

**SUPPLEMENTARY FILE S2**

(Sayari et al - *Ceratocystidaceae* Nonribosomal peptide synthetase gene clusters)

To confirm the order of genes within the different NRPS clusters identified, a PCR-based approach was used. For each cluster type, primers were designed that allow amplification of individual genes, as well as the regions between them. Correlation between predicted and observed fragment sizes were used as evidence that the specific cluster was correctly assembled.

For these PCRs, DNA was extracted from the five representative isolates: *Ceratocystis manginecans* (CMW17570), *Thielaviopsis musarum* (CMW1546), *Endoconidiophora polonica* (CMW20930), *Huntia bhutanensis* (CMW8217) *Davidsoniella virescens* (CMW17339) and *Bretziella fagacearum* (CMW2656). This was done using 14-day-old fungal cultures grown at room temperature on malt-extract-Agar (MEA; Merck) medium and the DNeasy Plant Mini Kit (Qiagen, Carlsbad, CA, USA).

The table below lists the primer sequences and expected amplicon sizes for all of the PCRs conducted in this study. Each PCR mixture contained 2.5Mmol MgCl<sub>2</sub>, 150μM of each dNTP, 0.1μmol of each primer, 1U of *Taq* polymerase and 10X reaction buffer (Roche Applied Science, Mannheim, Germany) in a total volume of 25μl. Amplification was carried out with the GeneAmp PCR system 9700 (Applied biosystems) with initial denaturation 94°C for 4min, followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec, 72°C for 1 min, and a final extension step at 72°C for 15 min. The sizes of the resulting PCR products were then estimated by making use of 1% (w/v) agarose (whiteheads scientific, South Africa) gel electrophoresis, GelRed™ (Biotium, Inc., Fremont, California) nucleic acid staining and an UV-transilluminator.

Table 1. Sequences, annealing temperatures and GC content for the primers used in this study.

**A. Primers for individual genes in the *Ceratocystis* monomodular group**

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)
Hypothetical (F)	CAACAACAGAAGGAATTCG	55	40
Hypothetical (R)	GGCCTAGTACTTTTTGAATG	54	40
Hypothetical-NRPS (F)	GAAACCCCTTTTTGCTCT	56	40
Hypothetical-NRPS (R)	GGATGGAGTTTGAGGTGTTG	57	50
NRPS (F)	GCTTTCAACACCTCAAACCTC	56	45
NRPS (R)	TAAACTACGATGCCTCTGTC	55	45
NRPS-Acyl CoA (F)	GAGGCATCGTAGTTTATGTA	54	40
NRPS-Acyl CoA (R)	CCGTACAAAACCTGATAGCTT	55	40
Acyl CoA (F)	ACTCAGACACGCATTGATAC	57	45
Acyl CoA (R)	GGTTCCTTTGACCTGTTTGT	57	45
Acyl CoA-Siderophore (F)	ACAAACAGGTCAAAGGAACC	57	45
Acyl CoA-Siderophore (R)	TATGCAGAGAGGTACGGATG	57	50
Siderophore (F)	CAACTCTCGCTCTCAACTG	57	53
Siderophore (R)	CCAATACGCATTCCACCCA	57	53
Siderophore-Hypothetical (F)	GAGAGAACGAGAAGGTTGTG	57	50
Siderophore-Hypothetical (R)	GACTTGGTGGTAGGGTAACT	57	50
Hypothetical (F)	TCTTAGTTACCCTACCACCA	55	45
Hypothetical (R)	CTTTGATTCCGCCTGCTA	54	50

**B. Primers for individual genes and inter-regions in the *Ceratocystis* multimodular group**

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)
Hypothetical (F)	TAAGTGTCTCTCGGGTTTCC	58	50
Hypothetical (R)	AATTCGAGAGATTGGGGAAG	57	45
Hypothetical-NRPS (F)	TATGCTTCTTTGACCGTACT	55	40
Hypothetical-NRPS (R)	AGGAACATGGAAGAAATGGT	57	40
NRPS (F)	ATTTATCTTATCCCGCACAC	55	40
NRPS (R)	CTTCAACCAGCAACCGA	58	56
NRPS-Oxygenase (F)	GGGAAAAGGCAGTGAGAGAA	58	50
NRPS-Oxygenase (R)	CCGTCCGGAAGCACTATCA	57	56
Oxygenase (F)	TCAGCTCGCCTGTCACTTA	56	53
Oxygenase (R)	CATCTACAATCTCGCCCGA	57	53
Oxygenase-Aspergillopepsin (F)	GGGCGAGATTGTAGATGCT	58	53
Oxygenase-Aspergillopepsin (R)	TGGCGTAGTCGTGTATCTG	57	53
Aspergillopepsin (F)	ACGACTACGCCATGAATATA	55	40
Aspergillopepsin (R)	GTTTGATTATAGCTACGGCT	54	40

**C. Primers for individual genes in the *Bretziella* monomodular group**

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)
NRPS (F)	GATGACAACCTACGGGAGCA	56	53
NRPS (R)	GTCTCCATCTCCACCATCC	57	58
NRPS-Acyl CoA (F)	GTCTCCATCTCCACCATCC	57	47
NRPS-Acyl CoA (R)	TCTTTGGACTTTGGTGGC	56	47
Acyl CoA (F)	CCAAAGTCCAAAAGATGTCC	56	45

Acyl CoA (R)	ACCCATTACGAATCTCATC	57	45
Acyl CoA-Siderophore (F)	GAAGATGATGCCTAGTGCTA	55	45
Acyl CoA-Siderophore (R)	TCCCGCGATCTAACCTTT	56	50
Siderophore (F)	CAAAGGTTAGATCGCGGG	57	56
Siderophore (R)	ACCGCAATACTTCCACA	58	50

#### D. Primers for individual genes in the *Bretziella* multimodular group

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)
NRPS (F)	ACCCTAACCTAACCTAAC	57	50
NRPS (R)	TTTCCAGAAAAACGCCAAAC	57	40
NRPS-Oxygenase (F)	CTGTTGGCGTTTTCTGGA	58	45
NRPS-Oxygenase (R)	GAAAGCAATGGGAGAGTTGA	58	45
Oxygenase (F)	GATATCAACTCTCCATTGC	55	45
Oxygenase (R)	CACTAGAACGAATAGCAAGA	55	40
Oxygenase-Glucanase D (F)	CCTCCTTCTGTTCTTGCTA	56	45
Oxygenase-Glucanase D (R)	TACGGCTACTCCCAAGAC	56	56
Glucanase D (F)	GTCTTGGGAGTAGCCGTA	55	56
Glucanase D (R)	TACTGCTACCCTCATGGC	56	56
NRPS (F)	ACCCTAACCTAACCTAAC	57	50
NRPS (R)	TTTCCAGAAAAACGCCAAAC	57	40
NRPS-Oxygenase (F)	CTGTTGGCGTTTTCTGGA	58	45
NRPS-Oxygenase (R)	GAAAGCAATGGGAGAGTTGA	58	45

#### E. Primers for individual genes in the *Huntia* monomodular group

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)
NRPS (F)	CTAAGCAATTCTCGCACG	58	50
NRPS (R)	GACTCGTCTGCCTTTTC	57	56
NRPS-Acyl CoA (F)	GCGAAGCTGAACCATTTT	55	44
NRPS-Acyl CoA (R)	GAAGAATGGAGAGAGCCG	57	56
Acyl CoA (F)	ATCGATACTCCAAACCCC	55	50
Acyl CoA (R)	AAATCACAACCCCAGCA	56	50
Acyl CoA-Siderophore (F)	GCAAGCGAACAAATATCG	55	44
Acyl CoA-Siderophore (R)	GGAAAGAGTGGTAGCTGG	55	56
Siderophore (F)	CCCAGCTACCCTTTTC	56	56
Siderophore (R)	TACAGCTTAGCCACCAGA	54	50
Siderophore-Hypothetical (F)	TCTGGTGGCTAAGCTGTA	55	50
Siderophore-Hypothetical (R)	AAGGAGGCTGAGAAAAG	55	44
Hypothetical (F)	CTACTTTTCTCAGCCTC	53	44
Hypothetical (R)	TCTTGATTTGACGAGTG	53	44

#### F. Primers for individual genes in the *Huntia* multimodular group

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)
NRPS (F)	CACCAATCACCAATTTCC	55	44
NRPS (R)	AACAAGAAAGCAACGTGC	57	44
NRPS-Oxygenase (F)	ATTGTGAAAGCCTAGTGC	55	44
NRPS-Oxygenase (R)	GAATGAGTGGGTAGTGGG	56	56
Oxygenase (F)	TCACCTTGACCTTGACAT	55	44
Oxygenase (R)	TAGTCTTGAAGACGGCA	54	44
Oxygenase-Hypothetical (F)	GACACTTGCTTTCCGTT	56	50
Oxygenase-Hypothetical (R)	TGTTGTCTTTGACGGTGG	58	50
Hypothetical (F)	GGAAGCCCAACCAATAGT	57	50
Hypothetical (R)	TAACCTCCTTCTCTGGC	57	56
Hypothetical-RNA Pol subunit 7 (F)	AGGGAGTTAAGAGAAGGG	54	50
Hypothetical-RNA Pol subunit 7 (R)	GGAAGTTGACCAGCAAGA	54	50
RNA Pol subunit 7 (F)	CAAGACCCCAACCAAGGA	57	56
RNA Pol subunit 7 (R)	CCACTGGCAAGCTTCAAA	56	50

#### G. Primers for individual genes in the *Davidsoniella* monomodular group

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)
NRPS (F)	CTTGAACCTCCGCCAACTC	57	56
NRPS (R)	CGATCTCGGTTGCGTCTA	56	56
NRPS-Acyl CoA (F)	CCATGGTGAAAGATTGCT	54	44
NRPS-Acyl CoA (R)	TAGACATTTTCTGGCGCT	55	44

Acyl CoA (F)	TCAGCGCCAGAAAATGTC	57	50
Acyl CoA (R)	GTAGGCGTGTCTTCGTA	56	50
Acyl CoA-Siderophore (F)	GCTACCCCTTCTATGTCTTC	56	50
Acyl CoA-Siderophore (R)	GTATGGGTGAGGAAGCACAA	57	50
Siderophore (F)	TCCCGCTCAGCAACTATC	57	56
Siderophore (R)	TGCGCATACCACCTATAACC	58	50
Siderophore-Hypothetical (F)	TGTAAGGTAGTTAGGGCGTG	57	50
Siderophore-Hypothetical (R)	TGCGAAAAAGGAGGACTCTA	57	45
Hypothetical (F)	TGCGAAAAAGGAGGACTCTA	55	45
Hypothetical (R)	CTTTGATTCCGCCTGCTA	54	50

#### H. Primers for individual genes in the *Davidsoniella* multimodular group

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)
Hypothetical (F)	TCTGATCACCCCATACCA	54	50
Hypothetical (R)	CTTTCAGCCACCCATTA	54	50
Hypothetical-NRPS (F)	TAGTAATGGGGTGGCTGAAA	55	45
Hypothetical-NRPS (R)	GAAAAATTGTTGCTGCCCTC	57	45
NRPS (F)	TCTTCTGCCCAATCCTCATC	57	50
NRPS (R)	TCTTCTACCAGACACCAAAA	56	45
NRPS-Oxygenase (F)	TTTGGTGTCTGGTAGGAAGA	54	45
NRPS-Oxygenase (R)	GTC AATATGGGGAGACAT	52	42
Oxygenase (F)	ATGTCTCCCCATATTGACG	57	47
Oxygenase (R)	ACTGCATCAACAATCTCAC	56	42
Oxygenase-Hypothetical (F)	GTGAGATTGTTGATGCAGTT	56	40
Oxygenase-Hypothetical (R)	GTCTGCCTTTGTCGCTTC	57	53
Hypothetical (F)	TCGAAGACGACAAAGGCAGA	57	50
Hypothetical (R)	CTCTTTTGTGCAGCTCTG	58	45

#### I. Primers for individual genes in the *Thielaviopsis* monomodular group

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)
Hypothetical (F)	CGGGCACAGGAAACAATA	57	50
Hypothetical (R)	ACAACCTCTTCAATCAGC	55	44
Hypothetical-NRPS (F)	GAAGCTGATTGAAGAGGT	55	44
Hypothetical-NRPS (R)	GCTGGAAATGATGCGGAA	55	50
NRPS (F)	TGCCTTCAGCTCTGCCAA	58	56
NRPS (R)	ACTCAAAAATGGTCCAGC	55	44
NRPS-Acyl CoA (F)	GCTGGACCATTTTTGAGTAA	55	44
NRPS-Acyl CoA (R)	TCGATGCGGGTCTGAGTA	57	56
Acyl CoA (F)	ATGCTTTCCATTCTCCAC	55	44
Acyl CoA (R)	TACAGCTTCGATTGGGC	57	50
Acyl CoA-Siderophore (F)	GCCCAAATCGAAGCTGTA	56	50
Acyl CoA-Siderophore (R)	GCGAATGGTGTGTTGACAT	56	44
Siderophore (F)	ATGTCAAACACCATTTCGC	56	44
Siderophore (R)	TTACTCGCCATAAGCCTT	55	44
Siderophore-Aerobactin (F)	CGCAGTGCAAAAAGAGCTA	56	50
Siderophore-Aerobactin (R)	CAGGCACGTCAAGAGAGA	55	56
Aerobactin (F)	GTCTCTTTGACGTGCCT	57	56
Aerobactin (R)	CATGCCTCCAACAACCTT	57	50

#### J. Primers for individual genes in the *Thielaviopsis* multimodular group

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)
NRPS (F)	TTAAACCCACTAACACGG	53	44
NRPS (R)	GGTCCCAATCTTTTACAC	52	44
NRPS-Oxygenase (F)	GTTGAGAATGGCCAGAGC	56	56
NRPS-Oxygenase (R)	GCAGGGAGAACATCGTCA	58	56
Oxygenase (F)	GATCATCTGGACCTCGAC	55	56
Oxygenase (R)	GAGCGGATGGAAGAACC	57	56
Oxygenase-Endothiaepsin (F)	CACTCTCTGTGCGTTCT	58	56
Oxygenase-Endothiaepsin (R)	CAACGAAGCCTCTGACAG	58	56
Endothiaepsin (F)	CAACGAAGCCTCTGACAG	57	44
Endothiaepsin (R)	TTCTTATGGCGCTCTT	57	50
Endothiaepsin-Hypothetical (F)	GTTGACAGAGGGAGACAT	55	50
Endothiaepsin-Hypothetical (R)	TGTTGCTGGCGTAGAAGA	56	50
Hypothetical (F)	CTCTTCTACGCCAGCAAC	57	56
Hypothetical (R)	TTGACTCCTCCTCCCTT	58	56

K. Primers for individual genes in the *Endoconidiophora* monomodular group

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)
Hypothetical (F)	AAATAGTGGTGTATCGGG	54	44
Hypothetical (R)	CTTCTGGATGCCTTCT	55	50
Hypothetical-NRPS (F)	GAAGAGGCATCCAGAAGG	57	56
Hypothetical-NRPS (R)	GATTTGATACGCCGCGAG	58	56
NRPS (F)	ATGCGCACCTTAAACTCT	57	44
NRPS (R)	GCAACTTTGGGGGTTCTG	57	56
NRPS-Acyl CoA (F)	CAGAACCCCAAAGTTGC	57	56
NRPS-Acyl CoA (R)	AGACATTTTCTGGCGCTG	58	50
Acyl CoA (F)	CAGCGCCAGAAAATGTCT	57	50
Acyl CoA (R)	TTAACCAAGTTCGTTGCC	58	56
Acyl CoA-Siderophore (F)	GGTAAAGAAGACCACGCT	57	50
Acyl CoA-Siderophore (R)	GGCAGGAGAAGCCATTAT	56	50
Siderophore (F)	TAATGGCTTCTCTGCC	57	56
Siderophore (R)	ACCTTTTCATTGTGCCT	57	44

L. Primers for individual genes in the *Endoconidiophora* multimodular group

Primer	Primer sequence (5'→3')	Annealing temperature (°C)	GC content (%)
RNA Pol II subunit 7 (F)	GGATGACTCAGGGCAGAA	57	56
RNA Pol II subunit 7 (R)	TCAAGGTCACGTTTTTCCAA	56	40
RNA Pol II subunit 7-Endothiaepsin (F)	CGAGACGCACTATTGGAA	56	50
RNA Pol II subunit 7-Endothiaepsin (R)	ACGAGAAGGAGGTTTATG	54	44
Endothiaepsin (F)	CGCCTTTCATAAACCTCC	55	50
Endothiaepsin (R)	GCCGTCGAAAACCACAAA	56	50
Endothiaepsin-Oxygenase (F)	GTAAACTGGAAAAGAAGGG	53	44
Endothiaepsin-Oxygenase (R)	GCTCCGGTGAAATTGTTG	55	50
Oxygenase (F)	GCATCAACAATTTACCG	54	44
Oxygenase (R)	TGTCTCCCCATATTGACG	54	50
Oxygenase-NRPS (F)	CGTCAATATGGGGAGACA	57	50
Oxygenase-NRPS (R)	TGGCCCATGGATAGTGGA	56	56
NRPS (F)	CACTATCCATGGGCCAAC	57	56
NRPS (R)	TCTTCATCGGCTACCTCT	55	50