773$ $0.560$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.773$ $0.560$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.572$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.773$ $0.560$ $0.770$ $0.$	Phafor_01499c	9	0.733	0.745	0.986	S	0.600	0.523	0.976	4	0.567	0.599	0.531	9	0.833	0.789	0.890	9	0.767	0.776	0.296
2 0.133 0.231 0.020* 3 0.100 0.239 0.000*** 3 0.286 0.506 0.066* 3 0.417 0.352 0.645 2 0.300 0.255 7 0.633 0.684 0.274 4 0.367 0.321 0.959 4 0.700 0.665 0.540 5 0.586 0.666 0.965 6 0.567 0.574 6 0.286 0.641 0.000*** 6 0.333 0.347 1.000 7 0.667 0.742 0.368 3 0.000 0.667 0.112° 3 0.000 0.472 3 0.367 0.445 0.671 3 0.433 0.515 0.615 3 0.367 0.403 0.255 1 0.000 0.067 0.112° 3 0.000 0.446 5 0.633 0.628 0.955 3 0.500 0.496 0.000*** 4 0.367 0.474 0.458 2 0.100 0.095 0.773 5 0.267 0.565 3 0.333 0.380 0.372 0.572 0.637 0.645 0.682 4 0.321 0.406 0.295 2 0.300 0.093 0.773 5 0.267 0.565 3 0.333 0.580 0.340 0.725 7 0.567 0.707 0.437 8 0.667 0.681 0.831 8 0.767 0.780 0.944 6 0.700 0.773 mber of alleles per population; $H_e = \text{expected heterozygosity}; HWE = P \text{ values of } \chi^2 \text{ test of Hardy-Weinberg equilibrium.}$	2 0.133 0.231 0.020* 3 0.100 0.239 0.000*** 3 0.286 0.506 0.006** 3 0.417 0.352 0.645 2 0.300 0.255 7 0.633 0.684 0.274 4 0.367 0.321 0.959 4 0.700 0.665 0.540 5 0.586 0.646 0.965 6 0.567 0.574 6 0.286 0.641 0.000*** 6 0.333 0.347 1.000 7 0.667 0.742 0.368 3 0.000 0.667 0.112° 3 0.000 0.472 3 0.367 0.445 0.671 3 0.433 0.515 0.615 3 0.367 0.474 0.458 2 0.100 0.000 4 0.600 0.446 5 0.633 0.628 0.955 3 0.500 0.496 0.000*** 4 0.367 0.474 0.458 2 0.100 0.000 4 0.666 5 0.633 0.628 0.955 3 0.500 0.496 0.000*** 4 0.321 0.406 0.295 2 0.300 0.339 0.573 5 0.567 0.572 3 0.333 0.628 0.955 3 0.500 0.496 0.000*** 4 0.367 0.474 0.458 2 0.100 0.095 0.773 5 0.567 0.572 9 0.800 0.840 0.725 7 0.567 0.707 0.437 8 0.667 0.681 0.831 8 0.767 0.780 0.944 6 0.700 0.773 mber of alleles per population: $H_e = \text{expected heterozygosity}; HWE = P \text{ values of } \chi^2 \text{ test of Hardy-Weinberg equilibrium.}$	Phafor_01817c	4	0.367	0.448	0.106	0	0.033	0.095	$0.000^{***}$	4	0.400	0.433	0.029*	1	0.000	0.000	°	5	0.500	0.536	0.875
7 0.633 0.684 0.274 4 0.367 0.321 0.959 4 0.700 0.665 0.540 5 0.586 0.646 0.965 6 0.567 0.574 0.574 6 0.286 0.641 0.000*** 6 0.333 0.347 1.000 7 0.667 0.742 0.368 3 0.000 0.667 0.112° 3 0.000 0.472 3 0.367 0.472 3 0.367 0.472 3 0.333 0.347 1.000 0.000 $$ 4 0.600 0.446 5 0.567 0.574 $$ 5 0.533 0.538 0.641 0.000 $$ 5 0.567 0.475 $$ 5 0.539 0.558 $$ 5 0.530 0.446 $$ 5 0.530 0.446 $$ 5 0.530 0.446 $$ 5 0.530 0.446 $$ 5 0.500 0.446 $$	7 0.633 0.684 0.274 4 0.367 0.321 0.959 4 0.700 0.665 0.540 5 0.586 0.646 0.965 6 0.567 0.574 6 0.286 0.641 0.000*** 6 0.333 0.347 1.000 7 0.667 0.742 0.368 3 0.000 0.667 0.112° 3 0.000 0.472 3 0.367 0.472 3 0.367 0.472 0.368 3 0.000 0.667 0.112° 3 0.000 0.472 5 0.533 0.345 0.445 0.615 0.333 0.357 0.496 0.955 3 0.515 0.000 *** 4 0.367 0.474 0.458 2 0.100 0.095 0.773 5 0.267 0.565 3 0.563 0.580 0.546 0.956 0.540 0.572 0.567 0.746 5 0.572 0.572 0.561 0.446 0.965 0.540 0.445 0.667 0.112° 3 0.000 0.472 0.368 3 0.533 0.533 0.533 0.533 0.533 0.530 0.373 5 0.500 0.446 0.565 3 0.533 0.538 0.538 0.538 0.572 0.500 0.474 0.458 2 0.100 0.095 0.773 5 0.567 0.572 0.572 0.566 0.566 0.572 0.581 0.841 0.555 1 0.000 0.000 4 0.567 0.572 0.572 0.567 0.572 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.567 0.572 0.569 0.572 0.569 0.572 0.567 0.561 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.566 0.566 0.566 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.566 0.566 0.566 0.566 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.572 0.569 0.566 0.566 0.566 0.566 0.566 0.566 0.566 0.566 0.566 0.566 0.566 0.572 0.569 0.566 0.566 0.566 0.566 0.566 0.557 0.568 0.566 0.568 0.566 0.568 0.566 0.568 0.566 0.568 0.568 0.568 0.568 0.568 0.568 0.568 0.568 0.568 0.572 0.578 0.57	Phafor_02245	0	0.133	0.231	0.020*	б	0.100	0.239	$0.000^{***}$	ŝ	0.286	0.506	$0.006^{**}$	З	0.417	0.352	0.645	0	0.300	0.255	0.334
6 0.286 0.641 0.000*** 6 0.333 0.347 1.000 7 0.667 0.742 0.368 3 0.000 0.667 0.112° 3 0.000 0.472 0.472 0.368 3 0.367 0.445 0.671 3 0.433 0.515 0.615 3 0.367 0.445 0.671 0.112° 3 0.000 0.446 5 0.445 0.671 3 0.433 0.515 0.615 3 0.367 0.474 0.458 2 0.100 0.095 0.773 5 0.267 0.656 3 0.333 0.333 0.338 0.378 5 0.700 0.637 0.437 0.437 0.406 0.295 2 0.300 0.339 0.524 5 0.500 0.572 0.572 0.866 0.700 0.840 0.725 7 0.567 0.437 8 0.667 0.681 0.831 8 0.767 0.778 0.944 6 0.700 0.773 mmber of alleles per population; $H_e = \text{expected heterozygosity}; H_o = \text{observed heterozygosity}; HWE = P \text{ values of } \chi^2 \text{ test of Hardy-Weinberg equilibrium.}$	6 0.286 0.641 0.000*** 6 0.333 0.347 1.000 7 0.667 0.742 0.368 3 0.000 0.667 0.112° 3 0.000 0.472 3 0.367 0.472 0.368 3 0.000 0.677 0.112° 3 0.000 0.472 5 0.367 0.445 0.671 3 0.433 0.515 0.615 3 0.367 0.446 0.446 5 0.600 0.446 5 0.445 0.671 3 0.500 0.496 0.000*** 4 0.367 0.474 0.458 2 0.100 0.009 0.773 5 0.567 0.656 3 0.333 0.338 0.338 0.373 5 0.567 0.572 9 0.800 0.840 0.725 7 0.567 0.682 4 0.321 0.406 0.295 2 0.300 0.339 0.524 5 0.500 0.773 5 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.572 0.773 10 0.676 0.572 9 0.800 0.840 0.725 7 0.567 0.637 0.437 8 0.667 0.681 0.831 8 0.767 0.780 0.944 6 0.700 0.773 15 0.570 0.572 0.773 15 0.500 0.773 15 0.566 0.770 0.572 0.566 0.572 0.601 0.446 0.572 0.581 0.581 0.831 18 0.767 0.780 0.944 6 0.770 0.773 15 0.570 0.773 15 0.570 0.773 15 0.570 0.773 15 0.570 0.773 15 0.570 0.773 15 0.570 0.572 0.566 0.572 0.667 0.681 0.831 18 0.767 0.780 0.944 6 0.770 0.773 15 0.570 0.773 15 0.770 0.773 15 0.750 0.773 15 0.750 0.773 15 0.770 0.773 15 0.750 0.773 15 0.770 0.773 15 0.750 0.773 15 0.770 0.773 15 0.770 0.773 15 0.770 0.773 15 0.770 0.773 15 0.770 0.773 15 0.750 0.773 15 0.770 0.773 15 0.750 0.773 15 0.770 0.773 15 0.770 0.773 15 0.770 0.773 15 0.750 0.770 0.773 15 0.	Phafor_02638	2	0.633	0.684	0.274	4	0.367	0.321	0.959	4	0.700	0.665	0.540	S	0.586	0.646	0.965	9	0.567	0.574	0.718
3 0.367 0.445 0.671 3 0.433 0.515 0.615 3 0.367 0.403 0.255 1 0.000 0.000 — 4 0.600 0.446 5 0.633 0.628 0.955 3 0.500 0.496 0.000**** 4 0.367 0.474 0.458 2 0.100 0.095 0.773 5 0.267 0.656 3 0.333 0.380 0.378 5 0.700 0.637 0.682 4 0.321 0.406 0.295 2 0.300 0.339 0.524 5 0.500 0.773 9 0.800 0.840 0.725 7 0.567 0.707 0.437 8 0.667 0.681 0.831 8 0.767 0.780 0.944 6 0.700 0.773 mber of alleles per population; $H_e = \text{expected heterozygosity}; H_0 = \text{observed heterozygosity}; HWE = P \text{ values of } \chi^2 \text{ test of Hardy-Weinberg equilibrium.}$	3 0.367 0.445 0.671 3 0.433 0.515 0.615 3 0.367 0.403 0.255 1 0.000 0.000 — 4 0.600 0.446 5 0.633 0.628 0.955 3 0.500 0.496 0.000*** 4 0.367 0.474 0.458 2 0.100 0.095 0.773 5 0.267 0.656 3 0.333 0.380 0.378 5 0.700 0.637 0.682 4 0.321 0.406 0.295 2 0.300 0.339 0.524 5 0.500 0.773 9 0.800 0.840 0.725 7 0.567 0.707 0.437 8 0.667 0.681 0.831 8 0.767 0.780 0.944 6 0.700 0.773 mmber of alleles per population; $H_e = \exp$ ected heterozygosity; $H_0 = \text{observed heterozygosity};$ HWE = $P$ values of $\chi^2$ test of Hardy–Weinberg equilibrium.	Phafor_02824	9	0.286	0.641	$0.000^{***}$	9	0.333	0.347	1.000	٢	0.667	0.742	0.368	З	0.000	0.667	$0.112^{c}$	б	0.000	0.472	$0.000^{**:}$
5 0.633 0.628 0.955 3 0.500 0.496 0.000*** 4 0.367 0.474 0.458 2 0.100 0.095 0.773 5 0.267 0.656 3 0.333 0.338 0.378 5 0.700 0.637 0.682 4 0.321 0.406 0.295 2 0.300 0.339 0.524 5 0.500 0.572 9 0.800 0.840 0.725 7 0.567 0.637 0.637 0.656 0.651 0.681 0.831 8 0.767 0.780 0.944 6 0.700 0.773 mmber of alleles per population; $H_e = \exp$ deterozygosity; $H_0 = observed$ heterozygosity; HWE = $P$ values of $\chi^2$ test of Hardy–Weinberg equilibrium.	5 0.633 0.628 0.955 3 0.500 0.496 0.000*** 4 0.367 0.474 0.458 2 0.100 0.095 0.773 5 0.267 0.656 3 0.333 0.380 0.378 5 0.700 0.637 0.682 4 0.321 0.406 0.295 2 0.300 0.339 0.524 5 0.500 0.572 9 0.800 0.840 0.725 7 0.567 0.707 0.437 8 0.667 0.681 0.831 8 0.767 0.780 0.944 6 0.700 0.773 unber of alleles per population; $H_e = \exp$ ected heterozygosity; $H_0 = \text{observed heterozygosity};$ HWE = $P$ values of $\chi^2$ test of Hardy–Weinberg equilibrium.	Phafor_03037	Э	0.367	0.445	0.671	б	0.433	0.515	0.615	ŝ	0.367	0.403	0.255	-	0.000	0.000		4	0.600	0.446	0.480
3 0.333 0.380 0.378 5 0.700 0.637 0.682 4 0.321 0.406 0.295 2 0.300 0.339 0.524 5 0.500 0.572 9 0.800 0.840 0.725 7 0.567 0.707 0.437 8 0.667 0.681 0.831 8 0.767 0.780 0.944 6 0.700 0.773 umber of alleles per population; $H_e = \expected$ heterozygosity; $H_o = observed$ heterozygosity; HWE = P values of $\chi^2$ test of Hardy–Weinberg equilibrium. Id locality information are provided in Appendix 1.	3 0.333 0.380 0.378 5 0.700 0.637 0.682 4 0.321 0.406 0.295 2 0.300 0.339 0.524 5 0.500 0.572 9 0.800 0.840 0.725 7 0.567 0.707 0.437 8 0.667 0.681 0.831 8 0.767 0.780 0.944 6 0.700 0.773 umber of alleles per population; $H_e = \expected$ heterozygosity; $H_o = b$ served heterozygosity; HWE = $P$ values of $\chi^2$ test of Hardy–Weinberg equilibrium.	Phafor_03754	S	0.633	0.628	0.955	б	0.500	0.496	$0.000^{***}$	4	0.367	0.474	0.458	0	0.100	0.095	0.773	2	0.267	0.656	$0.000^{**:}$
9 0.800 0.840 0.725 7 0.567 0.707 0.437 8 0.667 0.681 0.831 8 0.767 0.780 0.944 6 0.700 0.773 0. mber of alleles per population; $H_e = \exp$ expected heterozygosity; $H_o = \text{observed heterozygosity}$ ; $HWE = P$ values of $\chi^2$ test of Hardy–Weinberg equilibrium. d locality information are provided in Appendix 1.	9 0.800 0.840 0.725 7 0.567 0.707 0.437 8 0.667 0.681 0.831 8 0.767 0.780 0.944 6 0.700 ( $I_{\rm mber}$ of alleles per population; $H_{\rm e} =$ expected heterozygosity; $H_{\rm o} =$ observed heterozygosity; $HWE = P$ values of $\chi^2$ test of Hardy–Weinberg equilibrium.	Phafor_05461c	Э	0.333	0.380	0.378	S	0.700	0.637	0.682	4	0.321	0.406	0.295	0	0.300	0.339	0.524	2	0.500	0.572	0.759
Note: $A =$ number of alleles per population; $H_e =$ expected heterozygosity; $H_0 =$ observed heterozygosity; HWE = P values of $\chi^2$ test of Hardy–Weinberg equilibrium. <sup>a</sup> Voucher and locality information are provided in Appendix 1.	<i>Note:</i> $A =$ number of alleles per population; $H_e =$ expected heterozygosity; $H_o =$ observed heterozygosity; HWE = $P$ values of $\chi^2$ test of Hardy–Weinberg equilibrium.	Phafor_13597s	6	0.800	0.840	0.725	Г	0.567	0.707	0.437	8	0.667	0.681	0.831	×	0.767	0.780	0.944	9	0.700	0.773	0.885
<sup>a</sup> Voucher and locality information are provided in Appendix 1.	<sup>a</sup> Voucher and locality information are provided in Appendix 1.	Note: $A = num$	ber of	f alleles j	per popul	ation; $H_{\rm e} =$	expeci	ted heterc	)zygosity;	$H_0 = obser$	ved h	eterozygc	sity; HW	E = P values	of $\chi^{i}$	test of ]	Hardy-We	sinberg equil	ibriun			
	$bC^{-1}$	<sup>a</sup> Voucher and 1	ocalit	y inform	nation are	provided in	ι Appe	ndix 1.														

Genetic characterization of 15 newly developed polymorphic microsatellite loci in Phacelia formosula, P. gina-glenneae, and a Phacelia population of uncertain specific status.<sup>4</sup>

TABLE 2.

Results based on >50% missing data due to poor amplification.

Taper, 2006; van Oosterhout et al., 2006), and we encourage their use with these markers. These microsatellite markers constitute a valuable tool for fine-scale genetic investigations in the genus *Phacelia*, as well as conservation of rare *Phacelia* species.

### LITERATURE CITED

- ACKERFIELD, J. 2015. Flora of Colorado. Botanical Research Institute of Texas, Fort Worth, Texas, USA.
- APG IV. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* 181: 1–20.
- CHAPUIS, M. P., AND A. ESTOUP. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* 24: 621–631.
- KALINOWSKI, S., AND M. TAPER. 2006. Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. *Conservation Genetics* 7: 991–995.
- KEARSE, M., R. MOIR, A. WILSON, S. STONES-HAVAS, M. CHEUNG, S. STURROCK, S. BUXTON, ET AL. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)* 28: 1647–1649.
- LEVY, F., AND C. L. NEAL. 1999. Spatial and temporal genetic structure in chloroplast and allozyme markers in *Phacelia dubia* implicate genetic drift. *Heredity* 82: 422–431.
- LEVY, F., J. ANTONOVICS, J. E. BOYNTON, AND N. W. GILLHAM. 1996. A population genetic analysis of chloroplast DNA in *Phacelia*. *Heredity* 76: 143–155.

- LUEBERT, F., L. CECCHI, M. W. FROHLICH, M. GOTTSCHLING, C. M. GUILLIAMS, K. E. HASENSTAB-LEHMAN, H. H. HILGER, ET AL. 2016. Familial classification of the Boraginales. *Taxon* 65: 502–522.
- NATURESERVE. 2017. NatureServe Explorer: An online encyclopedia of life [web application]. Version 7.0. NatureServe, Arlington, Virginia, USA. Website http://explorer.natureserve.org [accessed 15 February 2017].
- PEAKALL, R., AND P. E. SMOUSE. 2006. GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.
- RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248–249.
- ROUSSET, F. 2008. GENEPOP'007: A complete reimplementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.
- ROZEN, S., AND H. SKALETSKY. 1999. Primer3 on the WWW for general users and for biologist programmers. *In* S. Misener and S. A. Krawetz [eds.], Methods in molecular biology, vol. 132: Bioinformatics methods and protocols, 365–386. Humana Press, Totowa, New Jersey, USA.
- SCHUELKE, M. 2000. An economic method for the fluorescent labeling of PCR fragments: A poor man's approach to genotyping for research and high-throughput diagnostics. *Nature Biotechnology* 18: 233–234.
- USDA, NRCS. 2017. The PLANTS Database. National Plant Data Team, Greensboro, North Carolina, USA. Website http://plants.usda.gov [accessed 17 February 2017].
- U.S. FISH AND WILDLIFE SERVICE. 2011. 5-Year review of *Phacelia formosula* (North Park phacelia), December 2011. U.S. Fish and Wildlife Service, Western Colorado Field Office, Grand Junction, Colorado, USA.
- VAN OOSTERHOUT, D., D. WEETMAN, AND W. F. HUTCHINSON. 2006. Estimation and adjustment of microsatellite null alleles in nonequilibrium populations. *Molecular Ecology Notes* 6: 255–256.

APPENDIX 1. Voucher and general location information for five *Phacelia* populations used in this study.

Species	Population	Locality <sup>a</sup>	Ν	Voucher (Accession no.) <sup>b</sup>
Phacelia formosula Osterh.	JC1	Jackson County, Colorado, USA	30	M. Islam 1487 (KHD00062092)
	JC2	Jackson County, Colorado, USA	30	N. D. Atwood 33622 (BRY-V 0050698)
	JC3	Jackson County, Colorado, USA	30	N. D. Atwood 33558 (BRY-V 0050700)
<i>Phacelia</i> sp.	LC	Larimer County, Colorado, USA	30	M. Islam 1489 (KHD00062091)
Phacelia gina-glenneae N. D. Atwood & S. L. Welsh	GC	Grand County, Colorado, USA	30	M. Islam 12-271 (KHD00051791)

N = number of individuals sampled.

<sup>a</sup>Detailed location information has been omitted due to the protected status of these species.

<sup>b</sup>One voucher was collected from each sampled population. Vouchers were deposited at the Kathryn Kalmbach Herbarium (KHD), Denver Botanic Gardens, Denver, Colorado, USA, or the S. L. Welsh Herbarium (BRY), Brigham Young University, Provo, Utah, USA.