CHARACTERIZATION OF FUNGUS-SPECIFIC MICROSATTELITE MARKERS IN THE LICHEN-FORMING FUNGUS *PARMELINA CARPORRHIZANS* (*PARMELIACEAE*)

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**Premise of the study:** Microsatellite loci were developed to study the lichen-forming fungus *Parmelia* (*Parmeliaceae*) in different habitats of western Europe and the Mediterranean for baseline studies to understand the effects of climate change on its distribution.

**Methods and Results:** We cultured *P. carporrhizans* from ascospores for genomic sequencing with Illumina HiSeq. We successfully developed 11 polymorphic microsatellite markers and associated primer sets and assessed them with 30 individuals from two of the Canary Islands. The average number of alleles per locus was 8.8. Nei’s unbiased gene diversity of these loci ranged from 0.53 to 0.91 in the tested populations. Amplification in two closely related species (*P. tiliacea, P. cryptotiliacea*) yielded only limited success.

**Conclusions:** The new microsatellite markers will allow the study of genetic diversity and population structure in *P. carporrhizans*. We propose eight markers to combine in two multiplex reactions for further studies on a larger set of populations.

**Key words:** Ascomycota; lichen-forming fungi; microsatellites; multiplex; *Parmelia carporrhizans*; population genetics.

*Parmelina carporrhizans* (Taylor) Poelt & Vězda (*Parmeliaceae*) is a sexually reproducing foliose lichen species that has long been considered synonymous with the morphologically similar *P. quercina* (Willd.) Hale. Thus, the geographic distribution and degree of conservation of both species are poorly known (Argüello et al., 2007; Clerc and Truong, 2008). These two species are largely allopatric but they occasionally overlap, being apparently parapatric depending on the climatic conditions. Hence they possibly may be used as indicators of climate change. *Parmelina carporrhizans* has an Atlantic-Mediterranean distribution in Europe. It is abundant in the central-western Iberian Peninsula in the humid supra- and mesomediterranean level on deciduous *Quercus* L. vegetation (Argüello et al., 2007; Nuñez-Zapata, 2013). The species also occurs across open forest and in isolated trees above the Canarian monteverde forest in central Macaronesia from 800 to 1500 m and is locally common on Gran Canaria. Further, *P. carporrhizans* is listed as “vulnerable” on the Red Lists of England and Wales (Church et al., 1996; Woods, 2010). Despite these conservation concerns, our knowledge of the population genetics of this species is currently limited.

We developed 11 microsatellite markers for high-resolution population studies in *P. carporrhizans* to provide a better understanding of its genetic diversity, gene flow, and population structure. The enhanced knowledge will allow us to implement an informed conservation plan and investigate potential impacts of climate change on this narrowly distributed species. In addition, we also investigate whether this set of high-resolution microsatellite markers can be applied to other closely related species in the genus *Parmelina* Hale.

**METHODS AND RESULTS**

We isolated the mycobiont of *P. carporrhizans* from ascospores of two thalli (deposited in the herbarium of the Universidad Complutense de Madrid [MAF], Madrid, Spain: MAF-Lich 19191 and MAF-Lich 19192) collected in Cuevas del Valle, Spain (40°18′28.4″N, 5°09′39.0″W), in October 2012, following the inverted Petri dish method (Ahmadjian, 1993). We germinated spores in Bold Medium (Deason and Bold, 1960), and after two weeks these were transferred to corn meal agar (CMA) and malt yeast (Honegger et al., 2004), where the cultures were grown for four months.

Prior to DNA extraction, we removed secondary metabolites with acetone, and then crushed the samples with pestles in liquid nitrogen and extracted genomic DNA with the DNeasy Plant Kit (QIAGEN, Redwood City, California, USA) according to the manufacturer’s instructions.

To confirm the identity of the mycobiont cultures, we amplified the internal transcribed spacer (ITS) region of the nuclear rDNA from the axenic cultured tissues. Genomic DNA (10–25 ng) was used for PCR amplifications. Primers, PCR, and cycle sequencing conditions were the same as described previously.
(Argüello et al., 2007). Sequencing was conducted on an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, California, USA) at Centro de Genómica y Proteómica del Parque Científico de Madrid. The identity of the sequences and specimens were confirmed using the MegaBLAST search function in GenBank. ITS sequences were deposited in GenBank (accession numbers KM357892 and KM357893).

From the extracted DNA, approximately 0.5 μg of genomic DNA was used to construct an Illumina library using the Nextera XT multiplex paired-end kit (Illumina, San Diego, California, USA). The library was paired-end sequenced using an Illumina HiSeq 2000 with 100 cycles (version 3 chemistry). Standard Illumina protocols (http://www.illumina.com/) were used to generate the library. Sequencing was carried out at the Stab Vida Laboratory (Madan Parque, Caparica, Portugal). Illumina reads were assembled to contigs using the “De novo assembly” option of the CLC Genomics Workbench version 6.0.4 (CLC Bio, Aarhus, Denmark). A total of 38,115,484 reads with an average length of 69.06 bases and a total of 2,632,336,717 bases were recovered. De novo assembly produced 31,035 contigs (N50 = 3615 bp) with an average of approximately 69.06 bases and a coverage, which totaled 36.2 Mbp of genome data.

We tested the 24 primer pairs with seven accessions of P. carporrhizans and four pairs amplified in P. tiliacea. We then tested this subset of 12 primer pairs for variability with 30 samples of P. carporrhizans from Gran Canaria and Tenerife (MAF-Lich Numbers 19123–19152; Appendix 1), as well as one accession of P. tiliacea and four pairs amplified in P. tiliacea. We deposited these 11 primer sequences in GenBank (Table 1); other primer pairs were excluded due to their low amplification rate (<60%). Our limited cross-species amplification results suggest that it may be possible to use some of these markers in other species of the Parmelina clade (Nuñez-Zapata, 2013).

TABLE 2. Number of alleles (A) and Nei’s unbiased genetic diversity (Hs) of the eight polymorphic microsatellite loci that amplified across all P. carporrhizans samples was determined by counting the number of alleles and calculating Nei’s unbiased haploid diversity (Table 2) using GenAlEx version 6.1 (Peakall and Smouse, 2006). The number of alleles ranged from four to 14, and the average unbiased diversity was 0.76, a relatively high number for

### Table 1. Overview of the microsatellite loci and associated primer sets successfully developed for Parmelina carporrhizans and deposited in the National Center for Biotechnology Information (NCBI) database.

| Locus | Primer sequences (5′-3′) | Repeat motif | Dye | T_a (°C) | Allele size range (bp) | GenBank accession no. |
|-------|--------------------------|--------------|-----|---------|----------------------|----------------------|
| Pear1 | F: *CATGAAATCATTCCGCTACCA | (AC)_{18} | FAM | 57      | 124–147             | KM875582             |
|       | R: GGAGGAGTGAAGGAAGACAA  |              |     |         |                      |                      |
| Pear2 | F: *TTACCATAGTGGAGTGATCCG | (GT)_{15}   | NED | 57      | 206–265              | KM875583             |
|       | R: CTGTATCGAAGAAGATCAG   |              |     |         |                      |                      |
| Pear3 | F: *GCGCGGCGATCGTATCCGG | (AT)_{17}   | PET | 57      | 109–249              | KM875584             |
|       | R: GCCCGCGCAATACAGAT      |              |     |         |                      |                      |
| Pear4 | F: *AGAGGAGGAGTGAAGGAAGA | (AAGAG)_{16} | VIC | 57      | 280–318              | KM875585             |
|       | R: GCTGCTCTTCGATGCATCA    |              |     |         |                      |                      |
| Pear5 | F: *TCGGGATGATGATTTCGAG  | (AG)_{18}   | FAM | 57      | 227–309              | KM875586             |
|       | R: TCTCTGCTAATGTGGAGAAGA |              |     |         |                      |                      |
| Pear6 | F: *GCATTCTGATGAGGCTGAAC | (CTT)_{15}  | NED | 57      | 203–270              | KM875587             |
|       | R: TGCAATCGAATCTAACATGG  |              |     |         |                      |                      |
| Pear7 | F: *GCGCGGCGATCGTATCCGG | (AAG)_{19}  | PET | 57      | 120–223              | KM875588             |
|       | R: GCAACGAGAAAGCAACCAAC  |              |     |         |                      |                      |
| Pear8 | F: *TCGGGATGATGATTTCGAG  | (GAT)_{20}  | VIC | 57      | 372–474              | KM875589             |
|       | R: GAGGGGTGATGTTTTTAAC   |              |     |         |                      |                      |
| Pear9 | F: *GAAATCTCACCACAGCTTC | (AAG)_{16}  | FAM | 57      | 89–165               | KM875590             |
|       | R: AACATTTTGCGTCATGG     |              |     |         |                      |                      |
| Pear10| F: *GCCCCCTCAATGAGAGGTC | (AC)_{16}   | FAM | 57      | 341–390              | KM875591             |
|       | R: CCTGCTCGGATGAAAGAT    |              |     |         |                      |                      |
| Pear11| F: *GATGACGAGGAGTTTCCAG | (ACTC)_{17} | FAM | 57      | 250–371              | KM875592             |
|       | R: GTCGGCGCTGCCTGATTTAC  |              |     |         |                      |                      |

Note: T_a = annealing temperature.

*Size range indicates allele size based on two populations collected in the Canary Islands (see Appendix 1).

*M13 tail: TGTAAAACGACGGCCAGT.

Hale (MAF-Lich 17252); see Appendix 1 for specific localities. Out of these 24 primers, only 12 pairs successfully amplified all of the P. carporrhizans samples, and four pairs amplified in P. tiliacea. We then tested this subset of 12 primer pairs for variability with 30 samples of P. carporrhizans from Gran Canaria and Tenerife (MAF-Lich Numbers 19123–19152; Appendix 1), as well as one accession each of P. tiliacea and P. cryptotiliacea Crespo & Núñez-Zapata (MAF-Lich 19403 and MAF-Lich 19402, respectively). Eight of these primer pairs (Pear1–Pear8) amplified all P. carporrhizans samples, while the other three (Pear9–Pear11) had 3.3–10% missing data. Four of these primer pairs (Pear3, Pear5, Pear7, Pear9) amplified in P. tiliacea and none amplified in P. cryptotiliacea. We deposited these 11 primer sequences in GenBank (Table 1); other primer pairs were excluded due to their low amplification rate (<60%). Our limited cross-species amplification results suggest that it may be possible to use some of these markers in other species of the P. carporrhizans clade (Núñez-Zapata, 2013). Analyses within the eight microsatellite loci that amplified across all P. carporrhizans samples was determined by counting the number of alleles and calculating Nei’s unbiased haploid diversity (Table 2) using GenAlEx version 6.1 (Peakall and Smouse, 2006). The number of alleles ranged from four to 14, and the average unbiased diversity was 0.76, a relatively high number for

### Table 2. Number of alleles (A) and Nei’s unbiased genetic diversity (Hs) of the eight polymorphic microsatellite loci that were amplified with 100% success across 30 samples from the Canary Islands.

| Locus | Total | Gran Canaria (n = 20) | Tenerife (n = 10) |
|-------|-------|----------------------|-------------------|
|       | A     | H_s                 | A                 | H_s               |
| Pear1 | 6     | 0.55                 | 4                 | 0.56              | 4                 | 0.53 |
| Pear2 | 4     | 0.64                 | 4                 | 0.73              | 2                 | 0.56 |
| Pear3 | 14    | 0.89                 | 11                | 0.87              | 6                 | 0.91 |
| Pear4 | 8     | 0.82                 | 7                 | 0.78              | 6                 | 0.87 |
| Pear5 | 9     | 0.78                 | 6                 | 0.68              | 7                 | 0.87 |
| Pear6 | 9     | 0.78                 | 8                 | 0.87              | 4                 | 0.71 |
| Pear7 | 12    | 0.89                 | 9                 | 0.90              | 6                 | 0.89 |
| Pear8 | 9     | 0.75                 | 8                 | 0.89              | 3                 | 0.60 |
| Average| 8.88  | 0.76                 | 7.13              | 0.79              | 4.75              | 0.74 |

http://www.bioone.org/loi/apps
just 30 individuals from a small geographic area. No identical multilocus genotypes were found among the samples as is expected for a sexually reproducing lichen-forming fungus.

CONCLUSIONS

We developed 11 polymorphic fungus-specific microsatellite markers to facilitate studies of population genetics in *P. carporrhizans*. Eight of the 11 microsatellite primer pairs are being used to analyze *P. carporrhizans* populations. The results from future population genetic studies will help inform us on population responses to global changes, clarify the mechanisms of speciation, as well as define populations of this narrowly distributed species for conservation purposes.

LITERATURE CITED

AHMADJIAN, V. 1993. The lichen symbiosis. John Wiley & Sons Inc., New York, New York, USA.

ARGÜELLO, A., R. DEL PRADO, P. CUBAS, AND A. CRESPO. 2007. *Parmelina quercina* (Parmeliaceae, Lecanorales) includes four phylogenetically supported morphospecies. *Biological Journal of the Linnean Society* 91: 455–467.

CHURCH, J. M., B. J. COPPIN, O. L. GILBERT, P. W. JAMES, AND N. F. STEWART. 1996. Red Data Books of Britain and Ireland: Lichens. Volume I: Britain. Joint Nature Conservation Committee, Peterborough, United Kingdom.

CLERC, P., AND C. TRUONG. 2008. Non-sorediate and non-isidiate *Parmelina* species (lichenized ascomycetes, Parmeliaceae) in Switzerland—*Parmelina atricha* (Nyl.) P. Clerc reinstated in the European lichen flora. *Sauteria* 15: 175–194.

DEASON, D. R., AND H. C. BOLD. 1960. Phycological studies. I. Exploratory studies of Texas soil algae. University of Texas Publication no. 6022. University of Texas, Austin, Texas, USA.

DEVKOTA, S., C. CORNEJO, S. WERTH, R. P. CHAUDHARY, AND C. SCHRÜDDEGER. 2014. Characterization of microsatellite loci in the Himalayan lichen fungus *Lobaria pindarensis* (Lobariaceae). *Applications in Plant Sciences* 2(5): 1300101.

FAIRCLOTH, B. C. 2008. MSATCOMMANDER: Detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources* 8: 92–94.

HONEGGER, R., U. ZIPPLER, H. GANSNER, AND S. SCHERRER. 2004. Mating systems in the genus *Xanthoria* (lichen-forming ascomycetes). *Mycological Research* 108: 480–488.

JONES, T. C., T. G. A. GREEN, I. D. HOGG, AND R. J. WILKINS. 2012. Isolation and characterization of microsatellites in the lichen *Buellia frigida* (Physciaceae), an Antarctic endemic. *American Journal of Botany* 99: e131–e133.

NUÑEZ-ZAPATA, J. 2013. Genetic variability, cryptic species and molecular phylogeny in the lichen-forming fungal genus *Parmelina* (Parmeliaceae, Ascomycota). Ph.D. thesis, Universidad Complutense de Madrid, Madrid, Spain.

PEARALL, R., AND P. E. SMOUSE. 2006. GenAIEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

ROZEN, S., AND H. SKALETSKY. 2000. 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. *Nature Biotechnology* 18: 233–234.

WOODS, R. G. 2010. A Lichen Red Data List for Wales. Plantlife, Salisbury, United Kingdom.

http://www.bioone.org/loi/apps
| Voucher no. | Species | Locality | Substrate | Geographic coordinates | Elevation (m) | Collectors | Collection date |
|------------|---------|----------|-----------|------------------------|--------------|------------|-----------------|
| 16476      | *P. carporrhizans* | Canakkale (Tr) | *Quercus* sp. | 40°06′N 26°55′E | 400 | A. Crespo, P. K. Divakar & M. Candan | 15/06/2007 |
| 17252      | *P. tiliacea* | Tenerife | Rock | 28°07′14″N 16′40′19″W | 982 | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 19/06/2009 |
| 19191      | *P. carporrhizans* | Avila (Es) | *Castanea sativa* | 40°18′28″N 05°00′39″W | 1007 | A. Crespo, D. Alors & C. Ruibal | 11/01/2012 |
| 19404      | *P. carporrhizans* | Tenerife | *Castanea sativa* | 28°27′11″N 16°24′55″W | 894 | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009 |
| 19405      | *P. carporrhizans* | Gran Canaria | *Pinus radiata* | 28°01′50″N 15°37′12″W | 1420 | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 19/06/2009 |
| 19406      | *P. carporrhizans* | Gran Canaria | *Prunus* sp. | 28°00′01″N 15°32′29″W | 954 | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 17/06/2009 |
| 19407      | *P. carporrhizans* | Tetouan (Ma) | Unidentified dead tree | 35°20′43″N 05°22′20″W | 687 | D. Alors & C. G. Boluda | 22/10/2013 |
| 19408      | *P. carporrhizans* | Gran Canaria | *Ulmus* sp. | 28°01′29″N 15°35′15″W | 1305 | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 18/06/2009 |
| 19402      | *P. cryptotiliacea* | Agadir (Ma) | *Quercus ilex* | 30°38′51″N 09°40′34″W | 711 | D. Alors & C. G. Boluda | 23/10/2013 |
| 19403      | *P. tiliacea* | Azilal (Ma) | *Quercus ilex* | 33°25′40″N 05°11′26″W | 1439 | D. Alors & C. G. Boluda | 20/10/2013 |
| 19123      | *P. carporrhizans* | Gran Canaria | *Pinus radiata* | 27°59′21″N 15°35′33″W | 1499 | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009 |
| 19124      | *P. carporrhizans* | Gran Canaria | *Pinus radiata* | 27°59′21″N 15°35′33″W | 1499 | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009 |
| 19125      | *P. carporrhizans* | Gran Canaria | *Pinus radiata* | 27°59′21″N 15°35′33″W | 1499 | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009 |
| 19126      | *P. carporrhizans* | Gran Canaria | *Pinus radiata* | 27°59′21″N 15°35′33″W | 1499 | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009 |
| 19127      | *P. carporrhizans* | Gran Canaria | *Pinus radiata* | 27°59′21″N 15°35′33″W | 1499 | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009 |
| 19128      | *P. carporrhizans* | Gran Canaria | *Pinus radiata* | 27°59′21″N 15°35′33″W | 1499 | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009 |
| 19129      | *P. carporrhizans* | Gran Canaria | *Pinus radiata* | 27°59′21″N 15°35′33″W | 1499 | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009 |
| Voucher no. | Species         | Locality     | Substrate     | Geographic coordinates | Elevation (m) | Collectors                      | Collection date |
|------------|-----------------|--------------|---------------|------------------------|---------------|---------------------------------|-----------------|
| 19130      | *P. carporrhizans* | Gran Canaria | *Pinus radiata* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19131      | *P. carporrhizans* | Gran Canaria | *Pinus radiata* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19132      | *P. carporrhizans* | Gran Canaria | *Pinus radiata* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19133      | *P. carporrhizans* | Gran Canaria | *Castanea sativa* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19134      | *P. carporrhizans* | Gran Canaria | *Castanea sativa* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19135      | *P. carporrhizans* | Gran Canaria | *Castanea sativa* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19136      | *P. carporrhizans* | Gran Canaria | *Castanea sativa* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19137      | *P. carporrhizans* | Gran Canaria | *Castanea sativa* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19138      | *P. carporrhizans* | Gran Canaria | *Castanea sativa* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19139      | *P. carporrhizans* | Gran Canaria | *Castanea sativa* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19140      | *P. carporrhizans* | Gran Canaria | *Castanea sativa* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19141      | *P. carporrhizans* | Gran Canaria | *Castanea sativa* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19142      | *P. carporrhizans* | Gran Canaria | *Castanea sativa* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19143      | *P. carporrhizans* | Gran Canaria | *Castanea sativa* | 27°59'21"N 15°35'33"W | 1499          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 22/06/2009     |
| 19144      | *P. carporrhizans* | Tenerife     | *Castanea sativa* | 28°27'11"N 16°24'55"W | 894           | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 23/06/2009     |
| 19145      | *P. carporrhizans* | Tenerife     | *Castanea sativa* | 28°27'11"N 16°24'55"W | 894           | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 23/06/2009     |
| 19146      | *P. carporrhizans* | Tenerife     | *Castanea sativa* | 28°27'11"N 16°24'55"W | 894           | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 23/06/2009     |
### APPENDIX 1. Continued.

| Voucher no. | Species         | Locality | Substrate<sup>a</sup> | Geographic coordinates | Elevation (m) | Collectors                                      | Collection date |
|-------------|-----------------|----------|------------------------|------------------------|--------------|------------------------------------------------|-----------------|
| 19147       | *P. carporrhizans* | Tenerife | *Castanea sativa*      | 28°27'11"N 16°24'55"W | 894          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 23/06/2009      |
| 19148       | *P. carporrhizans* | Tenerife | *Castanea sativa*      | 28°27'11"N 16°24'55"W | 894          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 23/06/2009      |
| 19149       | *P. carporrhizans* | Tenerife | *Castanea sativa*      | 28°27'11"N 16°24'55"W | 894          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 23/06/2009      |
| 19150       | *P. carporrhizans* | Tenerife | *Castanea sativa*      | 28°27'11"N 16°24'55"W | 894          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 23/06/2009      |
| 19151       | *P. carporrhizans* | Tenerife | *Castanea sativa*      | 28°27'11"N 16°24'55"W | 894          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 23/06/2009      |
| 19152       | *P. carporrhizans* | Tenerife | *Castanea sativa*      | 28°27'11"N 16°24'55"W | 894          | A. Crespo, P. Cubas, A. Santo & P. K. Divakar | 23/06/2009      |

**Note:** Tr = Turkey; Es = Spain; Ma = Morocco.

<sup>a</sup>The first eight samples were tested against all 24 microsatellite primer pairs. The last 32 samples were tested against a subset of 12 microsatellite primer pairs (see Methods and Results).

<sup>b</sup>Scientific authorities for substrate species: *Castanea sativa* Mill., *Pinus radiata* D. Don, *Prunus* L., *Quercus* L., *Quercus ilex* L., *Ulmus* L.