



Supplementary Fig. 1 Representations of Real-Time standard curves generated using tenfold serial dilutions  $(2ng\rightarrow0,004 ng)$  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<sup>2</sup> 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.

	(5'-CCATGGCTGTCTCGAGTATG-3')	
Species/Abbrv		
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2. 14802CP C platani	CONTREGERCIFICERETRICITE COCTATION ACCOUNT ACCOUNT ACCOUNT CONTRECT COUNT ACCOUNT	GGGT
3. 10844CPF c papillata	CCATGGCTGTCTCGAGTATCTACGGCTGTCCCAGTATATACATTTTGCGCCTCTCACTATATACACTTCCCCTTATCTCTCGCCTCTGCGCTGTCCCAGCGGCGATCATCGCCTTGTCGCCTCTCCCCAGCGGCGATCATCGCCTTGTCGCCTCCCAGCGGCGATCATCGCCTTGTCGCCTCCCAGCGGCGATCATCGCCTTGTCGCCTCCCAGCGGCGATCATCGCCTTGTCGCCTCCCAGCGGCGATCATCGCCTCCCAGCGGCGATCATCGCCTCCCAGCGGCGATCATCGCCTCCCAGCGGCGATCATCGCCTCCCAGCGGCGATCATCGCCTCCCAGCGGCGATCATCGCCTCCCAGCGGCGATCATCGCCTCCCAGCGGCGATCATCGCCTCCCAGCGGCGATCATCGCCTCCCAGCGGCGATCAGCGCGCGC	GGGI
4. 11536CP C eucalypticola	CCATGGCTGTCTCGAGTATCTACGGCTGTGCGCGTGTGAGGATTATACATITTAGGCGTCTCGCTGCAGTATGTCGCTGTGGGCGGGTGTGGGGGGGG	GGGT
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7. 14809CP C adelpha	CCATGGCTGTCICGAGTATGTACGTGTTTTAGCCTATTATACATTTIGGGCGCTCCCCTCATTAGTCTCCTCTTAGTCTCTCGCGGCGGGGGGGG	COGT
0. 15052CP C mangivora	CONTRECTOR CONSTANCES CONTINUES C	CCGI
9. 22443CP C curvata	CCATGGCTGTCTCGAGTATGTACTGCTTTTACGCTTTTACGCTTTGTCTCGCTCTCACTATAGTCTCCTCTTTAGTCTCTCTGCCGCGGCTGACCATGGCGCTGCGCCGCTGTCGCCGCGCGCG	GGGT
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11. 26375CP C cacaofunesta	CATEGOROTOTOTORAGTATOTACOCONTRACCOCONTRACATINGUCCOCOCONTRACCONTRAC	GOGI
12. 44102CP C lukuohia	caregoisticeseistere de transferente a contra contra a contra de transferente a contra de transferente de contra de transferente a contra de	CCCT
13. 5751CP C colombiana	_ccaregerererererererererererererererererer	CCGT
14. 11284 C. neglecta	CATEGOLETICESAGEATEGOLETICECCETERCCCETERCETERCETERCE	GEGI
15. 22445 C. diversiconidia	A CONTRECTOR DETAIL A CONTRECT AND A TETAR CONTRACTOR DE ATACENTA CONTRECTOR ANTE CONTRECTOR A CONTRECT A CONT	CCGI
16. 22092 C ecuadoriana	CCATEGOLGICICEAGTAIGTACTECTITIACCOLATEATACATI-TEGEOCTCIACTAATACTCICCTITACTCICCTATCIACGCCGCTEACCTEGECAGCGTGCCTCAACGCCCAATACCCTEATACCCTACGCCCCACGCCCACGCCCCCCACGCCCCCCCC	GGGI
17. 14797 C mangicola	confecterorceastaterectereterectereterectereteretereteret	CCCI
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3. 10844CPF c papillata	coarced teresteader a terestrated a transference a transference a transference a transference a terestated a terestate a transference a trans	COT
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9. 22442CP C Curvata		JUGI
10. 24174CP C FIMDFIAtomima		1001
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12. 44102CP C lukuonia		CO.T
14 11004 C poplaces		
15 22445 C diversionidia		COOT
16 22092 C equadoriana		COL
17 14797 C mangicola		COOT
ATT ATT - ManyAcore		-
	(5'- ACCCAGTGTAGGGTATTGST	C-3')

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  $(2ng \rightarrow 0,004 \text{ ng})$  of the plasmid (p) containing the targeted CP.RE region from *C. manginecans* Type 2 (CMW13851). R<sup>2</sup> 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 ( $1ng \rightarrow 0,002$  ng) of *C. manginecans* Type 2 (CMW13851) gDNA. R<sup>2</sup> 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).