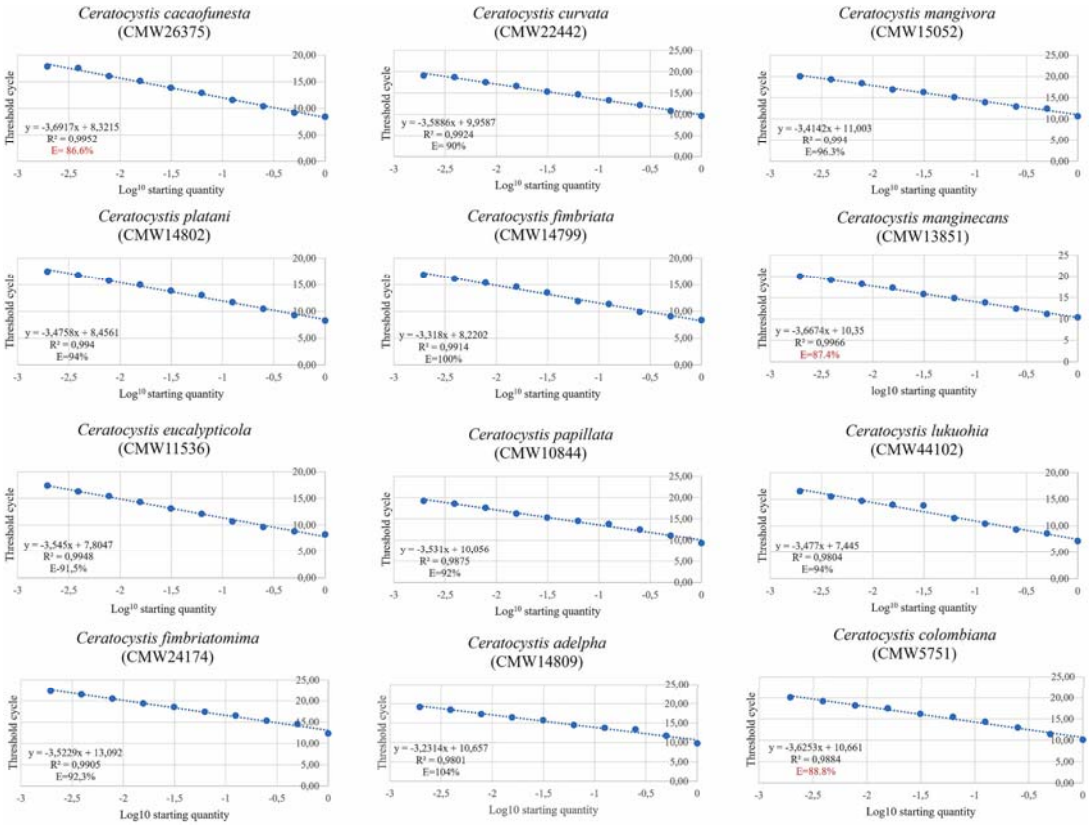
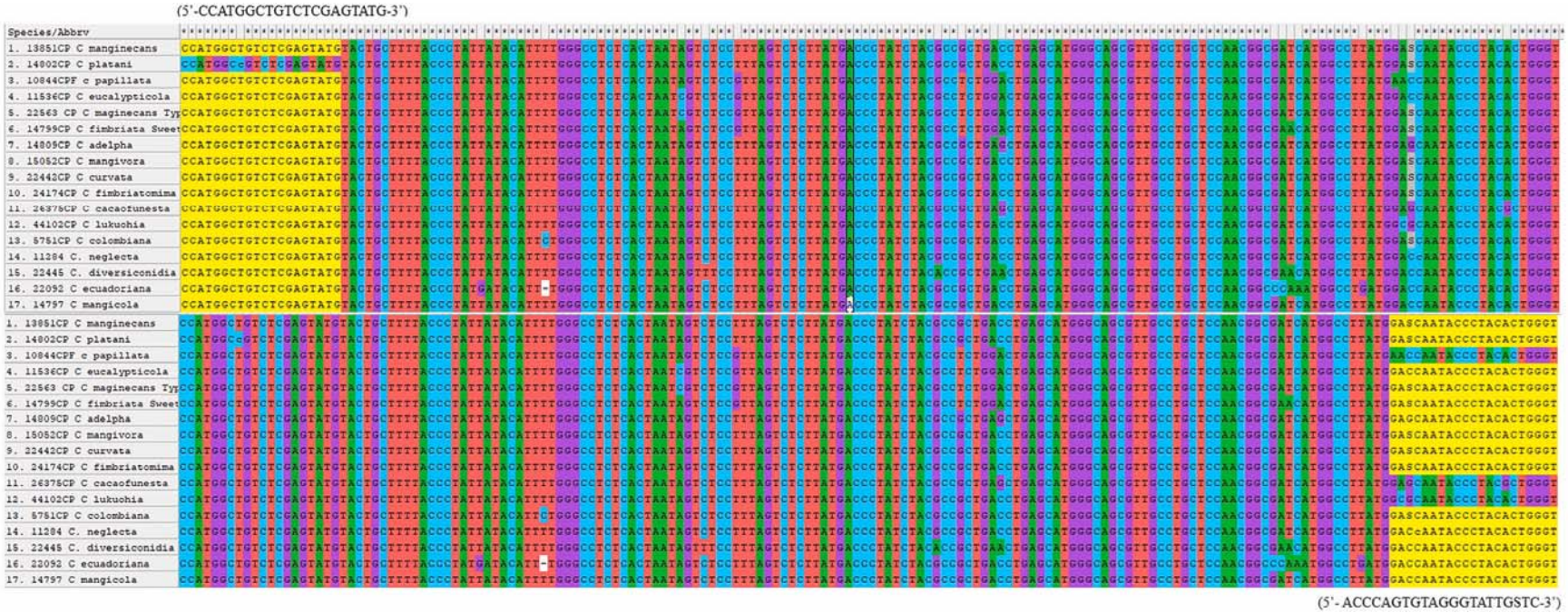
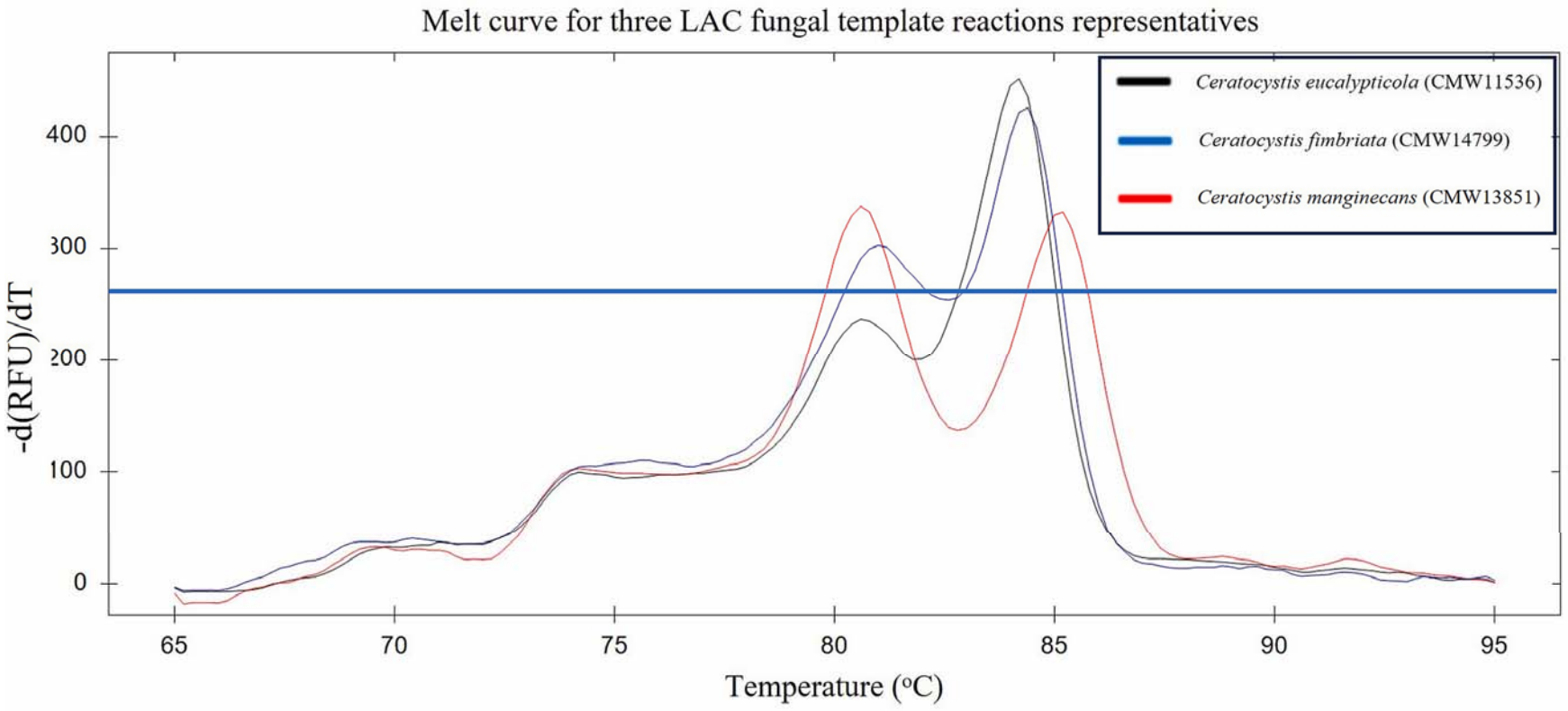
Appendix A. Supplementary data



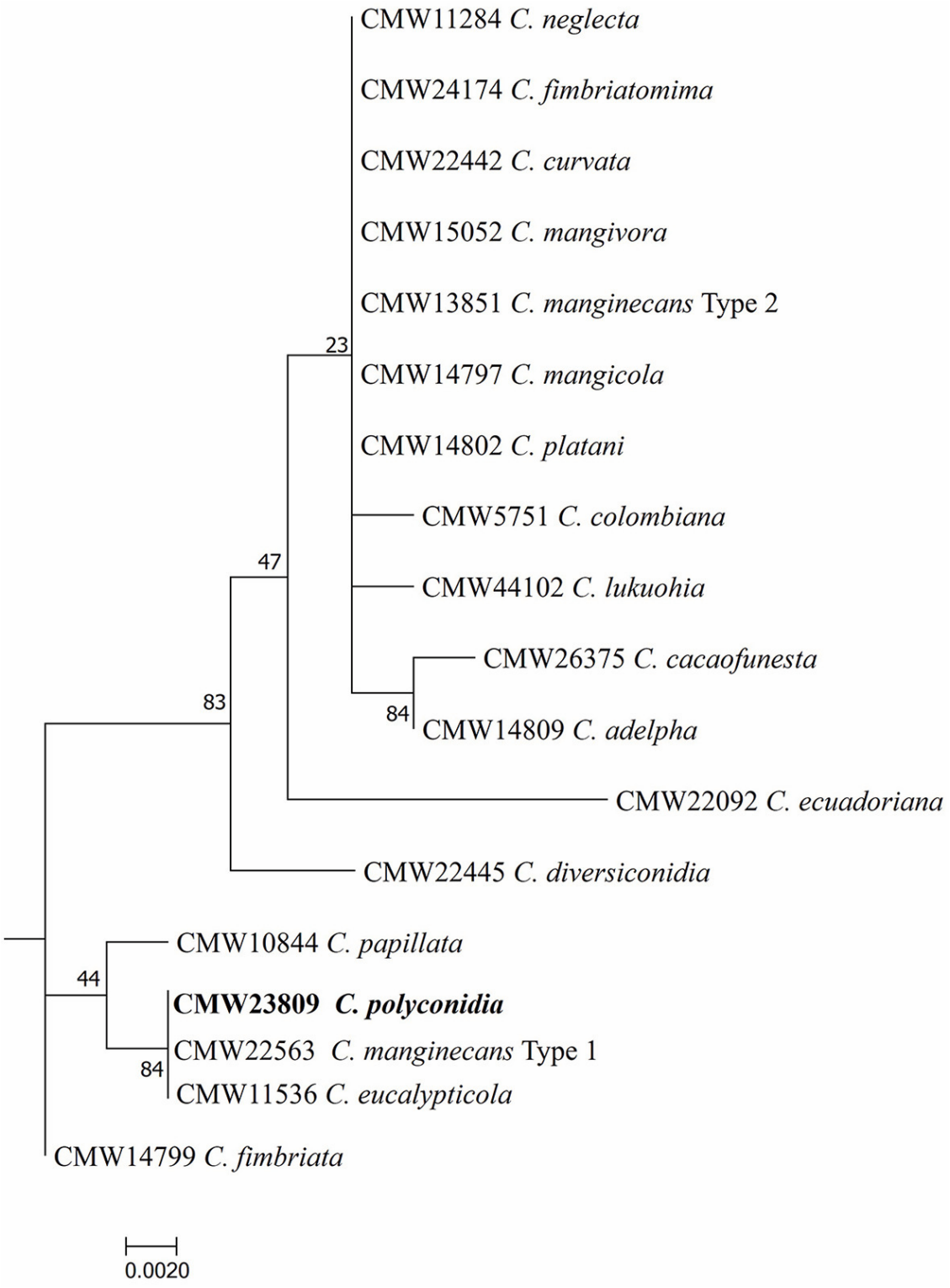
Supplementary Fig. 1 Representations of Real-Time standard curves generated using tenfold serial dilutions (2ng→0,004 ng) of the plasmid (p) containing the targeted CP.RE region from the 12 *Ceratocystis* species in the LAC in circular form. Efficiency (E) and R^2 define the level of quality of the procedures with values of 90-105% and 1 as best fit, respectively. Equations for the linear regression line (y=mx+b) were deduced from the values of the slope (m) and the y-intercept (b), which were output of the software. Standard curves generated using the target-containing plasmid were ideal for nine out of the 12 LAC species tested (Efficiency = 90-105% and R> 0.98) and nearly ideal for the remaining three species tested with calculated PCR efficiencies just below optimal (86-88%), and highlighted in red.



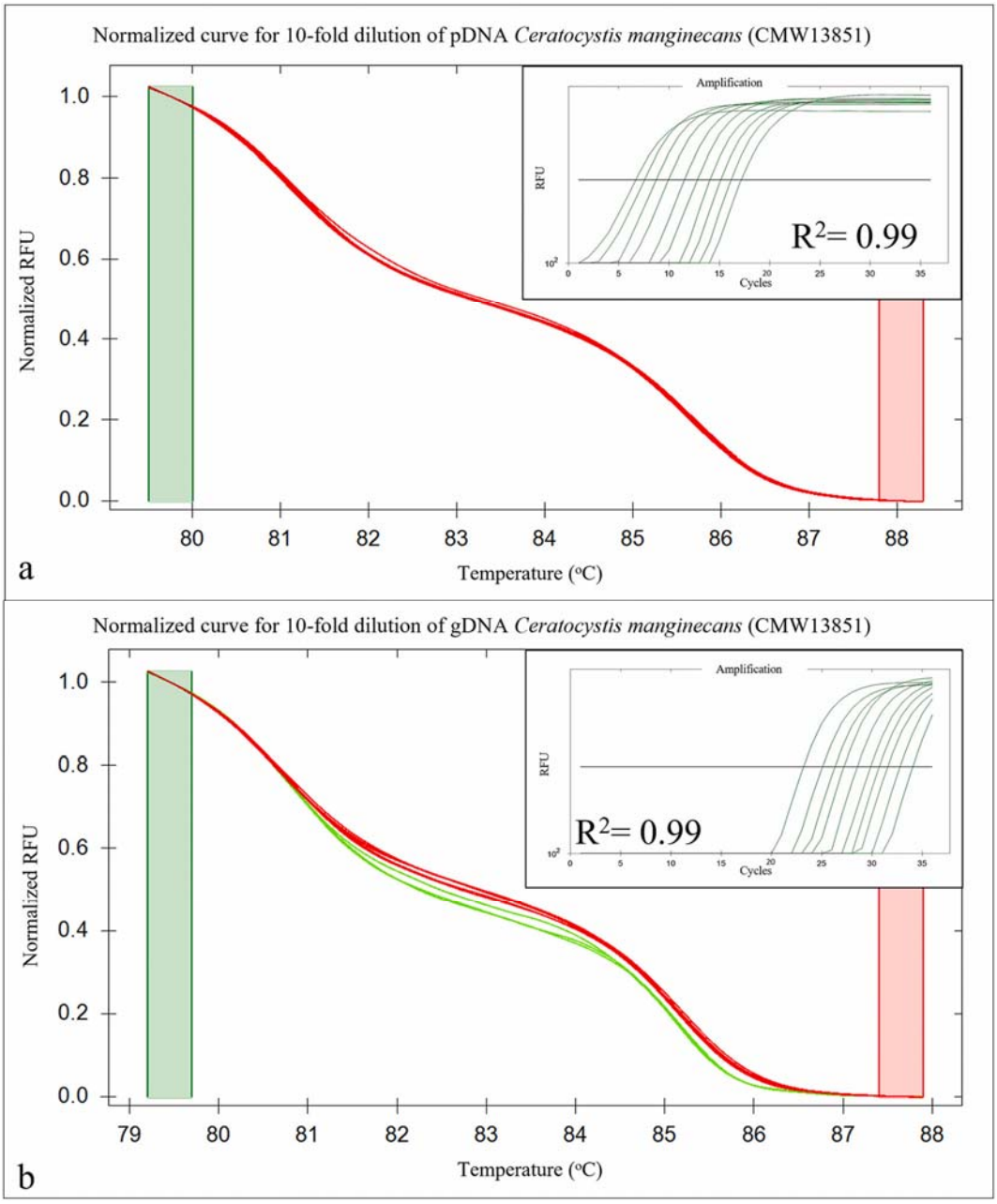
Supplementary Fig. 2 Binding sites of the CP.RE primers to all 16 LAC species. Primer sequences are shown in a 5' to 3' direction. Base pair mismatches in *C. cacaofunesta, C. platani*, *C. lukuohia* and *C. papillata* can also be observed.



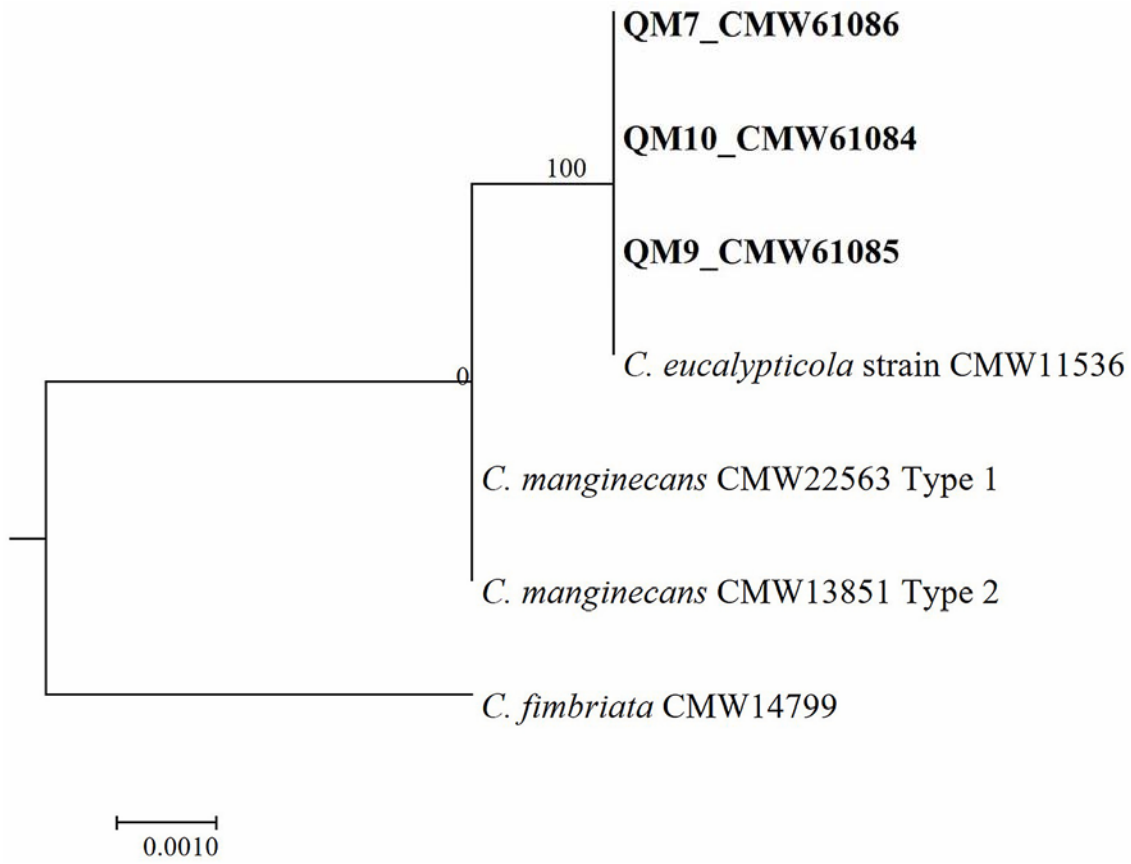
Supplementary Fig. 3 Melt curves of the three fungal template reaction representatives (*C. fimbriata*, *C. manginecans* and *C. eucalypticola*) visualized as the first negative derivative of the change in fluorescence [-d(RFU)/dT] plotted as a function of the temperature. Melt curves were consistent with *in-silico* testing using uMELT Quartz. Double melt curve peaks were expected due to the GC structure of the LAC-targeted amplicons.



Supplementary Fig. 4 Phylogenetic tree based on maximum likelihood (ML) analysis of CP.RE gene sequences for *Ceratocystis* species in the Latin American Clade and one off-target *Ceratocystis* species. *Ceratocystis polyconidia* formed a single distinct phylogenetic clade with *C. manginecans* (Type 1) and *C. eucalypticola.*



Supplementary Fig. 5 HRMA clustering reactions of pDNA and gDNA of all 12 LAC species at the 10 different concentrations tested a) Amplification curves obtained at different concentrations (2ng→0,004 ng) of the plasmid (p) containing the targeted CP.RE region from *C. manginecans* Type 2 (CMW13851). R^2 values are also depicted in the acceptable range. HRMA correctly clustered reactions of *C. manginecans* Type 2 (CMW13851) pDNA at the 10 different concentrations tested. 4b) Amplification curves obtained at different concentrations (1ng→0,002 ng) of *C. manginecans* Type 2 (CMW13851) gDNA. R^2 values are also depicted in the acceptable range. HRMA on positively detected reactions of *C. manginecans* Type 2 (CMW13851) fungal gDNA correctly clustered species from 2 ng to 0,06 ng (RED). HRMA on reaction concentrations lower than the average detection limit (>0,088 ng) of the fungal gDNA tested periodically clustered species incorrectly as depicted by the second curve (GREEN). However, the shape of the two curves are identical.



Supplementary Fig. 6 Phylogenetic tree based on maximum likelihood (ML) analysis of the MS204 gene sequences for *Ceratocystis fimbriata* (CMW14799), two *Ceratocystis manginecans* isolates each representing each ITS type (Type 2: CMW13851; Type 1 CMW 22563), *Ceratocystis eucalypticola* (CMW11536) and fungal isolates isolated from *Ceratocystis* infected wood samples **(Bold).** Fungal isolates from *Ceratocystis* infected wood samples clustered with *Ceratocystis eucalypticola* (CMW11536).