Supporting Information Appendix

SI Materials and Methods

***Cercospora* and *Colletotrichum spp.* genome sequencing**

Genomic DNA of *C. beticola* strain 09-40 was isolated using the CTAB method from mycelia scraped from the surface of V8 juice agar Petri plates (1). Library preparation of three genomic libraries with increasing insert size (500 bp, 5 Kbp and 10 Kbp) and subsequent paired-end (PE) and mate-pair (MP) genome sequencing was performed by BGI Americas Corporation (BGI) using the Illumina platform. A total of ~11,100,000 high-quality filtered sequence reads with an average length of 100 bp were generated for the 500 bp PE library, ~23,500,000 reads of 50 bp length were derived from the 5 Kbp MP library, and a total of ~16,800,000 reads of 90 bp length were obtained from the 10 Kbp MP library. Total sequence output was ~3.8 Gbp, corresponding to an estimated 100-fold coverage. In the first stage of the genome assembly, SOAPdenovo (version 2.223) was used with the following command: "*SOAPdenovo-63mer all —s config.txt —K 51 —R —o soapfinal.fa —p 8 —L 200*" to assemble contigs and scaffolds incorporating all three libraries as input. The libraries were ranked according to insert size, using the smallest insert library as ranked first. Only the smallest insert library was used for all stages of the assembly with option "*asm_flags=3*", the other two libraries were only used for scaffold assembly with option "*asm_flags=2*".

For the second stage of the genome assembly, optical mapping was used to scaffold sequence reads. Optical maps were generated on the Argus System by BGI and sequences were placed using MapSolver (version 3.2.2; Opgen). BioNano Genomics optical maps (genome maps) were generated on the Irys System (version 9; BioNano Genomics) at the Nucleomics Core facility (Vlaams Instituut voor Biotechnologie), and sequences were placed using the IrysView software. Recommendations by the software packages were followed for scaffold placement. The resulting alignment maps were subsequently compared visually, and accordingly an AGP-like (A Golden Path) file was constructed

manually, applying a majority-consensus ruling wherever necessary that details the placement (position and orientation) of all sequences (Supplementary File 1). Using the custom Perl script *parse_agp-like_to_fasta.pl*, the AGP-like file was used to construct 12 supercontigs (possibly chromosomes) ranging in size from ~227 Kbp to ~6.2 Mbp. We then applied Pilon (version 1.7; http://www.broadinstitute.org/software/pilon) for automated assembly improvement. To this end, the sequencing reads from all three libraries (MP libraries were reverse complemented prior to mapping) were aligned to the genome assembly using Bowtie (2) (version 2.1.0) implementing default parameters for end-to-end mapping (i.e. "*—end-to-end*, *--sensitive*", with the exception of insert length, that was adjusted accordingly for each library). Pilon was run subsequently with default parameters, incorporating the mapped PE library as "frags" and the mapped MP libraries as "jumps."

Repetitive sequences were identified by RepeatMasker (version open-4.0.3; http://www.repeatmasker.com) using two distinct libraries: 1) the RepBase repeat library (http://www.girinst.org/server/RepBase/index.php, obtained on October 23, 2013) and 2) a *C. beticola* specific repeat library constructed using the RepeatModeler (version 1.0.7; http://www.repeatmasker.org/RepeatModeler.html). A total of 45 repeat families were found by RepeatModeler, of which 13 could be classified.

Cercospora berteroae strain CBS 538.71 was obtained from Centraal Bureau voor Schimmelcultures (CBS) and cultivated on Petri plates containing potato dextrose agar (PDA; Difco). High-quality DNA was extracted using the CTAB method (3). Library preparation (500 bp) and subsequent paired-end (PE) genome sequencing was performed by BGI via the Illumina platform. A total of 31 million high-quality filtered sequence reads with an average length of 100 bp were generated. A draft genome assembly was constructed using SOAPdenovo (version 2.04), applying default parameters and K-mer length 51. Protein-coding gene models were predicted *ab initio* using the previously established training parameters in Augustus (version 3.2.1).

Colletotrichum fioriniae strains HC89 and HC91 were isolated previously from infected apples and cultivated on Petri plates containing potato dextrose agar (PDA; Difco). High-quality DNA was extracted using the CTAB method (3). Library preparation (500 bp) and subsequent paired-end (PE) genome sequencing was performed by BGI via the Illumina platform. Draft genome assemblies were constructed using SOAPdenovo (version 2.04), applying default parameters and K-mer length 51. Protein-coding gene models were predicted using the *Neurospora crassa* training parameters incorporated in Augustus (version 3.2.1).

Transcriptome sequencing

To aid in gene prediction and discover gene expression patterns under specific conditions, three RNA samples were prepared for RNA sequencing; one from *in vitro* cultured *C. beticola* tissue and two from *C. beticola* infected sugar beet seedlings at four and seven days post inoculation (DPI). Total RNA was extracted from flash-frozen fungal and/or plant material using a Qiagen total RNA extraction kit as described (4). Library preparation and sequencing were performed at BGI, which resulted in the generation of ~12,800,000, 44,500,000 and 44,800,000 PE reads of length 90 bp and fragment size of 200 bp for the *in vitro*, the 4DPI *in planta* and the 7 DPI *in planta* libraries respectively.

Genome-guided Trinity assembly (5) of sequencing reads was subsequently performed as described by the software manual, with minimal intron length of 20 bp and the GSNAP aligner. Transcript assemblies were subsequently generated using PASA2 (version r20140417). We applied another transcript assembly approach simultaneously by mapping the RNA-Seq reads to the reference genome using Tophat (version 2.0.8b; http://ccb.jhu.edu/software/tophat/index.shtml), converting these to raw transcript assemblies using Cufflinks (version 2.1.1; http://cufflinks.cbcb.umd.edu/) and final selection of the best assemblies using TransDecoder (version 2014-07-04; http://transdecoder.sourceforge.net/).

Gene prediction and curation

EvidenceModeler (6) was used to predict protein-coding genes by integrating protein-coding evidence from multiple sources according to specified weights. First, Augustus (7) parameters (version 2.5.5) were trained locally with the *autoAug.pl* script included in the Augustus software package, using the assembled Trinity transcripts sequences as training input as well as hints in the final gene prediction step. Secondly, GeneMarkES (8) (version 2.3c) was used in self training modus, optimizing coding parameters for the full genome sequence. Protein similarity was detected by the alignment of proteins from the related fungal species *Z. tritici*, *P. nodorum*, *L. maculans*, *C. heterostrophus* and *C. zeae-maydis* against the genome sequence using the Analysis and Annotation Tool (AAT) package (9) (version r03052011). Protein sequences from these related fungi were derived from the MycoCosm platform at the Joint Genome Institute (JGI; http://genome.jgi-psf.org/programs/fungi/index.jsf). For integration of the various protein-coding sources with the EvidenceModeler, we ranked all sources according to their expected accuracy and importance. From high to low the ranking was: genome-aligned Trinity transcripts (with PASA), Cufflinks-derived TransDecoder transcripts, trained Augustus *ab initio* predictions, aligned protein sequences and self-trained GeneMarkES *ab initio* predictions. These initial EvidenceModeler transcripts were substantially further improved by manual curation on the WebApollo (10) (version 2013-05-16) platform. Approximately 10,500 gene models were visually analyzed and updated according to the available evidence as needed. The updates included adjustments to splice sites (e.g. alternative donor or acceptor sites), exon usage, five prime and three prime UTRs (untranslated regions) and the addition of alternative transcripts where supported by RNA-Seq evidence. A majority-consensus ruling was used for the updates, considering all evidence tracks. For transfer of the final gene predictions to newer versions of the assembly, the map2assembly algorithm from Maker was used routinely (version 2.28; http://www.yandell-lab.org/software/maker.html).

To investigate the predicted gene models in more detail, we calculated a number of statistics based on the gene structures. For this purpose, all gene models were stored in GFF3 format according to

the specifications at The Sequence Ontology Project (http://www.sequenceontology.org/gff3.shtml). The *gff3_gene_prediction_file_validator.pl* Perl script, part of the EvidenceModeler package was routinely used for verification of GFF3 file format. GFF3 gene statistics, e.g. the mean/median gene length, the number of exons, CDSs or UTRs, were calculated using the custom Perl script, *GFF3stats.pl*.

Protein function characterization

For functional characterization of the predicted protein sequences, hardware-accelerated BLASTp on a DeCypher machine (TimeLogic; Carlsbad, USA) was used to identify homologous proteins in the non-redundant (nr) protein database obtained at the NCBI. InterProScan (version 5.44; http://www.ebi.ac.uk/Tools/pfa/iprscan5/) was used to identify conserved protein domains. The results of both analyses were imported into Blast2GO (11) and used to generate single, uniquely functional annotations for each protein as well as a list of all associated gene ontology (GO) terms.

Secondary metabolite cluster identification, characterization and visualization

Putative SM clusters were identified in the genome sequence of *C. beticola* and that of related fungi using antiSMASH2 (12) (version 2.1.0; https://bitbucket.org/antismash/antismash2/). To generate antiSMASH2-required EMBL formatted genome files, the GFF3 gene features files in combination with the respective genome sequences were converted to the EMBL sequence format using the custom Perl script *GFF3_2_EMBL.pl*. Subsequently, antiSMASH2 was run with default parameters, allowing for the identification of PKS, NRPS SM, Hybrid PKS-NRPS, terpene cyclase (TC), siderophore and lantipeptide SM clusters. SM clusters that showed similarity to a mixture of these clusters or only a minimal set of homologous protein domains were depicted as “other.” In addition, DMAT, for dimethylallyl tryptophan synthase clusters were identified by screening the InterProScan results for Pfam domain PF11991.

Secondary metabolite phylogenetic analyses

For phylogenetic analyses of the type I polyketide enzymes we used Mafft (version 7.187), applying global alignment (--globalpair) and a 1000 cycles of iterative refinement (--maxiterate 1000), to align full-length

sequences as well as selected domains of all PKS enzymes that were identified by antiSMASH2 in the genome sequences of the six Dothideomycetes: *C. beticola*, *D. septosporum*, *Z. tritici*, *L. maculans*, *P. tritici-repentis* and *P. nodorum*, and one Eurotiomycete: *Aspergillus nidulans*. In addition, previously characterized polyketide synthases (Supplementary Table 3) were included for reference. Prior to phylogenetic tree reconstruction, the alignments were trimmed with TrimAl (13) (version 1.2). Maximum likelihood phylogenetic trees were determined with RAxML (version 8.1.3), applying rapid bootstrapping (-# 100) and automated protein model selection (-m PROTGAMMAAUTO). Final trees were prepared online using EvolView (14). Species tree topologies were built with CVtree (15) webserver by uploading the predicted proteomes of 48 published Ascomycete fungi (Table S4).

For phylogenetic tree reconciliation analyses of the protein and species trees, the protein trees were pre-processed with treefixDTL (16) (version 1.1.10) to minimize errors introduced during tree reconstruction. TreefixDTL is able to correct phylogenetic trees in the presence of horizontal gene transfer. Reconciliation analyses as well as rooting were conducted in NOTUNG (17) according to the instructions (version 2.8 beta).

Secondary metabolite cluster alignment visualization

For comparative analyses of the secondary metabolite clusters, we used the R-package *genoPlotR* (18) (version 0.8.2; http://genoplotr.r-forge.r-project.org/). To this end, individual clusters were extracted from the genome sequence using the Perl script *get_seq_by_id.pl*, using the start or stop positions of the flanking genes in the cluster as extremities. The resulting sequences were then aligned using the bl2seq (BLAST two sequences) algorithm, part of the BLAST toolkit (version 2.2.26). Also, transcripts locations (CDS start and stop coordinates specifically) were extracted from the GFF3 files using *gff3CDS_2_genoplot.pl*, and adjusted according to the start and end coordinates of the extracted cluster sequence, by means of the Unix command line tool Awk. We then used custom R code (example in *genoPlotR.R*) to parse these input files and generate cluster alignment figures.

Genome-wide gene cluster microsynteny and protein identity analysis

Genome-wide gene-by-gene cluster analyses were performed using the custom Perl script *calClusterSimilarity.pl*, and plotted using ggplot2 in R using *synteny.R*. As input, this pipeline takes the typical output of an orthoMCL analysis, reformatted by *analyseOrthoMCL.pl*. In short, it requires each proteinId to have an associated clusterId. Furthermore, it requires properly formatted GFF3 files for each genome that are used to associate location of protein-coding genes and their flanks. Finally, the number of flanking genes to be used can be chosen freely, but must be set ODD. For the analyses presented in Figure 4, a cluster size of 30 was set. Genome-wide protein-by-protein best-BLASTp percent identities were derived from the similarities table prepared during orthoMCL analyses and subsequently plotted in R using *pairwise_pident_boxplots.R*.

All custom Perl and R scripts are deposited as part of auxiliary data on figshare with permanent doi here: https://doi.org/10.6084/m9.figshare.4056522.v1, and are also hosted on GitHub here: https://github.com/rdejonge/genomics.

Gene expression analysis

To investigate the expression of cercosporin cluster genes, *C. beticola* was grown in a 250 mL Erlenmeyer flask containing 100 mL potato dextrose broth (PDB; Difco) either in the light or dark, to promote and repress cercosporin production, respectively. Total RNA was isolated using TRIzol (ThermoFisher) following the manufacturer's instructions followed by an on-column Dnase treatment (Qiagen). Total RNA was used for cDNA synthesis using an oligo-(dT) primer and the SuperScript II reverse transcriptase kit (Invitrogen) following manufacturer's instructions. The resulting cDNA was used as a template for quantitative polymerase chain reaction (qPCR). Selected genes were queried for expression using the Power SYBR Green PCR Master Mix (Applied Biosytems) using a PTC-2000 thermal cycler (MJ Research) outfitted with a Chromo4 Real-Time PCR Detector (Bio-Rad) and MJ Opticon Monitor analysis software version 3.1 (Bio-Rad). Primers for gene expression analysis are listed in Table S10.

To investigate the expression of *Co. fioriniae CTB1 in planta*, apples were inoculated with *Co. fioriniae* as described (19) or mycelia were collected from *in vitro* cultures (non- and TSA-induced). Total RNA was isolated from infected apples as described by Gapper et al (20) except the RETROscript Reverse Transcription kit (Invitrogen, USA) was used for cDNA synthesis. Total RNA was isolated from *in vitro* cultures as described above. qPCR parameters were as described above. Gene expression was quantified using procedure described by Pfaffl (21) using primers listed in Table S10.

Transformation and disruption of target genes

Split-marker PCR constructs for targeted gene replacement were prepared as described (1) using genomic DNA of 10-73-4 and 09-40 wild-type *C. beticola* and pDAN as PCR templates. Selected mutants were complemented using pFBT005, which encodes resistance to nourseothricin and allowed us to clone our gene of interest between the ToxA promoter and TrpC terminator using PacI and NotI (Promega) restriction sites. Primers for split-marker and complementation constructs are listed in Table S9.

A 5 mm plug was taken from the actively growing zone of *C. beticola* on PDA. Liquid cultures were initiated by grinding the plug with 500 µL Fries medium (1), which was subsequently transferred to a 125 mL flask containing 50 mL Fries medium . Flasks were wrapped in aluminum foil and shaken at 150 rpm at 21 °C for four days. Cultures were then transferred to a sterile blender cup, ground for 10 s, and transferred to a 500 mL flask containing 200 mL Fries medium. Cultures were grown as described above for an additional 24 h after which mycelium was harvested with two layers of Miracloth (Calbiochem) using a Büchner funnel. The mycelium was rinsed with mycelium wash solution (0.7M KCl, 10mM $CaCl_2 \cdot 2H_2O$), broken into small pieces with a sterile spatula, transferred to a deep Petri dish containing 40 mL of osmoticum (20 g L^{-1} lysing enzymes from *T. harzianum* (Sigma-Aldrich) and 12.5 g L^{-1} Driselase (Sigma-Aldrich), and incubated at 30 °C for 6 to 14 h while shaking at 50 rpm. Protoplasts were harvested by filtering the osmoticum solution through two layers of Miracloth and collected by centrifugation (2000 x *g* for 5 min). After a washing step with 15 mL STC (1 M Sorbitol, 10 mM Tris-HCl pH 7.5, 10 mM $CaCl_2$),

protoplasts were adjusted to 10^8 mL^{-1} with STC:PEG (4:1) and divided into 200-µL aliquots. Subsequently, PEG-based transformation was performed essentially as described (1) using 70 µg of each construct or 70 µg of complementation plasmid linearized with ApaI (Promega) per 200 µL protoplast aliquot.

Cercosporin production

To screen for the ability to produce cercosporin, 10 mm plugs were transferred from the growing edge of *C. beticola* 10-73-4 wild-type, mutant and complemented mutant colonies to 100 mm x 15 mm Petri plates (Falcon; Oxnard, USA) that contained 10 mL PDA. After an incubation period of 6 days at 21 °C with a natural light-dark cycle, five 10 mm (dia) plugs of PDA containing *C. beticola* mycelium were collected and extracted with ethyl acetate for 18 h in the dark at 4 °C. The extracts were filtered and dried under a stream of nitrogen (30 °C). The dried extracts were re-dissolved in 25% acetonitrile in 1% aqueous acetic acid and fractionated by reverse-phase HPLC on a Waters 600 HPLC system fitted with a Waters radial pak column (8 mm x 120 mm, 8 µm). Injections were 200 µL. Solvent A was 1% (v/v) aqueous acetic acid and solvent B was acetonitrile. Starting conditions were 25% A 75% B, hold for 1 min, a linear gradient to 100% B in 19 min, hold for 5 min, and return to initial conditions in 5 min. Where needed, extracts were also subjected to thin-layer chromatography (TLC) on Silica HF Plates pre-coated with calcium phosphate and reactivated as described Balis and Payne (22). Plates were developed in hexane: 2-propanol (8:2, v/v) and visualized by UV fluorescence (302 nm) using a transilluminator (Alpha Innotech Corp; CA). For cercosporin isolation, multiple mycelial mats were collected and extracted two times with ethyl acetate (4 °C, dark). After filtration, the extracts were partitioned against an equal volume of reagent-grade water, residual water was removed by passage through anhydrous sodium sulfate, and the extracts were dried under a stream of nitrogen (30 °C). Cercosporin was purified by TLC as described above and quantified by spectrophotometry using published extinction coefficients (23).

Pre-cercosporin isolation and characterization

Mycelial plugs of *C. beticola ΔCTB9* were placed on top of eight "thin" potato dextrose agar (PDA, Difco) plates (3.0 mL PDA per 50 mm Petri plate). Cultures were incubated at 22 °C for one week under continuous light. Three separate methods were attempted to prepare crude secondary metabolite extractions. 1) PDA and mycelia were extracted with ethyl acetate for 4 min. The resulting supernatant was collected and frozen for further analysis. 2) PDA and mycelia were placed into a GenElute Maxiprep binding column (Sigma Aldrich) and centrifuged at 3500 x *g* for 10 min. The flow through was collected and frozen for further analysis. 3) PDA and mycelia were placed into a GenElute Maxiprep binding column along with 15 mL ethyl acetate. After a 30 s incubation, the column was centrifuged at 2400 x *g* for 10 min. The flow through was collected and frozen for further analysis. To obtain sufficient pre-cercosporin for isolation and ^{1}H-NMR analysis, primary extracts from all three methods were combined (less than 1 mg of cercosporin was obtained from eight 50 mm plates).

The combined extracts were re-suspended with water and acidified with conc. HCl. Pre-cercosporin was extracted quickly from this aqueous solution by partitioning thrice with ethyl acetate in dark, wrapping the glassware with aluminum foil. The combined ethyl acetate fractions were washed with brine, dried over anhydrous sodium sulfate and evaporated under vacuum at 30 °C. The reddish-brown residue was resuspended in methanol and filtered through 0.2 µm PTFE filters. The methanol extracts were initially analyzed by reverse phase HPLC on an Agilent model 1200 fitted with a Kinetex XB-C18 column (4.6 mm x 75 mm, 2.6 µm, Phenomenex). Injections of 1 µl were run at 1.25 mL/min on a linear gradient of 5% solvent C/95% solvent D to 95% solvent C/5% solvent D over 10.8 min, where solvent C was acetonitrile + 0.1% formic acid and solvent D was 0.1% formic acid. Chromatograms were monitored at 436, 280, and 210 nm, and UV-vis spectra were recorded over a range of 210-800 nm. High-resolution mass data were obtained from a Waters Acquity/Xevo-G2 UPLC-ESI-MS in positive ion mode.

To isolate sufficient pre-cercosporin for ^{1}H-NMR analysis, the filtered methanol extract prepared above was purified by reverse-phase HPLC on an Agilent model 1100 fitted with a Kinetex XB-C18 semi-

prep column (10 mm x 250 mm, 5 µm, Phenomenex). The crude extract (10 mg/mL in methanol) was injected (generally 500 µL) and run at 4 mL/min using the following method: 20% solvent C/80% solvent D for 3 min, 20-70% solvent C over 17 min, 70-95% solvent D over 5 min, where solvent C and D were as above. Chromatograms were recorded at 436, 280, and 210 nm. The metabolite of interest was collected from multiple injections, combined, and lyophilized to dryness. The purified pre-cercosporin was analyzed by UPLC-ESI-MS as described above and ^{1}H-NMR.

To isolate sufficient pre-cercosporin for ^{13}C NMR analysis, mycelial plugs of *C. beticola ΔCTB9* were placed on top of 320 "thin" PDA plates (3.0 mL PDA per 60 mm Petri plate) amended with 2 mM 1-^{13}C-sodium acetate (Sigma-Aldrich) and 2 mM 2-^{13}C-sodium acetate (Cambridge Isotope Laboratory; Tewksbury, MA). Cultures were incubated at 22 °C for one week. The following extraction procedures were carried out quickly under low light: PDA was collected from Petri plates and extracted with ethyl acetate. The ethyl acetate/mycelium mixture was transferred to a sterile blender cup, ground for 10 s, and placed into a GenElute Maxiprep binding column (Sigma Aldrich) and centrifuged at 3500 x *g* for 1 min. The flow through was collected and frozen until further analysis. The combined extracts were re-suspended with water and pre-cercosporin was extracted quickly from this aqueous solution by partitioning thrice with ethyl acetate in the dark, wrapping the glassware with aluminum foil. The combined ethyl acetate fractions were washed with brine, dried over anhydrous sodium sulfate and evaporated under vacuum at 30 °C. The reddish-brown residue was resuspended in methanol and filtered through 0.2 µm PTFE filters. The filtered methanol extract was purified by reverse-phase HPLC on a CombiFlash EZ prep model fitted with a Luna C18 prep HPLC column (50 mm x 250 mm, 10 µm). The crude extract (22.5 mg/mL in methanol) was injected all at once and run at 155 mL/min using the following method: 20% solvent C/80% solvent D for 2 min, 20-70% solvent C over 18 min, where solvent C was acetonitrile + 0.1% formic acid and solvent D was water + 0.1% formic acid. Chromatograms were

recorded at 400 and 280 nm. The metabolite of interest was collected from single injection and lyophilized to dryness.

4,6,9-trihydroxy-1,12-bis(2-hydroxypropyl)-2,7,11-trimethoxyperylene-3,10-dione (pre-cercosporin)

^{1}H NMR (400 MHz, $CDCl_3$, δ), (Data obtained at 5 °C on a Bruker AVANCE spectrometer, Fig. S9A): 15.24 (s, 1H), 14.93 (s, 1H), 9.25 (s, 1H), 6.92 (s, 1H), 6.87 (s, 1H), 4.28 (s, 3H), 4.19, (s, 3H), 4.18 (s, 3H), 3.57 – 3.51 (m, 2H), 3.42 – 3.36 (sym 5-line overlapping signal, 2H), 2.86 – 2.74 (m, 2H), 0.64 (d, *J* = 6.1 Hz, 3H), 0.60 (d, *J* = 6.1 Hz, 3H). ^{13}C NMR (125MHz, $CDCl_3$, δ), (Data obtained at 9 °C on a Bruker AVANCE II spectrometer with TCI Cryoprobe, Fig. S9B and S9C): 181.47, 181.24, 169.58, 169.17, 162.99, 161.21, 152.92, 152.53, 136.03, 134.32, 131.19, 129.18, 128.97, 127.84, 113.23, 112.46, 107.61, 106.75, 105.94, 101.53, 68.22, 68.20, 61.43, 57.95, 42.14, 41.88, 23.58, 23.35. UPLC-ESI-HRMS: calculated for $C_{29}H_{29}O_{10}$ [M+H$^+$]: 537.1761, found [M+H$^+$]: 537.1762.

Quantification of cercosporin recovery from apple

A standard curve for quantification of cercosporin by HPLC was prepared using cercosporin isolated from WT *C. beticola.* Crude cercosporin extraction was as described above. The crude extract was resuspended in methanol and further purified using a Sephadex LH20 column. Injections of 800 µL were applied to the column and developed using methanol as the mobile phase. Fractions were collected and analyzed by HPLC on an Agilent 1200 as described above for pre-cercosporin. Fractions containing pure cercosporin, as determined by absorbance at 210 nm, were pooled and evaporated to dryness.

The purified cercosporin was resuspended in methanol to a final concentration of 5 mM. For the standard curve, this stock solution was further diluted in methanol to 1, 10, 25, 50, and 100 µM. These standard solutions were prepared in triplicate and 10 µL of each was injected on to an Agilent 1200 equipped with an autosampler, and analyzed as before. 280 nm chromatograms were integrated using ChemStation (Agilent), and the integrated peak area was plotted against concentration in Prism

(GraphPad). The data was fit using linear regression, constraining the line to go through 0,0, to yield the standard curve (Fig. S11).

To assess the efficiency of cercosporin extraction from apple, 50 µL of 250 µM cercosporin (isolated as described above) in methanol was injected into organic Gala apples at 5 spots, 2 cm below the skin. Apples were incubated at room temperature for 1 h, 1.5 d, and 3 d. At each time point, the apples were cut up and flash frozen in liquid nitrogen. The frozen apples were then ground under liquid nitrogen, lyophilized to dryness, and re-ground. The ground apples were resuspended in acidified water (pH < 1) and extracted thrice with ethyl acetate. These extracts were pooled, washed with brine, and evaporated to dryness. The samples were then resuspended in 500 µL methanol for analysis by HPLC on an Agilent 1200, as described above for pre-cercosporin. Cercosporin recovery was quantified based on integrated peak area of the HPLC chromatogram at 280 nm, using the standard curve developed above (Fig. S12).

Extraction of *Colletotrichum higginsianum* infected *Arabidopsis* leaves

Arabidopsis thaliana was inoculated as described by O'Connell (24). Several methods were attempted to extract cercosporin from infected leaves. First, the leaves were ground under liquid nitrogen and lyophilized twice prior to soaking in water acidified with HCl (pH <1). After 10 min, solids were removed by gravity filtration and the filtrate was extracted thrice with ethyl acetate. The extractions were pooled, washed with brine, and evaporated to dryness. The crude extract was resuspended in 500 µL methanol and analyzed by HPLC and UPLC-ESI-MS as described above. Second, the leaves were ground and lyophilized as described above, and resuspended in methanol. The mixture was stirred at room temperature for 1 h. All solids were removed by vacuum filtration, and the resulting filtrate was evaporated to dryness. This crude extract was then analyzed for the presence of cercosporin by HPLC and UPLC-ESI-MS as before. For the final method, the leaves were ground, lyophilized, and a crude methanol

extract was prepared as described above. The crude extract was resuspended in water basified with sodium hydroxide (pH > 12), and extracted thrice with dichloromethane. The dichloromethane fractions were discarded, and the aqueous fraction was acidified with HCl to pH <1 and extracted thrice with ethyl acetate. The ethyl acetate fractions were pooled, washed with brine, and evaporated to dryness. The extracted metabolites were resuspended in 500 µL methanol and analyzed by HPLC and UPLC-ESI-MS as before.

SI Figures

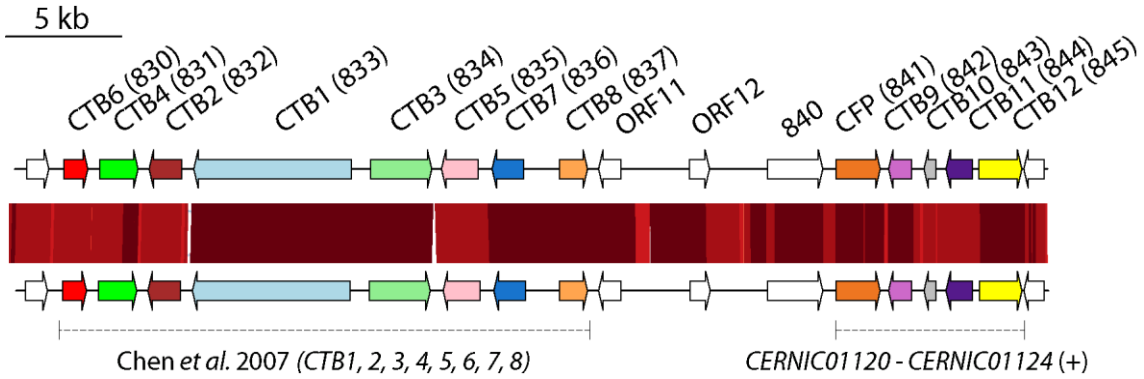


Figure S1: Synteny of the *C. beticola* and *C. nicotianae* cercosporin biosynthetic clusters. The cercosporin biosynthetic cluster in *C. beticola* (top) and flanking genes are fully syntenic with that in *C. nicotianae* (bottom). The displayed identifiers are transcript IDs and the corresponding sequences can be retrieved from ORCAE. *CTB1* until *CTB8* are named according to Chen et al. (25) and the region encoding these genes in *C. nicotianae* is indicated by the bottom dashed line. *CTB* orthologs are colored relative to the *C. beticola CTB* cluster genes to illustrate gene conservation.

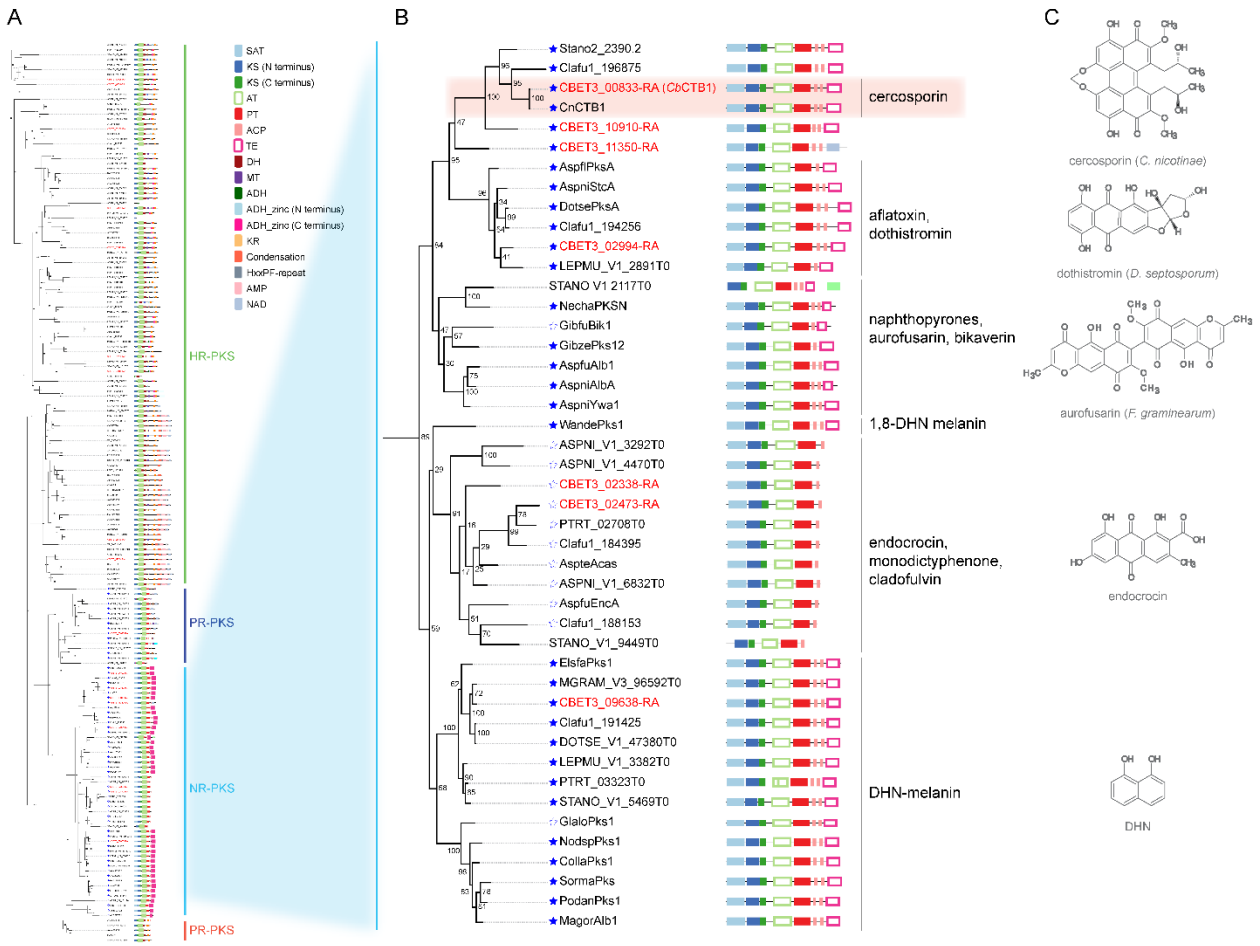


Figure S2: Phylogenetic analysis of *C. beticola* polyketide synthase enzymes. (**a**) Maximum likelihood phylogenetic tree of all predicted polyketide synthase (PKS) from the selected species set (*C. beticola* (CBET3), *D. septosporum* (DOTSE_V1), *Z. tritici* (MGRAM_V3), *L. maculans* (LEPMU_V1), *P. tritici-repentis* (PTRT) and *P. nodorum* (STANO_V1)) as well as several additional PKSs derived from Collemare *et al*. (26) (see Table S9 for full set), constructed by maximum-likelihood analysis of aligned full-length β-ketoacyl synthase domains. The domain architecture for each protein as determined by Pfam domain scanning is shown aligned to each protein accession and the legend shown at the top. PKSs with a starter unit (SAT) domain encompassing the canonical active-site motif GxCxG are marked by filled blue stars, whereas those without the

canonical active-site motif are marked by unfilled blue stars. The full version of the panel A PKS tree is part of the supplementary material published on figshare under doi: 10.6084/m9.figshare.4056522. (**b**) The magnified section from (**a**) encompassing seven of eight *C. beticola* non-reducing (NR)-PKSs (excludes more distantly related CBET3_04827-RA) and several well-known NR-PKSs with established molecular function. SAT = starter-unit:ACP transacylase, KS = β-ketoacyl synthase, AT = ACP transacylase, PT = product template, ACP = acyl carrier protein; TE/CYC = thioesterase, DH = dehydratase, MT = methyl transferase, ADH = alcohol dehydrogenase, ADH_zinc = alcohol zinc dehydrogenase, KR = ketoreductase, Condensation = condensation domain, HxxPF-repeat = HxxPF-repeated domain, AMP = AMP-binding enzyme, NAD = NAD-binding region. PKSs with a SAT domain encompassing the canonical active-site motif GxCxG are marked by filled blue stars, whereas those without are marked by unfilled blue stars. The final tree was prepared in EvolView (14). (**c**) Structures of characterized secondary metabolite compounds associated with the various subclades in (**b**).

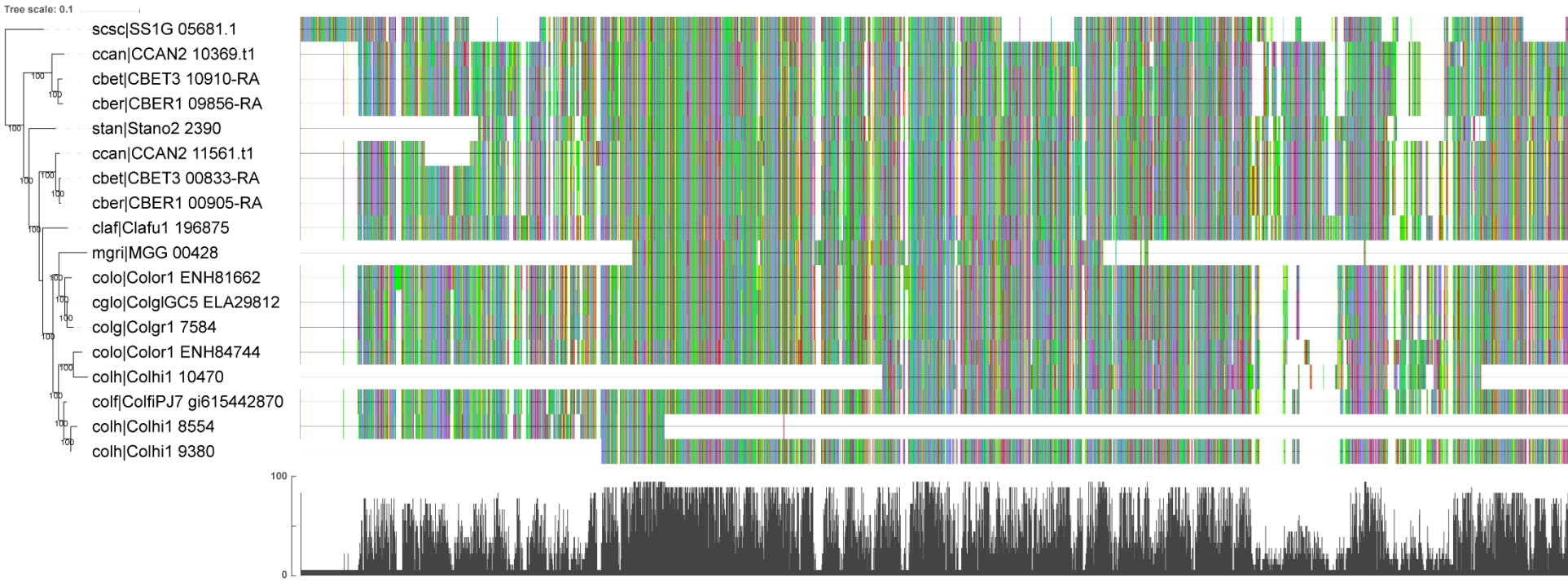


Figure S3A: CTB1 (CBET3_00833)

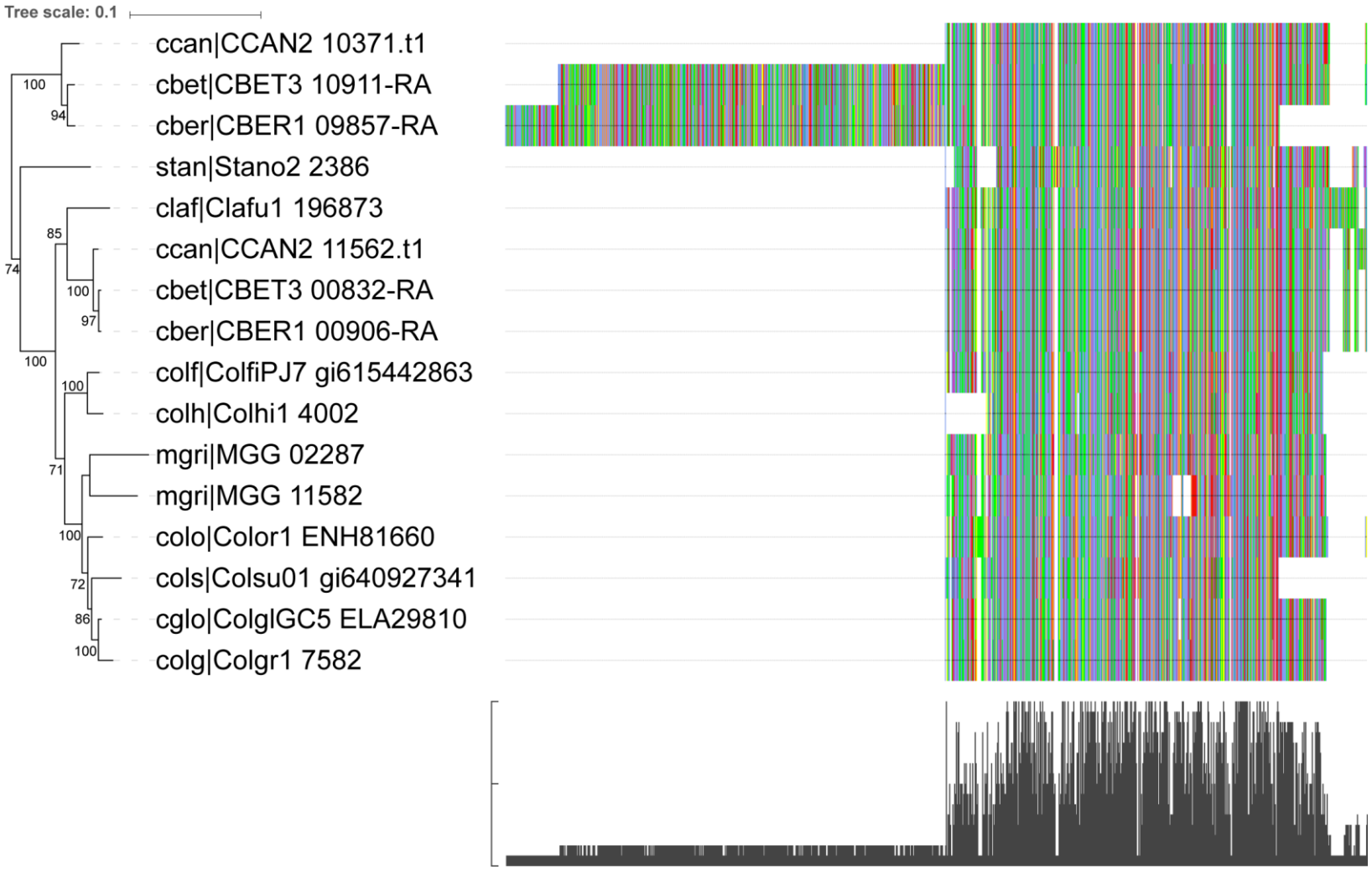


Figure S3B: CTB2 (CBET3_00832)

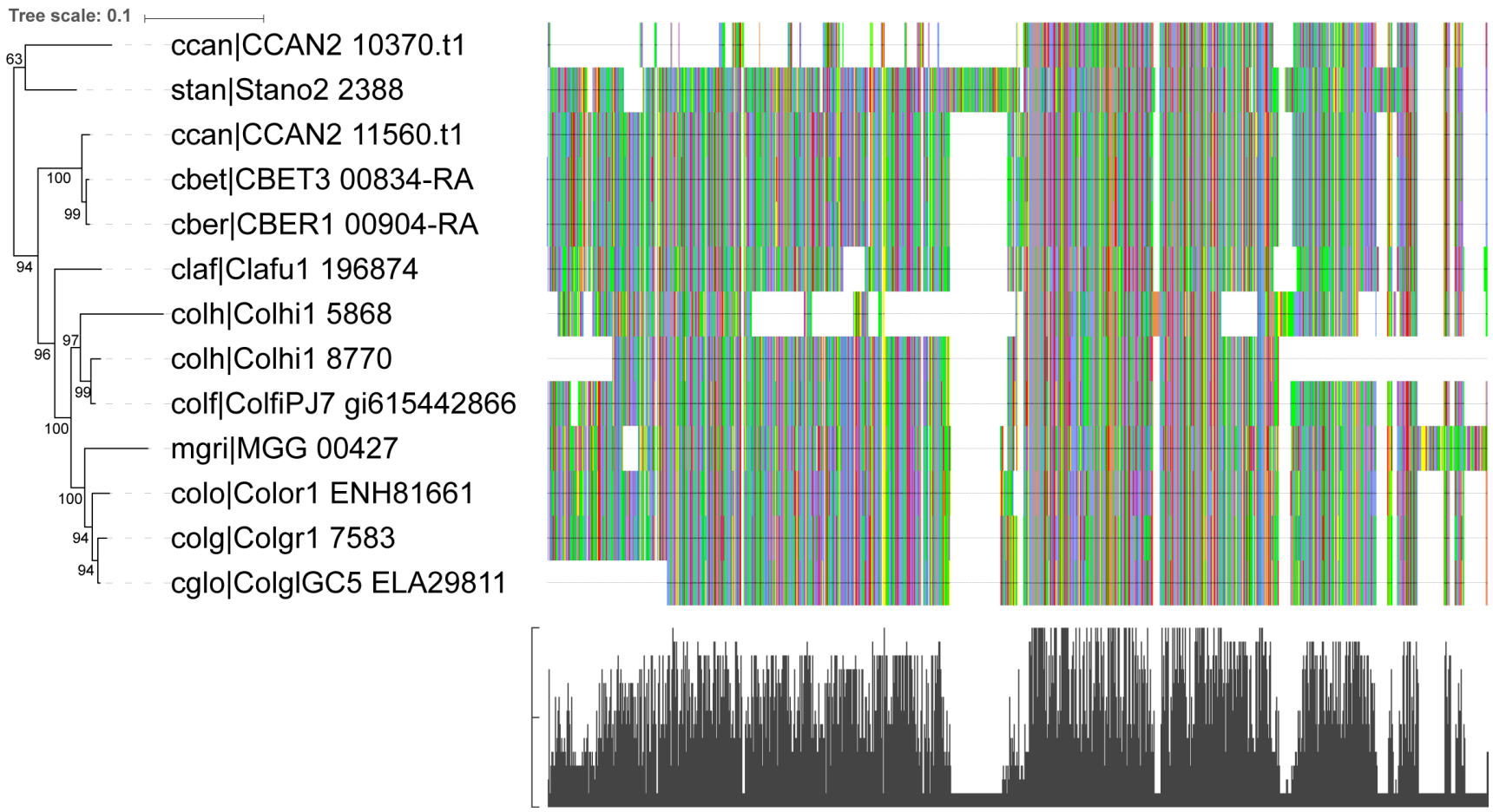


Figure S3C: CTB3 (CBET3_00834)

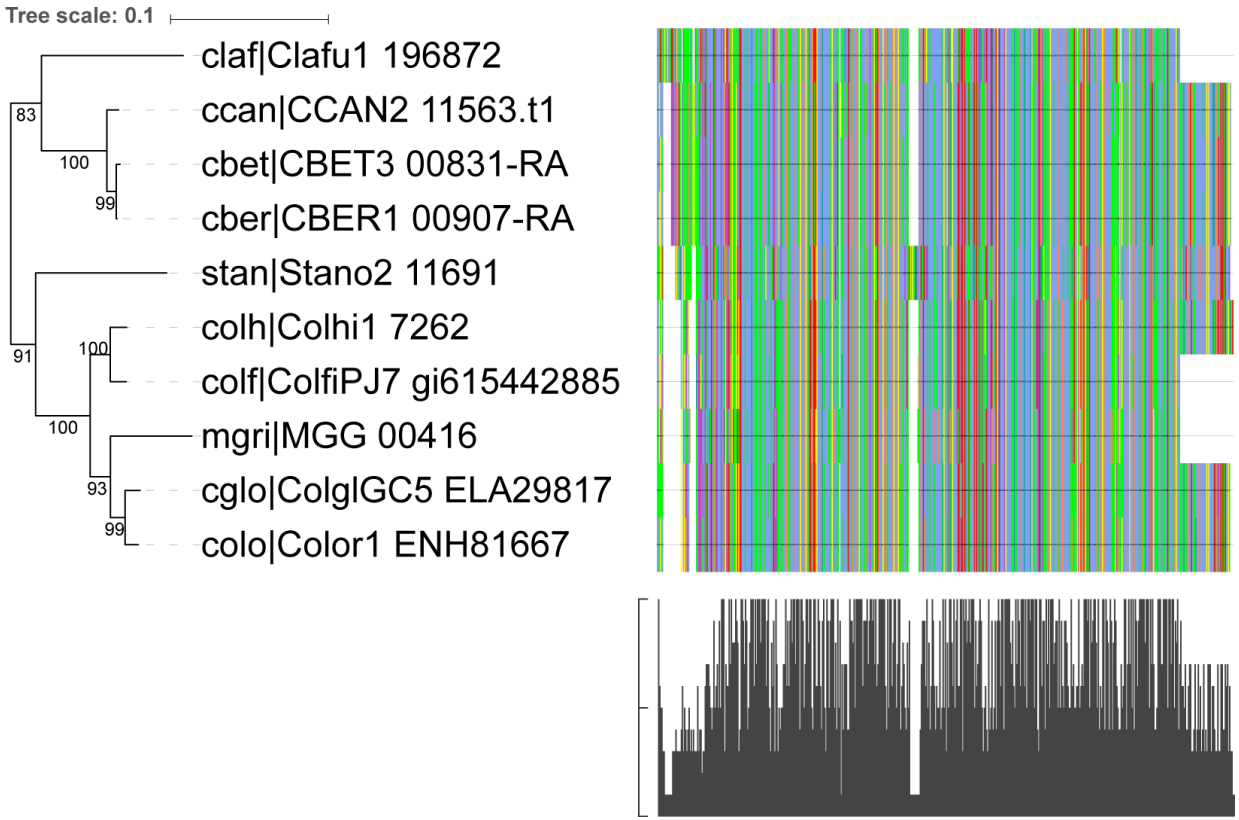


Figure S3D: CTB4 (CBET3_00831)

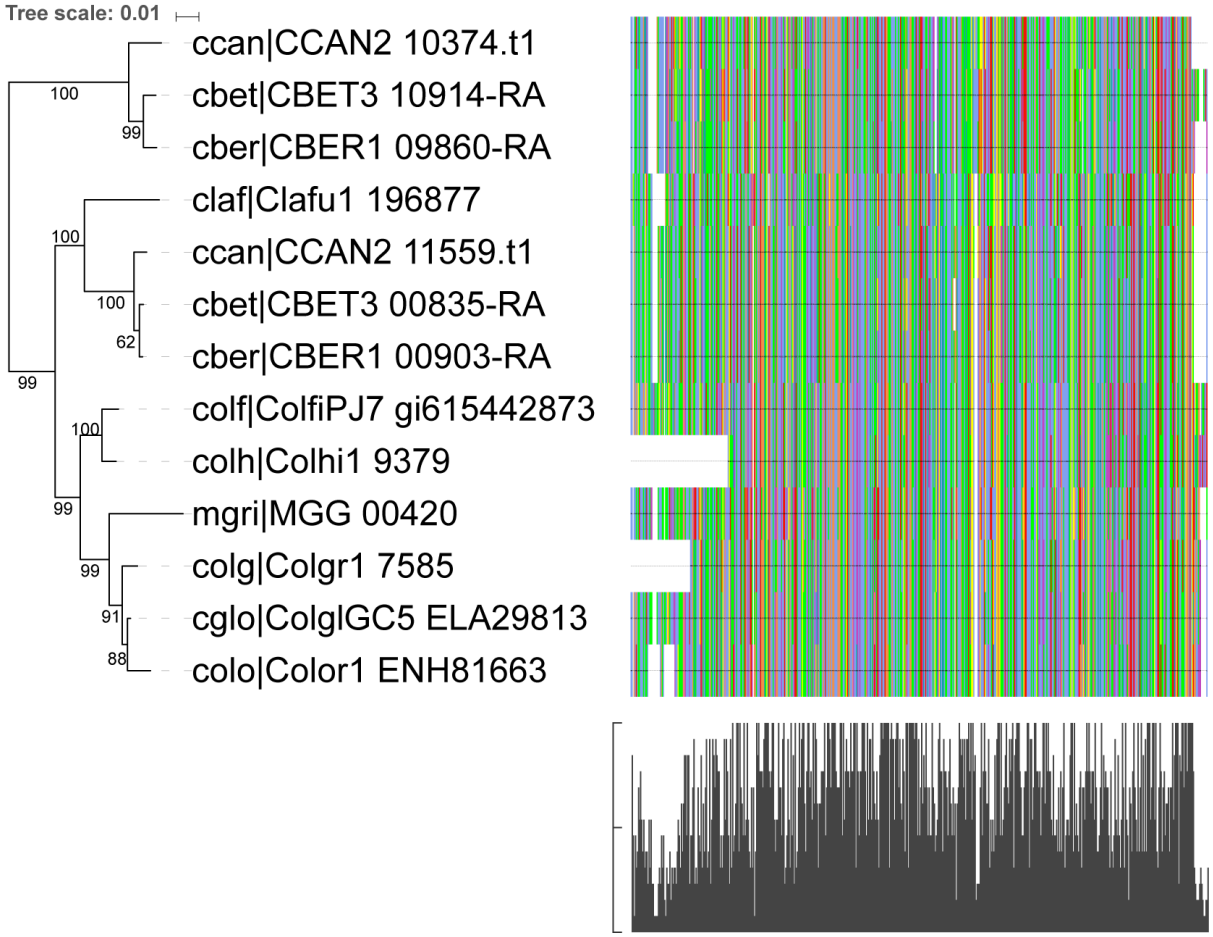


Figure S3E: CTB5 (CBET3_00835)

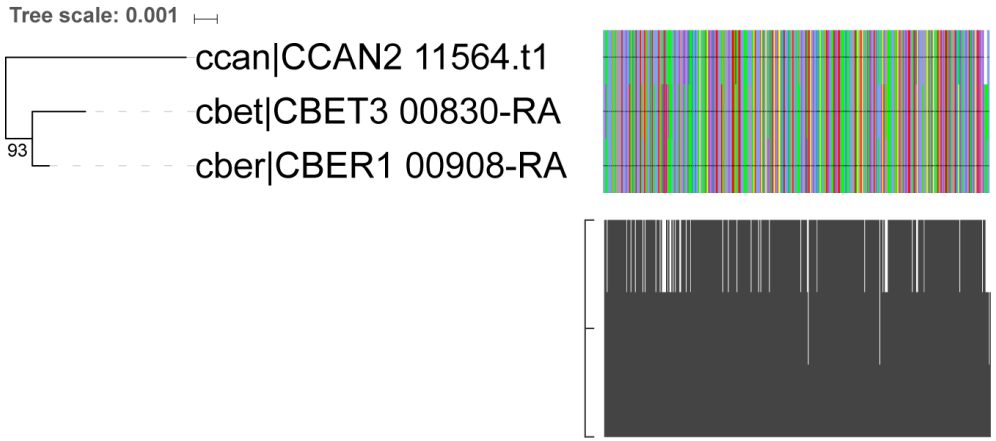


Figure S2F: CTB6 (CBET3_00830)

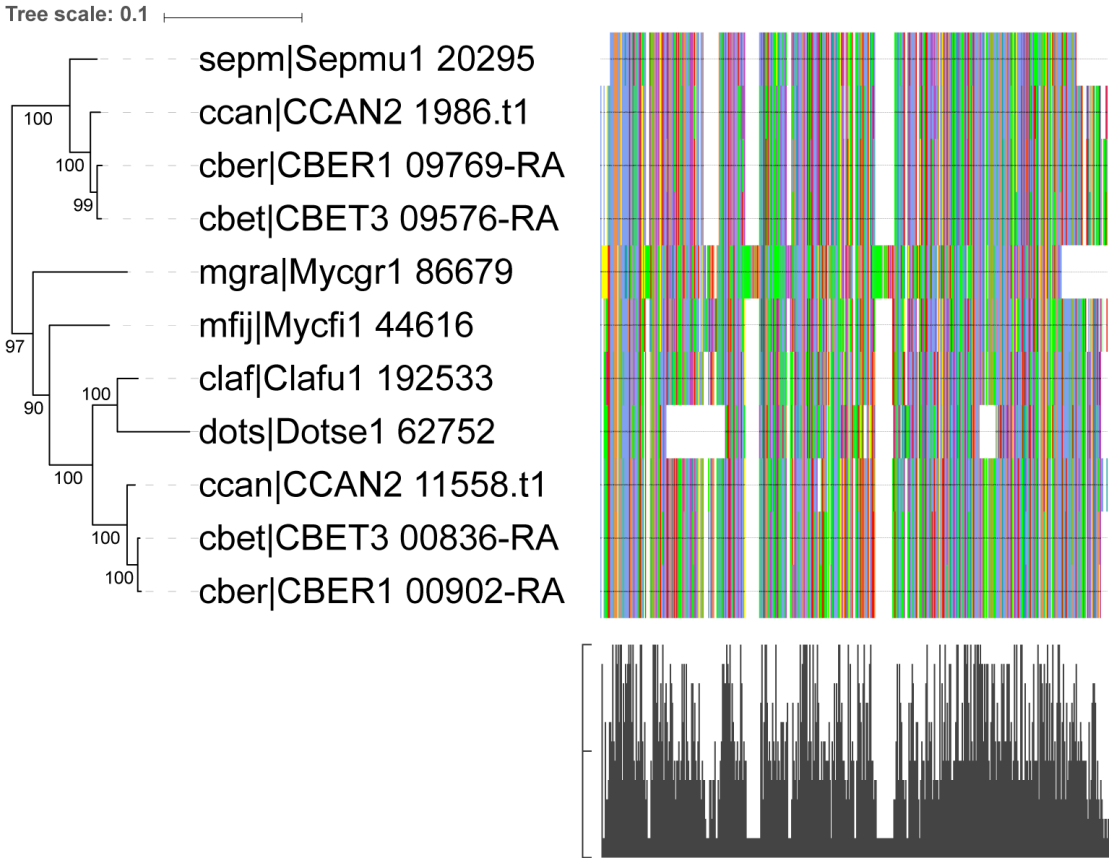


Figure S3G: CTB7 (CBET3_00836)

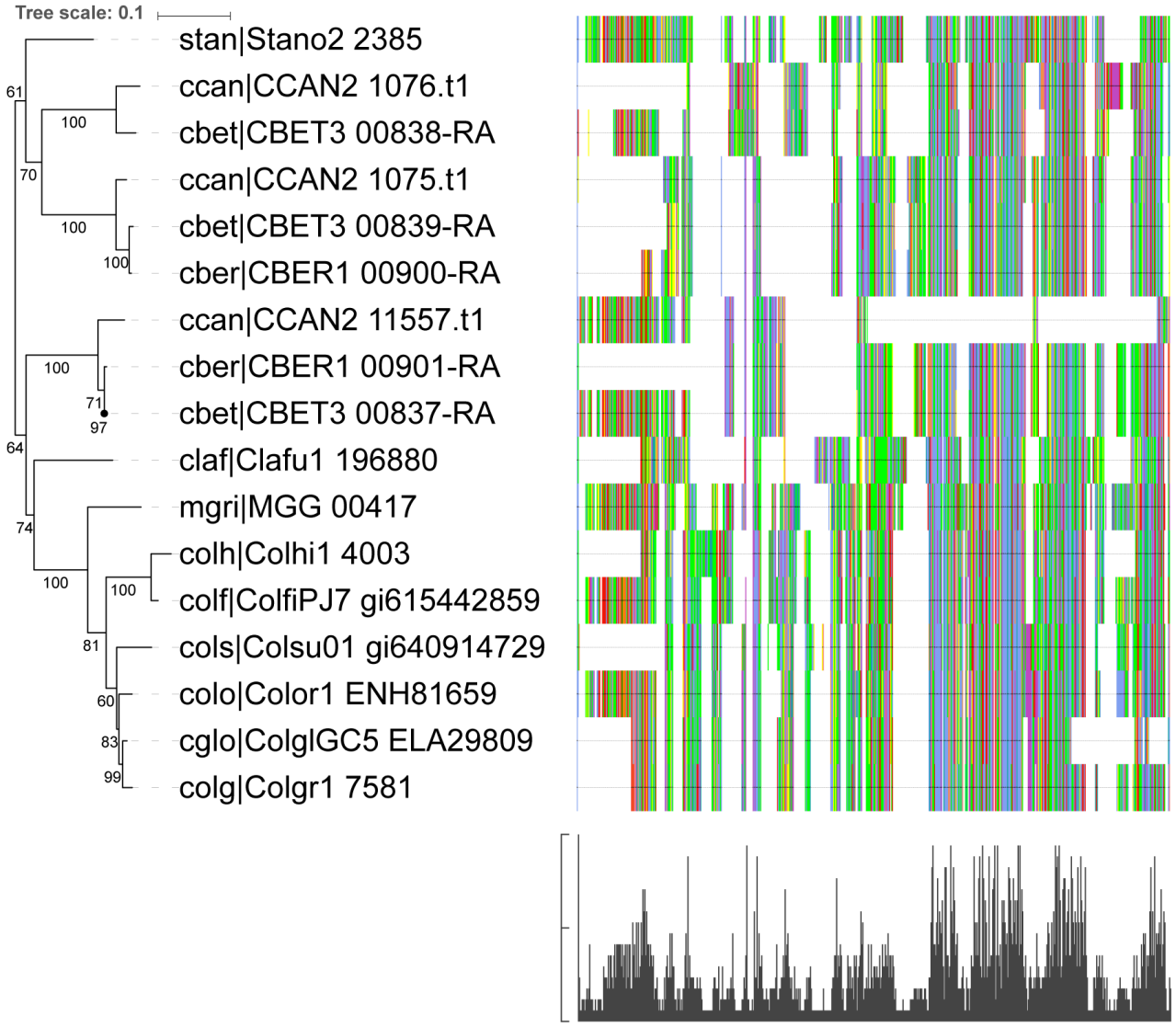


Figure S3H: CTB8 (CBET3_00837)

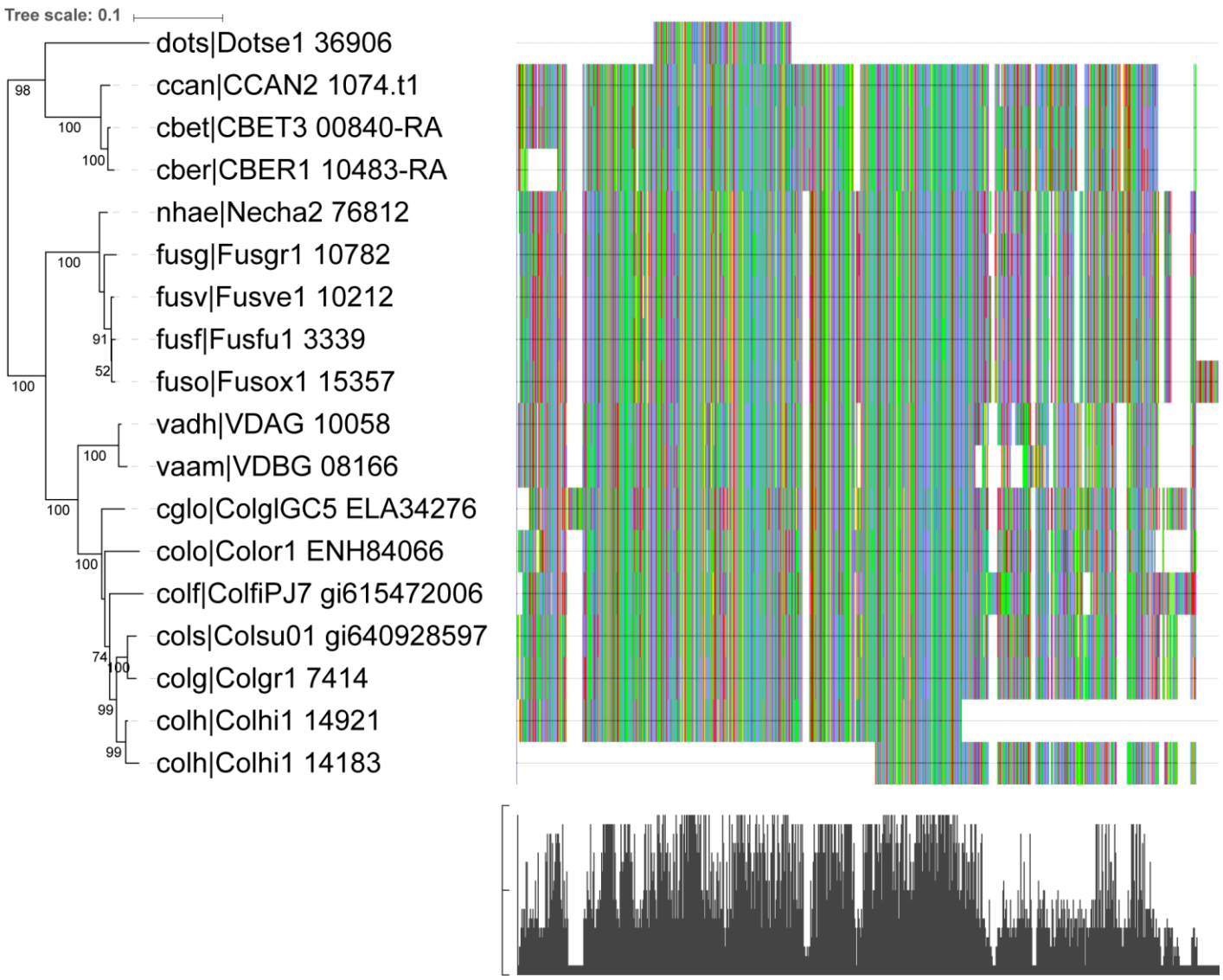


Figure S3I (CBET3_00840)

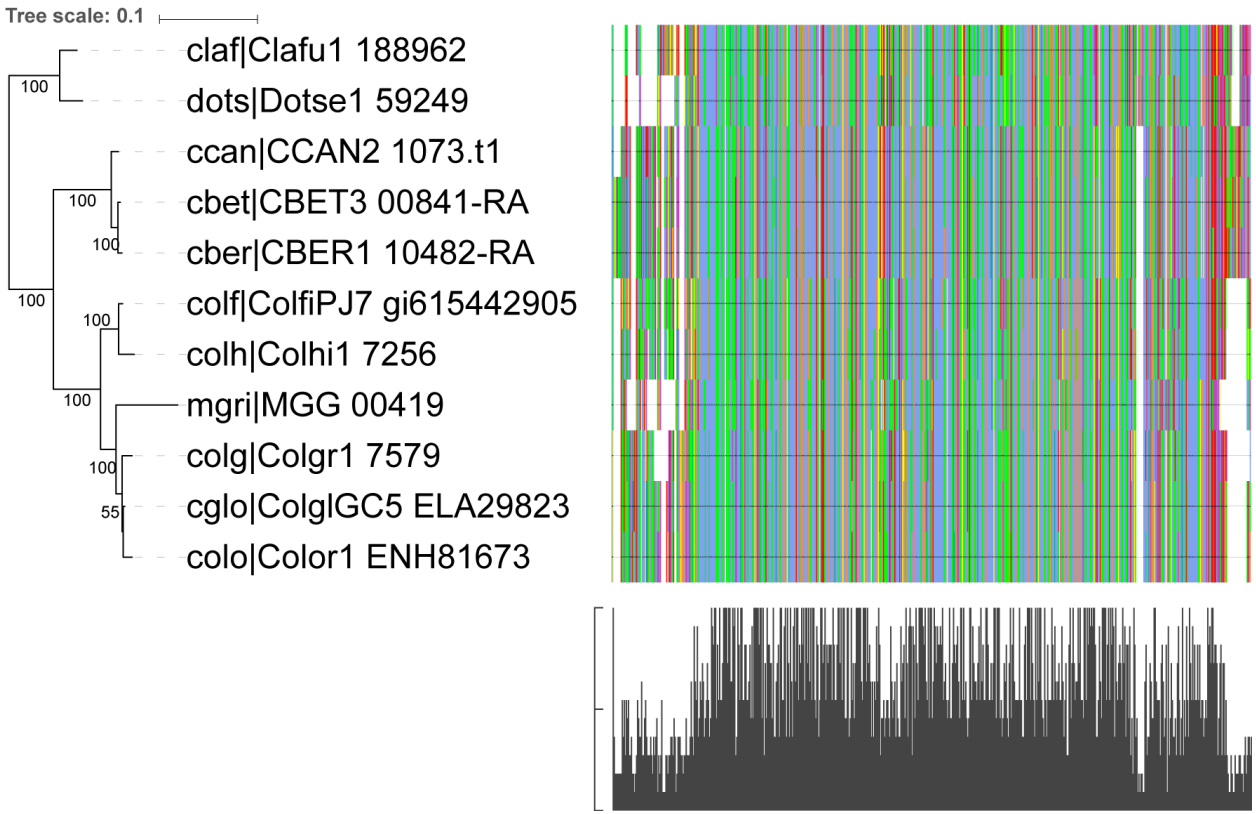


Figure S3J: CFP (CBET3_00841)

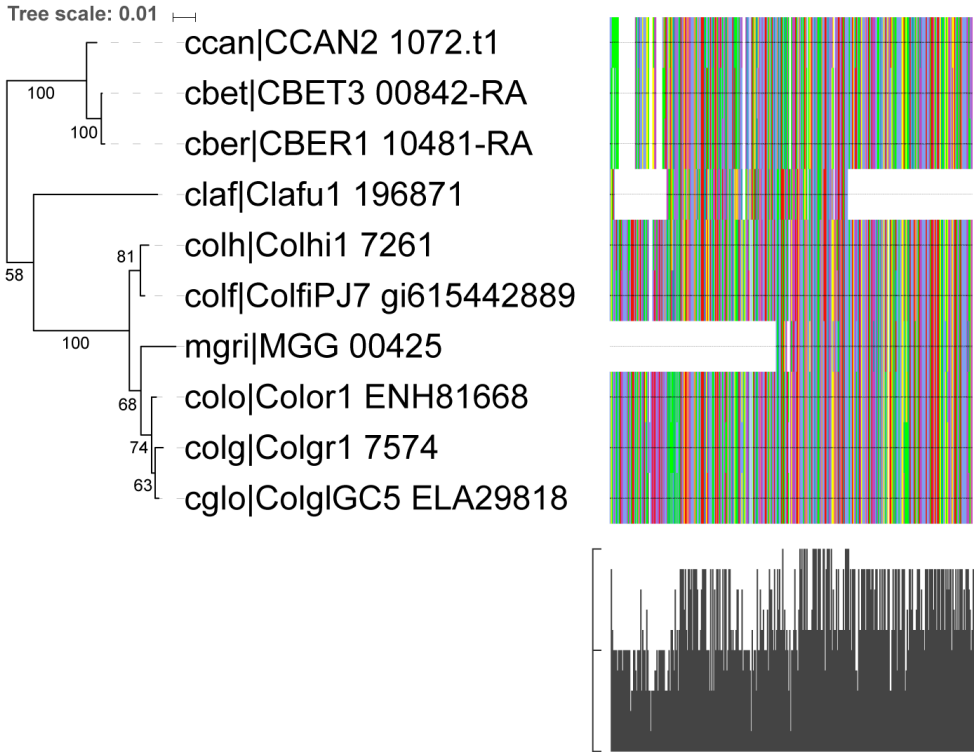


Figure S3K: CTB9 (CBET3_00842)

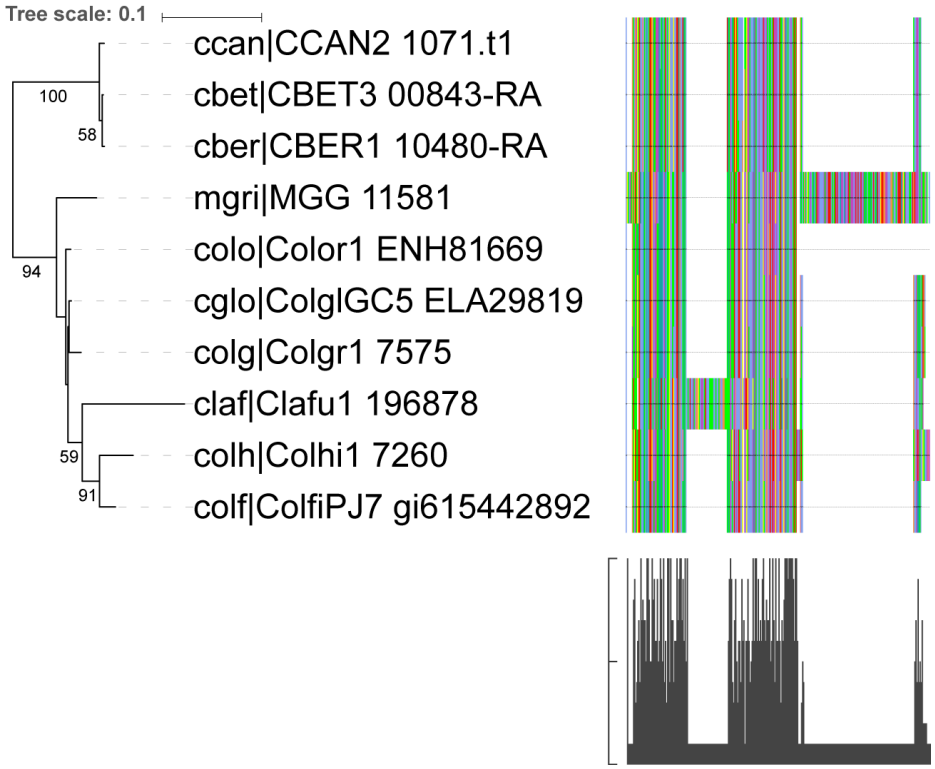


Figure S3L:CTB10 (CBET3_00843)

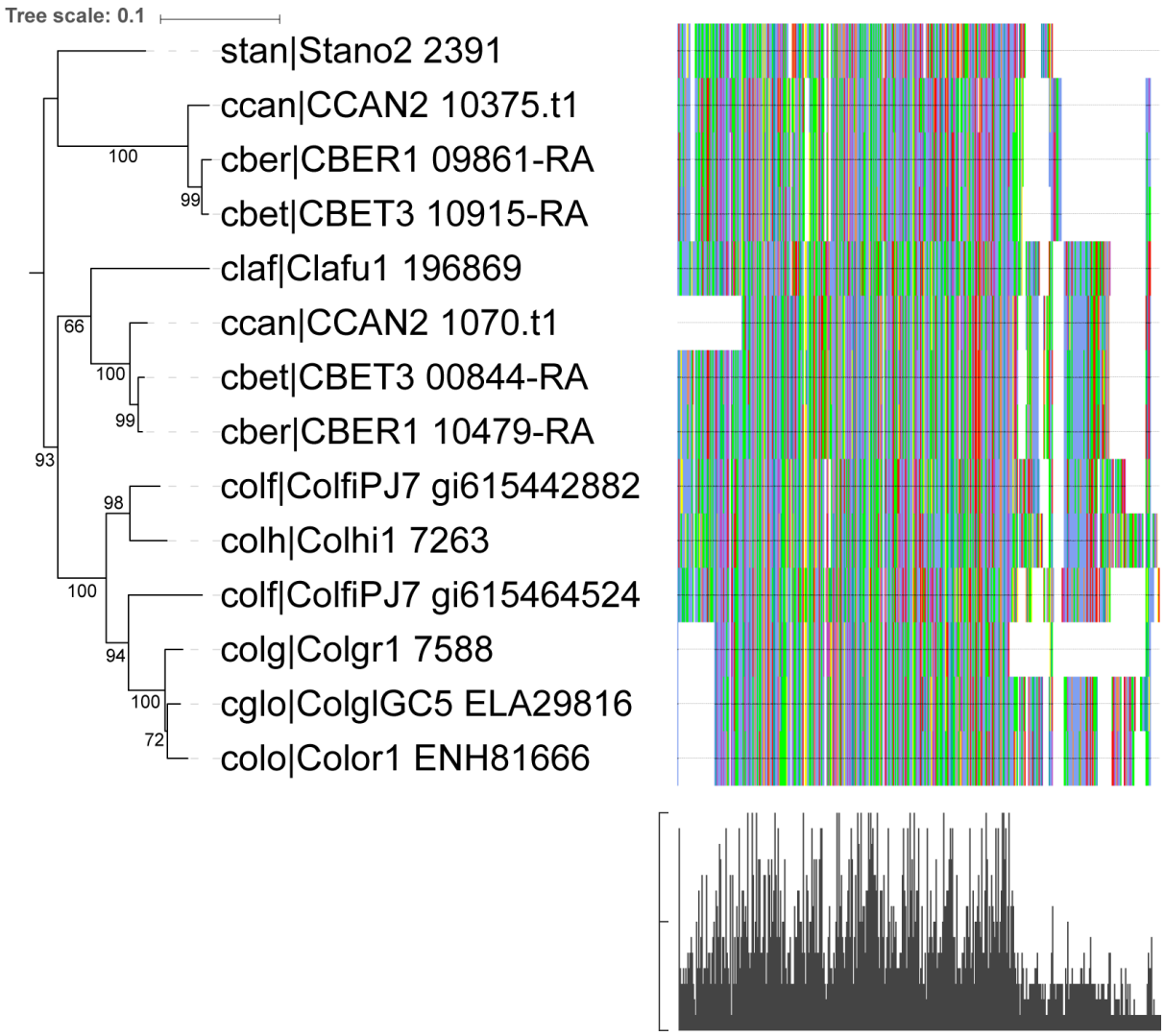


Figure S3M: CTB11 (CBET3_00844)

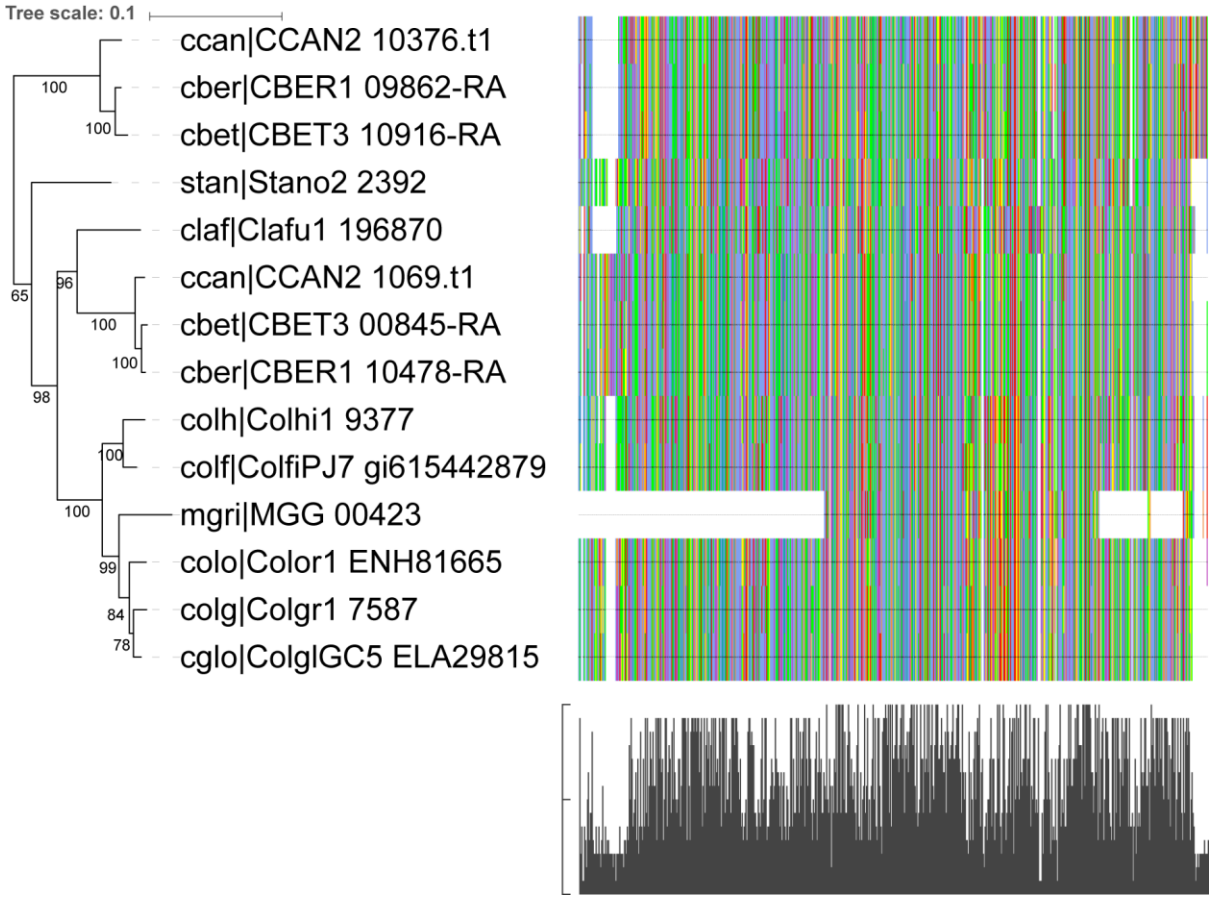


Figure S3N: CTB12 (CBET3_00845)

Figure S3: Phylogenetic analyses of CTB1-CTB12 homologs. Selected sub trees of maximum likelihood trees of CTB1-CTB12 orthologs (2A-2N) in *C. beticola* and 45 published genome sequences. The subtree with orthologous sequences were selected from the full phylogenetic tree which were constructed from a full-length multiple sequence alignment of CTB1-CTB12 proteins and determined by maximum likelihood using RAxML. The full phylogenetic trees for CTB1 – CTB12 are available as supplementary material on figshare under DOI: 10.6084/m9.figshare.4056522. For each CTB protein, the associated subtree protein alignment, after degapping (i.e. removing all gap-only positions), is shown adjacent to the tree.

Figures are prepared in iTOL (27). Amino acids are colored per the clustalx scheme, and the residue conservation graph is shown below the alignment ranging from 0 till 100%. Bootstrap support, determined by RAxML, ranging from 50 – 100 are indicated at the corresponding nodes. Species abbreviations are cbet (*C. beticola*), cber (*C. berteroae*), ccan (*C. canescens*), colf (*C. fioriniae*), colh (C*. higginsianum*), cglo (*C. gloeosporioides*), colg (*C. graminicola*), colo (*C. orbiculare*), mgri (*M. oryzae*), claf (*C. fulvum*), stan (*P. nodorum*), dots (*D. septosporum*), and sepm (*Septoria musiva*). A full list of species and the corresponding abbreviation can be found in Table S3.

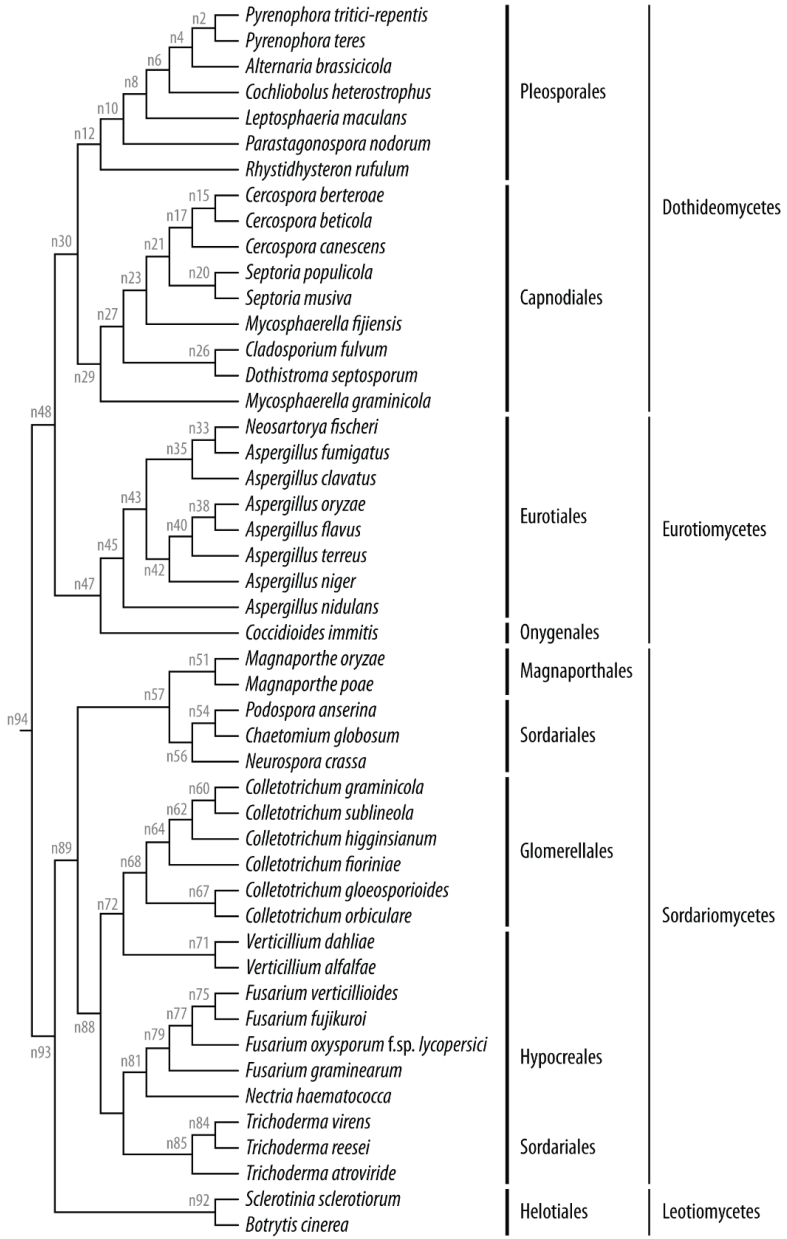
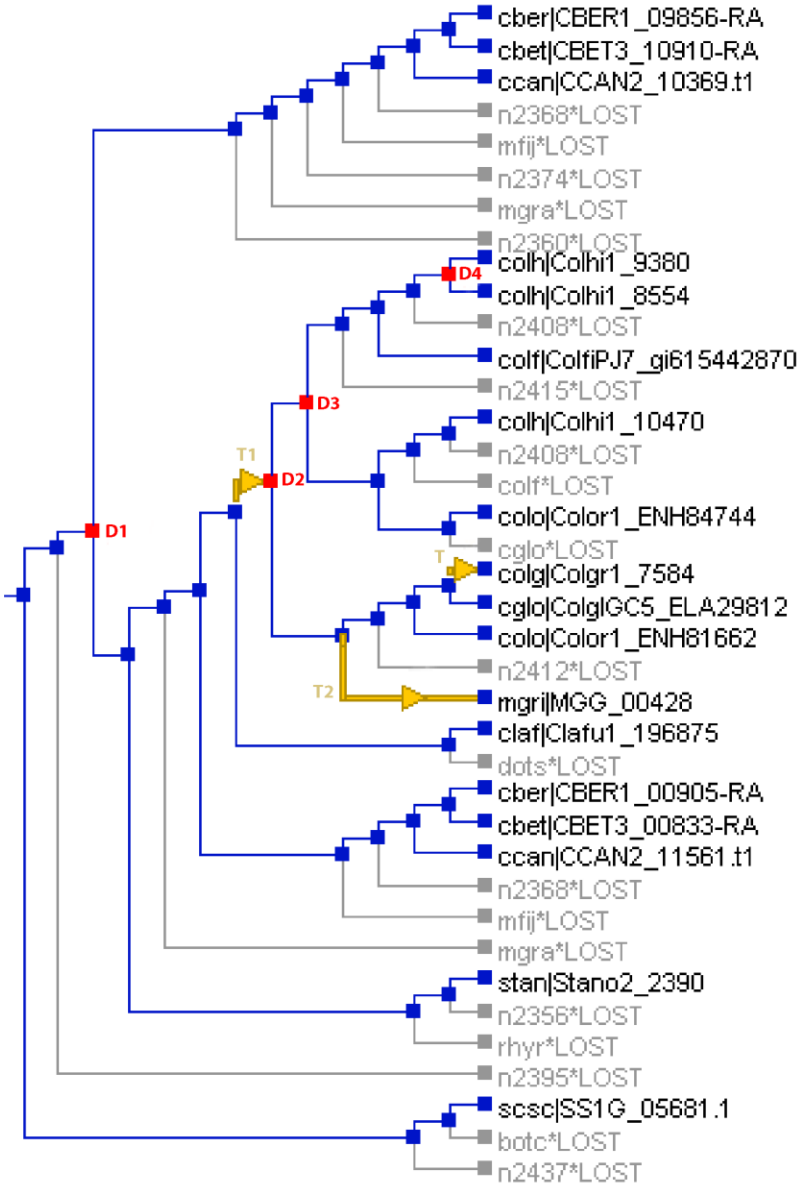

n2
n4
n6
n8
n10
n12
n30
n48
n94
n89
n88
n93
n27
n29
n23
n21
n17
n15
n20
n26
n43
n45
n47
n35
n33
n38
n40
n42
n57
n51
n54
n56
n64
n62
n60
n68
n67
n72
n71
n79
n77
n75
n81
n84
n85
n92
Pyrenophora tritici-repentis
Pyrenophora teres
Alternaria brassicicola
Cochliobolus heterostrophus
Leptosphaeria maculans
Parastagonospora nodorum
Rhystidhysteron rufulum
Cercospora berteroae
Cercospora beticola
Cercospora canescens
Septoria populicola
Septoria musiva
Mycosphaerella fijiensis
Cladosporium fulvum
Dothistroma septosporum
Mycosphaerella graminicola
Neosartorya fischeri
Aspergillus fumigatus
Aspergillus clavatus
Aspergillus oryzae
Aspergillus flavus
Aspergillus terreus
Aspergillus niger
Aspergillus nidulans
Coccidioides immitis
Magnaporthe oryzae
Magnaporthe poae
Podospora anserina
Chaetomium globosum
Neurospora crassa
Colletotrichum graminicola
Colletotrichum sublineola
Colletotrichum higginsianum
Colletotrichum fioriniae
Colletotrichum gloeosporioides
Colletotrichum orbiculare
Verticillium dahliae
Verticillium alfalfae
Fusarium verticillioides
Fusarium fujikuroi
Fusarium oxysporum f.sp. lycopersici
Fusarium graminearum
Nectria haematococca
Trichoderma virens
Trichoderma reesei
Trichoderma atroviride
Sclerotinia sclerotiorum
Botrytis cinerea
Pleosporales
Capnodiales
Eurotiales
Onygenales
Magnaporthales
Sordariales
Glomerellales
Hypocreales
Sordariales
Helotiales
Dothideomycetes
Eurotiomycetes
Sordariomycetes
Leotiomycetes

Figure S4: Phylogeny of *Cercospora* spp. and related Ascomycete fungi. Cladogram showing the phylogenetic relationship of *Cercospora* spp. and 45 other sequenced fungi. The unscaled tree was constructed using CVTree (15).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cbet|CBET3_10910-RA
ccan|CCAN2_10369.t1
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mfij*LOST
n2374*LOST
mgra*LOST
n2360*LOST
D4
colh|Colhi1_9380
colh|Colhi1_8554
n2408*LOST
colf|ColfiPJ7_gi615442870
n2415*LOST
D3
colh|Colhi1_10470
n2408*LOST
T1
D2
colf*LOST
colo|Color1_ENH84744
D1
cglo*LOST
T
colg|Colgr1_7584
cglo|ColgIGC5_ELA29812
colo|Color1_ENH81662
n2412*LOST
T2
mgri|MGG_00428
claf|Clafu1_196875
dots*LOST
cber|CBER1_00905-RA
cbet|CBET3_00833-RA
ccan|CCAN2_11561.t1
n2368*LOST
mfij*LOST
mgra*LOST
stan|Stano2_2390
n2356*LOST
rhyr*LOST
n2395*LOST
scsc|SS1G_05681.1
botc*LOST
n2437*LOST

Figure S5A: CTB1 (CBET3_00833) protein tree reconciliation. Total reconciliation score 44.0 (4 duplications, 3 transfers and 20 losses).

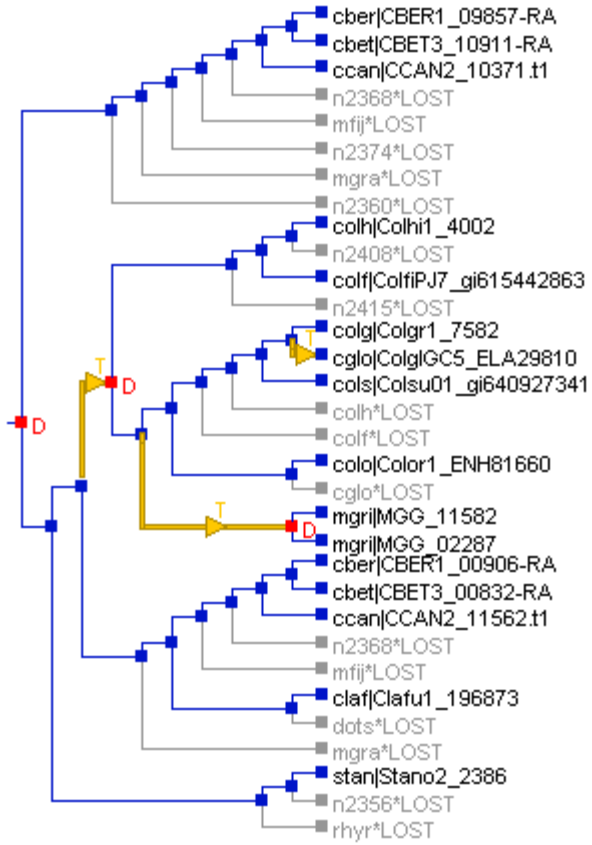


Figure S5B: CTB2 (CBET3_00832) protein tree reconciliation. Total reconciliation score 38.5 (3 duplications, 3 transfers and 16 losses).

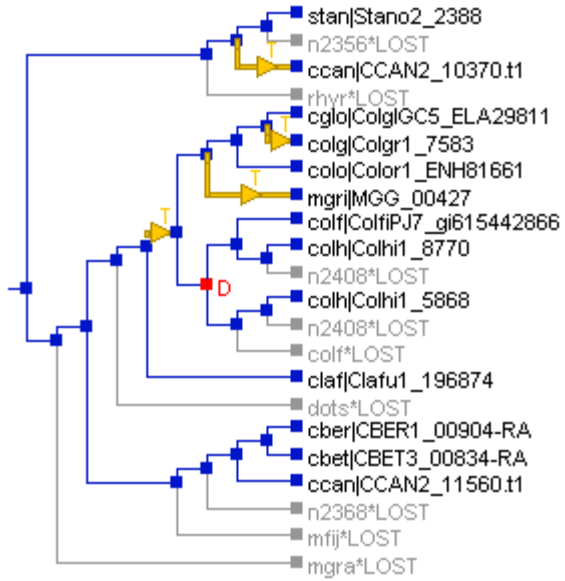


Figure S5C: CTB3 (CBET3_00834) protein tree reconciliation. Total reconciliation score 34.5 (1 duplication, 4 transfers and 9 losses).

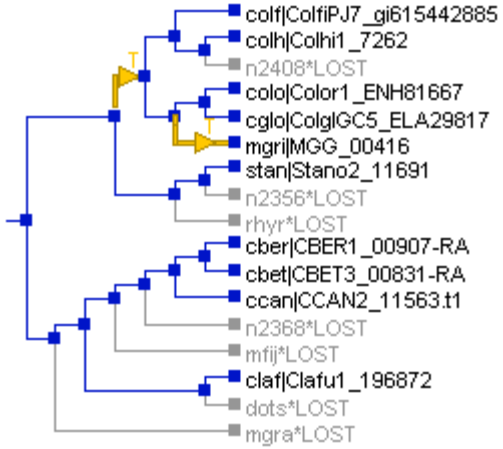


Figure S5D: CTB4 (CBET3_00831) protein tree reconciliation. Total reconciliation score 19.0 (0 duplications, 2 transfers and 7 losses).

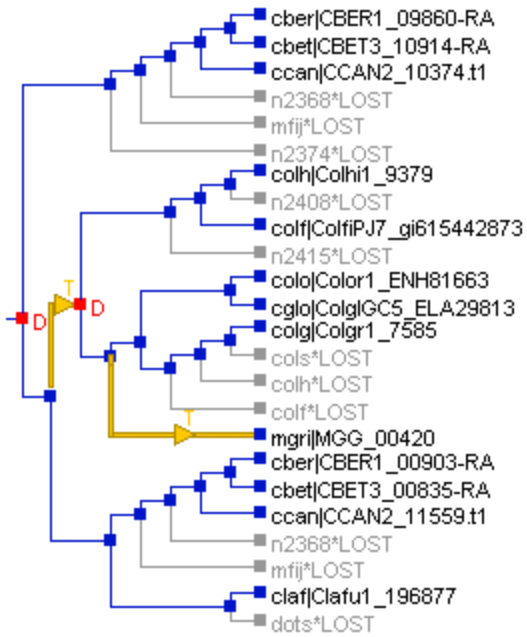


Figure S5E: CTB5 (CBET3_00835) protein tree reconciliation. Total reconciliation score 26.0 (2 duplications, 2 transfers and 11 losses).

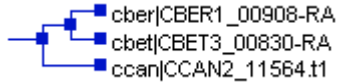


Figure S5F: CTB6 (CBET3_00830) protein tree reconciliation. Only observed in *Cercospora* spp., no reconciliation output.

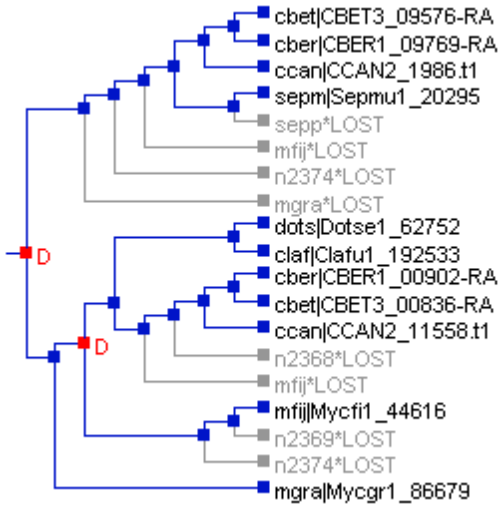


Figure S5G: CTB7 (CBET3_00836) protein tree reconciliation. Total reconciliation score 11.0 (2 duplications, 0 transfers and 8 losses).

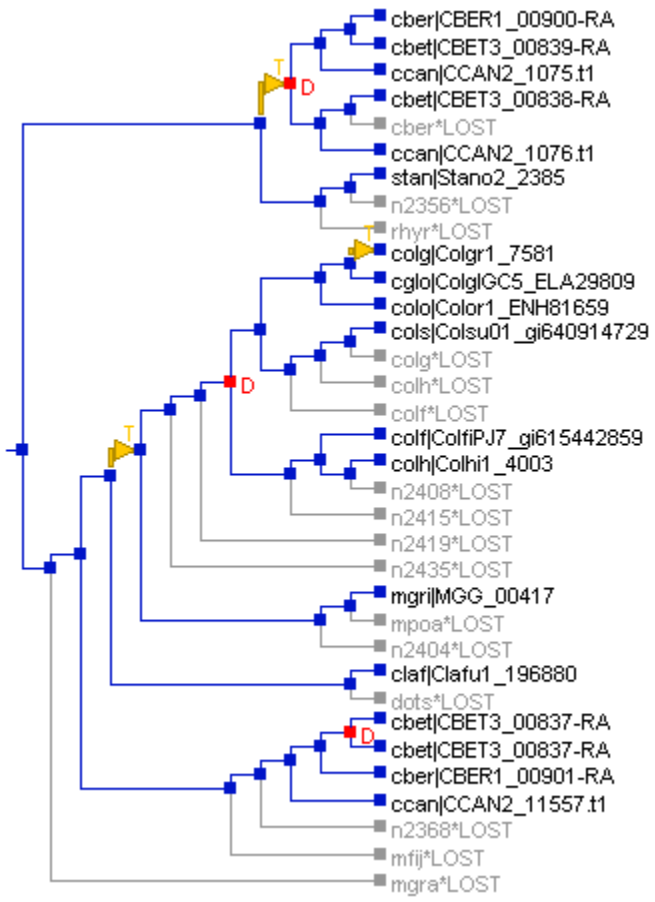


Figure S5H: CTB8 (CBET3_00837) protein tree reconciliation. Total reconciliation score 38.5 (3 duplications, 3 transfers and 16 losses).

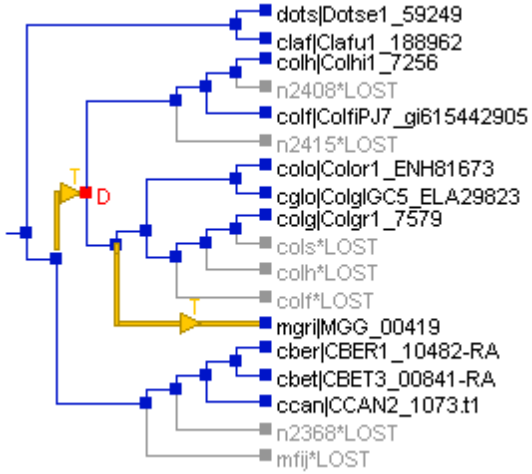


Figure S5I: CFP (CBET3_00841) protein tree reconciliation. Total reconciliation score 20.5 (1 duplication, 2 transfers and 7 losses).

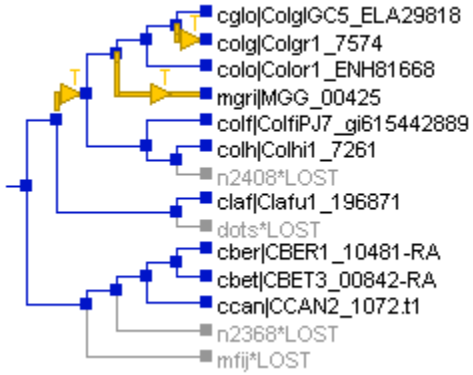


Figure S5J: CTB9 (CBET3_00842) protein tree reconciliation. Total reconciliation score 22.0 (0 duplications, 3 transfers and 4 losses).

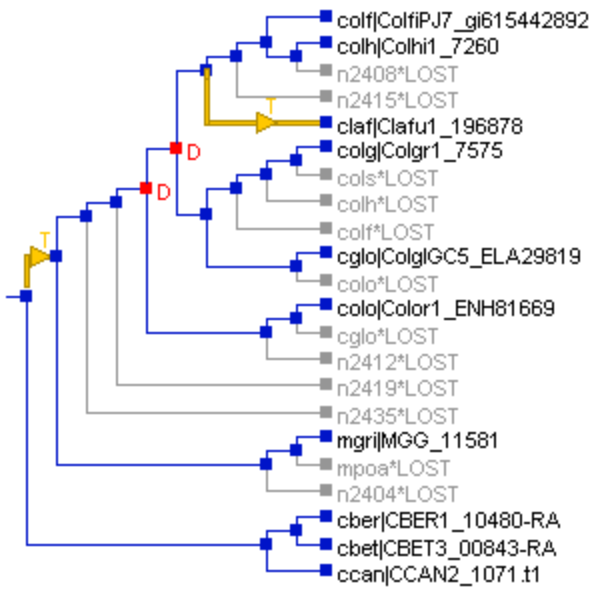


Figure S5K: CTB10 (CBET3_00843) protein tree reconciliation. Total reconciliation score 27.0 (2 duplications, 2 transfers and 12 losses).

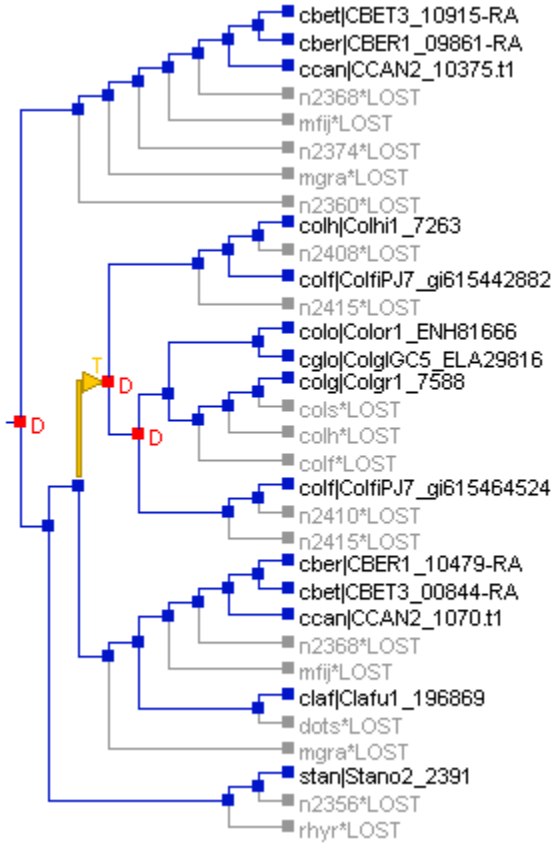


Figure S5L: CTB11 (CBET3_00844) protein tree reconciliation. Total reconciliation score 28.5 (3 duplication, 1 transfer and 18 losses).

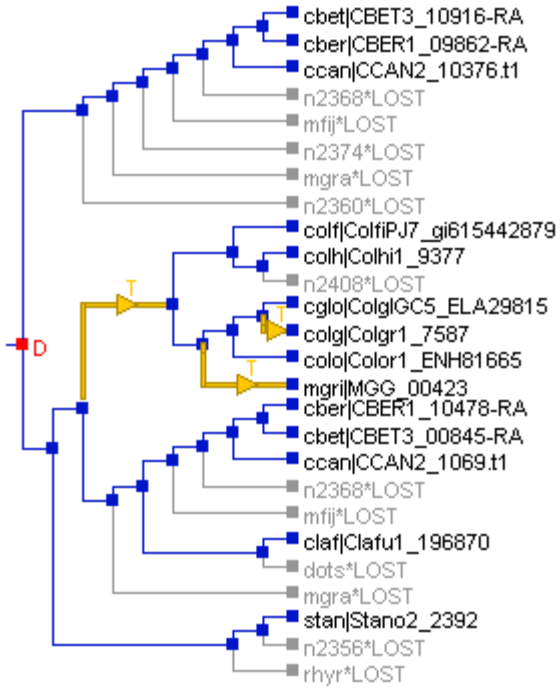


Figure S5M: CTB12 (CBET3_00845) protein tree reconciliation. Total reconciliation score 31.5 (1 duplication, 3 transfers and 12 losses).

Figure S5: Tree reconciliations of CTB1 – CTB12. The protein trees, generated using RAxML (Fig. S3) were midpoint rooted and subsequently reconciled in Notung with the species tree (Fig. S4) to infer the number of duplications, losses and transfers. Duplication nodes are marked with red squares, losses are in grey and transfers are highlighted by yellow arrows. Total reconciliation score is determined by summing all event costs according to the following event costs/weights: duplications (D): 1.5, transfers (T): 6.0, losses (L): 1.0 (ratio D:T:L is 1:4:0.67). A full list of species and the corresponding abbreviation can be found in Table S3.

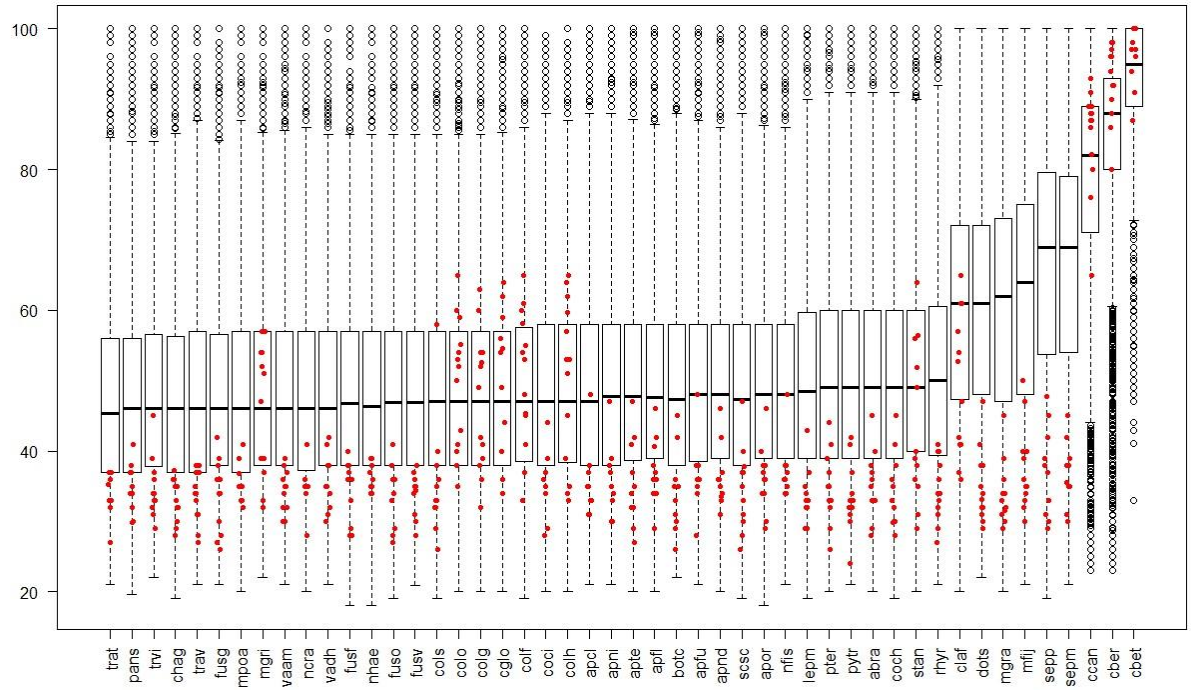


Figure S6: CTB protein sequence identity segregates from the genome-wide average. The genome-wide, protein-by-protein sequence identity between *C. beticola* and the 48 selected genomes (including *C. beticola* itself) is shown. Each boxplot represents the distribution of sequence identities for one genome-wide protein-by-protein comparison, based on best BLASTp match. The red dots represent the sequence identity between best BLASTp matches to CTB proteins. CTB protein similarities are within the highest quartile or above for *P. nodorum* (stan), *Colletotrichum* spp. (*colo*, *colg*, *cglo*, *colg*, and *colh*) and *M. oryzae* (*mgri*). A full list of species and the corresponding abbreviations can be found in Table S3.

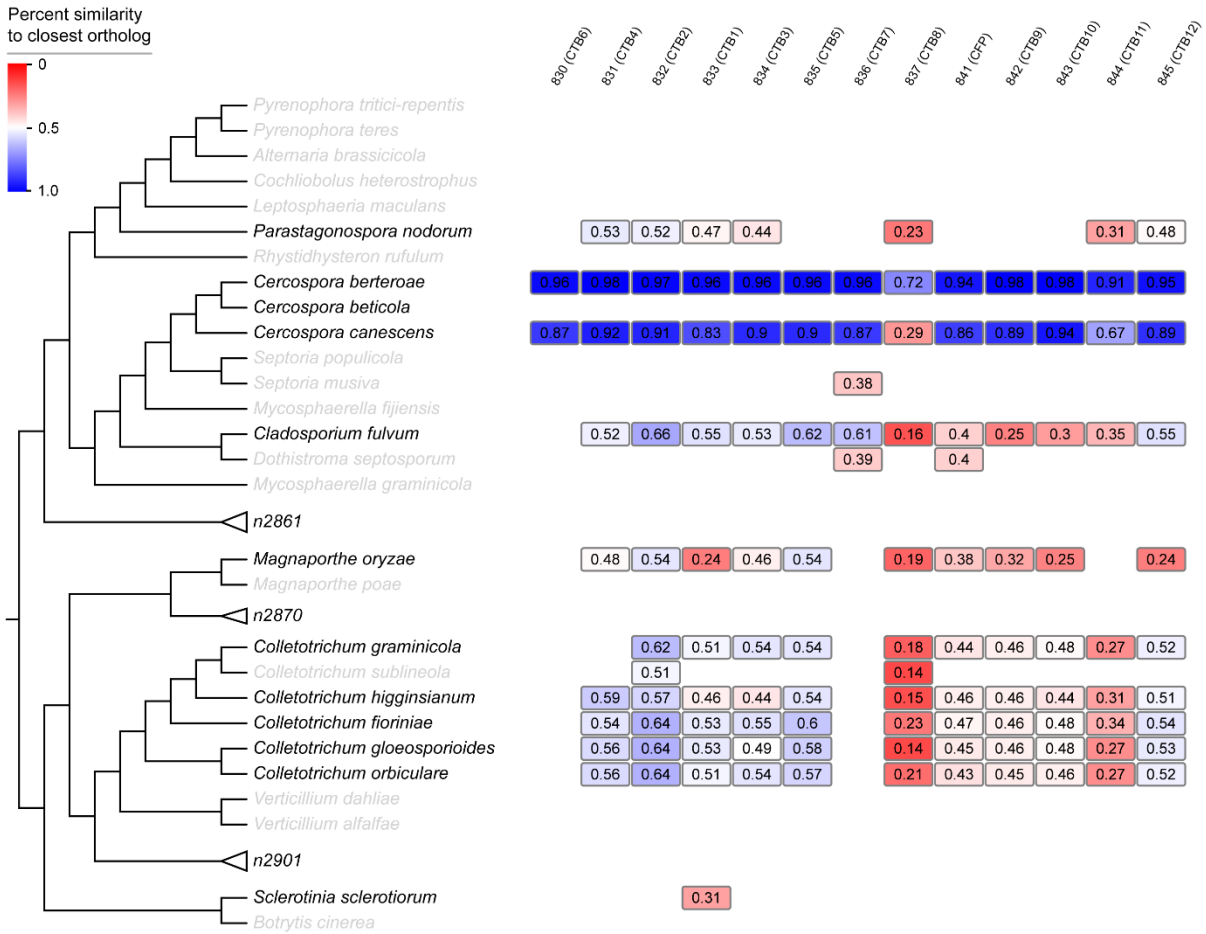


Figure S7: CTB orthologs pairwise identities in tree format. Pairwise identities of *C. beticola* CTB proteins and orthologs, identified by phylogenetic analysis of each specific protein family. Percentage identity is only shown for orthologs.

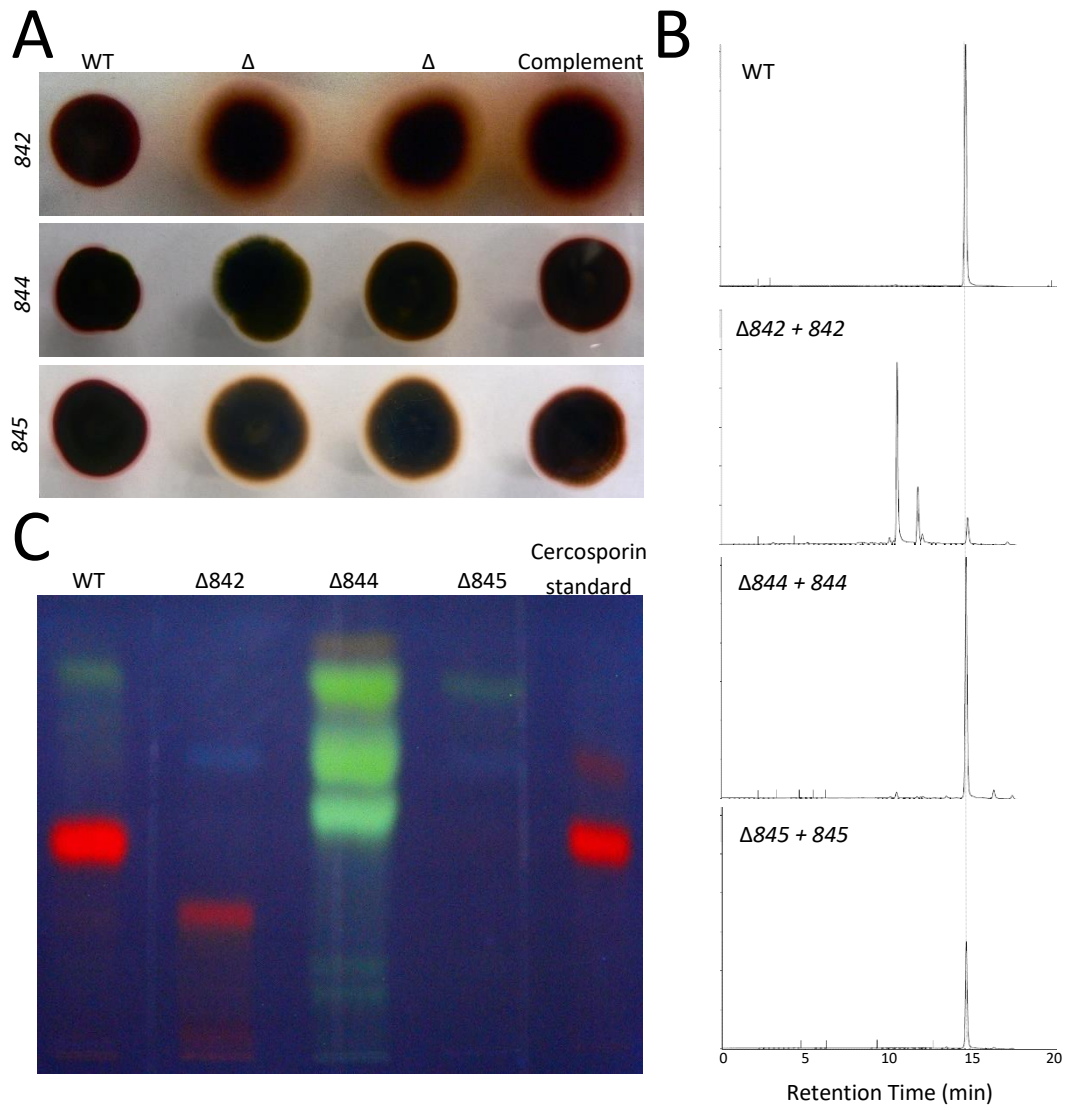


Figure S8: Analysis of cercosporin production in *CTB* mutants of *C. beticola*. Site-directed knock-out mutants in genes *CBET3_00842* (*CbCTB9*), *CBET3_00844* (*CbCTB11*), and *CBET3_00845* (*CbCTB12*) and their associated complements were assayed for cercosporin production (**a**) visually

by growth on Petri plates containing thin PDA where the red pigment around cultures is indicative of cercosporin, (**b**) comparing HPLC retention times recorded at 280 nm from extracts of *C. beticola* and *C. beticola* mutants, and (**c**) TLC. Cercosporin extracted from *C. beticola* strain 10-73-4 (WT) were used as controls in HPLC and TLC assays. Complemented mutants in HPLC assays are indicated as Δ842+842, Δ844+844, and Δ845+845. UPLC ESI-MS analyses of all mutants is shown in Figure 5A.

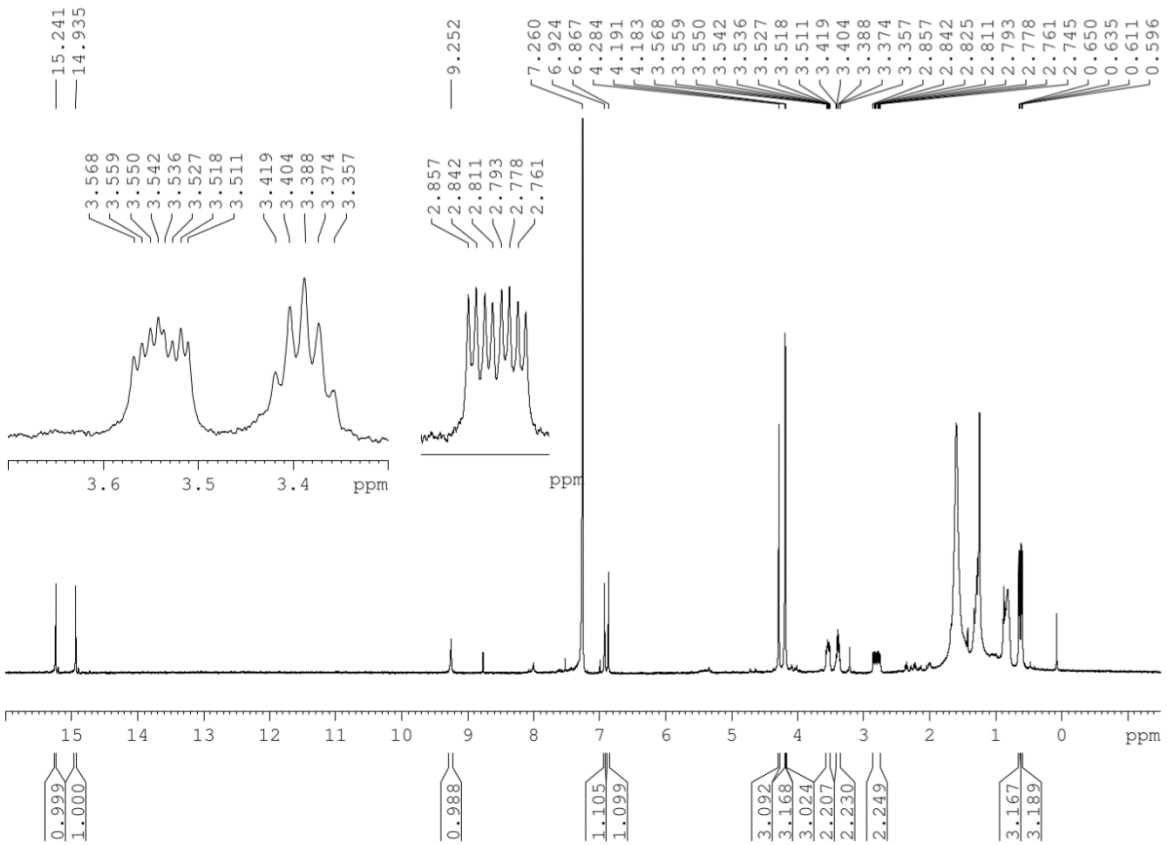


Figure S9a. ^{1}H-NMR spectrum of pre-cercosporin (2) in $CDCl_3$ (400 MHz).

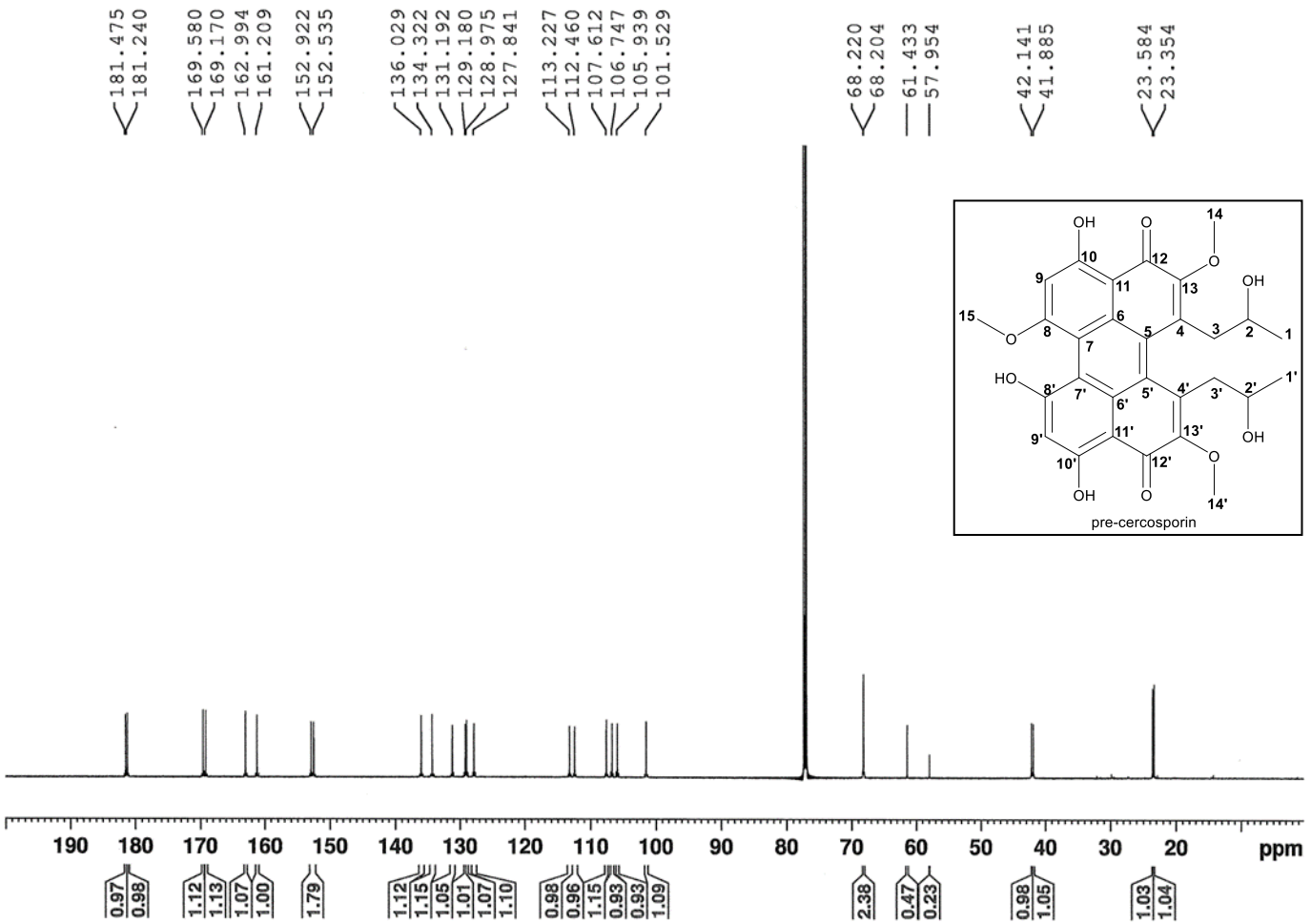
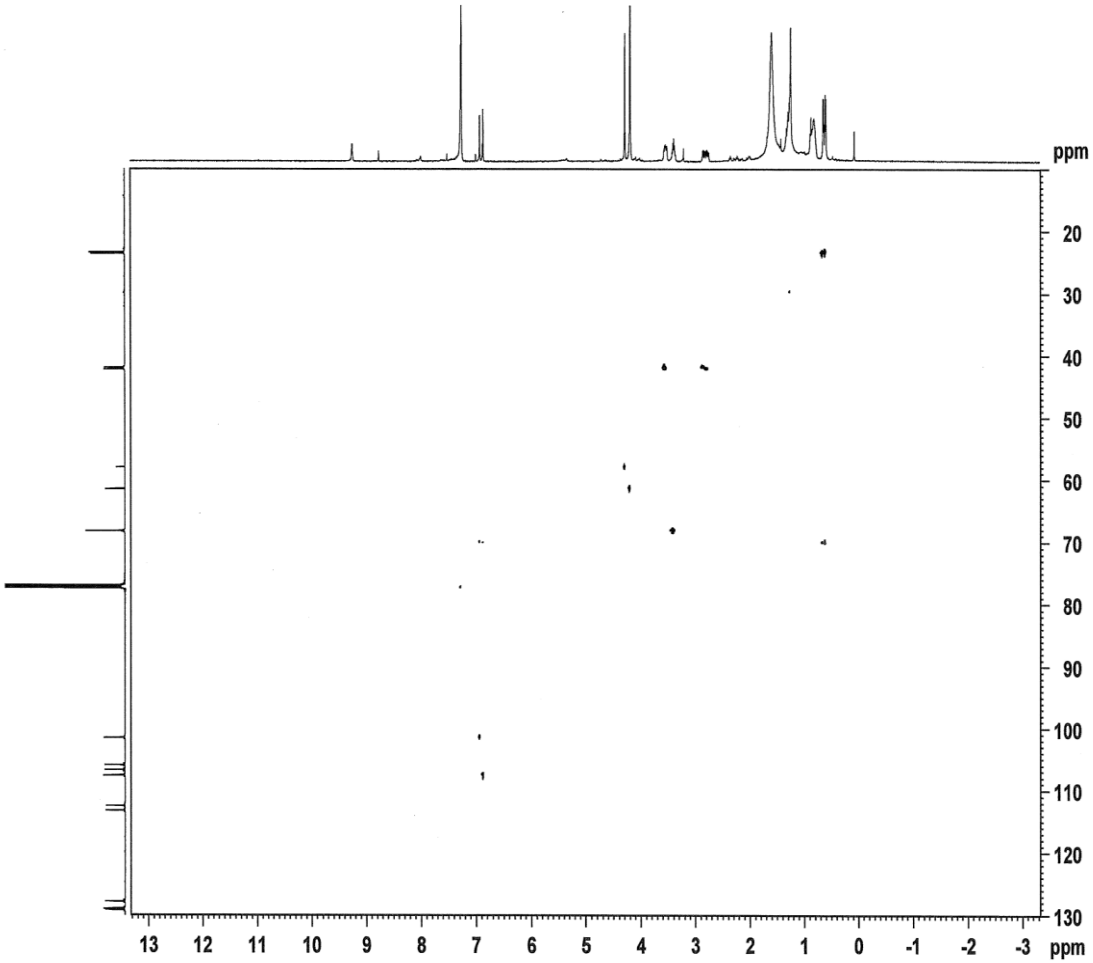


Figure S9b. ^{13}C-NMR spectrum of pre-cercosporin (2) in $CDCl_3$ (125 MHz).



δ_C	δ_H (*J* in Hz)	Atom #
23.35[a]	0.60, d (6.1)[a]	1
23.58[a]	0.64, d (6.1)[a]	1’
41.88[b]	2.86 – 2.74, m[b]	3
42.14[b]	3.57 – 3.51, m[b]	3’
57.95	4.28, s	15
61.43	4.18, s; 4.19, s	14, 14’
68.20[c]	3.42 – 3.36, m	2
68.22[c]	3.42 – 3.36, m	2’
101.53[d]	6.92, s[c]	9
105.94[e]		11
106.75[e]		11’
107.61[d]	6.87, s[c]	9’
112.46[f]		7
113.23[f]		7’
127.84[g]		4
128.97[g]		4’
129.18[h]		5
131.19[h]		5’
134.32[i]		6
136.03[i]		6’
152.53[j]		13
152.92[j]		13’
161.21[k]		8
162.99[k]		8’
169.17[l]		10
169.58[l]		10’
181.24[m]		12
181.47[m]		12’
	9.25, s	OH (C8’)
	14.93, s[d]	OH (C10)
	15.24, s[d]	OH (C10’)

Figure S9c. HSQC-NMR spectrum of pre-cercosporin (2) in $CDCl_3$ (600 MHz).

[a-m] Assignments within a column are interchangeable

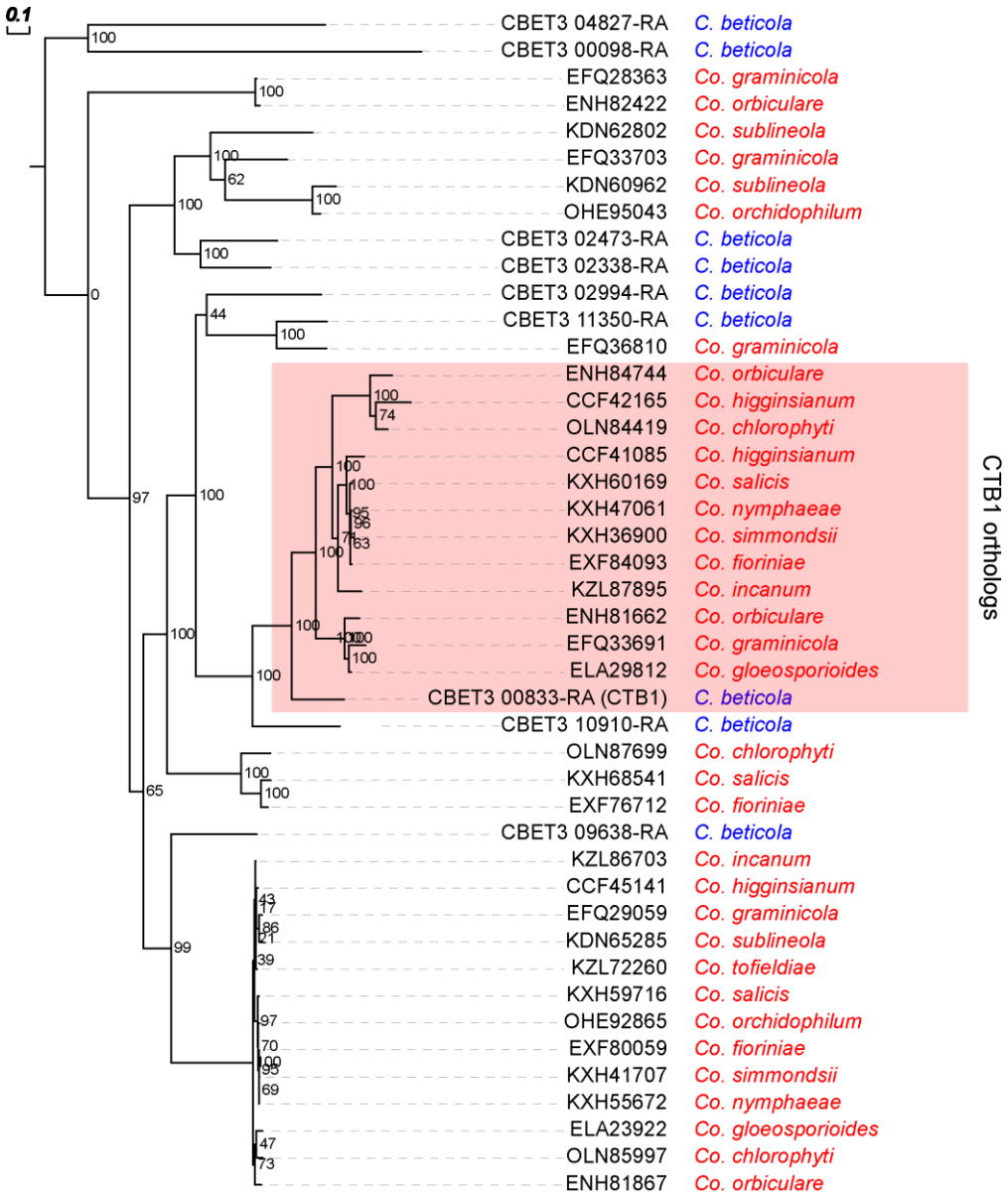

0.1
CBET3 04827-RA C. beticola
CBET3 00098-RA C. beticola
EFQ28363 Co. graminicola
ENH82422 Co. orbiculare
KDN62802 Co. sublineola
EFQ33703 Co. graminicola
KDN60962 Co. sublineola
OHE95043 Co. orchidophilum
CBET3 02473-RA C. beticola
CBET3 02338-RA C. beticola
CBET3 02994-RA C. beticola
CBET3 11350-RA C. beticola
EFQ36810 Co. graminicola
ENH84744 Co. orbiculare
CCF42165 Co. higginsianum
OLN84419 Co. chlorophyti
CCF41085 Co. higginsianum
KXH60169 Co. salicis
KXH47061 Co. nymphaeae
KXH36900 Co. simmondsii
EXF84093 Co. fioriniae
KZL87895 Co. incanum
ENH81662 Co. orbiculare
EFQ33691 Co. graminicola
ELA29812 Co. gloeosporioides
CBET3 00833-RA (CTB1) C. beticola
CBET3 10910-RA C. beticola
OLN87699 Co. chlorophyti
KXH68541 Co. salicis
EXF76712 Co. fioriniae
CBET3 09638-RA C. beticola
KZL86703 Co. incanum
CCF45141 Co. higginsianum
EFQ29059 Co. graminicola
KDN65285 Co. sublineola
KZL72260 Co. tofieldiae
KXH59716 Co. salicis
OHE92865 Co. orchidophilum
EXF80059 Co. fioriniae
KXH41707 Co. simmondsii
KXH55672 Co. nymphaeae
ELA23922 Co. gloeosporioides
OLN85997 Co. chlorophyti
ENH81867 Co. orbiculare
CTB1 orthologs

Figure S10: Phylogenetic analyses of *Co. higginsianum* CTB1 orthologs encoded by 13 *Colletotrichum* genomes. Maximum likelihood phylogenetic tree of *Co. higginsianum* CTB1 orthologs encoded by 13 *Colletotrichum* genomes, including *Co. higginsianum* itself, and all predicted non-reducing polyketide synthases from *C. beticola* (*SI Appendix*, Fig. S2). The highlighted clade confirms CTB1 orthologs in 10/13 analyzed *Colletotrichum* species.

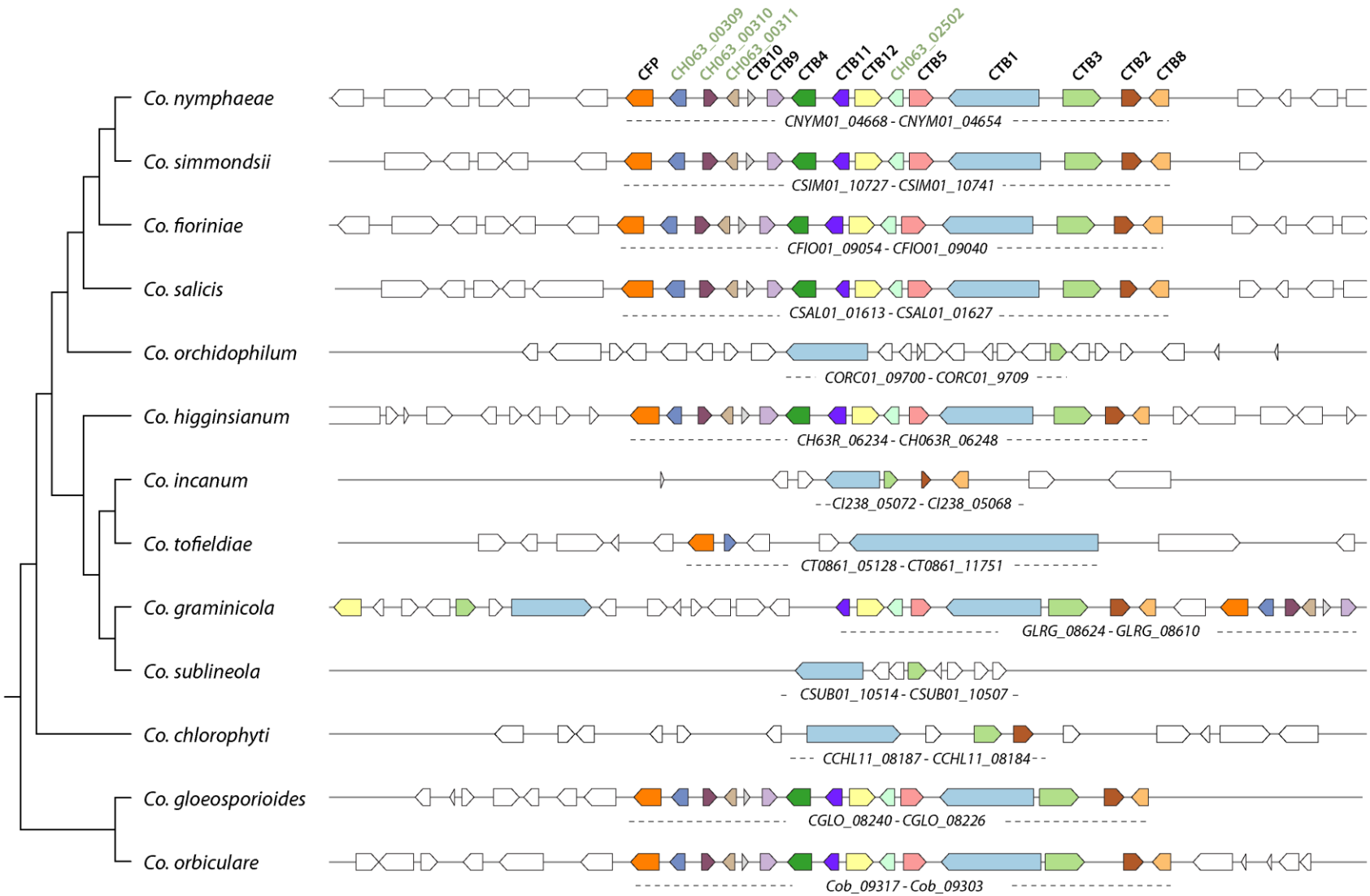

CFP
CH063_00309
CH063_00310
CH063_00311
CTB10
CTB9
CTB4
CTB11
CTB12
CH063_02502
CTB5
CTB1
CTB3
CTB2
CTB8
Co. nymphaeae
CNYM01_04668 - CNYM01_04654
Co. simmondsii
CSIM01_10727 - CSIM01_10741
Co. fioriniae
CFIO01_09054 - CFIO01_09040
Co. salicis
CSAL01_01613 - CSAL01_01627
Co. orchidophilum
CORC01_09700 - CORC01_9709
Co. higginsianum
CH63R_06234 - CH063R_06248
Co. incanum
CI238_05072 - CI238_05068
Co. tofieldiae
CT0861_05128 - CT0861_11751
Co. graminicola
GLRG_08624 - GLRG_08610
Co. sublineola
CSUB01_10514 - CSUB01_10507
Co. chlorophyti
CCHL11_08187 - CCHL11_08184
Co. gloeosporioides
CGLO_08240 - CGLO_08226
Co. orbiculare
Cob_09317 - Cob_09303

Figure S11. Synteny, rearrangement and species-specific erosion of the conserved *CTB* biosynthetic cluster in Colletotrichum. For all species, the displayed identifiers are gene IDs and the corresponding sequences can be retrieved from GenBank or Ensemble Fungi. *CTB* orthologs, labelled accordingly at the top, are colored relative to the *C. beticola CTB* cluster genes, see Figures 1 and 3; except for *CH063_00309*, *CH063_00310*, *CH063_00311* and *CH063_02502* which are unique for the *Colletotrichum CTB* cluster. Raw data for this plot was produced by MultiGeneBlast (28) using the *C. higginsianum CTB* cluster (see Figure 3) as query; and listed are the best records per genome, that were ordered by cumulative blast score. The species phylogeny was prepared by calculating pair-wise Mash (29) distances with k equals 9 and the amino acid alphabet using all the predicted protein sequences for each genome as input.

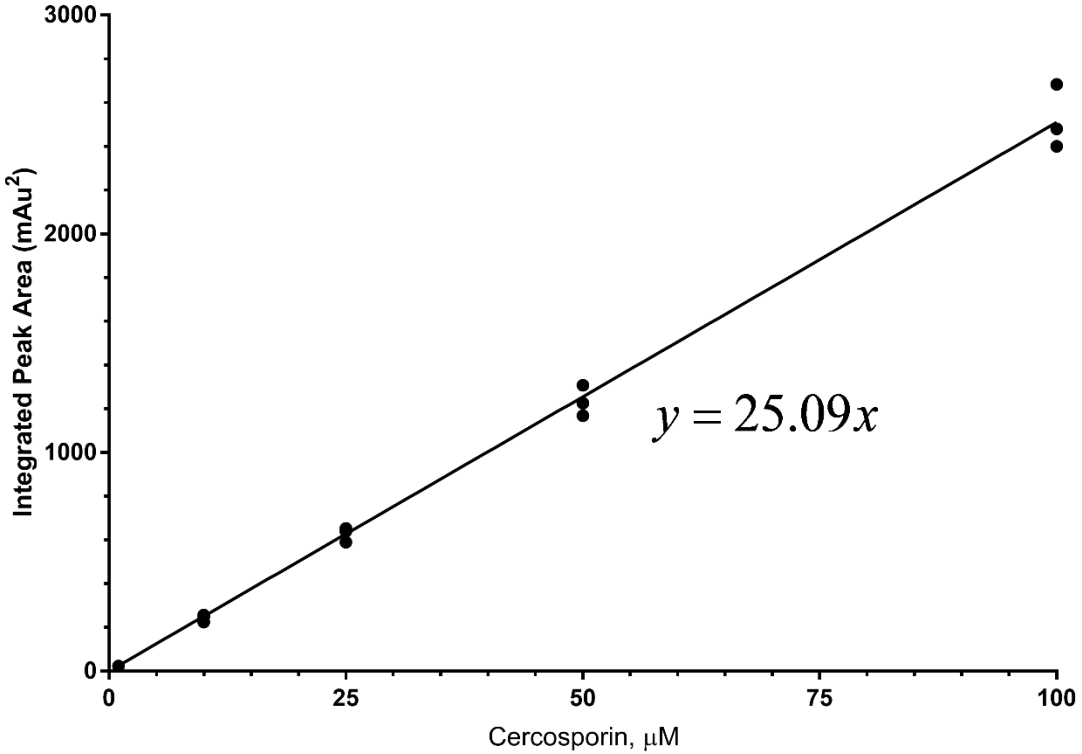


Figure S12: Cercosporin standard curve. Individual data points from three replicates are shown. Data was plotted and linear regression calculated in Prism (GraphPad).

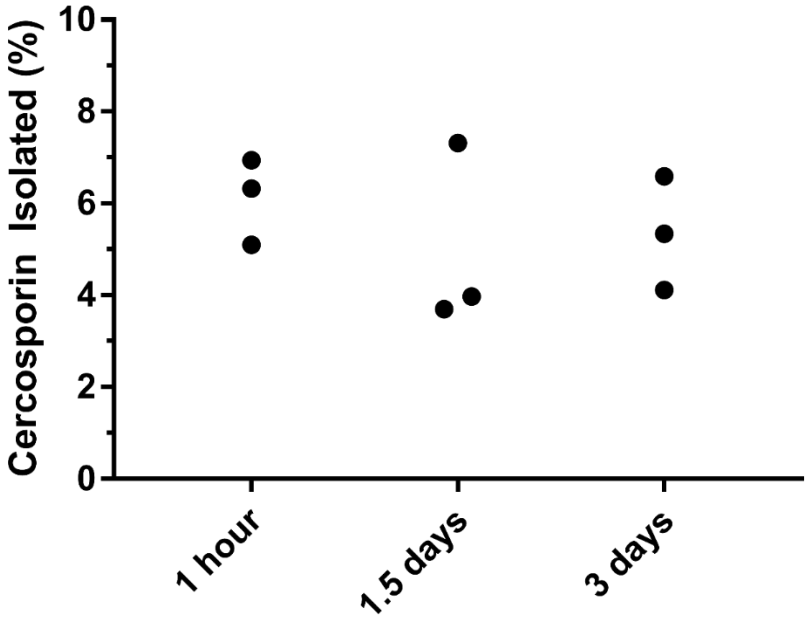


Figure S13. Recovery of cercosporin from apple tissue. An HPLC-based standard curve (Figure S12) was used to calculate the amount of cercosporin extracted from apples which had been infiltrated with the toxin after the indicated time period. Amount isolated is shown as a percentage of total cercosporin added to the apple. Individual data points from three replicates are shown.

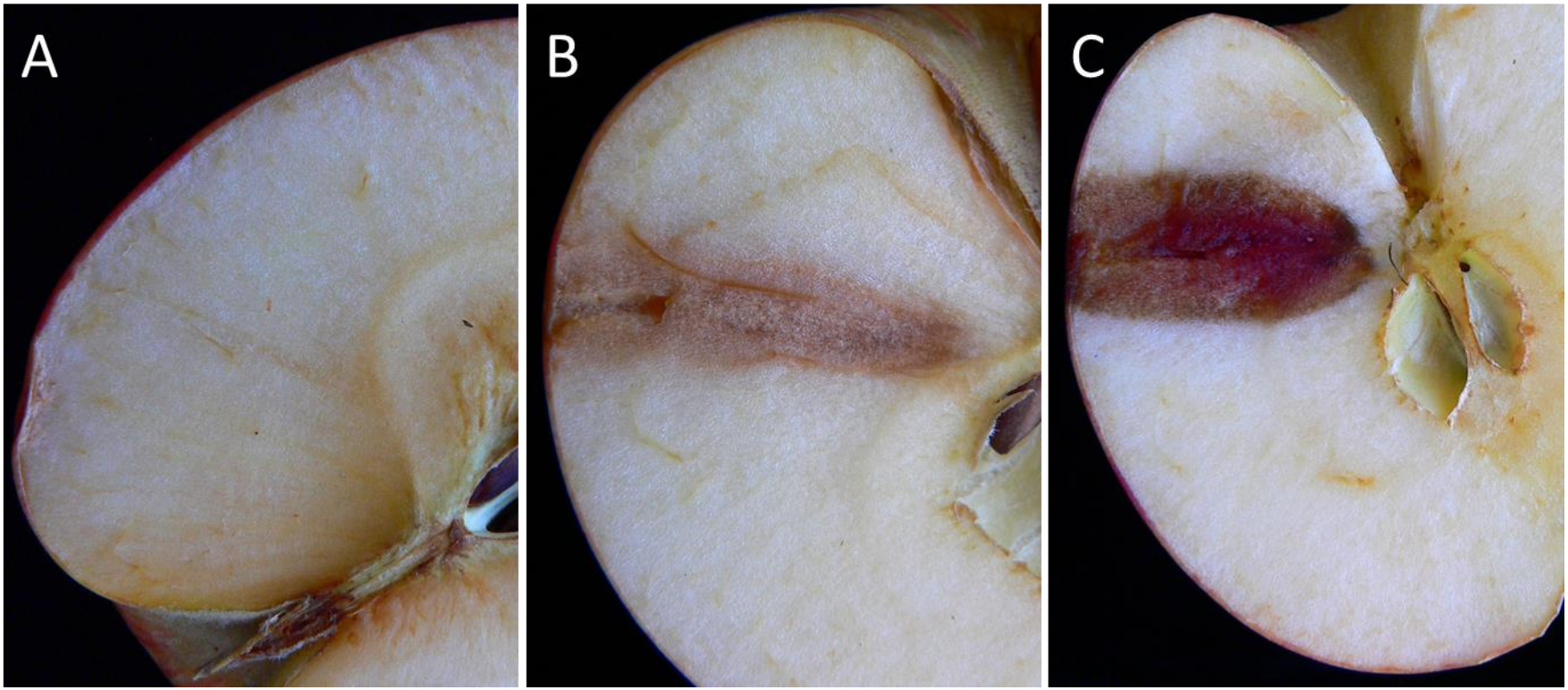


Figure S14. Symptomology of apple fruit infiltrated with (a) buffer control (50 µl), (b) 250 µM cercosporin (50 µl), or inoculated with (c) *Colletotrichum fioriniae* at five days post infiltration/inoculation. Although the amount of cercosporin produced *in planta* is dependent on many factors including *Cercospora* spp., inoculum concentration, and host plant, the cercosporin concentration in (b) may be higher than physiologically relevant.

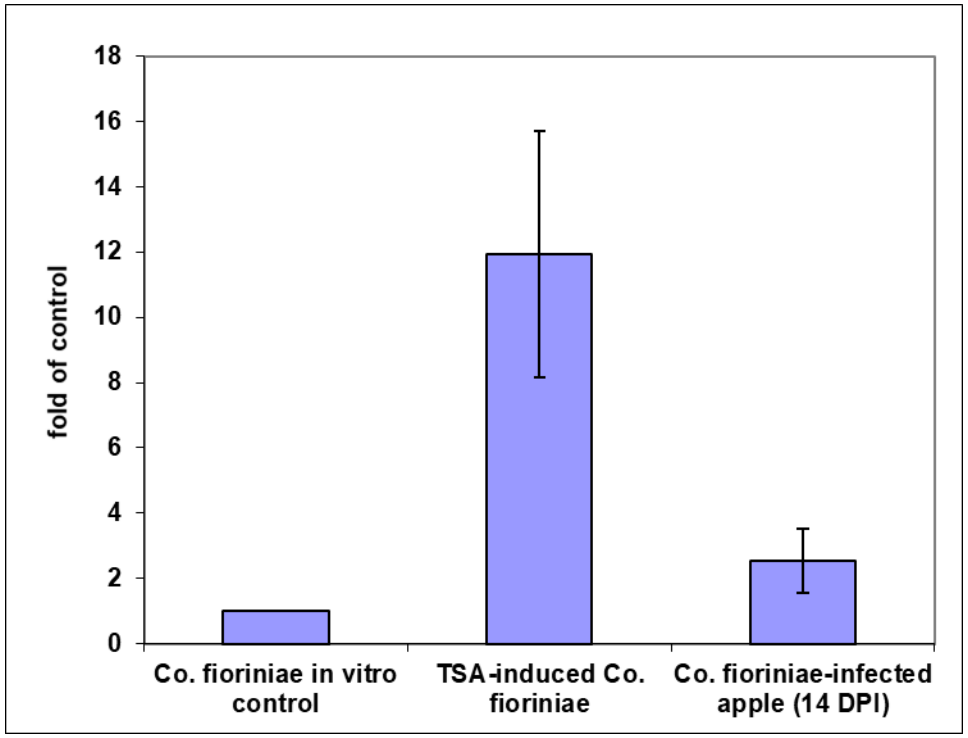


Figure S15. *Co. fioriniae CTB1* expression. Expression of *Co. fioriniae CTB1* was interrogated in *in vitro*-grown samples (control), trichostatin A- (TSA-) induced *Co. fioriniae in vitro* samples, and in apples infected with *Co. fioriniae* collected 14 days post inoculation (DPI) using qRT-PCR. One primer from each primer pair was designed to span an intron. Negative control samples in which no reverse transcriptase was added to the cDNA synthesis reaction yielded no detectable Ct values, confirming that gDNA did not contribute to expression levels in cDNA preparations. All qRT-PCR amplicons were sequenced and verified to be derived from cDNA.

SI Tables

Table S1: *Cercospora beticola* genome statistics and comparison to other related fungi.

Species*	Cbe	Cbr	Ccn	Dse	Zst	Lma	Ptr	Pno	Ani
Assembly statistics									
Total assembly length (Mbp)	37.1	37.4	34.0	30.2	39.7	44.9	37.8	37.2	30.5
Total length of gaps (Mbp)	3.2	0.3	0.0	0.1	0.0	1.1	0.6	0.2	0.7
No. of scaffolds/contigs	248	28,905	6,126	20	21	41	47	108	8
NG50 scaffolds (No.)	4	111	422	5	6	10	6	13	4
LG50 scaffolds (Mbp)	4.17	0.096	0.023	2.60	2.67	1.77	1.99	1.05	3.76
NG95 scaffolds (No.)	10	11,497	2,320	12	18	25	20	43	8
GC content (%)									
Overall (excl. gaps)	52.2	51.5	52.6	53.1	52.1	45.2	51.0	50.4	50.4
Coding (CDS)	53.8	53.9	54.3	54.6	55.6	54.1	53.6	54.6	53.4
Repeat content (Mbp)	0.51	ND***	ND***	1.08	6.98	15.93	0.80	2.88	1.07
Protein-coding genes									
Protein-coding genes (No.)	12,281	11,972	11,556	12,580	10,951	12,469	12,169	12,380	10,680
Mean gene length (bp)	1,885	1,584	1,556	1,896	1,602	1,446	1,616	1,468	1,736
Percentage coding	68.4	51.1	52.9	79.3	44.2	41.2	52.9	49.1	62.2
Mean gene density (No. genes/100 Kbp)	36.3	32.3	34.0	41.8	27.6	28.5	32.7	33.4	35.8
Mean CDS length (bp)	1,469	1,473	1,450	1,223	1,310	1,258	1,349	1,271	1,456
Exons**									
No. of exons	28,100	28,046	26,848	28,937	28,611	35,201	32,716	32,994	34,743
Exons/gene	2.3	2.3	2.3	2.3	2.6	2.8	2.7	2.7	3.3
Mean exon length (bp)	780	629	624	773	532	446	530	495	477
Introns**									
Introns (No. introns)	15,819	16,074	15,292	16,356	17,660	22,732	20,547	20,614	24,062
Introns/gene	1.3	1.3	1.3	1.3	1.6	1.8	1.7	1.7	2.3
Mean intron length (bp)	73	83	80	91	133	103	114	90	82

*Genomes are *Cercospora beticola* (Cbe), *Cercospora berteroae* (Cbr), *Cercospora canescens* (Ccn), *Dothistroma septosporum* (Dse), *Zymoseptoria tritici* (Zst), *Leptosphaeria maculans* (Lma), *Pyrenophora tritici-repentis* (Ptr), *Parastagonospora nodorum* (Pno), and *Aspergillus nidulans* (Ani).

**Only considering the longest transcript (if alternatives exist).
*** Not determined (ND).

Table S2: Number of secondary metabolism biosynthesis clusters in *Cercospora beticola* and other related fungi.

	NRPS	T1PKS	T3PKS	Hybrid[∞]	Terpene[*]	DMAT[†]	Siderophore	LanD[‡]	Other[$]	Total
Cercospora beticola	23	15	0	3	7	1	2	0	12	63
Dothistroma septosporum	5	3	0	1	4	1	0	0	10	24
Zymoseptoria tritici	8	10	1	1	4	0	1	0	7	32
Leptosphaeria maculans	4	9	1	2	6	1	0	0	7	30
Pyrenophora tritici-repentis	13	15	1	1	5	0	0	1	7	43
Parastagonospora nodorum	8	15	1	2	6	2	0	0	6	40
Aspergillus nidulans	10	23	0	2	13	7	0	0	11	66

[∞]Hybrid refers to gene clusters incorporating both NRPS and PKS key enzymes

[*]Terpene cyclases and hybrids (-NRPS and -PKS).

[†]Number of proteins that contain the PFAM domain PF11991.

[‡]Lantipeptide biosynthesis clusters

[$]Category refers to candidate NRPS clusters that lack key domains (antiSMASH: Blin et al. 2013)

Table S3: Fungal proteomes used in this study.

No.	Species Name (common)	Alternative name	Strain	Source	Reference	ShortID
1	*Alternaria brassicicola*		ATCC 96836	JGI	http://alternaria.vbi.vt.edu/index.html	abra
2	*Aspergillus clavatus*		NRRL 1	JGI (original FGI)	Fedorova et al. 2008	apcl
3	*Aspergillus flavus*		NRRL 3357	JGI (original FGI)	Arnaud et al. 2012	apfl
4	*Aspergillus fumigatus*		Af293	JGI (original FGI)	Fedorova et al. 2008	apfu
5	*Aspergillus nidulans*		FGSC A4	JGI (original FGI)	Galagan et al. 2005	apnd
6	*Aspergillus niger*		ATCC 1015	JGI	Pel et al. 2007	apni
7	*Aspergillus oryzae*		RIB40 / ATCC 42149	JGI (original FGI)	Machida et al. 2005	apor
8	*Aspergillus terreus*		NIH 2624	JGI (original FGI)	Arnaud et al. 2012	apte
9	*Botrytis cinerea*		B05.10	JGI (original FGI)	Amselem et al. 2011	botc
10	*Cercospora berteroae*		CBS538.71	This study	This study	cber
11	*Cercospora beticola*		09-40	This study	This study	cbet
12	*Cercospora canescens*		MTCC-I0835	BioProject PRJNA183604	BioProject PRJNA183604	ccan
13	*Chaetomium globosum*		CBS 148	FGI	Cuomo et al. 2015	chag
14	*Cladosporium fulvum*		CBS 131901	JGI	de Wit et al. 2012	claf
15	*Coccidioides immitis*		RS	JGI (original FGI)	Sharpton et al. 2009	coci
16	*Cochliobolus heterostrophus*		C5	JGI	Condon et al. 2013	coch
17	*Colletotrichum fioriniae*	*Colletotrichum acutatum Sensu Lato*	PJ7	BioProject PRJNA233987	Baroncelli et al. 2014	colf
18	*Colletotrichum gloeosporioides*	*Glomerella cingulata*	Nara GC5	Ensemble Fungi	Gan et al. 2013	cglo
19	*Colletotrichum graminicola*		M1.001	JGI (original FGI)	O'Connell et al. 2012	colg
20	*Colletotrichum higginsianum*		IMI349063	JGI (original FGI)	O'Connell et al. 2012	colh
21	*Colletotrichum orbiculare*		MAFF240422	Ensemble Fungi	Gan et al. 2013	colo
22	*Colletotrichum sublineola*		TX430BB	BioProject PRJNA246670	Baroncelli et al. 2014	cols
23	*Dothistroma septosporum*	*Mycosphaerella pini*	NZE10	JGI	de Wit et al. 2012	dots
24	*Fusarium fujikuroi*		IMI58289	JGI	Wiemann et al. 2013	fusf

25	*Fusarium graminearum*		PH-1	JGI (original FGI)	Cuomo et al. 2007	fusg
26	*Fusarium oxysporum* f. sp. *lycopersici*		4287	JGI (original FGI)	Ma et al. 2010	fuso
27	*Fusarium verticillioides*		7600	JGI (original FGI)	Ma et al. 2010	fusv
28	*Leptosphaeria maculans*		v23.1.3	JGI (original FGI)	Rouxel et al. 2011	lepm
29	*Magnaporthe oryzae*	*Magnaporthe grisae*	70-15	JGI (original FGI)	Dean et al. 2005	mgri
30	*Magnaporthe poae*		ATCC 64411	JGI (original FGI)	FGI	mpoa
31	*Mycosphaerella fijiensis*	*Pseudocercospora fijiensis*	CIRAD86	JGI	Ohm et al. 2012	mfij
32	*Mycosphaerella graminicola*	*Zymoseptoria tritici*	IPO323	JGI	Goodwin et al. 2011	mgra
33	*Nectria haematococca*	*Fusarium solani*	MPVI	JGI	Coleman et al. 2009	nhae
34	*Neosartorya fischeri*		NRRL 181	JGI	Fedorova et al. 2008	nfis
35	*Neurospora crassa*		OR74A	JGI (original FGI)	Galagan et al. 2003	ncra
36	*Podospora anserina*		DSM980	JGI (original FGI)	Espagne et al. 2008	pans
37	*Pyrenophora teres* f. *teres*		0-1	Ensemble Fungi	Ellwood et al. 2010	pter
38	*Pyrenophora tritici-repentis*		Pt-1C-BFP	JGI (original FGI)	Manning et al. 2013	pytr
39	*Rhystidhysteron rufulum*		CBS 306.38	JGI	Ohm et al. 2012	rhyr
40	*Sclerotinia sclerotiorum*		1980	JGI (original FGI)	Amselem et al. 2011	scsc
41	*Septoria musiva*	*Mycosphaerella populorum*	SO2202	JGI	Ohm et al. 2012	sepm
42	*Septoria populicola*	*Mycosphaerella populicola*	p0202b	JGI	Ohm et al. 2012; Dhillon et al. 2015	sepp
43	*Parastagonospora nodorum*	*Phaeosphaeria nodorum*	SN15	JGI (original FGI)	Oliver et al. 2012; Syme et al. 2013	stan
44	*Trichoderma atroviride*		IMI202040	JGI	Kubicek et al. 2011	trat
45	*Trichoderma reesei*		QM6a	JGI	Martinez et al. 2008	trav
46	*Trichoderma virens*		Gv29-8	JGI	Kubicek et al. 2011	trvi
47	*Verticillium alfalfae*		VaMS102	JGI (original FGI)	Klosterman et al. 2011	vaam
48	*Verticillium dahliae*		VdLS17	JGI (original FGI)	Klosterman et al. 2011	vadh

Table S4: Pairwise identity between CTB1-CTB12 homologs of multiple species*.

	CTB1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	scsc|SS1G_05681.1	ID	0.314	0.32	0.322	0.305	0.296	0.079	0.293	0.314	0.292	0.118	0.184	0.306	0.312	0.307	0.302	0.314	0.312
2	ccan|CCAN2_10369.t1	0.314	ID	0.717	0.712	0.418	0.431	0.099	0.359	0.424	0.396	0.15	0.215	0.409	0.419	0.414	0.428	0.441	0.442
3	cbet|CBET3_10910-RA	0.32	0.717	ID	0.864	0.417	0.427	0.1	0.356	0.424	0.398	0.153	0.21	0.419	0.429	0.42	0.428	0.445	0.446
4	cber|CBER1_09856-RA	0.322	0.712	0.864	ID	0.419	0.437	0.096	0.363	0.429	0.404	0.157	0.209	0.423	0.428	0.421	0.433	0.451	0.451
5	stan|Stano2_2390	0.305	0.418	0.417	0.419	ID	0.433	0.068	0.444	0.43	0.385	0.179	0.255	0.417	0.424	0.411	0.486	0.47	0.468
6	claf|Clafu1_196875	0.296	0.431	0.427	0.437	0.433	ID	0.107	0.445	0.534	0.473	0.189	0.23	0.507	0.515	0.5	0.542	0.548	0.548
7	colh|Colhi1_8554	0.079	0.099	0.1	0.096	0.068	0.107	ID	0.058	0.186	0.145	0	0.041	0.144	0.15	0.144	0.099	0.112	0.113
8	colh|Colhi1_9380	0.293	0.359	0.356	0.363	0.444	0.445	0.058	ID	0.695	0.51	0.295	0.334	0.513	0.54	0.518	0.476	0.456	0.457
9	colf|ColfiPJ7_gi615442870	0.314	0.424	0.424	0.429	0.43	0.534	0.186	0.695	ID	0.644	0.25	0.271	0.638	0.684	0.648	0.524	0.532	0.536
10	colo|Color1_ENH84744	0.292	0.396	0.398	0.404	0.385	0.473	0.145	0.51	0.644	ID	0.299	0.244	0.553	0.575	0.553	0.472	0.473	0.474
11	colh|Colhi1_10470	0.118	0.15	0.153	0.157	0.179	0.189	0	0.295	0.25	0.299	ID	0.144	0.215	0.218	0.209	0.208	0.2	0.202
12	mgri|MGG_00428	0.184	0.215	0.21	0.209	0.255	0.23	0.041	0.334	0.271	0.244	0.144	ID	0.282	0.287	0.274	0.249	0.241	0.239
13	colo|Color1_ENH81662	0.306	0.409	0.419	0.423	0.417	0.507	0.144	0.513	0.638	0.553	0.215	0.282	ID	0.842	0.786	0.499	0.514	0.513
14	cglo|ColglGC5_ELA29812	0.312	0.419	0.429	0.428	0.424	0.515	0.15	0.54	0.684	0.575	0.218	0.287	0.842	ID	0.883	0.513	0.528	0.526
15	colg|Colgr1_7584	0.307	0.414	0.42	0.421	0.411	0.5	0.144	0.518	0.648	0.553	0.209	0.274	0.786	0.883	ID	0.491	0.508	0.508
16	ccan|CCAN2_11561.t1	0.302	0.428	0.428	0.433	0.486	0.542	0.099	0.476	0.524	0.472	0.208	0.249	0.499	0.513	0.491	ID	0.828	0.828
17	cbet|CBET3_00833-RA	0.314	0.441	0.445	0.451	0.47	0.548	0.112	0.456	0.532	0.473	0.2	0.241	0.514	0.528	0.508	0.828	ID	0.959
18	cber|CBER1_00905-RA	0.312	0.442	0.446	0.451	0.468	0.548	0.113	0.457	0.536	0.474	0.202	0.239	0.513	0.526	0.508	0.828	0.959	ID

	CTB2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	cbet|CBET3_10911-RA	ID	0.79	0.414	0.244	0.254	0.24	0.254	0.211	0.258	0.256	0.23	0.226	0.245	0.257	0.257	0.255
2	cber|CBER1_09857-RA	0.79	ID	0.342	0.202	0.212	0.194	0.202	0.21	0.209	0.21	0.194	0.179	0.193	0.205	0.205	0.203
3	ccan|CCAN2_10371.t1	0.414	0.342	ID	0.491	0.527	0.498	0.519	0.428	0.527	0.53	0.479	0.465	0.461	0.504	0.508	0.506
4	stan|Stano2_2386	0.244	0.202	0.491	ID	0.516	0.508	0.514	0.403	0.495	0.495	0.467	0.457	0.466	0.506	0.516	0.512
5	colf|ColfiPJ7_gi615442863	0.254	0.212	0.527	0.516	ID	0.776	0.692	0.559	0.722	0.708	0.554	0.606	0.587	0.643	0.643	0.637
6	colh|Colhi1_4002	0.24	0.194	0.498	0.508	0.776	ID	0.646	0.532	0.65	0.656	0.515	0.552	0.554	0.575	0.574	0.576
7	colo|Color1_ENH81660	0.254	0.202	0.519	0.514	0.692	0.646	ID	0.651	0.836	0.789	0.651	0.662	0.578	0.632	0.641	0.636
8	cols|Colsu01_gi640927341	0.211	0.21	0.428	0.403	0.559	0.532	0.651	ID	0.681	0.656	0.505	0.514	0.469	0.511	0.511	0.513
9	cglo|ColglGC5_ELA29810	0.258	0.209	0.527	0.495	0.722	0.65	0.836	0.681	ID	0.891	0.637	0.658	0.577	0.632	0.637	0.634
10	colg|Colgr1_7582	0.256	0.21	0.53	0.495	0.708	0.656	0.789	0.656	0.891	ID	0.624	0.635	0.577	0.617	0.622	0.624
11	mgri|MGG_02287	0.23	0.194	0.479	0.467	0.554	0.515	0.651	0.505	0.637	0.624	ID	0.554	0.501	0.538	0.539	0.541
12	mgri|MGG_11582	0.226	0.179	0.465	0.457	0.606	0.552	0.662	0.514	0.658	0.635	0.554	ID	0.476	0.536	0.533	0.531
13	claf|Clafu1_196873	0.245	0.193	0.461	0.466	0.587	0.554	0.578	0.469	0.577	0.577	0.501	0.476	ID	0.645	0.656	0.652
14	ccan|CCAN2_11562.t1	0.257	0.205	0.504	0.506	0.643	0.575	0.632	0.511	0.632	0.617	0.538	0.536	0.645	ID	0.912	0.914
15	cbet|CBET3_00832-RA	0.257	0.205	0.508	0.516	0.643	0.574	0.641	0.511	0.637	0.622	0.539	0.533	0.656	0.912	ID	0.974
16	cber|CBER1_00906-RA	0.255	0.203	0.506	0.512	0.637	0.576	0.636	0.513	0.634	0.624	0.541	0.531	0.652	0.914	0.974	ID

	CTB3	1	2	3	4	5	6	7	8	9	10	11	12	13
1	ccan|CCAN2_10370.t1	ID	0.263	0.222	0.245	0.253	0.29	0.247	0.2	0.266	0.262	0.266	0.269	0.264
2	stan|Stano2_2388	0.263	ID	0.387	0.448	0.437	0.415	0.259	0.349	0.44	0.407	0.444	0.443	0.447
3	mgri|MGG_00427	0.222	0.387	ID	0.577	0.578	0.521	0.284	0.411	0.519	0.442	0.461	0.455	0.452
4	colo|Color1_ENH81661	0.245	0.448	0.577	ID	0.803	0.714	0.352	0.516	0.672	0.548	0.534	0.537	0.536
5	colg|Colgr1_7583	0.253	0.437	0.578	0.803	ID	0.79	0.335	0.526	0.686	0.545	0.533	0.537	0.534
6	cglo|ColglGC5_ELA29811	0.29	0.415	0.521	0.714	0.79	ID	0.303	0.538	0.634	0.484	0.484	0.488	0.482
7	colh|Colhi1_5868	0.247	0.259	0.284	0.352	0.335	0.303	ID	0.271	0.37	0.3	0.302	0.296	0.295
8	colh|Colhi1_8770	0.2	0.349	0.411	0.516	0.526	0.538	0.271	ID	0.688	0.461	0.43	0.439	0.43
9	colf|ColfiPJ7_gi615442866	0.266	0.44	0.519	0.672	0.686	0.634	0.37	0.688	ID	0.573	0.55	0.55	0.543
10	claf|Clafu1_196874	0.262	0.407	0.442	0.548	0.545	0.484	0.3	0.461	0.573	ID	0.53	0.534	0.533
11	ccan|CCAN2_11560.t1	0.266	0.444	0.461	0.534	0.533	0.484	0.302	0.43	0.55	0.53	ID	0.898	0.897
12	cbet|CBET3_00834-RA	0.269	0.443	0.455	0.537	0.537	0.488	0.296	0.439	0.55	0.534	0.898	ID	0.957
13	cber|CBER1_00904-RA	0.264	0.447	0.452	0.536	0.534	0.482	0.295	0.43	0.543	0.533	0.897	0.957	ID

	CTB4	1	2	3	4	5	6	7	8	9	10
1	stan|Stano2_11691	ID	0.568	0.54	0.573	0.579	0.507	0.461	0.535	0.533	0.535
2	colh|Colhi1_7262	0.568	ID	0.813	0.785	0.797	0.638	0.488	0.587	0.585	0.585
3	colf|ColfiPJ7_gi615442885	0.54	0.813	ID	0.729	0.737	0.707	0.527	0.538	0.536	0.534
4	cglo|ColglGC5_ELA29817	0.573	0.785	0.729	ID	0.915	0.672	0.475	0.564	0.56	0.56
5	colo|Color1_ENH81667	0.579	0.797	0.737	0.915	ID	0.678	0.485	0.564	0.564	0.564
6	mgri|MGG_00416	0.507	0.638	0.707	0.672	0.678	ID	0.518	0.484	0.482	0.48
7	claf|Clafu1_196872	0.461	0.488	0.527	0.475	0.485	0.518	ID	0.516	0.524	0.516
8	ccan|CCAN2_11563.t1	0.535	0.587	0.538	0.564	0.564	0.484	0.516	ID	0.917	0.927
9	cbet|CBET3_00831-RA	0.533	0.585	0.536	0.56	0.564	0.482	0.524	0.917	ID	0.984
10	cber|CBER1_00907-RA	0.535	0.585	0.534	0.56	0.564	0.48	0.516	0.927	0.984	ID

	CTB5	1	2	3	4	5	6	7	8	9	10	11	12	13
1	cbet|CBET3_10914-RA	ID	0.885	0.774	0.449	0.411	0.425	0.438	0.444	0.414	0.429	0.445	0.446	0.443
2	cber|CBER1_09860-RA	0.885	ID	0.785	0.436	0.408	0.436	0.437	0.442	0.416	0.435	0.443	0.444	0.441
3	ccan|CCAN2_10374.t1	0.774	0.785	ID	0.451	0.402	0.425	0.429	0.441	0.403	0.411	0.439	0.434	0.431
4	colf|ColfiPJ7_gi615442873	0.449	0.436	0.451	ID	0.739	0.617	0.712	0.682	0.655	0.549	0.598	0.602	0.6
5	colh|Colhi1_9379	0.411	0.408	0.402	0.739	ID	0.555	0.632	0.638	0.689	0.496	0.534	0.537	0.534
6	mgri|MGG_00420	0.425	0.436	0.425	0.617	0.555	ID	0.7	0.68	0.648	0.486	0.542	0.544	0.549
7	cglo|ColglGC5_ELA29813	0.438	0.437	0.429	0.712	0.632	0.7	ID	0.864	0.823	0.543	0.591	0.582	0.585
8	colo|Color1_ENH81663	0.444	0.442	0.441	0.682	0.638	0.68	0.864	ID	0.784	0.53	0.569	0.566	0.569
9	colg|Colgr1_7585	0.414	0.416	0.403	0.655	0.689	0.648	0.823	0.784	ID	0.489	0.533	0.538	0.541
10	claf|Clafu1_196877	0.429	0.435	0.411	0.549	0.496	0.486	0.543	0.53	0.489	ID	0.608	0.616	0.612
11	ccan|CCAN2_11559.t1	0.445	0.443	0.439	0.598	0.534	0.542	0.591	0.569	0.533	0.608	ID	0.903	0.911
12	cbet|CBET3_00835-RA	0.446	0.444	0.434	0.602	0.537	0.544	0.582	0.566	0.538	0.616	0.903	ID	0.964
13	cber|CBER1_00903-RA	0.443	0.441	0.431	0.6	0.534	0.549	0.585	0.569	0.541	0.612	0.911	0.964	ID

	CTB6	1	2	3
1	ccan|CCAN2_11564.t1	ID	0.868	0.885
2	cbet|CBET3_00830-RA	0.868	ID	0.963
3	cber|CBER1_00908-RA	0.885	0.963	ID

	CTB7	1	2	3	4	5	6	7	8	9	10	11
1	sepm|Sepmu1_20295	ID	0.682	0.682	0.679	0.353	0.438	0.384	0.261	0.385	0.38	0.385
2	ccan|CCAN2_1986.t1	0.682	ID	0.862	0.869	0.334	0.451	0.386	0.288	0.402	0.401	0.401
3	cber|CBER1_09769-RA	0.682	0.862	ID	0.943	0.341	0.454	0.39	0.279	0.4	0.394	0.401
4	cbet|CBET3_09576-RA	0.679	0.869	0.943	ID	0.341	0.454	0.395	0.284	0.405	0.399	0.399
5	mgra|Mycgr1_86679	0.353	0.334	0.341	0.341	ID	0.379	0.361	0.232	0.378	0.363	0.361
6	mfij|Mycfi1_44616	0.438	0.451	0.454	0.454	0.379	ID	0.481	0.336	0.473	0.485	0.488
7	claf|Clafu1_192533	0.384	0.386	0.39	0.395	0.361	0.481	ID	0.495	0.62	0.606	0.606
8	dots|Dotse1_62752	0.261	0.288	0.279	0.284	0.232	0.336	0.495	ID	0.392	0.392	0.385
9	ccan|CCAN2_11558.t1	0.385	0.402	0.4	0.405	0.378	0.473	0.62	0.392	ID	0.867	0.855
10	cbet|CBET3_00836-RA	0.38	0.401	0.394	0.399	0.363	0.485	0.606	0.392	0.867	ID	0.964
11	cber|CBER1_00902-RA	0.385	0.401	0.401	0.399	0.361	0.488	0.606	0.385	0.855	0.964	ID

	CTB8	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	ccan|CCAN2_1075.t1	ID	0.674	0.657	0.172	0.144	0.152	0.119	0.137	0.111	0.144	0.145	0.122	0.121	0.173	0.032	0.168	0.154
2	cbet|CBET3_00839-RA	0.674	ID	0.86	0.177	0.15	0.153	0.108	0.139	0.108	0.145	0.14	0.131	0.117	0.151	0.025	0.171	0.151
3	cber|CBER1_00900-RA	0.657	0.86	ID	0.165	0.156	0.165	0.121	0.147	0.121	0.157	0.134	0.134	0.127	0.168	0.032	0.159	0.154
4	ccan|CCAN2_1076.t1	0.172	0.177	0.165	ID	0.481	0.151	0.12	0.132	0.109	0.147	0.138	0.142	0.133	0.129	0.022	0.152	0.125
5	cbet|CBET3_00838-RA	0.144	0.15	0.156	0.481	ID	0.201	0.178	0.197	0.147	0.171	0.134	0.142	0.188	0.145	0.081	0.14	0.182
6	stan|Stano2_2385	0.152	0.153	0.165	0.151	0.201	ID	0.2	0.2	0.13	0.166	0.135	0.157	0.219	0.192	0.112	0.151	0.228
7	mgri|MGG_00417	0.119	0.108	0.121	0.12	0.178	0.2	ID	0.361	0.258	0.293	0.234	0.218	0.304	0.152	0.074	0.134	0.192
8	colo|Color1_ENH81659	0.137	0.139	0.147	0.132	0.197	0.2	0.361	ID	0.562	0.597	0.407	0.307	0.437	0.149	0.09	0.147	0.209
9	cglo|ColglGC5_ELA29809	0.111	0.108	0.121	0.109	0.147	0.13	0.258	0.562	ID	0.698	0.407	0.29	0.302	0.137	0.05	0.127	0.139
10	colg|Colgr1_7581	0.144	0.145	0.157	0.147	0.171	0.166	0.293	0.597	0.698	ID	0.467	0.338	0.352	0.171	0.045	0.172	0.175
11	cols|Colsu01_gi640914729	0.145	0.14	0.134	0.138	0.134	0.135	0.234	0.407	0.407	0.467	ID	0.261	0.262	0.158	0.021	0.16	0.137
12	colh|Colhi1_4003	0.122	0.131	0.134	0.142	0.142	0.157	0.218	0.307	0.29	0.338	0.261	ID	0.552	0.165	0.034	0.163	0.153
13	colf|ColfiPJ7_gi615442859	0.121	0.117	0.127	0.133	0.188	0.219	0.304	0.437	0.302	0.352	0.262	0.552	ID	0.148	0.093	0.167	0.23
14	claf|Clafu1_196880	0.173	0.151	0.168	0.129	0.145	0.192	0.152	0.149	0.137	0.171	0.158	0.165	0.148	ID	0.028	0.172	0.158
15	ccan|CCAN2_11557.t1	0.032	0.025	0.032	0.022	0.081	0.112	0.074	0.09	0.05	0.045	0.021	0.034	0.093	0.028	ID	0.089	0.286
16	cber|CBER1_00901-RA	0.168	0.171	0.159	0.152	0.14	0.151	0.134	0.147	0.127	0.172	0.16	0.163	0.167	0.172	0.089	ID	0.722
17	cbet|CBET3_00837-RA	0.154	0.151	0.154	0.125	0.182	0.228	0.192	0.209	0.139	0.175	0.137	0.153	0.23	0.158	0.286	0.722	ID

	CFP	1	2	3	4	5	6	7	8	9	10	11
1	claf|Clafu1_188962	ID	0.735	0.399	0.392	0.403	0.394	0.386	0.376	0.403	0.395	0.398
2	dots|Dotse1_59249	0.735	ID	0.414	0.406	0.408	0.397	0.392	0.398	0.405	0.399	0.394
3	colf|ColfiPJ7_gi615442905	0.399	0.414	ID	0.855	0.676	0.712	0.689	0.558	0.48	0.474	0.469
4	colh|Colhi1_7256	0.392	0.406	0.855	ID	0.648	0.677	0.67	0.54	0.468	0.464	0.462
5	colg|Colgr1_7579	0.403	0.408	0.676	0.648	ID	0.887	0.845	0.587	0.452	0.437	0.435
6	cglo|ColglGC5_ELA29823	0.394	0.397	0.712	0.677	0.887	ID	0.911	0.598	0.459	0.449	0.449
7	colo|Color1_ENH81673	0.386	0.392	0.689	0.67	0.845	0.911	ID	0.593	0.445	0.432	0.43
8	mgri|MGG_00419	0.376	0.398	0.558	0.54	0.587	0.598	0.593	ID	0.4	0.382	0.387
9	ccan|CCAN2_1073.t1	0.403	0.405	0.48	0.468	0.452	0.459	0.445	0.4	ID	0.861	0.853
10	cbet|CBET3_00841-RA	0.395	0.399	0.474	0.464	0.437	0.449	0.432	0.382	0.861	ID	0.943
11	cber|CBER1_10482-RA	0.398	0.394	0.469	0.462	0.435	0.449	0.43	0.387	0.853	0.943	ID

	CTB9	1	2	3	4	5	6	7	8	9	10
1	claf|Clafu1_196871	ID	0.23	0.226	0.227	0.115	0.234	0.237	0.253	0.25	0.25
2	colo|Color1_ENH81668	0.23	ID	0.932	0.949	0.456	0.824	0.841	0.447	0.45	0.447
3	colg|Colgr1_7574	0.226	0.932	ID	0.946	0.437	0.807	0.836	0.452	0.457	0.455
4	cglo|ColglGC5_ELA29818	0.227	0.949	0.946	ID	0.447	0.827	0.852	0.453	0.459	0.456
5	mgri|MGG_00425	0.115	0.456	0.437	0.447	ID	0.452	0.447	0.307	0.318	0.318
6	colh|Colhi1_7261	0.234	0.824	0.807	0.827	0.452	ID	0.945	0.458	0.461	0.456
7	colf|ColfiPJ7_gi615442889	0.237	0.841	0.836	0.852	0.447	0.945	ID	0.461	0.464	0.458
8	ccan|CCAN2_1072.t1	0.253	0.447	0.452	0.453	0.307	0.458	0.461	ID	0.892	0.883
9	cbet|CBET3_00842-RA	0.25	0.45	0.457	0.459	0.318	0.461	0.464	0.892	ID	0.981
10	cber|CBER1_10481-RA	0.25	0.447	0.455	0.456	0.318	0.456	0.458	0.883	0.981	ID

	CTB10	1	2	3	4	5	6	7	8	9	10
1	colo|Color1_ENH81669	ID	0.622	0.681	0.373	0.838	0.794	0.378	0.446	0.462	0.462
2	colh|Colhi1_7260	0.622	ID	0.706	0.382	0.643	0.622	0.321	0.419	0.44	0.44
3	colf|ColfiPJ7_gi615442892	0.681	0.706	ID	0.413	0.735	0.705	0.326	0.477	0.484	0.484
4	claf|Clafu1_196878	0.373	0.382	0.413	ID	0.409	0.392	0.216	0.281	0.298	0.298
5	cglo|ColglGC5_ELA29819	0.838	0.643	0.735	0.409	ID	0.911	0.394	0.463	0.477	0.477
6	colg|Colgr1_7575	0.794	0.622	0.705	0.392	0.911	ID	0.374	0.463	0.477	0.477
7	mgri|MGG_11581	0.378	0.321	0.326	0.216	0.394	0.374	ID	0.239	0.247	0.247
8	ccan|CCAN2_1071.t1	0.446	0.419	0.477	0.281	0.463	0.463	0.239	ID	0.939	0.931
9	cbet|CBET3_00843-RA	0.462	0.44	0.484	0.298	0.477	0.477	0.247	0.939	ID	0.977
10	cber|CBER1_10480-RA	0.462	0.44	0.484	0.298	0.477	0.477	0.247	0.931	0.977	ID

	CTB11	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	ccan|CCAN2_10375.t1	ID	0.712	0.718	0.282	0.209	0.223	0.21	0.253	0.195	0.203	0.192	0.196	0.236	0.23
2	cber|CBER1_09861-RA	0.712	ID	0.871	0.274	0.224	0.226	0.195	0.236	0.195	0.196	0.172	0.189	0.226	0.221
3	cbet|CBET3_10915-RA	0.718	0.871	ID	0.274	0.224	0.226	0.198	0.228	0.193	0.196	0.185	0.176	0.224	0.213
4	stan|Stano2_2391	0.282	0.274	0.274	ID	0.295	0.269	0.248	0.283	0.244	0.256	0.244	0.255	0.305	0.302
5	colf|ColfiPJ7_gi615442882	0.209	0.224	0.224	0.295	ID	0.59	0.374	0.363	0.413	0.405	0.256	0.297	0.342	0.342
6	colh|Colhi1_7263	0.223	0.226	0.226	0.269	0.59	ID	0.362	0.347	0.4	0.38	0.222	0.258	0.311	0.323
7	colf|ColfiPJ7_gi615464524	0.21	0.195	0.198	0.248	0.374	0.362	ID	0.379	0.361	0.356	0.232	0.246	0.31	0.318
8	colg|Colgr1_7588	0.253	0.236	0.228	0.283	0.363	0.347	0.379	ID	0.612	0.617	0.224	0.281	0.272	0.276
9	cglo|ColglGC5_ELA29816	0.195	0.195	0.193	0.244	0.413	0.4	0.361	0.612	ID	0.757	0.22	0.274	0.268	0.277
10	colo|Color1_ENH81666	0.203	0.196	0.196	0.256	0.405	0.38	0.356	0.617	0.757	ID	0.235	0.284	0.274	0.271
11	claf|Clafu1_196869	0.192	0.172	0.185	0.244	0.256	0.222	0.232	0.224	0.22	0.235	ID	0.3	0.349	0.355
12	ccan|CCAN2_1070.t1	0.196	0.189	0.176	0.255	0.297	0.258	0.246	0.281	0.274	0.284	0.3	ID	0.666	0.666
13	cbet|CBET3_00844-RA	0.236	0.226	0.224	0.305	0.342	0.311	0.31	0.272	0.268	0.274	0.349	0.666	ID	0.914
14	cber|CBER1_10479-RA	0.23	0.221	0.213	0.302	0.342	0.323	0.318	0.276	0.277	0.271	0.355	0.666	0.914	ID

	CTB12	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	ccan|CCAN2_10376.t1	ID	0.81	0.788	0.463	0.439	0.448	0.438	0.448	0.447	0.224	0.441	0.426	0.434	0.432
2	cber|CBER1_09862-RA	0.81	ID	0.903	0.46	0.443	0.447	0.446	0.457	0.458	0.217	0.456	0.433	0.441	0.439
3	cbet|CBET3_10916-RA	0.788	0.903	ID	0.458	0.435	0.444	0.446	0.454	0.457	0.207	0.453	0.43	0.434	0.433
4	stan|Stano2_2392	0.463	0.46	0.458	ID	0.503	0.518	0.503	0.505	0.518	0.224	0.498	0.485	0.478	0.479
5	colh|Colhi1_9377	0.439	0.443	0.435	0.503	ID	0.831	0.703	0.704	0.707	0.311	0.5	0.503	0.513	0.514
6	colf|ColfiPJ7_gi615442879	0.448	0.447	0.444	0.518	0.831	ID	0.711	0.72	0.731	0.314	0.528	0.522	0.536	0.533
7	colo|Color1_ENH81665	0.438	0.446	0.446	0.503	0.703	0.711	ID	0.845	0.864	0.331	0.518	0.524	0.52	0.52
8	colg|Colgr1_7587	0.448	0.457	0.454	0.505	0.704	0.72	0.845	ID	0.897	0.32	0.522	0.518	0.517	0.518
9	cglo|ColglGC5_ELA29815	0.447	0.458	0.457	0.518	0.707	0.731	0.864	0.897	ID	0.335	0.528	0.523	0.525	0.523
10	mgri|MGG_00423	0.224	0.217	0.207	0.224	0.311	0.314	0.331	0.32	0.335	ID	0.235	0.234	0.236	0.23
11	claf|Clafu1_196870	0.441	0.456	0.453	0.498	0.5	0.528	0.518	0.522	0.528	0.235	ID	0.552	0.551	0.552
12	ccan|CCAN2_1069.t1	0.426	0.433	0.43	0.485	0.503	0.522	0.524	0.518	0.523	0.234	0.552	ID	0.886	0.896
13	cbet|CBET3_00845-RA	0.434	0.441	0.434	0.478	0.513	0.536	0.52	0.517	0.525	0.236	0.551	0.886	ID	0.948
14	cber|CBER1_10478-RA	0.432	0.439	0.433	0.479	0.514	0.533	0.52	0.518	0.523	0.23	0.552	0.896	0.948	ID

*The short species codes preceding the protein IDs are identical to those depicted in Table S3.

Table S5: Comparing gene tree-species phylogeny reconciliation analyses

	Reconciliation scores*			Confirmed Transfers SetA		Confirmed Transfers SetB		Confirmed Transfers SetC	
CTB Protein	SetA**	SetB**	SetC**	T1***	T2***	T1***	T2***	T1***	T2***
CTB1	44	48.5	54.5	1	1	1	0	1	0
CTB2	38.5	45.5	47	1	1	1	0	0	0
CTB3	34.5	44	46.5	1	1	1	0	0	0
CTB4	19	25	25	1	1	1	1	0	0
CTB5	26	29	29	1	1	0	0	0	0
CTB6	0	0	0	0	0	0	0	0	0
CTB7	11	11	11	0	0	0	0	0	0
CTB8	38.5	45	48.5	1	0	1	0	0	0
CFP	20.5	20.5	27	1	1	1	0	0	0
CTB9	22	30	32.5	1	1	1	0	0	0
CTB10	27	31	37	1	0	0	0	0	0
CTB11	28.5	28.5	35	1	0	1	0	0	0
CTB12	31.5	39.5	40	1	1	1	0	0	0

*Higher reconciliation scores are considered less parsimonious.

**SetA costs/weights: duplications: 1.5, transfers: 6.0, losses: 1.0 (ratio D:T:L : 1:4:0.67). SetB costs as following: duplications: 1.5, transfers: 9.0, losses: 1.0 (ratio D:T:L : 1:6:0.67). SetC costs: duplications: 1.5, transfers: 15.0, losses: 1.0 (ratio D:T:L : 1:10:0.67).

*** T1 refers to the horizontal transfer between *Cercospora*/*Cladosporium* ancestor and the Glomerellales. T2 refers to the horizontal transfer between the Glomerellales and *Magnaporthe oryzae*.

Table S6: Comparative topology test results for Dothideomycete- or Sordariomycete-constrained topologies

		Monophyletic Dothideomycetes	Monophyletic Sordariomycetes
CTB protein ID	***C. beticola* protein ID**	**AU* (p-value)**	**AU* (p-value)**
CTB6	CBET3_00830	3.00E-44	2.00E-09
CTB4	CBET3_00831	5.00E-37	2.00E-04
CTB2	CBET3_00832	3.00E-35	3.00E-68
CTB1	CBET3_00833	1.00E-08	4.00E-04
CTB3	CBET3_00834	4.00E-06	2.00E-05
CTB5	CBET3_00835	7.00E-45	3.00E-05
CTB7	CBET3_00836	1.00E-05	3.00E-80
CTB8	CBET3_00837	2.00E-06	7.00E-07
CFP	CBET3_00841	4.00E-50	1.00E-10
CTB9	CBET3_00842	9.00E-56	3.00E-06
CTB10	CBET3_00843	1.00E-34	6.00E-51
CTB11	CBET3_00844	0.001	2.00E-157
CTB12	CBET3_00845	3.00E-42	4.00E-04

*****The difference in likelihood scores between constrained and non-constrained trees was evaluated by the Approximately Unbiased (AU) test to determine if the best topology represents a significantly better explanation of the data compared to the constraint topology. In all cases, the null hypothesis that the best topology is not statistically significantly better than the constrained topology is rejected.

Table S7: *CTB* cluster and flanking gene description and relative transcript abundance under cercosporin-inducing growth conditions.

C. beticola Gene ID	Gene	Genomic Location	CDS length (bp)	Amino acids	Predicted function	Subcellular localization*	Interpro Domains	FC ratio (dark to light)
CBET3_00828		Chr1:2250591-2251627	672	223	Microsomal signal peptidase 25 kda subunit	TM (2)	IPR009582	1.3
CBET3_00829		Chr1:2251755-2252870	786	261	DUF850 domain-containing protein	TM (3)	IPR008568; IPR002809	1.0
CBET3_00830	*CTB6*	Chr1:2253404-2254513	1074	357	NADPH-dependent oxidoreductase	Inside	IPR001509; IPR016040	5.8
CBET3_00831	*CTB4*	Chr1:2255060-2256755	1539	512	Major Facilitator Superfamily (MFS) Transporter	TM (12)	IPR011701; IPR016196; IPR020846	12.7
CBET3_00832	*CTB2*	Chr1:2257252-2258693	1389	462	*O*-methyltransferase	Inside	IPR001077; IPR011991	NA
CBET3_00833	*CTB1*	Chr1:2259245-2266283	6615	2204	Polyketide synthase	Inside	IPR001031; IPR001227; IPR009081; IPR014030; IPR014031; IPR014043; IPR016035; IPR016038; IPR016039; IPR018201; IPR020801; IPR020802; IPR020806; IPR020841	47.8
CBET3_00834	*CTB3*	Chr1:2266958-2269866	2616	871	*O*-methyltransferase / FAD-dependent monooxygenase	Inside	IPR001077; IPR002938; IPR003042; IPR011991	13.0
CBET3_00835	*CTB5*	Chr1:2270302-2271941	1584	527	Oxygen, FAD/FMN-dependent oxidoreductase	Inside	IPR006094; IPR016166; IPR016167; IPR016169	177.5
CBET3_00836	*CTB7*	Chr1:2272445-2274194	1260	419	FAD/FMN-dependent oxidoreductase	Inside	IPR002938; IPR003042	9.0
CBET3_00837	*CTB8*	Chr1:2275576-2276820	1194	397	Zinc finger transcription factor	Inside	IPR001138; IPR002409; IPR013700	10.0
CBET3_00838	*ORF11*	Chr1:2277314-2278312	999	332	Truncated transcription factor	Inside	IPR001138; IPR002409	11.3
CBET3_00839	*ORF12*	Chr1:2281328-2282265	906	301	Truncated transcription factor	Inside		2.3
CBET3_00840		Chr1:2284800-2287322	2124	707	Phenylalanine ammonia-lyase	Inside	IPR001106; IPR005922; IPR008948; IPR022313; IPR024083	112.5
CBET3_00841	*CFP*	Chr1:2287911-2289886	1824	607	Major Facilitator Superfamily (MFS) transporter	TM (12)	IPR011701; IPR016196; IPR020846	83.8
CBET3_00842	*CTB9*	Chr1:2290261-2291287	975	324	alpha-ketoglutarate-dependent dioxygenase	Inside		1459.9
CBET3_00843	*CTB10*	Chr1:2291840-2292765	399	132	Dehydratase	Inside	IPR009799; IPR011008	16.2
CBET3_00844	*CTB11*	Chr1:2292823-2294000	1125	374	Beta-ig-h3 fasciclin	TM/Outside (1)	IPR000782	4.5
CBET3_00845	*CTB12*	Chr1:2294299-2296209	1911	636	Laccase / multi-copper oxidase	Outside	IPR001117; IPR002355; IPR008972; IPR011706; IPR011707	2.4
CBET3_00846		Chr1:2296295-2297194	900	299	U1 zinc finger domain-containing protein	Inside	IPR000690; IPR003604; IPR013085	0.9

CBET3_00847	Chr1:2297434-2300205	1437	478	Protein phosphatase pp2a	Inside	IPR000009; IPR001680; IPR015943; IPR017986; IPR018067; IPR019775	78.2
CBET3_00848	Chr1:2300653-2302047	567	188	DNA (cytosine-5)-methyltransferase 3b isoform 1	TM (1)		1.2

*Subcellular localization determined by computational prediction using the Phobius webserver (30). Transmembrane localized proteins are depicted by “TM”, intracellular proteins as “Inside”, extracellular proteins as “Outside” and membrane bound, extracellular proteins, i.e. CTB11, are depicted as TM/Outside. The number of TMs is indicated between brackets.

Table S8: CTB orthologs found in annotated *Colletotrichum* genomes that are available at Ensemble Fungi[#,$]

CTB C. higginsianum	CTB ref	*Ensemble Fungi Ref.*	Cochl	Cofio	Coglo	Cogra	Cohig	Coinc	Conym	Coorb	Coorc	Cosal	Cosim	Cosub	Cotof	Gene trees (Ensemble Fungi)
CH063_00308	CFP	CCF39163	0	1	1	1	1	0	1	1	0	1	1	0	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_00308
CH063_00309	-	CCF39164	0	1	1	1	1	1	1	1	0	1	1	0	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_00309
CH063_00310	-	CCF39165	0	1	1	1	1	1	1	1	1	1	1	0	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_00310
CH063_00311	-	CCF39166	0	1	1	1	1	1	1	1	0	1	1	0	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_00311
CH063_00312	CTB10	CCF39167	0	1	1	1	1	0	1	1	0	1	1	0	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_00312
CH063_00313	CTB9	CCF39168	0	1	1	1	1	1	1	1	0	1	1	0	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_00313
CH063_00314	CTB4	CCF39169	0	1	1	0	1	0	1	1	0	1	1	0	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_00314
CH063_00315	CTB11	CCF39170	0	1	1	1	1	0	1	1	0	1	1	0	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_00315
CH063_02500	CTB12	CCF41082	0	1	1	1	1	0	1	1	0	1	1	0	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_02500
CH063_02502	-	CCF41083	0	1	1	1	1	0	1	1	0	1	1	0	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_02502
CH063_02504	CTB5	CCF41084	0	1	1	1	1	0	1	1	0	1	1	0	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_02504
CH063_02506	CTB1	CCF41085	3*	3*	2*	5*	3*	2*	2*	4*	2	3*	2*	3	1	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_02506
CH063_11016	CTB3	CCF40455	1	1	1	1	1	1	1	1	1	1	1	0	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_11016
CH063_07427	CTB2	CCF35700	1	1	1	1	1	1	1	1	0	1	1	1	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_07427
CH063_07428	CTB8	CCF35701	0	1	1	1	1	1	1	1	0	1	1	1	0	http://fungi.ensembl.org/Colletotrichum_higginsianum/Gene/Compara_Tree?g=CH063_07428

[#] Species abbreviations are: Cochl (*Co. chlorophyti*), Cofio (*Co. fioriniae*), Coglo (*Co. gloeosporioides*), Cogra (*Co. graminicola*), Cohig (*Co. higginsianum*), Coinc (*Co. incanum*), Conym (*Co. nymphaeae*), Coorb (*Co. orbiculare*), Coorc (*Co. orchidophilum*), Cosal (*Co. salicis*), Cosim (*Co. simmondsii*), Cosub (*Co. sublineola*) and Cotof (*Co. tofieldiae*).

[$] CH063_00309, CH063_00310, CH063_00311 and CH063_02502 are not found in the *Cercospora CTB* cluster.

*CTB1 ortholog confirmed by phylogenetic analysis (*SI Appendix*, Fig. S10)

Table S9: Polyketide synthase (PKS) accession codes used in this study

Protein name	*Species*	ProteinId*	Study Id	Alternative Id
AltbrDep5	*Alternaria brassicicola*	ACZ57548.1		
AltsoPksN	*Alternaria solani*	BAD83684.1		
Aspcla023380	*Aspergillus clavatus*	XP_001269050.1		
AspclaCssA	*Aspergillus clavatus*	XP_001270543.1		
AspflCpaA	*Aspergillus flavus*	BAI43678.1		
AspflPksA	*Aspergillus flavus*	AAS90093.1		
AspfuAlb1	*Aspergillus fumigatus*	AAC39471.1		
AspfuEncA	*Aspergillus fumigatus*	XP_746435.1		
AspfuPsoA	*Aspergillus fumigatus*	ABS87601.1		
AspniStcA	*Aspergillus nidulans*	Q12397.1**	ASPNI_V1_3885T0	
AspniYwa1	*Aspergillus nidulans*	CAA46695.2	ASPNI_V1_1846T0	
AspniAlbA	*Aspergillus niger*	EHA28527.1		
AsporCpaA	*Aspergillus oryzae*	BAK26562.1		
AsppaPksL1	*Aspergillus parasiticus*	Q12053.1**		
Aspte00325	*Aspergillus terreus*	EAU38971.1		
AspteAcas	*Aspergillus terreus*	XP_001217072.1		
AspteLDKS	*Aspergillus terreus*	AAD34559.1		
AspteLNKS	*Aspergillus terreus*	Q9Y8A5.1**		
AspweAomsas	*Aspergillus westerdijkiae*	AAS98200.1		
BeabaTenS	*Beauveria bassiana*	CAL69597.1		
BotciBcBOA6	*Botrytis cinerea*	CAP58786.1		
BotciBcBOA9	*Botrytis cinerea*	CBX87032.1		
Bysni6Msas	*Byssochlamys nivea*	AAK48943.1		
CnCTB1	*Cercospora nicotianae*	AAT69682.1		CerniCtb1
Chagl15110	*Chaetomium globosum*	XP_001221381.1		
HPS1	*Cladosporium fulvum*	192259***	Clafu1_192259	
PKS1	*Cladosporium fulvum*	191425***	Clafu1_191425	
PKS2	*Cladosporium fulvum*	186350***	Clafu1_186350	
PKS3	*Cladosporium fulvum*	188474***	Clafu1_188474	
PKS4	*Cladosporium fulvum*	188483***	Clafu1_188483	

PKS5	*Cladosporium fulvum*	184292***	Clafu1_184292	
PKS6	*Cladosporium fulvum*	184395***	Clafu1_184395	
PKS7	*Cladosporium fulvum*	196875***	Clafu1_196875	
PKS8	*Cladosporium fulvum*	196070***	Clafu1_196070	
PKS9	*Cladosporium fulvum*	188153***	Clafu1_188153	
PKSA	*Cladosporium fulvum*	194256***	Clafu1_194256	
Cocim06629	*Coccidioides immitis*	XP_001242733.1		
CochePks1	*Cochliobolus heterostrophus*	AAB08104.3		
CochePks2	*Cochliobolus heterostrophus*	ABB76806.1		
ColfiPJ7_gi615442870	*Colletotrichum fioriniae*	XP_007592254		
ColglGC5_ELA29812	*Colletotrichum gloeosporioides*	L2FTZ2		CGGC5_9796
Colgr1_7584	*Colletotrichum graminicola*	7584***		GLRG_08620
Colhi1_10470****	*Colletotrichum higginsianum*	10470***		
Colhi1_CH063_02506****	*Colletotrichum higginsianum*	H1VLI2**		CH063_02506
CollaPks1	*Colletotrichum lagenaria*	BAA18956.1		
Color1_ENH81662	*Colletotrichum orbiculare*	N4VD65**		Cob_09306
Color1_ENH84744	*Colletotrichum orbiculare*	N4VBL7**		Cob_07065
DotsePksA	*Dothistroma septosporum*	EME39092.1	DOTSE_V1_48387T0	
ElsfaPks1	*Elsinoë fawcettii*	ABU63483.1	EfPKS1	
FusheEqiS	*Fusarium heterosporum*	AGO86662.1		
Fusox9586	*Fusarium oxysporum*	EGU88865.1		
FusoxFum1	*Fusarium oxysporum*	ACB12550.1		
GibfuBik1	*Gibberella fujikuroi*	CAB92399.1		
GibmoFusS	*Gibberella moniliformis*	AAT28740.1		
GibmoPks1	*Gibberella moniliformis*	AAR92208.1		
GibzeFsl1	*Gibberella zeae*	XP_390640.1		
GibzePks12	*Gibberella zeae*	AAU10633.1		
GibzePks13	*Gibberella zeae*	ABB90282.1		
GibzePks4	*Gibberella zeae*	ABB90283.1		
GlaloPks1	*Glarea lozoyensis*	AAN59953.1		
HypsuHpm3	*Hypomyces subiculosus*	ACD39762.1		
HypsuHpm8	*Hypomyces subiculosus*	ACD39767.1		

MagorAce1	*Magnaporthe oryzae*	CAG28797.1		
MagorAlb1	*Magnaporthe oryzae*	XP_003715434.1		
MagorSyn2	*Magnaporthe oryzae*	CAG28798.1		
MagorSyn6	*Magnaporthe oryzae*	CAG29113.1		
MGG_00428****	*Magnaporthe oryzae*	G4NCB1**		
MetroNgs1	*Metarhizium robertsii*	ACS68554.1		
MonpiMkA	*Monascus pilosus*	ABA02239.1		
MonpiMkB	*Monascus pilosus*	ABA02240.1		
MonpuPksCT	*Monascus purpureus*	BAD44749.1		
NechaPKSN	*Nectria haematococca*	AAS48892.1		
NodspPks1	*Nodulisporium sp. ATCC74245*	AAD38786.1		
PenbrMpaC	*Penicillium brevicompactum*	ADY00130.1		
PenciMlcA	*Penicillium citrinum*	BAC20564.1		
PenciMlcB	*Penicillium citrinum*	BAC20566.1		
PenexCheA	*Penicillium expansum*	CAO91861.1		
PengrMsas	*Penicillium griseofulvum*	P22367.1**		
PodanPks1	*Podospora anserina*	XP_001910795.1		
SS1G_05681.1	*Sclerotinia sclerotiorum*	A7EK35**		
SormaPks	*Sordaria macrospora*	CAM35471.1		
Stano2_2390.2****	*Stagonospora nodorum*	EAT83782.2	Stano2_2390.2	SNOG08614
Uncre03815	*Uncinocarpus reesii*	EEP78969.1		
WandePks1	*Wangiella dermatidis*	AAD31436.3		

*NCBI protein database Id

**Uniprot protein database Id

***Protein ID from the Doe Joint Genome-Institute resource (http://www.jgi.doe.gov)

****Updated gene model (this study)

Table S10: Primers used in this study[1]

Primer	Specificity/role	Sequence (5’ to 3’)
MDB-1267	CBET3_00840 qPCR F	GCCAATGCATCACGGCTTAG
MDB-1268	CBET3_00840 qPCR R	CCAAGCTCATGCATCGCTTC
MDB-1269	CBET3_00841 qPCR F	CTGGGATCCCTGGCATGTTT
MDB-1270	CBET3_00841 qPCR R	GACAGCGAAGACCACAAGGA
MDB-1271	CBET3_00842 qPCR F	TCTGCGATGCTCGATCAGTC
MDB-1272	CBET3_00842 qPCR R	GGAGTCTTGGGAGGAGCAAC
MDB-1273	CBET3_00843 qPCR F	GTCACCAAGAAGCCAGACCA
MDB-1274	CBET3_00843 qPCR R	ACATTCTCTTCTGGCTGGCC
MDB-1275	CBET3_00844 qPCR F	TGGTGGATCGAGTCGAGTCT
MDB-1276	CBET3_00844 qPCR R	CCGTGACTGGTAAGCCTTGT
MDB-1277	CBET3_00845 qPCR F	AGAGAAGGCCAGACACGTTG
MDB-1278	CBET3_00845 qPCR R	ACAAACGACACCATCTCGCT
MDB-1279	CBET3_00846 qPCR F	GAAGAAGGAGGTCACGGGTG
MDB-1280	CBET3_00846 qPCR R	CTTCACTCCGGCCTTGTCAT
MDB-1281	CBET3_00847 qPCR F	ACGACGGCCGATACATTCTC
MDB-1282	CBET3_00847 qPCR R	TGACATTCTTGGCGTCTCCC
MDB-1283	CBET3_00848 qPCR F	AAAGCCCTGTTACCAGTCCG
MDB-1284	CBET3_00848 qPCR R	TACCGTCTCTCATCCTGCCA
MDB-277	Split-marker M13F (HYG-F)	GACGTTGTAAAACGACGGCCAGTG
MDB-258	Split-marker: HY (NLC37) (HY-R)	GGATGCCTCCGCTCGAAGTA
MDB-259	Split-marker: YG (NLC38) (YG-F)	CGTTGCAAGACCTGCCTGAA
MDB-278	Split-marker M13R (HYG-R)	CACAGGAAACAGCTATGACCATGA
MDB-1145	HY-R2 (split marker)	GGCAGGTAGATGACGACCAT
MDB-1293	CBET3_00841-00845 1F	ACGCAGACCCAATAGATCGA
MDB-1294	CBET3_00841-00845 2R	CACTGGCCGTCGTTTTACAACGTCTTGCCATTGTCAGTGTGTGG
MDB-1295	CBET3_00841-00845 3F	TCATGGTCATAGCTGTTTCCTGTGGAGATGGTGTCGTTTGTGCC
MDB-1296	CBET3_00841-00845 4R	CACCGGCATATTCAGCCTTG
MDB-1412	CBET3_00841-00845 5F	CGTTCCTGGAATCACCAGAT

MDB-1454	CBET3_00840 1F	TCGCATCCACAATCCGTTTC
MDB-1455	CBET3_00840 2R	CACTGGCCGTCGTTTTACAACGTCTGGGTCACTGCGAGTCATAA
MDB-1456	CBET3_00840 3F	TCATGGTCATAGCTGTTTCCTGTGACGCAGACCCAATAGATCGA
MDB-1457	CBET3_00840 4R	TGAACAACGATCTCCCTGCT
MDB-1458	CBET3_00840 5F	ATTTGAGAAACGCGAGCCTG
MDB-1459	CBET3_00841 1F	TGCGCTCATTGGATAGGAGT
MDB-1460	CBET3_00841 2R	CACTGGCCGTCGTTTTACAACGTCGGCCAGCCGGAGATATACTT
MDB-1461	CBET3_00841 3F	TCATGGTCATAGCTGTTTCCTGTGATTGGTATCGGCGTCAGTCT
MDB-1462	CBET3_00841 4R	ACATCTCACACTGCTTTGCC
MDB-1463	CBET3_00841 5F	GAGGACAGATGTTGCGCTTC
MDB-1464	CBET3_00842 1F	GTACACGACCCCTACTCAGC
MDB-1465	CBET3_00842 2R	CACTGGCCGTCGTTTTACAACGTCAGAATGCCCCAGACATGACA
MDB-1466	CBET3_00842 3F	TCATGGTCATAGCTGTTTCCTGTGCATCTCAGCAACTTCGCCAA
MDB-1467	CBET3_00842 4R	GTTCTCCCATGCAAGTCAGG
MDB-1468	CBET3_00842 5F	TGGAGCTGTCGATGATCTCC
MDB-1469	CBET3_00843 1F	TTTACGAGCTAGCCTTCGGT
MDB-1470	CBET3_00843 2R	CACTGGCCGTCGTTTTACAACGTCCAGAAGCACAACGATGCTCA
MDB-1471	CBET3_00843 3F	TCATGGTCATAGCTGTTTCCTGTGATGGGAGCATTGACCTTGGA
MDB-1472	CBET3_00843 4R	GCGCCGGTATCCTGTTAGAT
MDB-1473	CBET3_00843 5F	CCTGACTTGCATGGGAGAAC
MDB-1289	CBET3_00844 1F	ATACCGGCGCTTCGATTCT
MDB-1290	CBET3_00844 2R	CACTGGCCGTCGTTTTACAACGTCCGTGGAAAGAGAGGCGAGAT
MDB-1291	CBET3_00844 3F	TCATGGTCATAGCTGTTTCCTGTGGATACAGCACTCGATTCGGC
MDB-1292	CBET3_00844 4R	CATCAACCTTACGACCGCTG
MDB-1385	CBET3_00844 6R	AGTCTCTTTGATGGCGTGGA
MDB-1474	CBET3_00845 1F	TACTGCTCTTGGTCTCAGGC
MDB-1475	CBET3_00845 2R	CACTGGCCGTCGTTTTACAACGTCCCTCGTCATCTGGTGCAATG
MDB-1476	CBET3_00845 3F	TCATGGTCATAGCTGTTTCCTGTGGAGATGGTGTCGTTTGTGCC
MDB-1477	CBET3_00845 4R	CACCGGCATATTCAGCCTTG
MDB-1478	CBET3_00845 5F	AGTTGCCTTTGAAGTCAGCG
MDB-1479	CBET3_00846 1F	CTTAAAGACCACTGCAGGCG

MDB-1480	CBET3_00846 2R	CACTGGCCGTCGTTTTACAACGTCTTGCGGAGATTCTGGCAGA
MDB-1481	CBET3_00846 3F	TCATGGTCATAGCTGTTTCCTGTGGCTCGTATCAACCTCATGCG
MDB-1482	CBET3_00846 4R	AACTTTCCTTGAGCTTGCGG
MDB-1483	CBET3_00846 5F	CGAGTGTAGCTTGAGACTGC
MDB-1484	CBET3_841 complementation F	*TTAATTAA*ATGGCAAGCCCAGCGCGATC
MDB-1494	CBET3_841 complementation R	*GCGGCCGC*TCACACTGCTTTGCCCGCGAT
MDB-1450	CBET3_842 complementation F	*TTAATTAA*ATGACAAGTACTATCACGAC
MDB-1451	CBET3_842 complementation R	*GCGGCCGC*CTACTCAGCCTCCTGATCTT
MDB-1486	CBET3_843 complementation F	*TTAATTAA*ATGGGATCCATCGGAGAGCCAA
MDB-1487	CBET3_843 complementation R	*GCGGCCGC*CTAGAAAGTGAACTCCTCAAT
MDB-1448	CBET3_844 complementation F	*TTAATTAA*ATGCATTTTCCAGCGCTCGC
MDB-1449	CBET3_844 complementation R	*GCGGCCGC*CTACACCTTCTCATAATTGC
MDB-1488	CBET3_845 complementation F	*TTAATTAA*ATGTGGTCTGTTCGACTCTAC
MDB-1489	CBET3_845 complementation R	*GCGGCCGC*TCACGACAAAGCAGGCGGCT
MDB-1497	CBET3_841 intern 1 F	GTGCTTCTCATTCTGGGCAG
MDB-1498	CBET3_841 intern 2 F	TACGATCTGACAGTGCCGAA
MDB-1499	CBET3_841 intern 3 F	ATCGGGCAAAGTGTACTCCA
MDB-1500	CBET3_844 intern 1 F	GTCGTGGAAGCAGACATTCC
MDB-1501	CBET3_845 intern 1 F	CATTGCACCAGATGACGAGG
MDB-1502	CBET3_845 intern 2 F	TGTAGATGGGCAGTACGTGT
MDB-1503	CBET3_845 intern 3 F	CGTAACAGCAGGTCCATTCG
MDB-1849	Co. fioriniae TEF F	AGAAGTTCGAGAAGGAGGCT
MDB-1850	Co. fioriniae TEF R	ACGGTGACATAGTACTTGGGA
MDB-1845	Co. fioriniae CTB1 F	CCATTCGCAAAGACTCCCAG
MDB-1846	Co. fioriniae CTB1 R	GTTCTTGAGTTACGGCGTAGG

1 F indicates forward primers, R indicates reverse primers. Overlapping sequences in splitmarker primers for targeted gene replacement are underlined. Forward and reverse primers with a PacI and NotI restriction enzyme site respectively for generating over expression constructs are indicated in italics.

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