**Table S1.** List of GenBank accessions used to assess areas of DNA polymorphism among *Colletotrichum* spp. (n=1,487). Available as a Microsoft Office Excel spreadsheet.

**Table S2.** Highest annealing temperature (°C) at which target and non-target species amplified for each primer-probe set (— = never amplified at any temperature). Target amplifications are in **bold**.

| Taxon | CHLAD | FICAL | FRLAD | GLG | HEAP | NOLAD | NYMG | SIAP | THTUB |
|---|---|---|---|---|---|---|---|---|---|
| *Colletotrichum acutatum* species complex | | | | | | | | | |
| *C. acutatum* s.s. | — | 60 | — | — | — | — | — | — | — |
| *C. fioriniae* | 72 | **69** | — | — | — | — | 69 | — | — |
| *C. godetiae* | — | — | — | — | — | — | — | — | — |
| *C. johnstonii* | — | — | — | — | — | — | — | — | — |
| *C. lupini* | — | — | — | — | — | — | 69 | — | — |
| *C. nymphaeae* | — | 67 | — | — | — | 68 | **69** | — | — |
| *C. pyricola* | — | — | — | — | — | — | — | — | — |
| *C. salicis* | — | — | — | — | — | — | — | — | — |
| *Colletotrichum gloeosporioides* species complex | | | | | | | | | |
| *C. chrysophilum* | **72** | 67 | 70 | 65 | — | — | — | — | — |
| *C. fructicola* | 72 | — | **70** | — | — | — | — | — | — |
| *C. gloeosporioides* s.s. | 68 | 67 | 68 | **68** | — | — | — | — | — |
| *C. henanense* | 70 | — | 68 | — | **65** | — | — | — | — |
| *C. kahawae* clade | 68 | — | — | — | — | — | — | — | — |
| *C. noveboracense* | — | — | — | 67 | — | **68** | — | — | — |
| *C. siamense* | 68 | — | 68 | 67 | — | — | 69 | **70** | — |
| *C. theobromicola* | 72 | — | — | — | — | — | — | — | **65** |
| Other fungi | | | | | | | | | |
| *Botryosphaeria dothidea* | — | — | — | — | — | — | — | — | — |
| *Diaporthe* sp. | — | 67 | — | — | — | — | — | — | — |
| *Diplocarpon coronariae* | — | — | — | — | — | — | — | — | — |
| *Erysiphe necator* | — | — | — | — | — | — | — | — | — |
| *Neonectria ditissima* | — | — | — | — | — | — | — | — | — |
| *Penicillium expansum* | — | — | — | — | — | — | — | — | — |
| *Pestalotiopsis maculans* | — | — | — | — | — | — | — | — | — |
| *Phomopsis viticola* | — | — | — | — | — | — | — | — | — |
| *Plasmopara viticola* | — | — | — | — | — | — | — | — | — |
| Plants | | | | | | | | | |
| *Malus domestica* | — | — | — | — | — | — | — | — | — |
| *Vitis vinifera* | — | — | — | — | — | — | — | — | — |

**Table S3.** Real-time PCR standard curve Cq values and effect of apple DNA on Cq (NA = no amplification during assay with apple DNA, nt = not tested because it was below LoD).

| Species | Primer-probe set | [DNA] (ng/µL) | Cq mean ± SD | Ratio fungal DNA: apple DNA | Cq mean ± SD |
|---|---|---|---|---|---|
| *C. chrysophilum* | CHLAD | 1 | 24.09 ± 0.04 | 1:1 | 22.92 ± 2.48 |
| | | 0.1 | 27.44 ± 0.16 | 1:10 | 28.81 ± 1.54 |
| | | 0.05 | 28.35 ± 0.14 | 1:20 | 29.31 ± 1.01 |
| | | 0.01 | 31.07 ± 0.03 | 1:100 | 32.74 ± 0.69 |
| | | 0.005 | 32.52 ± 0.12 | 1:200 | 32.28 ± 0.65 |
| | | 0.001 | 34.77 ± 0.49 | 1:1,000 | 38.14 ± 0.45 |
| | | 0.0005 | 37.03 ± 1.21 | 1:2,000 | NA |
| | | | | | *P* = 0.18299 |
| *C. fioriniae* | FICAL | 1 | 25.75 ± 0.15 | 1:1 | 23.51 ± 0.15 |
| | | 0.1 | 28.95 ± 0.11 | 1:10 | 27.46 ± 0.14 |
| | | 0.05 | 29.99 ± 0.10 | 1:20 | 29.20 ± 0.35 |
| | | 0.01 | 32.28 ± 0.37 | 1:100 | 32.15 ± 0.63 |
| | | 0.005 | 34.00 ± 0.22 | 1:200 | 37.46 ± 0.27 |
| | | 0.001 | 35.92 ± 0.29 | 1:1,000 | 37.38 ± 0.00 |
| | | 0.0005 | 36.85 ± 0.39 | 1:2,000 | 36.41 ± 0.00 |
| | | | | | *P* = 0.82347 |
| *C. fructicola* | FRLAD | 1 | 32.31 ± 0.17 | 1:1 | 31.15 ± 0.07 |
| | | 0.1 | 34.95 ± 0.76 | 1:10 | 34.27 ± 0.29 |
| | | 0.05 | 36.08 ± 0.66 | 1:20 | 35.35 ± 0.21 |
| | | 0.01 | 38.09 ± 0.74 | 1:100 | 38.37 ± 1.52 |
| | | 0.005 | 39.32 ± 0.83 | 1:200 | 37.62 ± 0.00 |
| | | 0.001 | NA | 1:1,000 | nt |
| | | 0.0005 | NA | 1:2,000 | nt |
| | | | | | *P* = 0.07049 |
| *C. gloeosporioides* | GLG | 1 | 22.35 ± 0.22 | 1:1 | 22.58 ± 0.20 |
| | | 0.1 | 25.92 ± 0.18 | 1:10 | 25.65 ± 0.30 |
| | | 0.05 | 26.45 ± 0.28 | 1:20 | 26.58 ± 0.55 |
| | | 0.01 | 28.2 ± 0.17 | 1:100 | 29.99 ± 0.39 |
| | | 0.005 | 29.16 ± 0.34 | 1:200 | 31.51 ± 0.70 |
| | | 0.001 | 31.57 ± 0.55 | 1:1,000 | NA |
| | | 0.0005 | 31.90 ± 0.52 | 1:2,000 | NA |
| | | | | | *P* = 0.47416 |
| *C. henanense* | HEAP | 1 | 23.54 ± 0.14 | 1:1 | 22.83 ± 0.12 |
| | | 0.1 | 27.06 ± 0.15 | 1:10 | 26.60 ± 0.18 |
| | | 0.05 | 27.86 ± 0.22 | 1:20 | 27.48 ± 0.17 |
| | | 0.01 | 30.85 ± 0.23 | 1:100 | 31.58 ± 0.26 |
| | | 0.005 | 31.32 ± 0.14 | 1:200 | 31.32 ± 0.60 |
| | | 0.001 | 34.40 ± 1.01 | 1:1,000 | 34.41 ± 0.52 |
| | | 0.0005 | 34.89 ± 0.48 | 1:2,000 | 34.87 ± 0.18 |
| | | | | | *P* = 0.52883 |

| | | | | | |
|---|---|---|---|---|---|
| *C. noveboracense* | NOLAD | 1 | 25.30 ± 0.86 | 1:1 | 25.10 ± 0.46 |
| | | 0.1 | 29.11 ± 0.14 | 1:10 | 28.67 ± 0.25 |
| | | 0.05 | 30.48 ± 0.17 | 1:20 | 29.86 ± 0.26 |
| | | 0.01 | 32.64 ± 0.31 | 1:100 | 32.08 ± 0.26 |
| | | 0.005 | 33.96 ± 0.28 | 1:200 | 33.53 ± 0.47 |
| | | 0.001 | 36.22 ± 0.31 | 1:1,000 | 34.94 ± 0.00 |
| | | 0.0005 | 36.73 ± 1.22 | 1:2,000 | nt |
| | | | | | *P* = 0.05563 |
| *C. nymphaeae* | NYMG | 1 | 23.84 ± 0.31 | 1:1 | 23.58 ± 0.10 |
| | | 0.1 | 27.03 ± 0.06 | 1:10 | 27.22 ± 0.06 |
| | | 0.05 | 28.33 ± 0.09 | 1:20 | 28.22 ± 0.13 |
| | | 0.01 | 30.72 ± 0.42 | 1:100 | 30.79 ± 0.27 |
| | | 0.005 | 31.94 ± 0.34 | 1:200 | 33.16 ± 0.53 |
| | | 0.001 | 35.40 ± 2.46 | 1:1,000 | 35.36 ± 0.45 |
| | | 0.0005 | 36.53 ± 1.08 | 1:2,000 | NA |
| | | | | | *P* = 0.44967 |
| *C. siamense* | SIAP | 1 | 23.72 ± 0.18 | 1:1 | 23.33 ± 0.35 |
| | | 0.1 | 26.89 ± 0.44 | 1:10 | 26.63 ± 0.29 |
| | | 0.05 | 27.95 ± 0.08 | 1:20 | 27.58 ± 0.24 |
| | | 0.01 | 30.28 ± 0.19 | 1:100 | 31.11 ± 0.08 |
| | | 0.005 | 32.08 ± 0.04 | 1:200 | 32.41 ± 0.60 |
| | | 0.001 | 34.06 ± 0.93 | 1:1,000 | 35.60 ± 1.71 |
| | | 0.0005 | 35.30 ± 0.81 | 1:2,000 | 35.20 ± 0.00 |
| | | | | | *P* = 0.44250 |
| *C. theobromicola* | THTUB | 1 | 28.81 ± 0.17 | 1:1 | 29.35 ± 0.36 |
| | | 0.1 | 31.78 ± 0.23 | 1:10 | 31.71 ± 0.50 |
| | | 0.05 | 32.59 ± 0.22 | 1:20 | 33.71 ± 0.41 |
| | | 0.01 | 36.41 ± 1.37 | 1:100 | 35.97 ± 0.75 |
| | | 0.005 | 37.07 ± 1.36 | 1:200 | 37.44 ± 1.07 |
| | | 0.001 | NA | 1:1,000 | nt |
| | | 0.0005 | NA | 1:2,000 | nt |
| | | | | | *P* = 0.31748 |

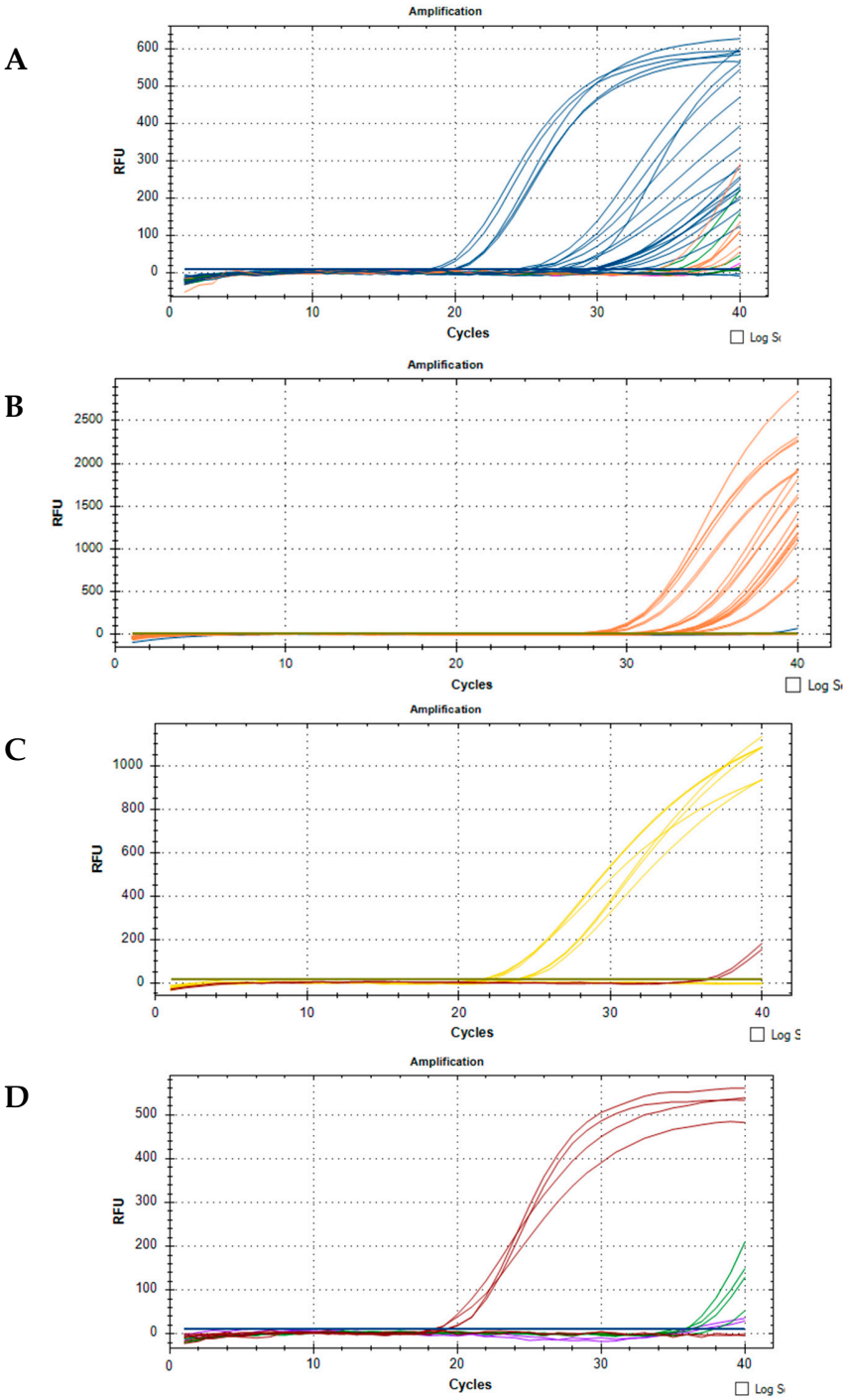


**Figure S1.** Amplification plots showing high Cq values and low RFU values of non-specific amplifications: A) CHLAD primer-probe set, B) FRLAD, C) NOLAD, and D) NYMG. Blue = *C. chrysophilum* (A, B), green = *C. fioriniae* (A, D), orange = *C. fructicola* (A, B), purple = *C. lupini* (D), yellow = *C. noveboracense* (C), dark red = *C. nymphaeae* (C, D), and pink = *C. theobromicola* (A). No-template controls are in the target species color.

| | 5′ CHLADF2 | 22 bp | CHLADP | 59 bp | CHLADR | 3′ |
|---|---|---|---|---|---|---|
| ***C. chrysophilum*** | CATCGTGGCTGTAATTTTGGATGTTTC | ... | GACACCAGTCGCCTTGACGTGG | ... | ATCGGCCGTGACCACCAGCGAAGGATTCGGCAAG | |
| *C. aenigma* | **TG**TC**A**TG**A**CTG**G**A**GC**TT**CC**GA**G**GT**C**TC | ... | **A**ACACCAGTCGCCTTGACGTGG | ... | ATCGGCCGTGACCACCA**A**CGA**G**GG**G**TTCG**C**CA**GC** | |
| *C. camelliae* | C**G**TCGTG**A**CTG**G**AA**C**TT**CC**GA**GA**TC**T**G | ... | **A**ACACCAGT**T**GCCTTGACGTGG | ... | **G**TCGGCCGTGACCACCAGCGA**G**GG**G**TTCGGCA**GC** | |
| *C. fructicola* | **TC**TC**A**TG**A**C**A**GG**A**G**C**TT**CC**GA**GA**TTTC | ... | **A**ACACCAGTCGCCTT**A**ACGTG**A** | ... | **G**TCGGCCGTGACCACCA**AT**GA**G**GG**G**TTCGGCA**GC** | |
| *C. gloeosporioides* | C**G**TC**A**TG**A**CTG**A**AA-TT**GT**G**G**TG**C**TTC | ... | GACACCAGTCGCCTTGACGTGG | ... | **G**TCGGCCGTGACCACCAG**T**GA**G**GG**G**TTCGGCA**GC** | |
| *C. noveboracense* | C**G**TC**A**TG**A**CTG**G**AATTT**GT**GATGTT**C**C | ... | **A**ACACC**T**GTCGC**T**TTGACGTGG | ... | ATCGGCCGTGACCACCA**A**CGA**G**GG**G**TTCGGCA**GC** | |
| *C. nupharicola* | C**G**TC**A**TG**A**CTG**G**AATTT**GT**GATGTT**C**C | ... | **A**ACACC**T**GTCGC**T**TTGACGTGG | ... | ATCGGCCGTGACCACCA**A**CGA**G**GG**G**TTCGGCA**GC** | |
| *C. siamense* | C**G**TC**A**TG**A**CTG**A**AA-TT**GT**G**GC**GTTTC | ... | **A**ACACCAGTCGCCTTGACGTGG | ... | **G**TCGGCCGTGACCACCAGCGAAGG**G**TTCGGCAAG | |
| *C. theobromicola* | **T-A**CA**T**C**A**GG**A**G**T**TT**CTCTT**C**T**CGG** | ... | **A**ACACCAGTCGCCTTGACGTGG | ... | **G**TCGGC**G**GTGAC**G**AC**G**AGCGA**G**GG**G**TTCGGCA**GC** | |
| *C. tropicale* | C**G**TC**A**TG**A**CTG**A**AA-T**CGT**G**GC**GT**G**TC | ... | **A**ACACCAGTCGCCTTGACGTGG | ... | ATCGGCCGT**T**ACCAC**T**AGCGAAGG**G**TTCGGCAAG | |
| *C. viniferum* | **TG**TC**A**TG**A**CTG**G**A**GC**TT**CC**GA**G**GT**C**TC | ... | **A**ACACCAGTCGCCTTGACGTGG | ... | ATCGGCCGTGACCACCAG**T**GAAGG**G**TTCGGCAAG | |

| | 5′ FICALF | 101 bp | FICALP | 3 bp | FICALR | 3′ |
|---|---|---|---|---|---|---|
| ***C. fioriniae*** | TTTACGCAGCAACCACTGGCAACCATC | ... | TTCAAGGTGAGAAGATCTGGCGCAA | ... | GCATGTAGATAGTGCTAATCAGAGAC | |
| *C. acutatum* | T**C**TAC**A**CA**A**CAAC**T**ACTGGCAA**T**CA**CG** | ... | TTCAAGGT**A**AGAAGA**G**CT**A**GT**G**CAA | ... | GCA**C**GT**G**GATA**A**TGCT**G**A**C**CAGAG**GA** | |
| *C. aenigma* | T**GG**AC**ATGCGG**A**ATC**CTG**CGT**------- | ... | TTCAAGGT**TG**G**CTACGAC**GGT**G**CT**T** | ... | **CGC**TG**CGAT**T**GA**TG**TT**GA**CTCTGAGT** | |
| *C. chrysophilum* | T**GG**AC**AC GCGG**A**ATC**CTG**CGT**------- | ... | TTCAAGGT**TG**G**CTACGAC**GGT**G**CT**T** | ... | **CGC**TG**CGAT**T**GA**TG**TT**GA**CTCTGAGT** | |
| *C. conoides* | T**GG**AC**ATGCGG**A**ATC**CTG**CGT**------- | ... | TTCAAGGT**TG**G**CTACGAC**GGT**G**CT**T** | ... | **CGC**TG**CGATCGA**TG**TT**GA**CTCTGAA**T | |
| *C. fructicola* | T**GG**AC**AC GCGG**A**ATC**CTG**CGT**------- | ... | TTCAAGGT**TG**G**CTACGAC**GGT**G**CT**T** | ... | **CGC**TG**CGAT**T**GA**TG**TT**GA**CTCTGAGT** | |
| *C. gloeosporioides* | T**GG**AC**ATGCGG**A**ATT**CTG**CGC**------- | ... | TTCAAGGT**TG**G**CTATGAC**GGT**G**CT**T** | ... | **CGC**TG**CGATCGAC**G**TT**GA**CTCT-AA**T | |
| *C. henanense* | T**GG**AC**ATGCGG**A**ATC**CTG**CAT**------- | ... | TTCAAGGT**TG**G**CTACGGCA**GT**G**TTT** | ... | **CGC**TG**CGATCG**GTGCT**GA**CTCCGC**AT** | |
| *C. hymenocallidis* | T**GG**AC**ATGCGG**A**ATC**CTG**CGT**------- | ... | TTCAAGGT**TG**G**CTACGAC**GGT**G**CT**T** | ... | **CGC**TG**CGATATA**TG**TT**GA**CTCTGA**AT** | |
| *C. nymphaeae* | T**C**TAC**A**CA**A**CAAC**T**ACTGGCAA**T**CA**CG** | ... | TTCAAGGT**A**AGAAGA**G**CT**A**GT**G**CAA | ... | GCA**C**GT**G**GATA**A**TGCT**G**A**C**CAGAG**GA** | |
| *C. siamense* | T**GG**AC**ATGCGG**A**ATC**CTG**CGT**------- | ... | TTCAAGGT**TG**G**CTACGAC**GGT**G**CT**T** | ... | **CGC**TG**CGATATA**TG**TT**GA**CTCTGA**AT** | |
| *C. theobromicola* | TT**G**AC**ATACGGAAAT**CC**GTGT**------- | ... | TTCAAGGT**C**AG**CTACGAGAAT**G**TTT** | ... | **TGC**TG**CG**G**TCGA**TG**TT**GA**CTCTGA**AC | |

| | 5′ FRLADF2 | 22 bp | FRLADP | 59 bp | FRLADR | 3′ |
|---|---|---|---|---|---|---|
| ***C. fructicola*** | TCTCATGACAGGAGCTTCCGAGATTTC | ... | AACACCAGTCGCCTTAACGTGA | ... | GTCGGCCGTGACCACCAATGAGGGGTTCGGCAGC | |
| *C. aenigma* | T**G**TCATGAC**T**GGAGCTTCCGAG**GT**C**T**C | ... | AACACCAGTCGCCTT**G**ACGTG**G** | ... | **A**TCGGCCGTGACCACCAA**C**GAGGGGTTCG**C**CAGC | |
| *C. camelliae* | **CG**TC**G**TGAC**T**GGA**A**CTTCCGAGAT**C**T**G** | ... | AACACCAGT**T**GCCTT**G**ACGTG**G** | ... | GTCGGCCGTGACCACCA**GC**GAGGGGTTCGGCAGC | |
| *C. chrysophilum* | **CA**TC**G**TG**G**C**T**G**T**A**AT**TTT**G**GA**TG**TTTC | ... | **G**ACACCAGTCGCCTT**G**ACGTG**G** | ... | **A**TCGGCCGTGACCACCA**GC**GA**A**GG**A**TTCGGCA**AG** | |
| *C. gloeosporioides* | **CG**TCATGAC**T**G**AAA**-TT**GT**G**GTGC**TTC | ... | **G**ACACCAGTCGCCTT**G**ACGTG**G** | ... | GTCGGCCGTGACCACCA**G**TGAGGGGTTCGGCAGC | |
| *C. noveboracense* | **CG**TCATGAC**T**GGA**AT**TT**GT**GA**TG**TT**C**C | ... | AACACC**T**GTCGC**T**TT**G**ACGTG**G** | ... | **A**TCGGCCGTGACCACCAA**C**GAGGGGTTCGGCAGC | |
| *C. nupharicola* | **CG**TCATGAC**T**GGA**AT**TT**GT**GA**TG**TT**C**C | ... | AACACC**T**GTCGC**T**TT**G**ACGTG**G** | ... | **A**TCGGCCGTGACCACCAA**C**GAGGGGTTCGGCAGC | |
| *C. siamense* | **CG**TCATGAC**T**G**AAA**-TT**GT**G**GCG**TTT**C** | ... | AACACCAGTCGCCTT**G**ACGTG**G** | ... | GTCGGCCGTGACCACCA**GC**GA**A**GGGTTCGGCA**AG** | |

*C. theobromicola* T-ACACATCAGGAGTTTCTCTTCTCGG...AACACCAGTCGCCTTGACGTGG...GTCGGCGGTGACGACGAGCGAGGGGTTCGGCAGC
*C. tropicale* CGTCATGACTGAAA-TCGTGGCGTGTC...AACACCAGTCGCCTTGACGTGG...ATCGGCCGTTACCACTAGCGAAGGGTTCGGCAAG
*C. viniferum* TGTCATGACTGGAGCTTCCGAGGTCTC...AACACCAGTCGCCTTGACGTGG...ATCGGCCGTGACCACCAGTGAAGGGTTCGGCAAG

5' GLGF 45 bp GLGP 5 bp GLGR 3'

*C. gloeosporioides* CTCCAAGCTCGWCATGACTTCAC...GCCGCCCGCGTTTAGTACAC...GGCCATYATGAATTAATGCCAATTGAAATC
*C. aenigma* CTCCAAACTCGCCATGACTTCAC...GCCACCCGCGTTTGGTAAAC...GGCAGTCATGAATGGAGGCCAATTGAAACC
*C. alienum* CTCCAAACTCGCCATGACTTCAC...GCCACCCGCGTTTGGTAAAC...GGCCGTCATGAATGGAGGCCAATTGAAACC
*C. aotearoa* CTCCAAACTCGTCATGACTCTCA...GCCGCCCGCATCTGGTAGAC...GGCTATCTTGACTTGATGCCAATTGAAACC
*C. camelliae* CTCCAAACTCGCCATGACTTCGC...GCCACCCGCGTTTGGTAATT...GGCCGTCATGAATGGAGGCCAATTGAAACC
*C. chrysophilum* CTCCAAACTCGCCATGACTTCAC...GCCACCCGCGTTTGGTAAAC...GGCCGTCATGAATGGAGGCCAATTGAAACC
*C. conoides* CTGCAAACTCGCCATGACTTCAC...GCCACCCGCGTTTGGTAAAC...GGCAGTCATGAATGGAGGCCAATTGAAACC
*C. fructicola* CTCCAAACTCGCCATGACTTCAC...GCCACCCGCGTTTGGTAATT...GGCCGTCATGAATGGAGGCCAATTGAAACC
*C. henanense* CTCCAAACTCGTCATGACT-CTC...GCCGCCCGCATCTGGTAGAC...GGCTATCGTGACTTGATGCCAATTGAAACC
*C. musae* CTCCAAACTCGCCATGACTTCAC...GCCACCCGCGTTTGGTAATT...GGCCGTCATGAATGGAGGCCAATTGAAACC
*C. queenslandicum* CTCCAAACTCGCCATGACTTCAC...GCCACCCGCGTTTGGTAAAC...GGCCGTCATGAATGGAGGCCAATTGAAACC
*C. siamense* CTCCAAACTCGCCATGACTTCAC...GCCACCCGCGTTTGGTAAAC...GGCCGTCATGAATGGAGGCCAATTGAAACC
*C. theobromicola* CTCCAAACTCGCCACTACTTCAC...CCCGCCTGTATTTGGCAGAC...GGCCAACATGAATTGATGCCAATTGATACC
*C. tropicale* CTCCAAAATCGCCATGACTTCAC...GCCACCCGCGTTTGGTAATT...GGCCGTCATGAATGGAGGCCAATTGAAACC

5' HEAPF 19 bp HEAPP 50 bp HEAPR 3'

*C. henanense* TGACTTGGTCATCGATTCGCTTCCCG...CCTTGCGCCAGAAACCAACCCACCT...CGAATCGAGAACCATCCTCGC
*C. aenigma* TCGTCGATTC--------ACTTCCCG...C-CTGCGCGAGAAACCAACAGACCT...GGAACCAAGAATCATCCTGGA
*C. aeschynomenes* TCATCGATTC---------TTTGCCG...CCCTGCACCAGAAAACAACAGACCT...GGAACCAAGAATCATCCTGGC
*C. alatae* TCATCAGTTC--------CATTCCCG...CCCTGCGCCAGAAACCAACAGATCT...TGAATCAAGAATCATCCTGGG
*C. alienum* TCGTCGATTC--------ACTTCCCG...C-CTGCGCGAGAAACCAACAGACCT...GGAACCAAGAGTCATCCTGGA
*C. aotearoa* TCATCGATTC--------GCTTCCCG...CCTTGCGCCAGAAACCAACACACCT...CGAACCAAGAACCATCCTGGT
*C. camelliae* TCATCGATTC--------GCTTCCCG...CCTTGCGCCAGAAACCCACAAACCT...CGAACCAAGAACCATCCTGGT
*C. chrysophilum* TCGTCGATTC--------ACTTCCCG...C-CTGCGCGAGAAACCAACAGACCT...GGAACCAAGAATCATCCTGGA
*C. clidemiae* TCATCGATTC--------GCTTCCCG...CCTTGCGCCAGAAACCCACAAACCT...CGAACCAAGAACCATCCTGGT
*C. conoides* TCGTCGATTC--------ACTTCCCG...C-CTGCGCGAGAAACCAACAGACCT...GGAACCAAGAATCATCCTGGA
*C. fructicola* TCGTCGATTC--------ACTTCCCG...C-CTGCGCGAGAAACCAACAGACCT...GGAACCAAGAATCATCCTGGA
*C. gloeosporioides* TCATCGATTC--------CCTTCCTA...CCCTGCGCCGGAAACCAACAGACCT...CGAACCAAGAATCATCCTGGC
*C. horii* TCGTCGATTC--------CCTTCCCG...CTCTGCGCCAGAGATCAAAAAACCT...CAAACCGAGAACCATCCTGGA
*C. jiangxiense* TCATCGATTC--------GCTTCCCG...CCTTGCGCCAGAAACCCACAAACCT...CGAACCAAGAACCATCCTGGT
*C. musae* TCGTCGATTC--------ACTTCCCG...C-CTGCGCGAGAAACCAACAGACCT...GGAACCAAGAATCATCCTGGA

```
C. noveboracense      TCGTCGATTC--------ACTTCCCG...C-CTGCGCGAGAAACCAACAGACCT...GGAACCAAGAATCATCCTGGA
C. perseae            TCGTCGATTC--------ACTTCCCG...C-CTGCGCGAGAAACCAACAGACCT...GGAACCAAGAATCATCCTGGA
C. psidii             TCATCGATTC--------GCTTCGCG...CCTTGCGCCAGAAACCCACAAACCT...CGAACCAAGAACCATCCTGGT
C. queenslandicum     TCATCGATTC--------CCTTCCCG...CCCTGCGCCAGAAACCAATAGACCT...GGACCCAAGAATCATCCTGGC
C. salsolae           CCATCGATTT--------CCTTCCCG...CCCTGCGCCAGAAACCAACAGACCT...GGAACCAAGAATCATCCTGGC
C. siamense           CCATCGATTC--------CCTTCCCG...CCCTGCGCCAGAAACCAACAGACCT...GGAACCAAGAATCATCCTGGC
C. theobromicola      CCAGCAACGC--------CCTTCCCG...CTCCGTGACAGAAACCAACGGGCGT...CAAACTCACAACCCACTTGGC
C. ti                 TCATCGATTC--------GCTTCCCG...CCTTGTGCCAGAAACCAACACACCT...CGAACCAAGAACCATCCTGGT
C. tropicale          TCATCGATTC--------CCTTCTCG...CCCTGCGCCAGAGACCAACGGGCCT...CGAACCAAGAATCATCCTGGT
C. viniferum          TCGTCGATTC--------ACTTCCCG...C-CTGCGCGAGAAACCAACAGACCT...GGAACCAAGAATCATCCTGGA
C. xanthorrhoeae      TCATCGATTC--------CCTTCCCG...CTCTGCGCCAGAAACCAACAGACCT...CAAATCAAGAACCATCCTGGT

                      5'          NOLADF          2 bp          NOLADP          280 bp          NOLADR          3'
C. noveboracense      GGGGAAGTA-TAGTCAGCGCATTG...CGTCATGACTGGAATTTGTGATGTTCC...GTCGAACGAACGAGAGACGGCGATTA
C. aenigma            AACGAGGGA-CTGTCAGCGAATTG...TGTCATGACTGGAGCTTCCGAGGTCTC...GTCGAACGAACGAGAGACGGCGATTA
C. camelliae          GACGAGAGAATTGTCAGCGCATTG...CGTCGTGACTGGAACTTCCGAGATCTG...GCCGAGCGAACGAGAGACGACAAATA
C. chrysophilum       GACGAGAGG-TTGTCAGCGCATTG...CATCGTGGCTGTAATTTTGGATGTTTC...ATCGAGCGAAGGAGAGACGACAAATC
C. fructicola         AACGAGGGA-CTGTCAGCGCATTG...TCTCATGACAGGAGCTTCCGAGATTTC...ACCGGGCGAAGGAGGGACGACAAATC
C. gloeosporioides    GACGAGAGG-TTGTCAGCGCATTG...CGTCATGACTGAAA-TTGTGGTGCTTC...ATCGAGCGAACGAGAGACGACGAGTC
C. nupharicola        GGGGAAGTA-TAGTCAGCGCATTG...CGTCATGACTGGAATTTGTGATGTTCC...GTCGAACGAACGAGAGACGGCGATTA
C. siamense           GACGAGAAG-TTGTCAGCGCATTG...CGTCATGACTGAAA-TTGTGGCGTTTC...ATCGACCGAACGAGAGACGACGAATC
C. theobromicola      GAGAGGGATGTTGTCAGCGCATTG...T-ACACATCAGGAGTTTCTCTTCTCGG...GTCGAACGAACGAGAGACGACGGATG
C. tropicale          GACGAGAAG-TTGTCAGCGCATTG...CGTCATGACTGAAA-TCGTGGCGTGTC...ATCGAGCGAAGGAGGGACGACGAATA
C. viniferum          AACAAGGGA-CTGTCAGCGGATTG...TGTCATGACTGGAGCTTCCGAGGTCTC...ATCGACCGAGCGAGAGACGATGAATC

                      5'          NYMGF          54 bp          NYMGP          24 bp          NYMGR          3'
C. nymphaeae          GATAACACCAGCTTCGTCGATAT--C...GATTGGGC---TTGTTGTAACGACACG...GCCGAGACAAAACTTGCTGACAGA
C. acutatum           GATAACACCAGCTTCGTCGGTAC--C...GATTGGGC---TCGTTGTAATGATGCG...GCCGAGACAAAATTTGCTGACAGA
C. cuscutae           GATAACACCAGCTTCGTCAATAT--C...GATTGGGC---TTGTTGTAACAACACG...GCCGAGACAAAACTTGCTGACAGA
C. fioriniae          AATAACACCAGCTTCATCGGTAA--C...GATTGGGCTCGTTGTTGTAATGATACG...GCCCAAACAAAATTTGCTGACAGA
C. kinghornii         ACGATAACACCACCCTACTCGGTAAC...GATTTGGC---TCGTTGCAATGGCACG...CCCAAGACAAAATTTGCTGACAGA
C. kniphofiae         GATAACACCACCCCAATCGGTTA--C...GATTTGGC---TTGTTGCAATGGCACC...TCCCAAGAAAAGATTTATTAACAG
C. lupini             GATAACACCAGCTTCGTCGGTAC--C...GATTGGGC---TTGTTGTAATGACACG...GCCGAGACAAAACTTGCTGACAGA
C. orientalis         AATAACACCAGCTTCATCGGTAA--C...GATTGGGCTCGTTGTTGTAATGATACG...GCCCAAACAAAATTTGCTGACAGA
C. pyricola           GATAACACCATCCTAATCGGTAA--C...GATTTGGC---TCGTTGCAATGGCAAG...TCCGAGACAAAATTTGCTGACAGA
C. scovillei          GATAACACCAGCTTCGTCGATAT--C...GATTGGGC---TTGTTGTAACGACACG...GCCGAGACAAAATTTGCTGACAGA
```

| | 5’ SIAPF | 1 bp | SIAPP | 96 bp | SIAPR | 3’ |
|---|---|---|---|---|---|---|
| *C. siamense* | ACTGATATCGGCGCTGCCAG | ... | CGACCTAAGGTTGTCTTTGTGTCCTAG | ... | CACATCTGGCCATCGATTC | --------CCTTC |
| *C. aenigma* | A**TA**GATATCGGCGCTGCCAG | ... | C**C**ACCT**T**AGGT**C**GTC**C**TTGTGT**T**CTAG | ... | C**G**CATCTGG**T**C**G**TCGATTC | --------**A**CTTC |
| *C. aeschynomenes* | AC**C**GATATCGGCGCTGCCAG | ... | CGACCT**C**AGGTTGTCTTTGTGTCCTAG | ... | CACATCTGG**T**CATCGATTC | ---------**-TTG** |
| *C. alatae* | AC**C**GATATCGGCGCTG**TT**AG | ... | CGAC**A**TG**A**AG**C**TGTCT**C**T**A**TGTCCTAG | ... | C**G**CATCTG**AT**CATC**AG**TTC | --------C**A**TTC |
| *C. alienum* | A**TA**GATATCGGCGCTGCCA**A** | ... | CGAC**TTT**AGGT**C**GTC**C**TTGTGT**T**CTAG | ... | C**G**CATCTGG**T**C**G**TCGATTC | --------**A**CTTC |
| *C. aotearoa* | AC**C**GATATCGGCGCTGC**T**AG | ... | CGAC**A**T**T**AGGTTGTCTTTGT**A**TCCTAG | ... | **AAG**ATCTGG**T**CATCGATTC | --------**G**CTTC |
| *C. camelliae* | AC**C**GATATCGGCGCTGC**T**AG | ... | CGAC**A**T**T**AGGTTGTCTTTGT**A**TCCTAG | ... | **AAG**ATCTGG**T**CATCGATTC | --------**G**CTTC |
| *C. chrysophilum* | A**TA**GATATCGGCGCTGCCA**A** | ... | CGAC**TTT**AGGT**C**GTC**C**TTGTGT**T**CTAG | ... | C**G**CATCTG**TT**C**G**TCGATTC | --------**A**CTTC |
| *C. clidemiae* | AC**C**GATATCGGCGCTGC**T**AG | ... | CGAC**A**T**T**AG**T**TTGTCTTTGT**A**TCCTAG | ... | **AAG**ATCTGG**T**CATCGATTC | --------**G**CTTC |
| *C. conoides* | A**TA**GATATCGGCGCTGCCAG | ... | CGAC**TTT**AGGT**C**GTC**C**TTGTGT**T**CTAG | ... | C**G**CATCTG**TT**C**G**TCGATTC | --------**A**CTTC |
| *C. fructicola* | A**TA**GATATCGGCGCTGCCA**A** | ... | CGAC**TTT**AGGT**C**GTC**C**TTGTGT**T**CTAG | ... | C**G**CATCTG**TT**C**G**TCGATTC | --------**A**CTTC |
| *C. gloeosporioides* | AC**C**GATATCGGCGCTGCCAG | ... | C**C**ACCT**T**AGGTTGTCTTTGTG**C**CCT**T**G | ... | **TG**CATCTGG**T**CATCGATTC | --------CCTTC |
| *C. henanense* | AC**C**GATATCG**A**CGCT**A**C**TT**G | ... | CG**G**C**A**T**C**AGGT**G**GTCTTT**TT**A**T**CCTAG | ... | **A**ACATCTGG**TG**A**CTTGG**TC**ATCGATTCG**CTTC |
| *C. horii* | AC**C**GATA**G**CGGCGCTGC**T**AG | ... | CGAC**A**T**G**AGA**A**TGT**G**TTTGT**A**TCC-AG | ... | C**G**CATCTGG**T**C**G**TCGATTC | --------CCTTC |
| *C. jiangxiense* | AC**C**GATA**G**CGGCGCTGC**T**AG | ... | CGAC**A**T**T**AGGTTGTCTTTGT**A**TCCTAG | ... | **AAG**ATCTGG**T**CATCGATTC | --------**G**CTTC |
| *C. musae* | A**TA**GATATCGGCGCTGCCAG | ... | CGAC**TCT**AGGT**C**GTC**C**TTGTGT**T**CTAG | ... | C**G**CA**C**CTGG**T**C**G**TCGATTC | --------**A**CTTC |
| *C. noveboracense* | A**TA**GATATCGGCGCTGCCA**A** | ... | CGAC**TTT**AGGT**C**G**CC**TTGTGT**T**CTAG | ... | C**G**CATCTG**TT**C**G**TCGATTC | --------**A**CTTC |
| *C. perseae* | A**TA**GATATCGGCGCTGCCAG | ... | CGAC**TTT**AGGT**C**GTC**C**TTGTGT**T**CTAG | ... | C**G**CA**C**CTGG**T**C**G**TCGATTC | --------**A**CTTC |
| *C. psidii* | AC**C**GATATCGGCGCTGC**TAC** | ... | CGAC**A**T**T**AG**T**TTGTCTTTGT**A**TCCTAG | ... | **AAG**ATCTGG**T**CATCGATTC | --------**G**CTTC |
| *C. queenslandicum* | AC**C**GATATCG**A**CGCTGCCAG | ... | C**A**ACCT**CAA**GTTGTCTTT**A**TGTCCTAG | ... | CACATCTGG**T**CATCGATTC | --------CCTTC |
| *C. salsolae* | ACTGATATCGGCGCTGCCAG | ... | CGACCT**C**AGGTTGTCTTTGTGTCCTAG | ... | CACATCTGGCCATCGATT**T** | --------CCTTC |
| *C. theobromicola* | ACT**A**AT**G**TC**C**GCGC**AA**C**T**AG | ... | CGAC**A**T**TG**GGTTGTCTTTGT**A**TCC**CA**T | ... | C**G**C**C**TCTGGCCA**G**C**AA**C**G**C | --------CCTTC |
| *C. ti* | AC**C**GATATCGGCGCTGCCAG | ... | CG**C**C**A**T**T**AGGTTGTCTTTGT**A**TCCTAG | ... | **AAG**ATCTGG**T**CATCGATTC | --------**G**CTTC |
| *C. tropicale* | A**TC**GA**C**ATCGGCGCTGC**T**AG | ... | CGACCT**C**AGG**C**TGTC**C**TTGTGTCCTAG | ... | C**G**CATCTG**CT**CATCGATTC | --------CCTTC |
| *C. viniferum* | A**TA**GATATCGGCGCTGCCAG | ... | C**C**ACCT**C**AGGT**C**GTC**C**TTGTGT**T**CTAG | ... | C**G**CATCTGG**T**C**G**TCGATTC | --------**A**CTTC |
| *C. xanthorrhoeae* | AC**C**GATATCGGCGCTGC**T**AG | ... | CGAC**A**T**TG**GG**C**TGTCTTTGT**A**TCCTAG | ... | C**GG**A**G**CTGG**T**CATCGATTC | --------CCTTC |

| | 5’ THTUBF | 14 bp | THTUBP | 106 bp | THTUBR | 3’ |
|---|---|---|---|---|---|---|
| *C. theobromicola* | CTTTCACCCGAGTTCCATGTTCACC | ... | CGTCAATC--CGACCCCCTACTGCG | ... | GCAGGGCTAAGGGCTAATC--TCTCGC | |
| *C. aenigma* | **T**TTT**T**ACCCGA**C**TTC**T**ATG**CA**CA**A**C | ... | **T**GTCAATC**AT**CGAC**G**CCC**A**ACT**CT**G | ... | GC**G**GGG-------CTAA**C**C--TC**CTTG** | |
| *C. aeschynomenes* | **T**TTT**T**ACCCGA**C**TTC**T**ATG**C**TCA**A**C | ... | **T**GTCAATC**AT**CGAC**GT**CC**A**ACT**CT**G | ... | GC**G**GGG-------CTAA**C**C--TC**CTTG** | |
| *C. alatae* | **T**TTT**T**ACCCGA**C**TTCCATG**C**TCACC | ... | CGTCAA**CCAT**CGAC**TT**CCTACT**CT**G | ... | GC**G**GGG-------CTAA**C**C--TC**CTTG** | |
| *C. alienum* | **T**TTT**T**ACCCGA**C**TTC**T**ATG**CA**CA**A**C | ... | **T**GTCAATC**AT**CGAC**G**CCC**A**ACT**CT**G | ... | GC**G**GGG-------CTAA**C**C--TC**CTTG** | |
| *C. aotearoa* | **G**TTT**T**AC-------CCATG**C**TCACC | ... | CGTCAATC**AT**CGACC**T**CCTACT**CT**G | ... | GC**G**GGG-------CTAA**C**C--TCT**TTG** | |

*C. asianum* **T**TTT**T**ACCCGA**C**TTCCATG**C**TCA**A**C...**T**GTCAATC**AT**CGACC**T**CC**A**ACT**CT**G...GC**G**GGG-------CTAA**C**C--TC**CTTG**
*C. camelliae* **G**TTT**T**AC-------CCATG**A**TCACC...CGTCAATC**AT**CGACC**T**CCTACT**CT**G...GC**G**GGG-------CTAA**C**C--TCT**TTG**
*C. changpingense* **T**TTT**T**ACCCGA**C**TTC**T**ATG**C**TCA**A**C...**T**GTCAATC**AT**CGAC**GT**CC**A**ACT**CT**G...GC**G**GGG-------CTAA**C**C--TC**CTTG**
*C. chrysophilum* **T**TTT**T**ACCCGA**C**TTC**T**ATG**CA**CA**A**C...**T**GTCAATC**AT**CGAC**T**CCC**A**ACT**CT**G...GC**G**GGG-------CTAA**C**C--TC**CTTG**
*C. cigarro* **G**TTT**T**AC-------CCATG**C**TCACC...CGTCAATC**AT**CGACC**T**CCTACT**CT**G...GC**G**GGG-------CTAA**C**C--TCT**TTG**
*C. cordylinicola* **G**TTT**T**AC-------CCATG**C**TCACC...CGTCAATC**AT**CGACC**T**CCTACT**CT**G...GC**G**GGG-------CTAA**C**C--TCT**TTG**
*C. endophyticum* **T**TTT**T**ACCCGA**C**TTCCATG**C**TCA**A**C...**T**CTCAATC**AT**CGACC**T**CCTACT**C**CG...GC**G**GGG-------CTAA**C**C--TC**CTTG**
*C. fructicola* **T**TTT**T**ACCCGA**C**TTC**T**ATG**CA**CA**A**C...**T**GTCAATC**AT**CGAC**G**CCC**A**ACT**CT**G...GC**G**GGG-------CTAA**C**C--TC**CTTG**
*C. gloeosporioides* **T**TTT**T**ACCCGA**CC**TC**TT**TG**C**TCA**A**C...**T**GTCAATC**AT**CGACC**T**CCTA**GT**C**T**G...GC**G**GGG-------CTAA**C**C--TC**CTTG**
*C. grevilleae* CTTTCACCCGAGTTCCATGTTCACC...CGTCAATC--CGACCCCCTACTGCG...GCAGGG-------CTAATC--TCTCGC
*C. grossum* CTTTCACCCGAGTTCCATGTTCACC...CGTCAATC--CGACCCCCTACTGCG...GCAGGG-------CTAATC--TCTCGC
*C. henanense* **G**TTT**T**AC-------CCATG**C**TCACC...CGTCAATC**AT**CGACC**T**CCTACT**CT**G...GC**G**GGG-------CTAA**C**C--TCT**TTG**
*C. horii* **T**TTT**T**ACCCGA**C**TTCCATG**C**TCA**A**C...C**T**TCAATT**AT**CGAC**GT**CCTACT**CT**G...GC**G**GGG-------CTAA**CCAGAG**T**TCT**
*C. jiangxiense* **G**TTT**T**AC-------CCATG**C**TCACC...CGTCAATC**AT**CGACC**T**CCTACT**CT**G...GC**G**GGG-------CTAA**C**C--TCT**TTG**
*C. kahawae* **G**TTT**T**AC-------CCATG**C**TCACC...CGTCAATC**AT**CGACC**T**CCTACT**CT**G...GC**G**GGG-------CTAA**C**C--TCT**TTG**
*C. musae* **T**T--------------ATG**CA**CA**A**C...**T**GTCAATC**AT**CGAC**G**CCC**A**ACT**CT**G...GC**G**GGG-------CTAA**C**C--TC**CTTG**
*C. noveboracense* **T**TTT**T**ACCCGA**C**TTC**T**ATG**CA**CA**A**C...**T**GTCAATC**AT**CGAC**G**CCC**A**ACT**CT**G...GC**G**GGG-------CTAA**C**C--TC**CTTG**
*C. proteae* **T**TTT**G**ACCCGA**C**TTCCATG**C**TCA**A**C...**T**GTCAATC**AT**CGAC**T**CC**A**TACT**CT**G...GC**G**GGG-------CTAA**C**C--TC**CGTG**
*C. queenslandicum* **T**TTT**T**ACCCGA**C**TTCCATG**C**TCA**A**C...**T**GTCAATC**AT**CGAC**GT**CC**A**ACT**CT**G...GC**G**GGG-------CTAA**C**C--TC**CTTG**
*C. salsolae* **T**TTT**T**ACCCGA**C**TTC**T**ATG**C**TCA**A**C...**T**GT**T**AATC**AT**CGAC**GT**CC**A**ACT**CT**G...GC**G**GGG-------CTAA**C**C--TC**CTCG**
*C. siamense* **T**TTT**T**ACCCGA**C**TTC**T**ATG**C**TCA**A**C...**T**GTCAATC**AT**CGAC**GT**CC**A**ACT**CT**G...GC**G**GGG-------CTAA**C**C--TC**CTTG**
*C. tropicale* **T**TTT**T**ACCCGA**T**TTC**T**ATG**C**TCA**A**C...**T**GTCAATC**AT**CGAC**GT**CC**A**ACT**CT**G...GC**G**GGG-------CTAA**C**C--TC**CTTG**
*C. viniferum* **T**TTT**T**ACCCGA**C**TTC**T**ATG**CA**CA**A**C...**T**GTCAATC**AT**CGAC**G**CC**AA**ACT**CT**G...GC**G**GGG-------CTAA**C**C--TC**CTTG**
*C. wuxiense* **G**TTT**T**AC-------CCATG**A**TCACC...CGTCAATC**AT**CGACC**T**CCTACT**CT**G...GC**G**GGG-------CTAA**C**C--TCT**TTG**

**Figure S2.** Alignments of available *Colletotrichum* accessions at primer and probe sites for each primer-probe set. **Bold** indicates mismatches with the target species, "-" is a gap, and "…" is excluded sequence. For *C. chrysophilum* ladA, *C. fructicola* ladA, C. *gloeosporioides* GAPDH, *C. noveboracense* ladA, *C. nymphaeae* GAPDH, and *C. theobromicola* THTUB, accessions outside their species complex were excluded due to high sequence dissimilarity at these primer and probe sites.