Supplementary Information

Genetic Analysis of *Platanus* samples

DNA extraction, amplification and sequence analysis

Genomic DNA from contemporary samples was extracted following a CTAB extraction protocol described previously (Seelenfreund et al. 2011; González-Lorca et al., 2015), based on Lodhi et al. (1994). DNA quality of all samples was determined with the 260 nm/280 nm absorbance ratio using Nanodrop TM 2000 and DNA concentration was determined by fluorescence, using the Quant-iTM PicoGreenTM dsDNA Assay Kit (#7589), as indicated by the provider. Integrity of extracted DNA was assessed by electrophoresis on 0.8% agarose gels.

To identify the *Platanus* species, samples were analyzed using the nuclear ribosomal ITS-1 region with primers ITS-5B 5'- TCGCGAGAAGTCCACTGAA-3' and ITS-4 5'- GCTTAAACTCAGCGGGTAGC-3', as described by Blattner (1999). Amplification was performed as described in Seelenfreund et al. (2011). Briefly, PCR-reaction mixtures consisted of 2.5 mM $MgCl_2$, 0.625 mM dNTPs, 0.25 µM of each primer and 0.2 U/µl of GoTaqR Flexi DNA Polymerase (Promega, Madison, WI, USA), in a final volume of 20 µl. In all experiments, blank reactions were performed by adding the appropriate amounts of sterile distilled water to the reaction mixture. All PCR reactions were set up in a UV-treated PCR cabinet.

The amplification program consisted of an initial denaturation step at 94ºC during 5 min, followed by 32 cycles with a denaturation step at 94 ºC for 1 min; an annealing stage at 60 ºC for 1 min, an extension at 72ºC for 1 min and a final extension at 72ºC for 5 min. Amplicons were analyzed by electrophoresis on 1.5% agarose gels, dyed with GelRed Nucleic Acid Gel Stain (Biotium, Inc.) and visualized under UV light. Amplicons were sequenced at Macrogen Inc. (Seoul, South Korea), electropherograms were checked using Bio Edit 7.1.3.0 software and polymorphisms were

determined by sequence alignment using the Clustal W algorithm (Thompson et al., 1994) of the CLC Sequence Viewer software, as previously described (González-Lorca et al., 2015).

References:

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