

**Supplementary Figure 1.** Homology among the *MAT* locus of three pathogenic *Sporothrix* species. The diagram was prepared from the output of Artemis Comparison Tool.

CLUSTAL 2.1 multiple sequence alignment

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Sbra_tMAT1-1-1 -----M-PPIT---RLPSTPR 12
Sglo_tMAT1-1-1 -----M-PQTT---RLPSTPR 12
Ssch_MAT1-1-1 -----NGASQRQNTFRPLNAFMARSFYWKMLPSIPQ 32
                                     *          *** *;

Sbra_tMAT1-1-1 QRSS-----HRQGSNSSGGESSTLMRTDVSQ-PFTLAVHLEKG----- 50
Sglo_tMAT1-1-1 QRSS-----PRQGSNSSGGESSTSFIQTDASQ-SFTLPVHLETR----- 50
Ssch_MAT1-1-1 KERSGVLTSLWNEDPYKSKWTTMAARVYSYLRAELGKDAVSLPSFLKHASFILGMPPLDAY 92
                                     :. *          * : . : . : : : : . : * . * :

Sbra_tMAT1-1-1 -----ILADREC----- 57
Sglo_tMAT1-1-1 -----VLADREC----- 57
Ssch_MAT1-1-1 LQQRGFALLENEAGHLTLIRPPRPSAVTPESTDTSDDGSDQYDSDVDTGTATILTSFGAT 152
                                     * :. * .

Sbra_tMAT1-1-1 -QDSRDEPMVLPTLTFC---TDTTQEIIRGTSSCNLIHREAVDMLDLSCERETIEV---SR 110
Sglo_tMAT1-1-1 -QDSRDEPMVLSALTFC---KDTTREIRGACSCNQIYREAVDSKDRSSEWKKVEV---SR 110
Ssch_MAT1-1-1 FQDTIQTDVAMSTVAHCPTPNESQMIANGTSDPDGIAGKEDATKYLFAEHQLLQAVFDSG 212
                                     ** : . : : : : * . : : . * : : * : . * : * : : : . *

Sbra_tMAT1-1-1 VGCEL-----EPETDSTASSIT 127
Sglo_tMAT1-1-1 VGCEL-----ERETDSASSVT 127
Ssch_MAT1-1-1 LDADLSNLRKGDKMIEKKQWLVLQKLYSMAHEMLVGGKNILVNHVEFEGRQVGNVASSA 272
                                     : . : *          : . . . * * :

Sbra_tMAT1-1-1 YQRAN-GKDDSNPTDSSVKDILRRASQAGHLDDETIVASENDELSSLQYR-----I 179
Sglo_tMAT1-1-1 YQRAN-GKDDSNPTDSSVKDILRRASQTGHSDDERIVTSENDELSSLQYR-----I 179
Ssch_MAT1-1-1 SRRASVGLRNIDEIHDRSYSNASGVVYQEHSSDNDTSVAEHNTNMETCDNNHHSCTGTS 332
                                     : * . * : : * * : . * * : : * : * : : : .

Sbra_tMAT1-1-1 VPEHRNASPNNATIEDGKEWLDRELERIYNTESDTSCHCGGVGGDHLSTGDQSTTVRSR 239
Sglo_tMAT1-1-1 VPAHRNOSPNDATIEDGQEWLDRELERIYNTSNDTSCHRGG----- 221
Ssch_MAT1-1-1 ADANQISHNPNNTIEDGKEWLDREMERIYNTESDTSCHRGGVSGDHLSTGDHTMTVRSR 392
                                     . : : . * : * * * : * * * : * * * : * * * : * *

Sbra_tMAT1-1-1 RPDEVHDTVSTNGTTLQDANGSARLNASALTSSQFPNNGRLEILNSVLSTHLDTMGSDNE 299
Sglo_tMAT1-1-1 -----VLSIHLDTMGSDNE 235
Ssch_MAT1-1-1 RPDEVHDISTTNGTTVQNTNDSTRLNALAFTSSRFPNNGRFEIPNSVLSTHLDTMGSDNE 452
                                     * * * * * * * * *

Sbra_tMAT1-1-1 ATNYMDRVARLSASSASASLAASSAAATAAAPASFPSSS-----STSGHLRSSSGRCLG 353
Sglo_tMAT1-1-1 AANYMDRVARRSASSASASLATSSSTATAAAP--FSCTS-----SSGHMRSSSSGSCLG 287
Ssch_MAT1-1-1 ATNYMDRVARLSASSASASLAESSATAAAPASFPSSSFSASPSSTSGHMRSLSGSCLG 512
                                     * : * * * * * * * * * * * : * * * * * * * * * * * * * * * * * * * *

Sbra_tMAT1-1-1 KRRRDSTDIGSETLAGGDCPTMPEDVDFAVSHDGGPDEPSEKAKGRPEGAI SPRKKTR 413
Sglo_tMAT1-1-1 KRRRGSTDIGSETLTGGDCCLTMTEDVDQVVDHGGPDEPSEKAKGRPEGAI SPRKKPR 347
Ssch_MAT1-1-1 KRRRESTDIGDGLAGGDCPTMPENVDPADSHDGGPDEPSEKAKGRPEGAI SPRKKSR 572
                                     * * * * * * * . * : * * * * * * * * * * * * * * * * * * * * * * * * *

Sbra_tMAT1-1-1 RREAINGYKHKMKTPEPPFHERPSVSVFKKGYSEN LAKTLTAVTQELEELQPLSTGIAAAS 473
Sglo_tMAT1-1-1 RREAINGYKHKMKTPEPPFHKRPSVSVFKKGYSEN LAKTLTAVTQELEELQPLSTGMAAAS 407
Ssch_MAT1-1-1 RREPIINGYKHKMKTPEPPFHKRPSVSVFKKGYSEN LAKTLTAVTQELEELQPLSTGIAAAS 632
                                     * * * . * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

Sbra_tMAT1-1-1 TAVTADTTDIADNTATTDTAQFPQPVVCTQAPSPFPTRPQPALSASQAMPTATSMQAPQF 533
Sglo_tMAT1-1-1 TAVTADT-----TATTDVQFRPLVSYTQDFSSFPTRPLRATFVSQTVPFSATAMQSLQS 461
Ssch_MAT1-1-1 TAVTADTTIADNTATTDTAQFPQPVVCTQAPSPFPTRPQPAISVSQAMPTATAMQFLQS 692
                                     * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

Sbra_tMAT1-1-1 MEVVPFHQHFSPMQSILPMLSLPPM-----PMPFVVSDEEMRSYQDMQALNAMRAI 584
Sglo_tMAT1-1-1 MEVVPPLLHFPPMQNIHSMLSLPPMTSMPSLVPFAPFASVVSDEEMRSYQDMQAMH--MRAI 520
Ssch_MAT1-1-1 TEVVPFHQHFPPMQNLLPMLSMPPM-----PMPFVVSDEEMRSYQDMQAMNAMRPI 743
                                     * * * . * : * * * * * * * * * * * * * * * * * * * * * * * * * * *

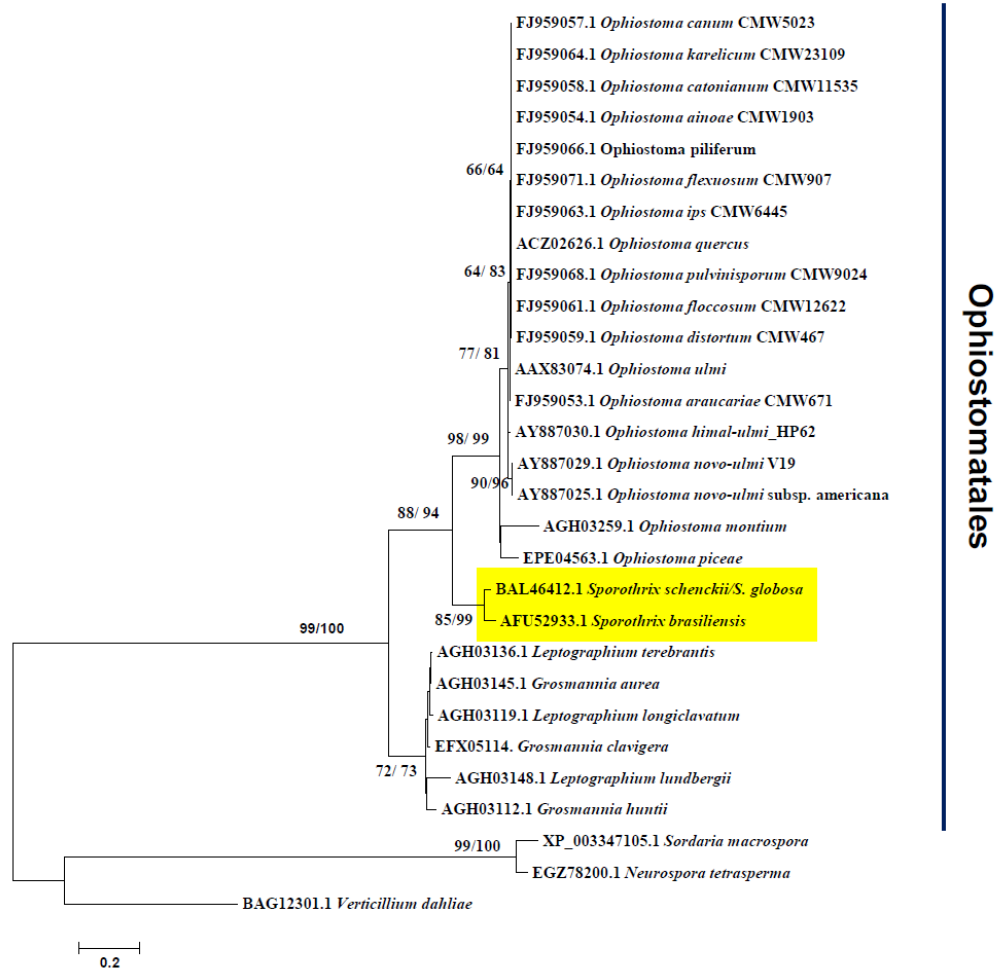
Sbra_tMAT1-1-1 EDLQAMDTVQAMQGLPGMSGVADDIPLMPAIPDPAMAHALQMPMPHGLPFTPTDYDHN 644
Sglo_tMAT1-1-1 EDLQAMDVQAMQGLPGMSGVADDIPLMPAIPDPAMAHALRPIPLPHGLPHTPTDY--HN 579
Ssch_MAT1-1-1 EDLQAMDTEQAMQGLPGMSGVADDIPLMPAIPDPAMAHALQMPMPHGLPFTPTDYDHN 803
                                     * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

Sbra_tMAT1-1-1 TINQAINHDAAYTAGYNSVPPPPSLVPAGHGLHSPEVDDYIEVDDYFGVAAPMDMDFAD 704
Sglo_tMAT1-1-1 TIHQNVNHDAAYTTGYNVPPPPSLVPAGGGLNSPEDEDYIEVDDYFGVAGPMD--FTD 637
Ssch_MAT1-1-1 TISQTIHDAAYTTGYDSVPPPPSLVPAGHGLHSPADDDYIEVDDYFGMAGPMDMDFAD 863
                                     * * * : * * * * * * * : * * * * * * * * * * * * * * * * * * * * * * *

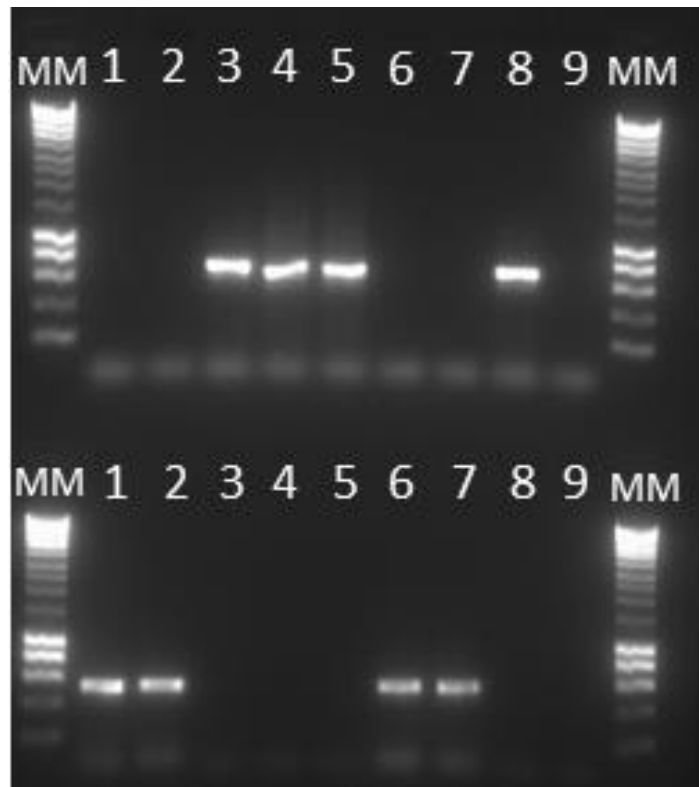
Sbra_tMAT1-1-1 PEAMHRFMSFCEELGTSHDGLDTSSEFPWI-- 734
Sglo_tMAT1-1-1 PGEMHRFMSFCEELGTSHDVLDTSSEGFQW-- 666
Ssch_MAT1-1-1 PGAMHRFMSFCEELGTSHDVFDTSSEDFQWI-- 893
                                     * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

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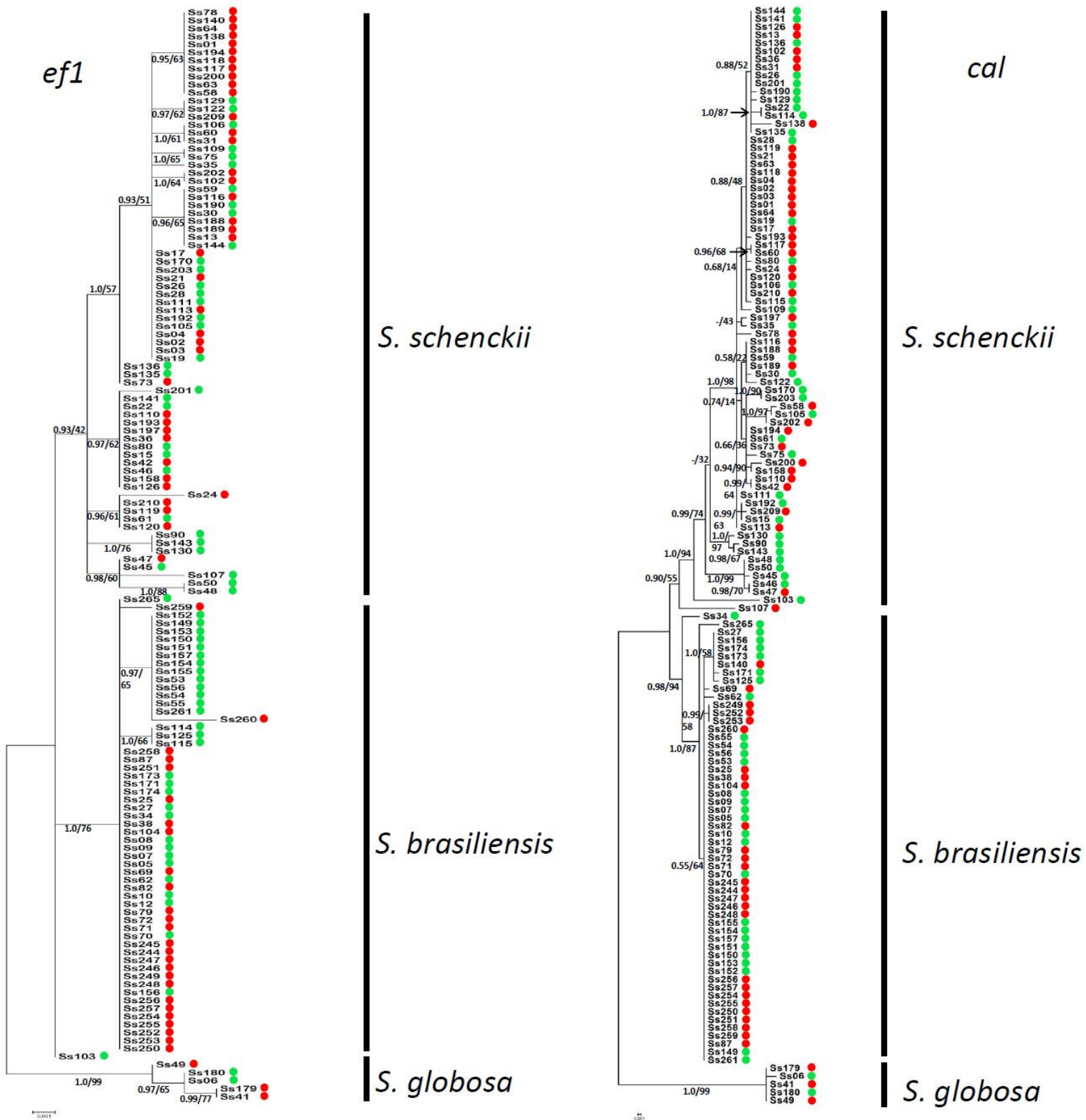
**Supplementary Figure 2** - The amino acid alignment of truncated *MAT1-1-1* in *MAT1-2* idiomorph of *S. brasiliensis* and *S. globosa* to full length *MAT1-1-1* in *MAT1-1* of *S. schenckii* idiomorph by Clustal W. The comparison indicates the absence of alpha-box domain (in yellow) in truncated *MAT1-1-1*.



**Supplementary Figure 3.** Phylogenetic relationships among Ophiostomatales fungi inferred from the HMG domain (225 amino acid characters) of the MAT1-2-1 based on Neighbor Joining (MEGA software) and Maximum Likelihood (PhyML) analysis. Number on branches indicated bootstrap support (1000 replicates) by NJ (> 60%) and ML (>60%).



**Supplementary Figure 4** – PCR amplifications of *MAT1-1-1* or *MAT1-2-1* from *S. brasiliensis* (Lanes 1-2) *S. schenckii* (Lanes 3 to 6) and *S. globosa* (Lanes 8-9) revealing that all contain a unique copy of *MAT* locus in their genomes suggesting to be heterothallic species. MM = 1Kb Molecular Marker. Lane 9 corresponds to the negative control of PCR reaction



**Supplementary Figure 5.** Phylogenetic analysis using partial nucleotide sequences of the calmodulin (*cal* - A) and elongation factor-1 alpha (*efl* - B). Trees were generated by Bayesian and ML searches. The majority-rule consensus trees and branch lengths

were deduced by Bayesian inferences sampled by MCMC and were elected to demonstrate the tree topology. Bootstrap and posterior probabilities values were added to respective concordant branches. Each species are indicated at each respective position at the phylogenetic tree. Cal and ef1 accessions numbers are indicated in the Supplementary table 2.