

**Supplementary Materials:** Figure S1; Figure S2; Table S1; Table S2

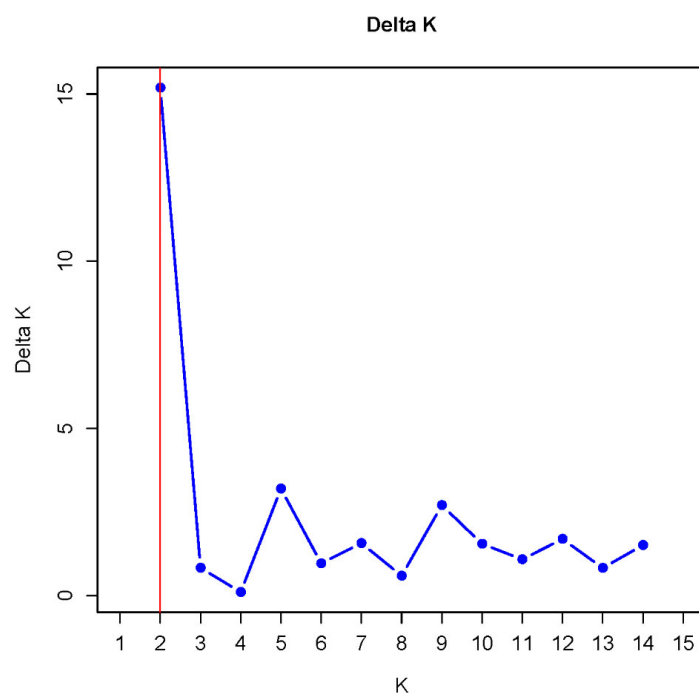


**Figure S1.** Inference of the most probable number of genetic clusters ( $K$ ) using STRUCTURE. The  $\Delta K$  statistic calculated according to the method of Evanno et al. [69] (second-order rate of change in the log-likelihood) is plotted as a function of  $K$ , based on 12 replicate runs for each  $K$  value.

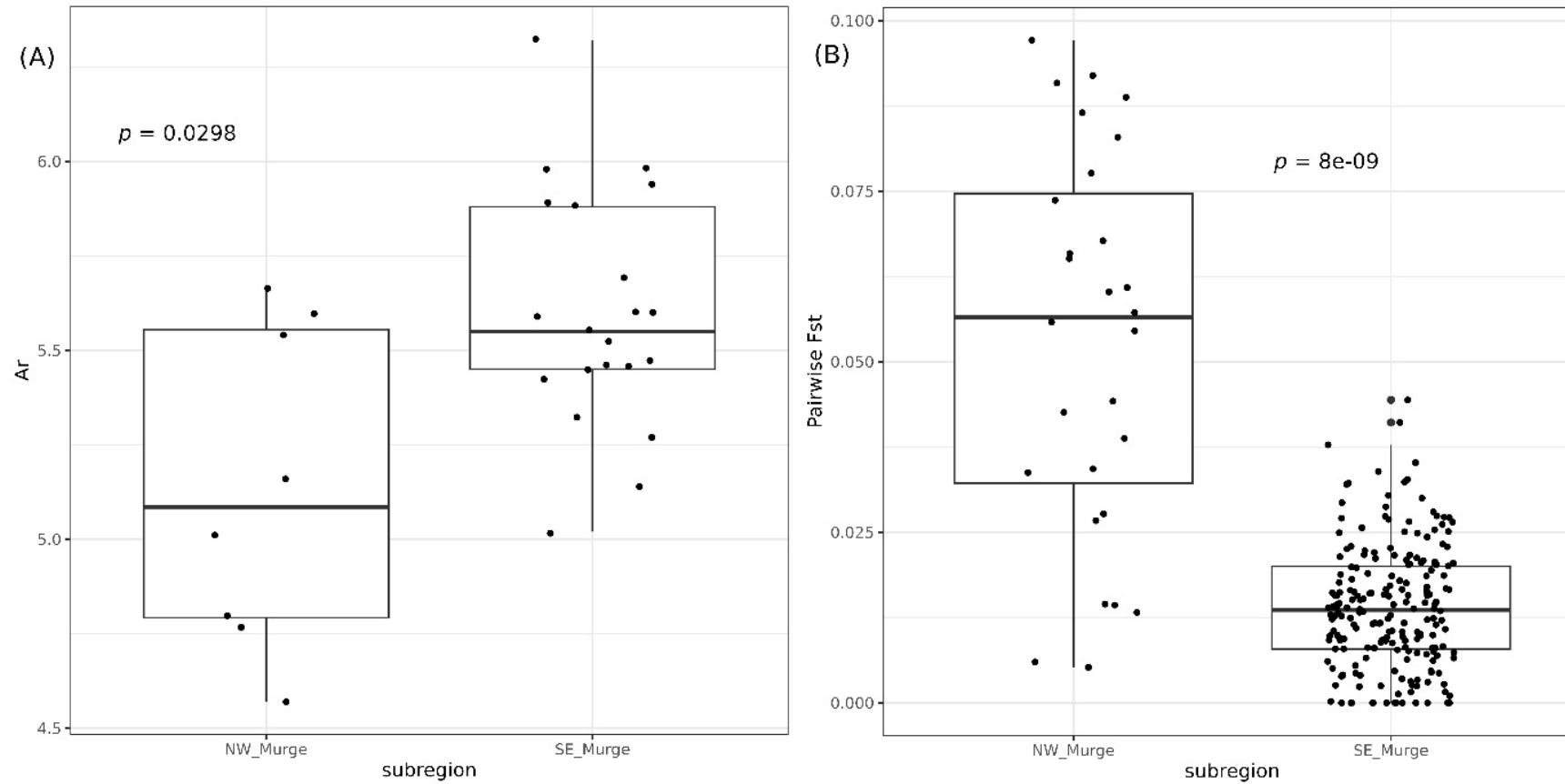


**Figure S2.** Comparison of genetic diversity and differentiation between the North-Western (NW) and South-Eastern (SE) Murge subregions. (A) Allelic richness (Ar) per population, calculated using rarefaction with a standardized sample size of  $N = 9$ , showing significantly higher values in SE Murge (Wilcoxon rank-sum test,  $p < 0.05$ ). (B) Distribution of pairwise  $F_{ST}$  values within each subregion, showing significantly higher genetic differentiation among populations in NW Murge (Brunner–Munzel test,  $p < 0.001$ ). The number of pairwise comparisons was  $N = 28$  for NW\_Murge and  $N = 210$  for SE\_Murge.

**Table S1.** Description of the 30 *Quercus trojana* forest stands sampled in southern Italy, covering the North-Western (NW) Murge and South-Eastern (SE) Murge subregions: identification code, sampling location, number of trees, geographical coordinates (decimal degrees), and elevation. Populations in bold correspond to the 14 newly sampled populations included in this study. The remaining 16 populations were previously collected and analysed using six SSR markers by Carabeo et al. [18].

| Population name                | Code | Location                                                  | Province (Region)   | No. trees | Longitude | Latitude | Elevation (m asl) |
|--------------------------------|------|-----------------------------------------------------------|---------------------|-----------|-----------|----------|-------------------|
| <i>NW Murge region</i>         |      |                                                           |                     |           |           |          |                   |
| <b>Bosco Acquatetta</b>        | BAQ  | National Park of Alta Murgia <sup>1</sup>                 | B-A-T (Apulia)      | 17        | 16.1325   | 41.0205  | 545               |
| Masseria Trullo di Mezzo       | TDM  | National Park of Alta Murgia <sup>1</sup>                 | Bari (Apulia)       | 20        | 16.3124   | 40.9662  | 604               |
| <b>Bosco Pulicchie</b>         | BPL  | National Park of Alta Murgia <sup>1</sup>                 | Bari (Apulia)       | 12        | 16.4382   | 40.8999  | 545               |
| <b>Jazzo Pietre Tagliate</b>   | JPT  | National Park of Alta Murgia <sup>1</sup>                 | Bari (Apulia)       | 5         | 16.5219   | 40.9785  | 387               |
| Lama Corriere                  | MAC  | North-Western Murge                                       | Bari (Apulia)       | 25        | 16.5373   | 40.8923  | 436               |
| Giustino                       | GIU  | National Park of Alta Murgia <sup>1</sup>                 | Bari (Apulia)       | 14        | 16.7441   | 40.8419  | 462               |
| <b>Contrada Murgia Timone</b>  | CMT  | Regional Park of the Murgia Materana <sup>1</sup>         | Matera (Basilicata) | 17        | 16.6312   | 40.6710  | 417               |
| <b>Rene delle Mandorle</b>     | REM  | North-Western Murge                                       | Bari (Apulia)       | 9         | 16.4031   | 40.7357  | 434               |
| Parata                         | PAR  | North-Western Murge                                       | Bari (Apulia)       | 13        | 16.7487   | 40.7715  | 467               |
| <i>SE Murge region</i>         |      |                                                           |                     |           |           |          |                   |
| <b>Masseria Selva Malvezzi</b> | MSM  | Regional Park of the Murgia Materana <sup>1</sup>         | Matera (Basilicata) | 27        | 16.7031   | 40.6064  | 361               |
| <b>Selva San Vito</b>          | SSV  | Regional Natural Park of Terra delle Gravine <sup>1</sup> | Taranto (Apulia)    | 31        | 16.8113   | 40.6199  | 349               |
| <b>Gravinella</b>              | GRA  | Regional Natural Park of Terra delle Gravine <sup>1</sup> | Taranto (Apulia)    | 15        | 16.9392   | 40.6833  | 246               |
| <b>Bosco Sant'Antuono</b>      | BSA  | Regional Natural Park of Terra delle Gravine <sup>1</sup> | Taranto (Apulia)    | 15        | 17.0992   | 40.6632  | 406               |
| <b>Masseria Signora</b>        | SIG  | Natural Reserve of the Murge Sud-orientali <sup>1</sup>   | Taranto (Apulia)    | 20        | 17.1791   | 40.6715  | 445               |
| <b>Masseria Gorgofreddo</b>    | GOF  | Natural Reserve of the Murge Sud-orientali <sup>1</sup>   | Taranto (Apulia)    | 20        | 17.2718   | 40.6926  | 452               |
| <b>Bosco di Monteferraro</b>   | BMF  | South-Eastern Murge                                       | Bari (Apulia)       | 20        | 17.0583   | 40.9125  | 254               |
| Santuario                      | SAN  | South-Eastern Murge                                       | Bari (Apulia)       | 20        | 17.0415   | 40.7980  | 388               |
| Galeone                        | GAL  | South-Eastern Murge                                       | Taranto (Apulia)    | 20        | 17.2171   | 40.7361  | 472               |
| Colucci                        | COL  | South-Eastern Murge                                       | Taranto (Apulia)    | 20        | 17.2031   | 40.6809  | 489               |

|                           |     |                     |                   |    |         |         |     |
|---------------------------|-----|---------------------|-------------------|----|---------|---------|-----|
| Palazzolo                 | PAL | South-Eastern Murge | Taranto (Apulia)  | 20 | 17.2238 | 40.7128 | 468 |
| Bosco Pianelle            | PIA | South-Eastern Murge | Taranto (Apulia)  | 18 | 17.2165 | 40.6707 | 480 |
| Mangiato                  | MAN | South-Eastern Murge | Taranto (Apulia)  | 20 | 17.2626 | 40.7441 | 420 |
| Croce Grande              | CRO | South-Eastern Murge | Taranto (Apulia)  | 19 | 17.2480 | 40.6776 | 513 |
| Locorotondo               | LOC | South-Eastern Murge | Bari (Apulia)     | 14 | 17.3165 | 40.7501 | 406 |
| <b>Masseria Palesi</b>    | MPL | South-Eastern Murge | Taranto (Apulia)  | 20 | 17.3057 | 40.6648 | 427 |
| Ciccio la Sorte           | SOR | South-Eastern Murge | Taranto (Apulia)  | 17 | 17.3761 | 40.6611 | 384 |
| Verdurizzo                | VER | South-Eastern Murge | Taranto (Apulia)  | 20 | 17.3869 | 40.6476 | 383 |
| Presidente                | PRE | South-Eastern Murge | Taranto (Apulia)  | 19 | 17.4140 | 40.6526 | 366 |
| Papariello                | PAP | South-Eastern Murge | Brindisi (Apulia) | 19 | 17.4201 | 40.6710 | 327 |
| <b>Masseria Termetrio</b> | TER | South-Eastern Murge | Brindisi (Apulia) | 20 | 17.4558 | 40.7509 | 307 |

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<sup>1</sup>Protected areas.

**Table S2.** Genetic characterization and polymorphism of the 10 selected SSR markers grouped into multiplexes A, B, C, and D for 546 *Quercus trojana* individuals across 30 populations. For each locus, the following parameters are reported: observed (Ao) and effective (Ae) number of alleles, number of private alleles (Pa), Shannon's index (I), observed (Ho) and expected (He) heterozygosity, unbiased expected heterozygosity (uHe), within-population inbreeding coefficient ( $F_{IS}$ ), total-population inbreeding coefficient ( $F_{IT}$ ), among-population genetic differentiation ( $F_{ST}$ ), and polymorphic information content (PIC).

| Locus              | Dye   | Size<br>(bp) | Ao | Ae   | Pa | I    | Ho   | He   | uHe  | $F_{IS}^1$ | $F_{IT}^1$ | $F_{ST}^1$ | PIC  |
|--------------------|-------|--------------|----|------|----|------|------|------|------|------------|------------|------------|------|
| <b>Multiplex A</b> |       |              |    |      |    |      |      |      |      |            |            |            |      |
| <i>QpZAG110</i>    | 6-FAM | 202-266      | 25 | 8.47 | 1  | 2.27 | 0.81 | 0.87 | 0.89 | 0.095***   | 0.115***   | 0.022**    | 0.92 |
| <i>QrZAG7</i>      | VIC   | 113-167      | 20 | 7.99 | 1  | 2.24 | 0.92 | 0.87 | 0.89 | -0.033     | -0.006     | 0.026***   | 0.91 |
| <i>QrZAG11</i>     | NED   | 251-261      | 6  | 1.69 | 2  | 0.60 | 0.18 | 0.40 | 0.41 | 0.563***   | 0.598***   | 0.080***   | 0.35 |
| <b>Multiplex B</b> |       |              |    |      |    |      |      |      |      |            |            |            |      |
| <i>CsCAT2</i>      | 6-FAM | 211-251      | 16 | 1.82 | 3  | 0.92 | 0.43 | 0.42 | 0.43 | -0.005     | 0.027      | 0.032***   | 0.44 |
| <i>CsCAT14</i>     | 6-FAM | 117-153      | 15 | 4.99 | 1  | 1.79 | 0.83 | 0.79 | 0.81 | -0.013     | 0.007      | 0.020*     | 0.80 |
| <b>Multiplex C</b> |       |              |    |      |    |      |      |      |      |            |            |            |      |
| <i>MSQ13</i>       | NED   | 220-246      | 8  | 1.50 | 3  | 0.53 | 0.32 | 0.30 | 0.31 | -0.020     | 0.034      | 0.053***   | 0.30 |
| <i>QrZAG96</i>     | VIC   | 143-169      | 6  | 1.79 | 1  | 0.76 | 0.44 | 0.42 | 0.44 | 0.032      | 0.046      | 0.015      | 0.40 |
| <b>Multiplex D</b> |       |              |    |      |    |      |      |      |      |            |            |            |      |
| <i>QpZAG9</i>      | VIC   | 195-257      | 17 | 2.18 | 8  | 1.02 | 0.50 | 0.51 | 0.53 | 0.064*     | 0.091***   | 0.029**    | 0.52 |
| <i>QpZAG36</i>     | NED   | 203-245      | 12 | 3.67 | 3  | 1.45 | 0.72 | 0.72 | 0.74 | 0.030      | 0.050*     | 0.021      | 0.73 |
| <i>QrZAG20</i>     | 6-FAM | 148-195      | 21 | 6.46 | 1  | 2.01 | 0.57 | 0.83 | 0.86 | 0.347***   | 0.365***   | 0.027*     | 0.89 |

<sup>1</sup>Levels of significance for the unbiased estimates of Wright's fixation indices were tested using a non-parametric approach [54] with 1000 permutations: \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ .