Supplementary material

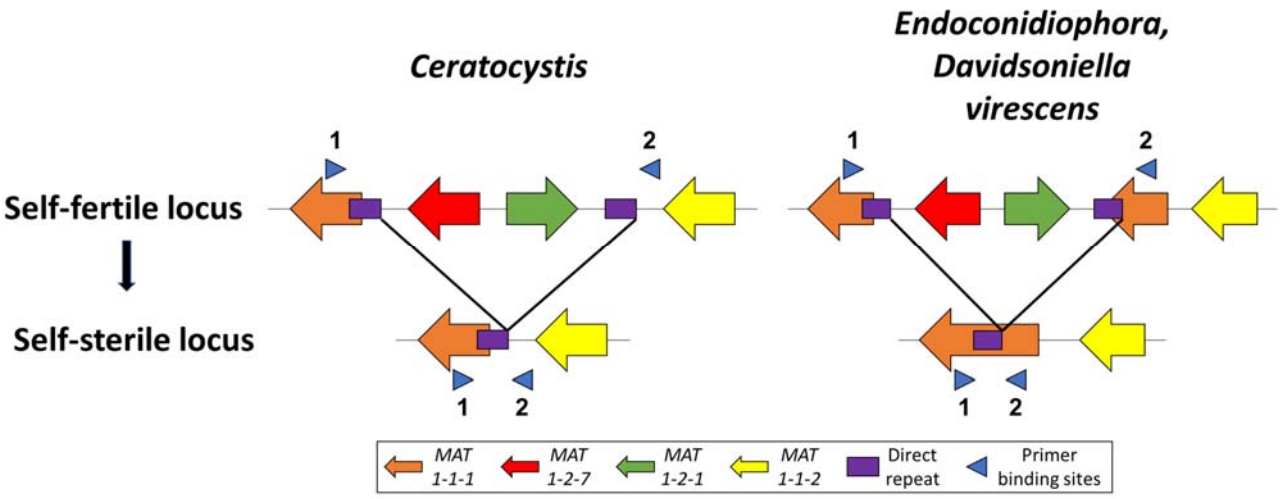

Figure S1: The experimental strategy used to confirm that unidirectional mating-type switching had taken place in species of *Ceratocystis, Davidsoniella virescens* and *Endoconidiophora*. The two diagrams depict the self-fertile and self-sterile versions of the respective species, and blue arrows indicate the expected primer binding sites in each version. The primer combinations would produce products that differ in sizes between the two versions. For primer details and expected sizes, see Table S2. Figure is not drawn to scale.

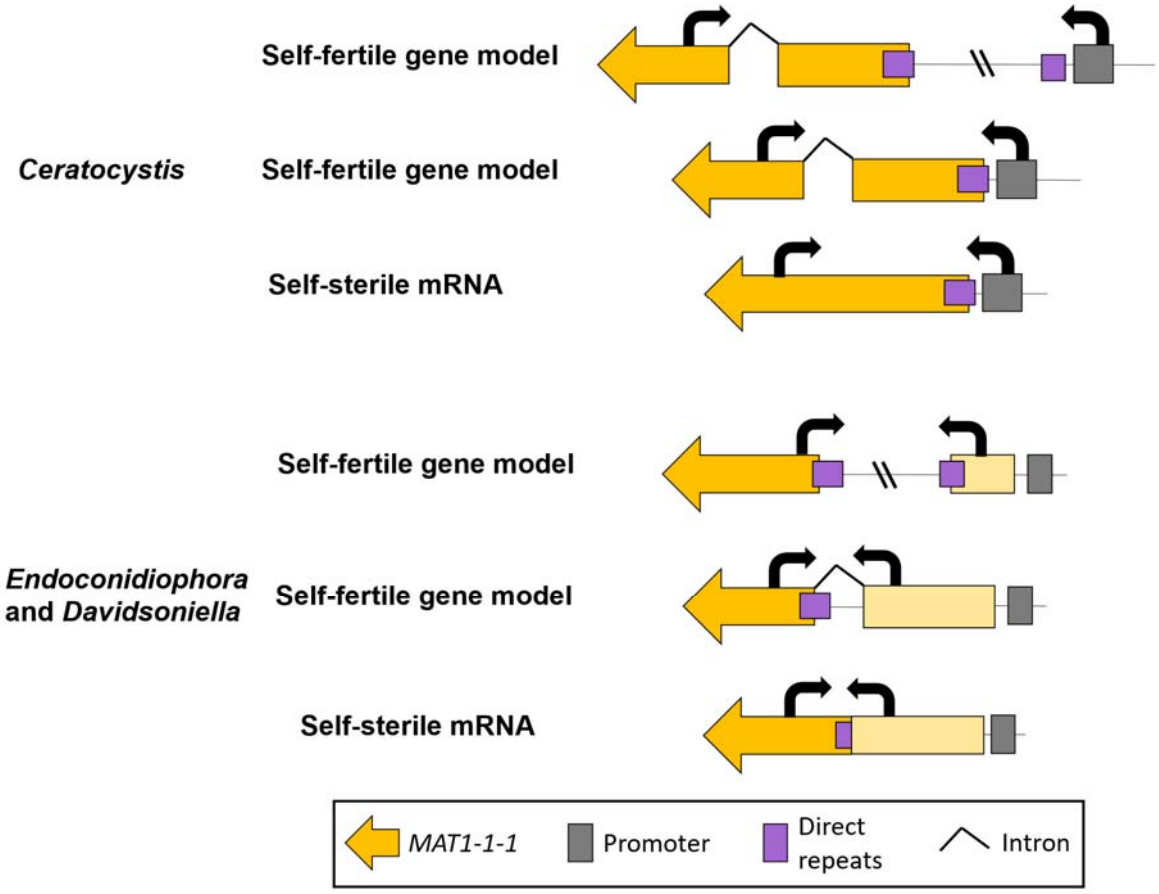

Figure S2: Strategy used to detect expression of the *MAT1-1-1* gene from the self-sterile locus using a cDNA-based PCR approach. For *Ceratocystis* species, the primer set was designed to flank the region that is deleted as well as a predicted intron. The cDNA (mRNA) origin of the amplified fragment was confirmed when the intron was successfully spliced. Similarly, in *Endoconidiophora* and *Davidsoniella* the primer set was designed to flank both the region that is deleted as well as an intron present in the self-sterile *MAT1-1-1* gene. This again meant cDNA produced from mRNA could be confirmed by screening for the absence of the intron sequence.

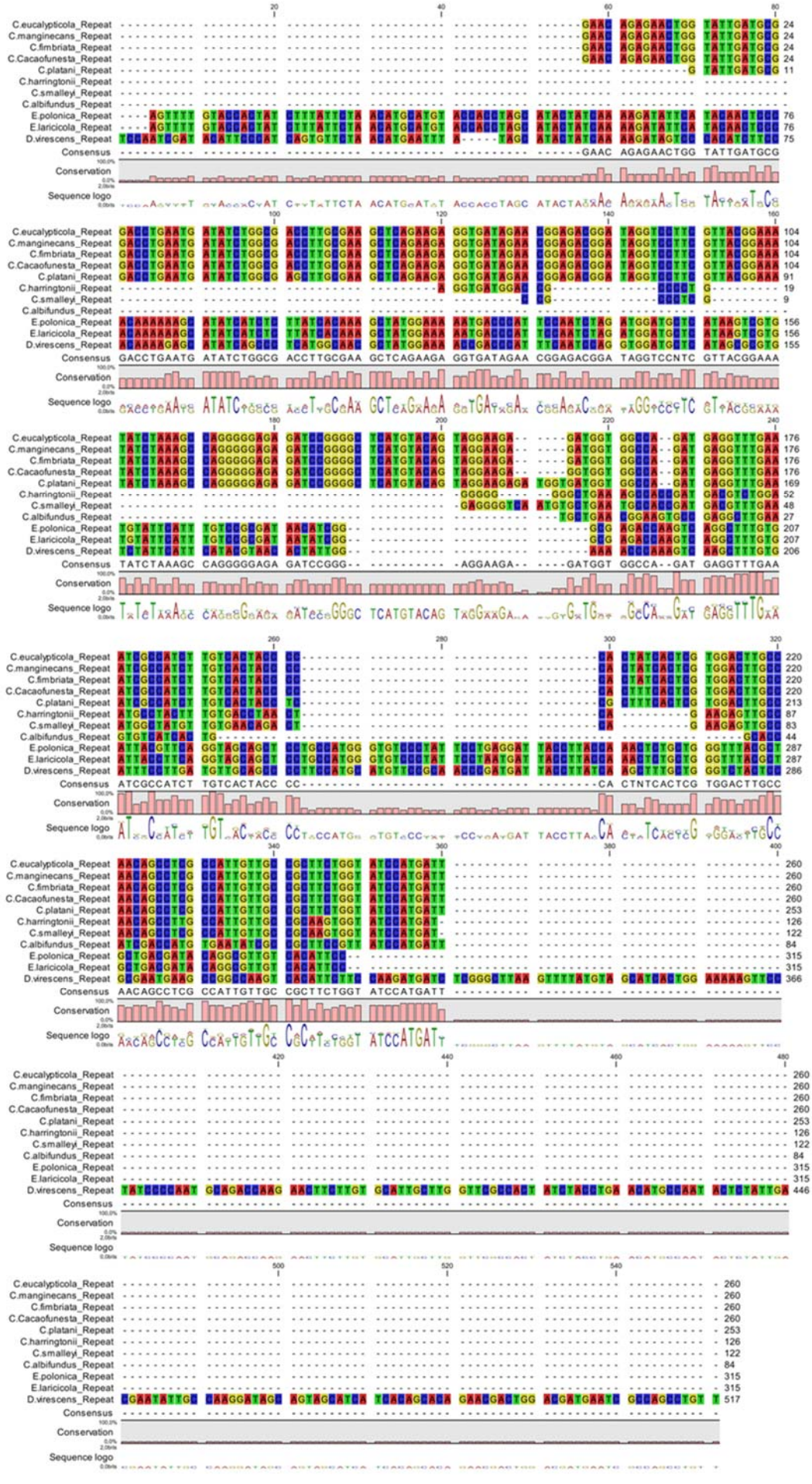
C.eucalypticola_Repeat
C.manginecans_Repeat
C.fimbriata_Repeat
C.Cacaofunesta_Repeat
C.platani_Repeat
C.harringtonii_Repeat
C.smalleyi_Repeat
C.albifundus_Repeat
E.polonica_Repeat
E.laricicola_Repeat
D.virescens_Repeat
Consensus
Conservation
Sequence logo

Figure S3: An alignment of the direct repeat sequence among the species included in the current study.

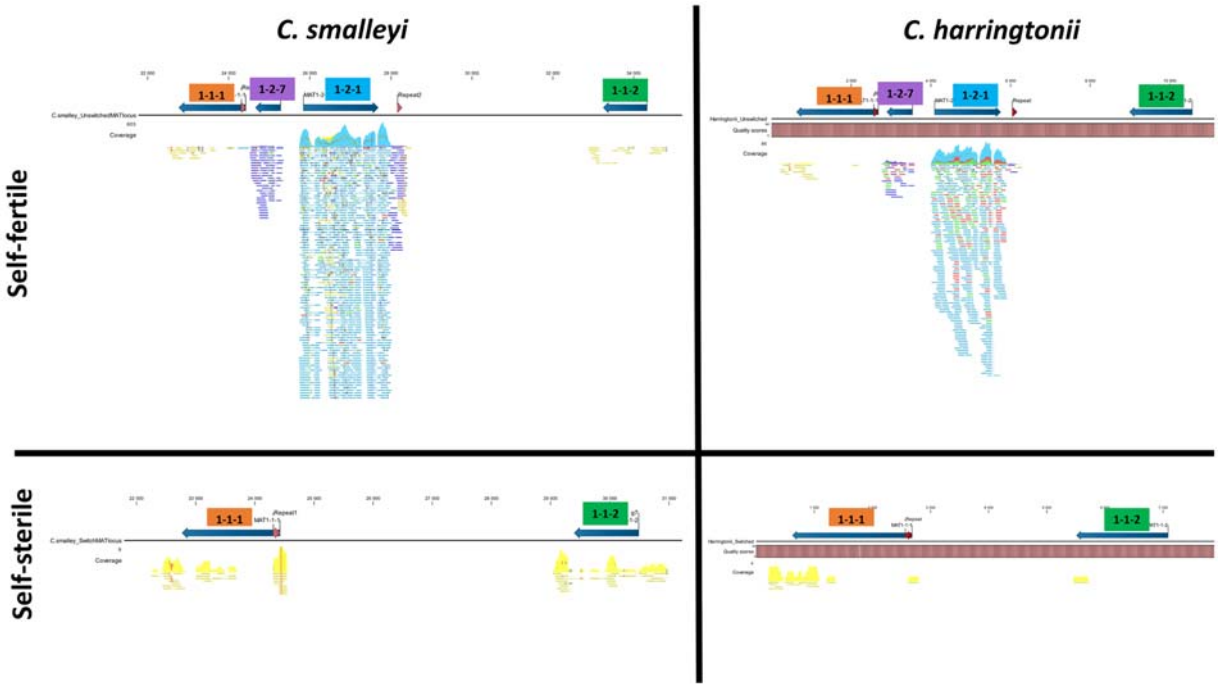

Figure S4: Illustration of the mappings from the partial RNA-seq analysis for *C. smalleyi* (left) and *C. harringtonii* (right). The gene models are shown for *MAT1-1-1* (orange), *MAT1-2-7* (purple), *MAT1-2-1* (blue) and *MAT1-1-2* (green). The mapped reads are shown for the self-fertile (top) and self-sterile (bottom) loci beneath each image.

Table S1: The representative isolates used to confirm the presence and structure of a self-sterile (switched) *MAT1* locus.

Species	Representative isolate's number (CMW[1])
C. manginecans	1750
C. eucalypticola	9998
C. cacaofunesta	26375
C. platani	26380
C. albifundus	4068
C. harringtonii	14789
C. smalleyi	14800
E. laricicola	20928
E. polonica	20930
D. virescens	17339

[1]The CMW culture collection of the Forestry and Agricultural Biotechnology Institute (FABI)

Table S2: The primer combinations and expected fragment sizes of the assay to detect unidirectional mating-type switching.

Species	Primer 1 and sequence (5' to 3')	Primer 2	Expected self-fertile amplicon size	Expected self-sterile amplicon size
C. albifundus	Albi_111F GCCTGTACTCGATGAAATT	Albi_MAT1-2-1F[1] CCAAGATCTTTTCCATCCTA	4 070 bp	722 bp
C. cacaofunesta	Cerato_Primer17[2] TTAGCCGGACGCTTATCATT	Cerato_Primer27 GAGTCTCCCGCTTCTTGTTG	4 125 bp	540 bp
C. eucalypticola	Cerato_Primer17[2] TTAGCCGGACGCTTATCATT	Cerato_Primer27 GAGTCTCCCGCTTCTTGTTG	4 123 bp	540 bp
C. fimbriata	Cerato_Primer17[2] TTAGCCGGACGCTTATCATT	Cerato_Primer27 GAGTCTCCCGCTTCTTGTTG	4 121 bp	540 bp
C. harringtonii	Small_Harr_SWTest_F AAGTGTTGGGCGGTAGAT	Small_Harr_Switched_F ATCTAGGTTCCGTTTTCAGT	5 055 bp	1 577 bp
C. manginecans	Cerato_Primer17[2] TTAGCCGGACGCTTATCATT	Cerato_Primer27 GAGTCTCCCGCTTCTTGTTG	4 125 bp	540 bp
C. platani	Cerato_Primer17[2]	Cerato_Primer27	4 137 bp	546 bp

	TTAGCCGGACGCTTATCATT	GAGTCTCCCGCTTCTTGTTG		
C. smalleyi	Small_Harr_SWTest_F AAGTGTTGGGCGGTAGAT	Small_Harr_Switched_F ATCTAGGTTCCGTTTTCAGT	4 898 bp	1 045 bp
E. laricicola	Endo_P3 CGACAGGAGCTAGTTCTGGT	Endo_P4 GGGTTCATTGCTTTTCGTGG	8 471 bp	656 bp
E. polonica	Endo_P3 CGACAGGAGCTAGTTCTGGT	Endo_P4 GGGTTCATTGCTTTTCGTGG	5 876 bp	665 bp
D. virescens	Dvir_Primer10 GACGAGTGTTGGCCAAAAGT	Vir_111_F GCTGCTCAAAATGTCAATCG	6 025 bp	1 327 bp

[1]Primers from Lee et al., 2015.

[2]Primers from Wilken et al., 2014

Table S3: Primers used to complete mating-type loci by joining contig sequences.

Species	Primer sets	Primer sequence (5'-3')
C. eucalypticola	Cf_Mt1_R1[1]	GAC CGC GAT TCT AAC CAA AA
	Cerato_26_F	CCT GTC CCA ACC ATC TCT TC
E. laricicola *E. polonica*	Endo_P5	AGT ACT CAT GCC GCC TCA TC
	Endo_P1	GCA ATG CCA TTT ATC CCA GC
C. smalleyi	Small_P102	AGT CCA TGA AAA CAG CGT GT
	Small_Harr_112_F	AAT CTC TCT TCG GGG CTA
C. harringtonii	Small_Harr_SWTest_F	AAG TGT TGG GCG GTA GAT
	Harring_P201	AAG TGA GTC CAT TCC CTG CA
D. virescens	DVir_Primer6	CCA ATC GAT ACA TTC CCA TCA GT
	Dvir_Primer10	GAC GAG TGT TGG CCA AAA GT

[1]Primers from (Wilken *et al.* 2014)