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Supporting Information for:

Identification of cyclosporin C from *Amphichorda felina* using a *Cryptococcus neoformans* differential temperature sensitivity assay

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Table S1. Features of the *Amphichorda felina* TTI-0347 draft genome

Predicted genome size (Mbp)	32.09
Number of scaffolds	30,421
Total size of scaffolds	35,697,800
Longest scaffold	369,970
Percent GC content	54.19
N_{50} scaffold length	107,143

Table S2. Gene positions, functions, and % identity and similarity of genes shared between the cyclosporin biosynthetic gene clusters of *A. felina* TTI-0347 (NRRL 66746) and *T. inflatum* NRRL 8044.

No.	Start	End	name	Coverage	Identity	Similarity
1	2859	1	Hypothetical protein	100%	87%	91%
2	5050	3732	bZIP transcription factor	100%	80%	83%
3	6927	8405	F-Box domain protein	100%	87%	90%
4	55043	9189	Cyclosporin synthetase (*simA*)	100%	92%	95%
5	55629	56862	Alanine racemase	100%	93%	97%
6	59728	60709	Cyclophilin	100%	93%	96%
7	65554	61295	ABC Transporter	97%	94%	96%
8	66945	68201	Esterase-like protein	100%	80%	85%
9	70087	68327	Cytochrome b2-like protein	95%	92%	95%
10	79609	70770	PKS	100%	91%	94%
11	80999	82103	Hypothetical protein	75%	95%	97%
12	83899	85773	Cytochrome P450	79%	95%	98%
13	87537	86278	Aminotransferase	100%	92%	96%

Note: The data is based on the comparison of the amino acid predicted by Augustus (http://bioinf.uni-greifswald.de/augustus/submission.php). See Figure 5 for gene numbering. The start and end means the location of start codon and end codon on the biosynthetic gene cluster of *A. felina* TTI-0347.

Figure S1. *Amphichorda felina* from Texas. A. Synnemata of *A. felina* TTI-0347 (NRRL 66746) formed on deer dung incubated in a humid chamber. B. Synnemata of *A. felina* TTI-0469 (NRRL 66747) among conidiophores *of Aspergillus clavatus* formed on racoon dung incubated in a humid chamber.

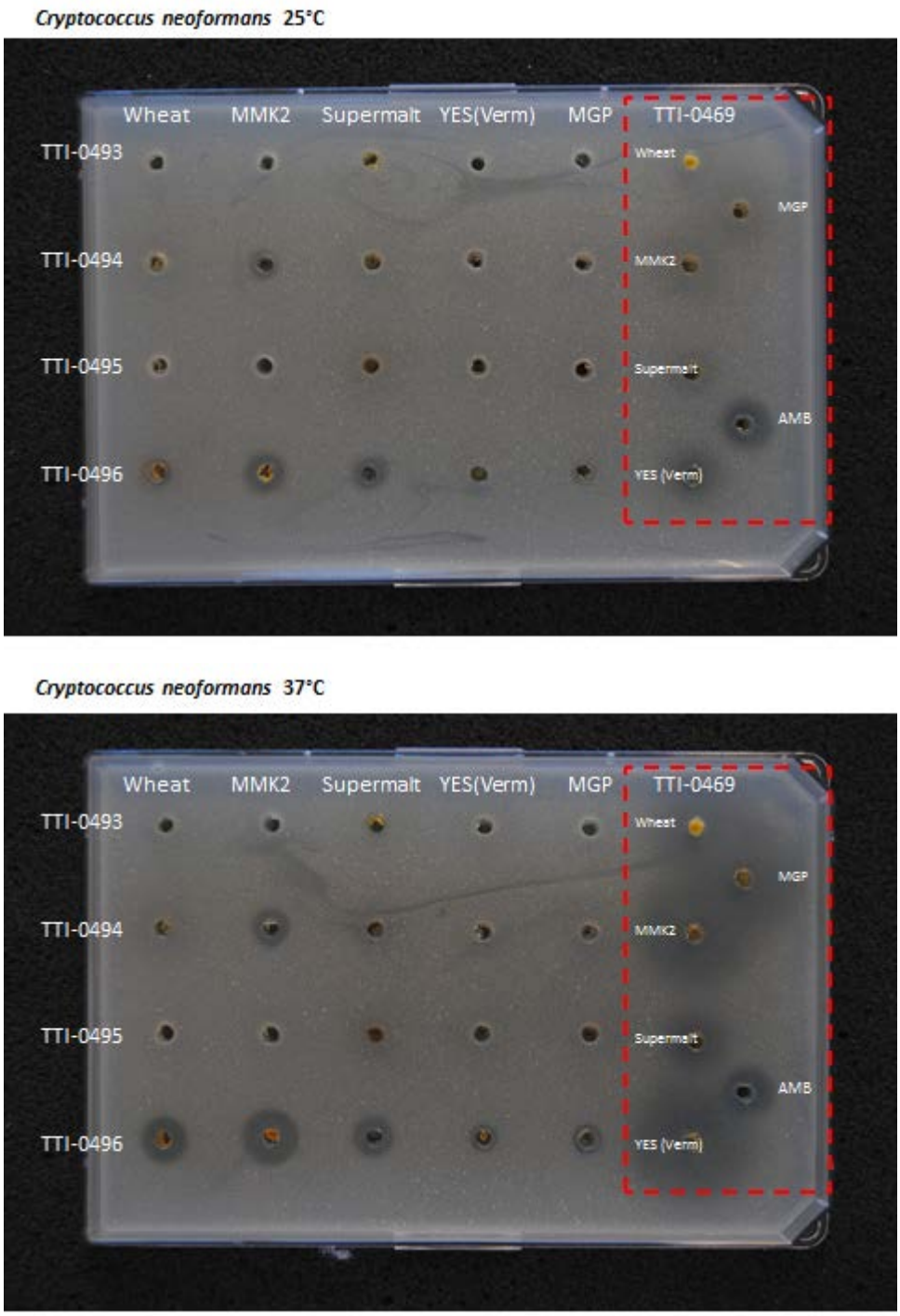
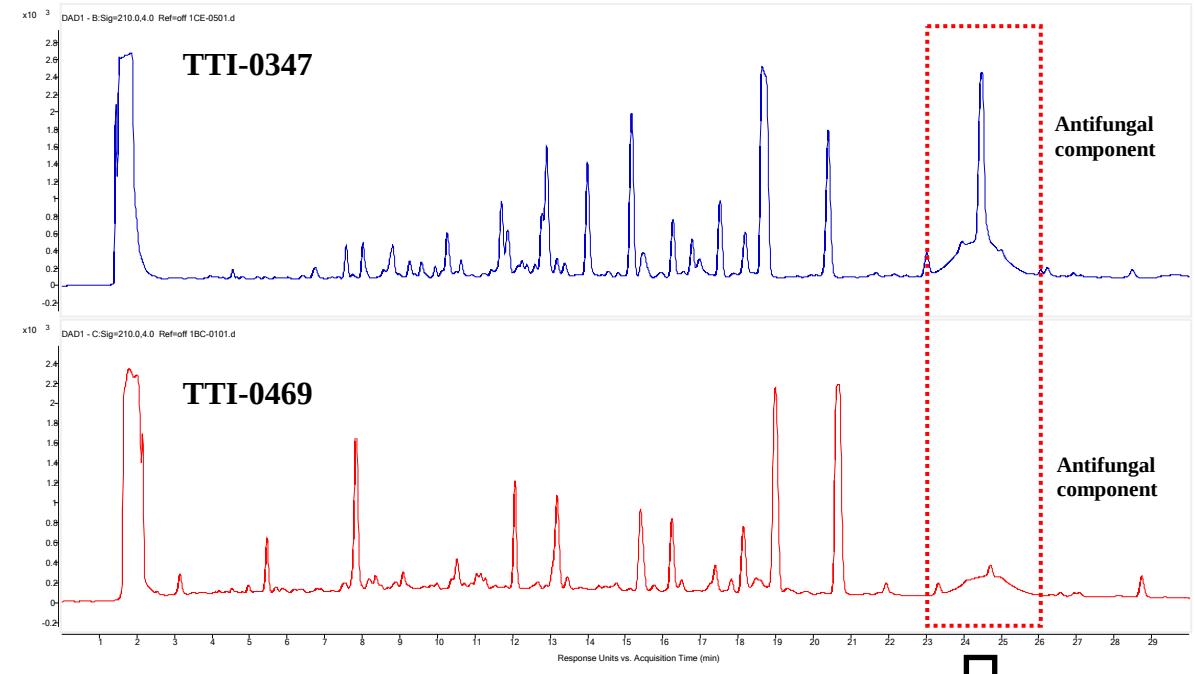


Figure S2. Two-plate temperature assay of MEK extracts of *A. felina* TTI-0469 (NRRL 66746) grown on five media (extracts and amphotericin B control were applied in the wells indicated within red line). Other wells are assays of other fungal extracts.

A)



B)

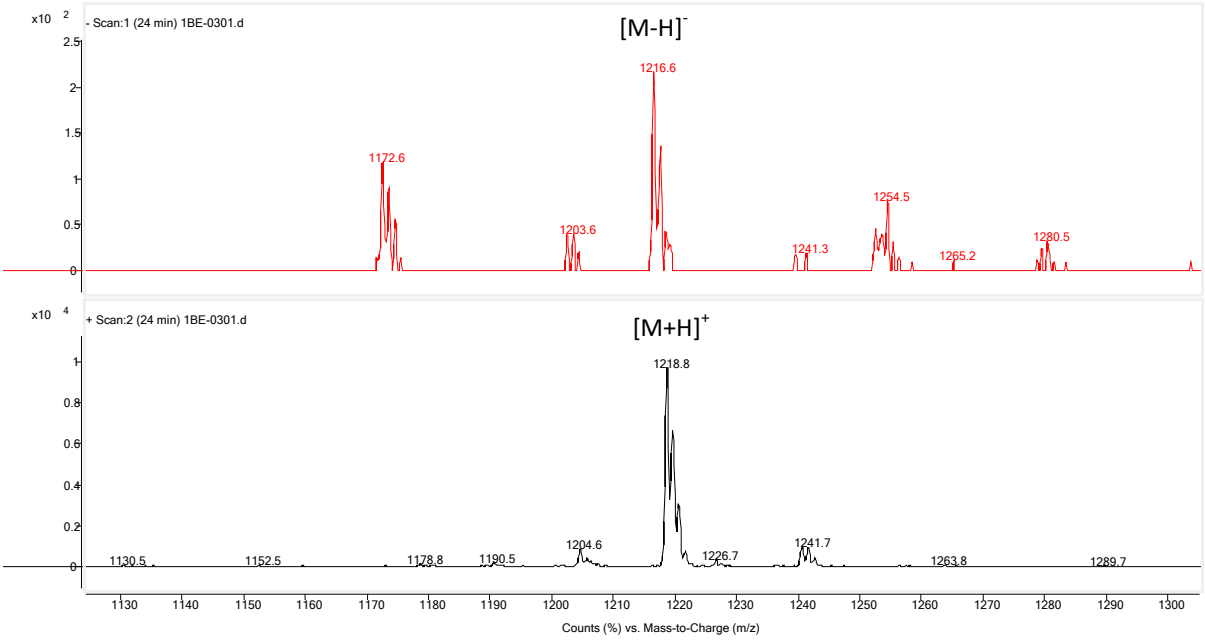
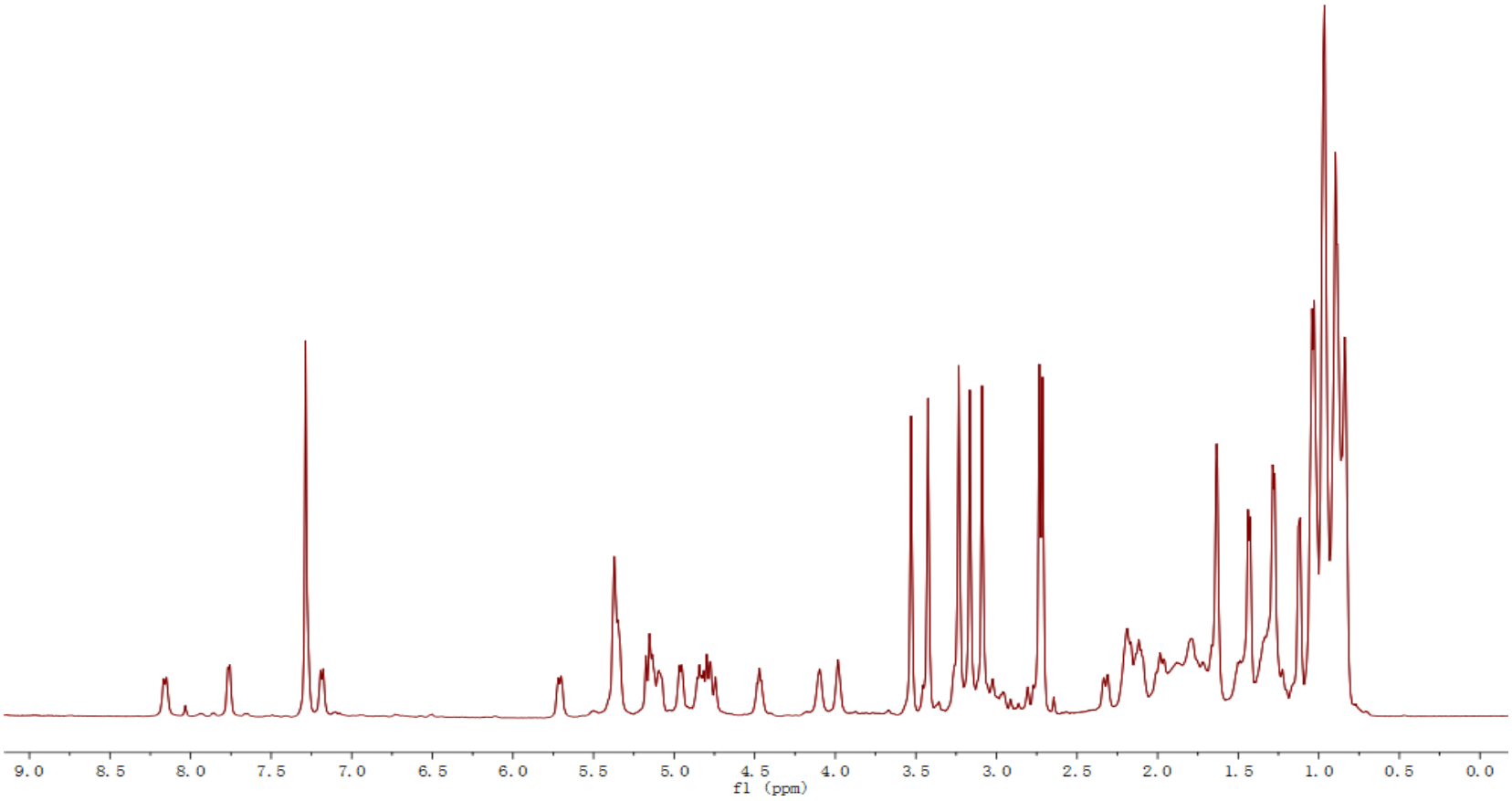


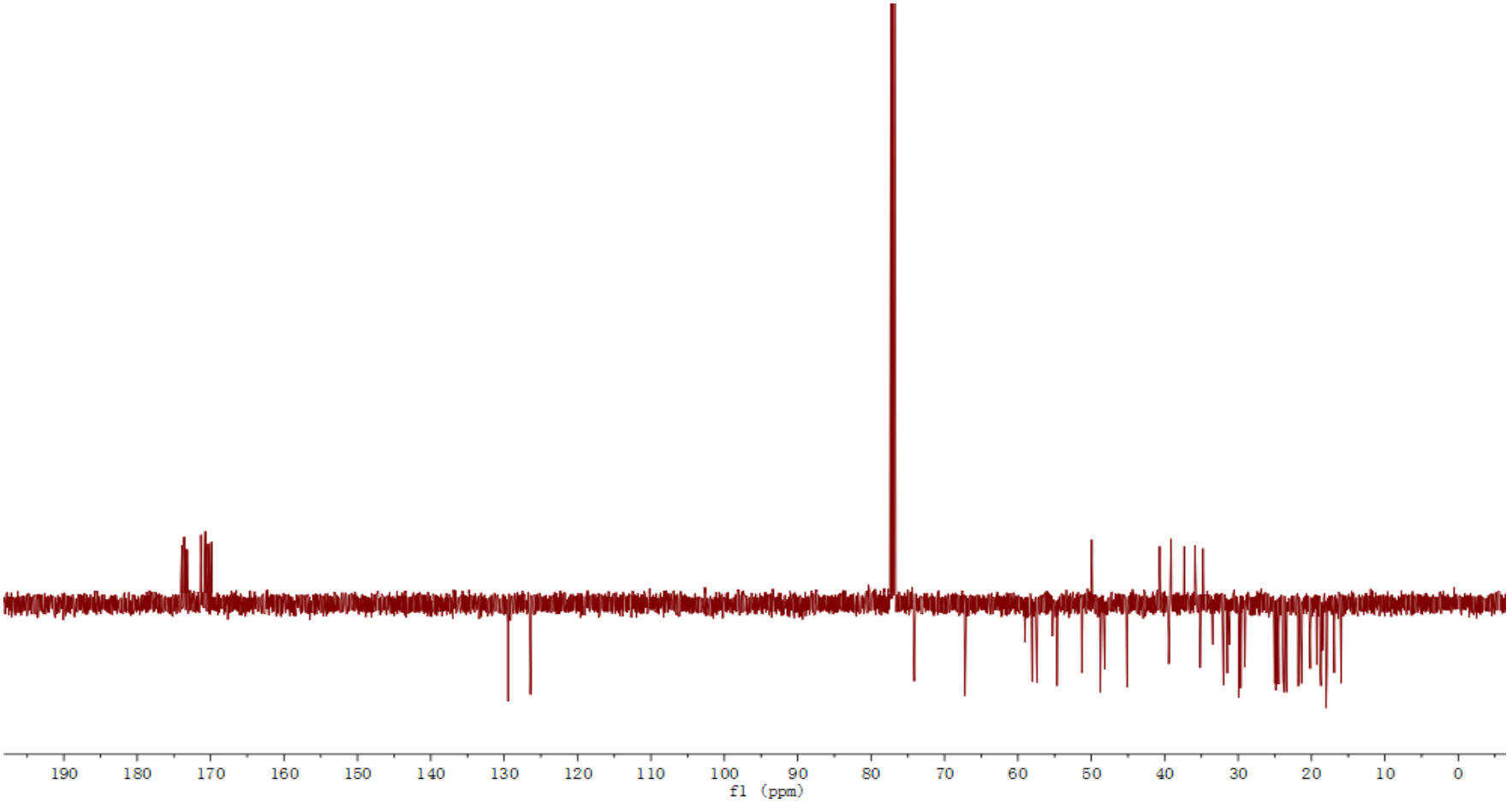
Figure S3. A. LC-MS Bioassay guided identification of the activity component for TTI-0347 and TTI-0469 grown 14 d on MMK2 medium. B. LC-MS spectra of cyclosprorin C from TTI-0347.

Figure S4.

A) ^{1}H NMR spectrum of cyclosporin C (CsC, 500 MHz, $CDCl_3$) from TTI-0347.



B) ^{13}C NMR spectrum (APT) of cyclosporin C (CsC, 125 MHz, $CDCl_3$) from TTI-0347.



C) HRESI mass spectrum of cyclosporin C from TTI-0347.

ID	Formula	Expected m/z	Observed m/z	Accuracy (ppm)
CSC	$C_{62}H_{111}N_{11}O_{13}$	1218.8436 $(M+H^+)$ 1240.8255 $(M+Na^+)$	1218.8412$(M+H^+)$ 1240.8223 $(M+Na^+)$	1.9ppm 2.5ppm

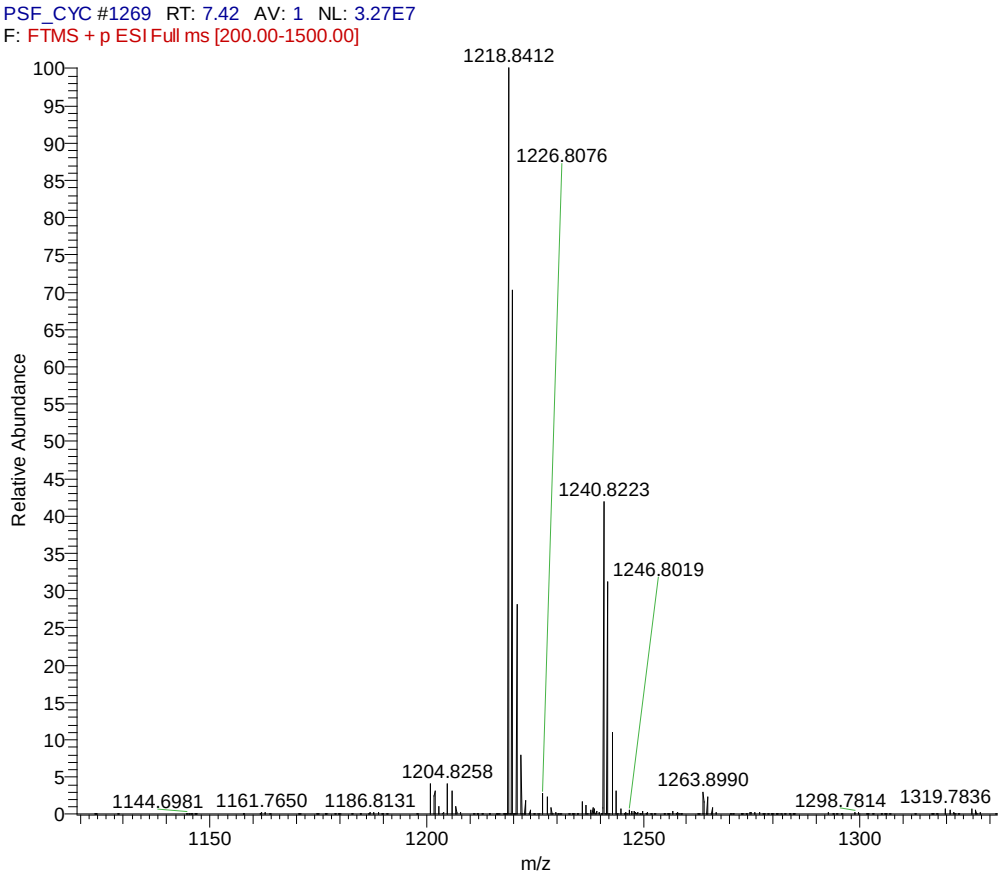
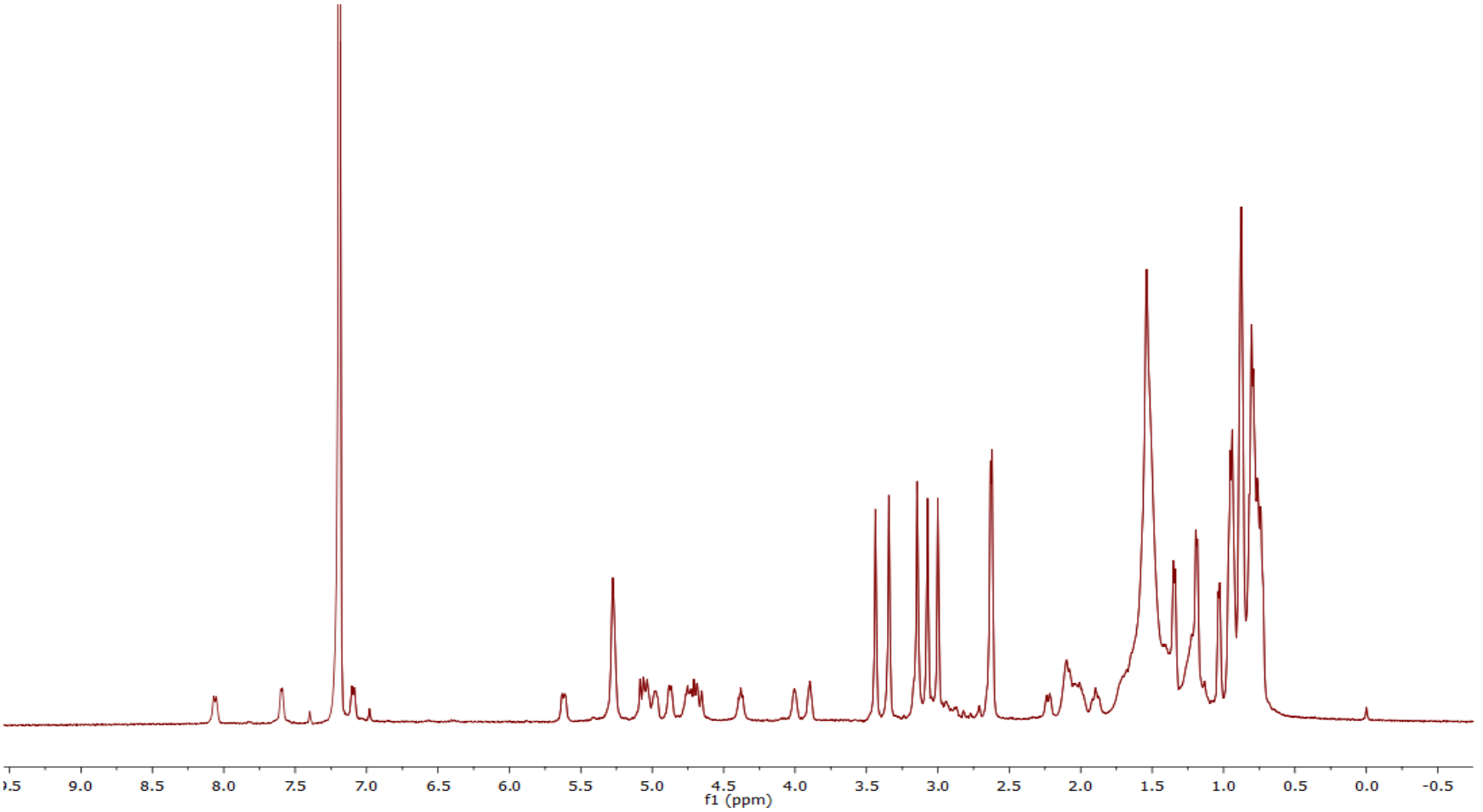


Figure S5. ^{1}H NMR spectrum of the commercial cyclosporin C Standard (500 MHz, $CDCl_3$)



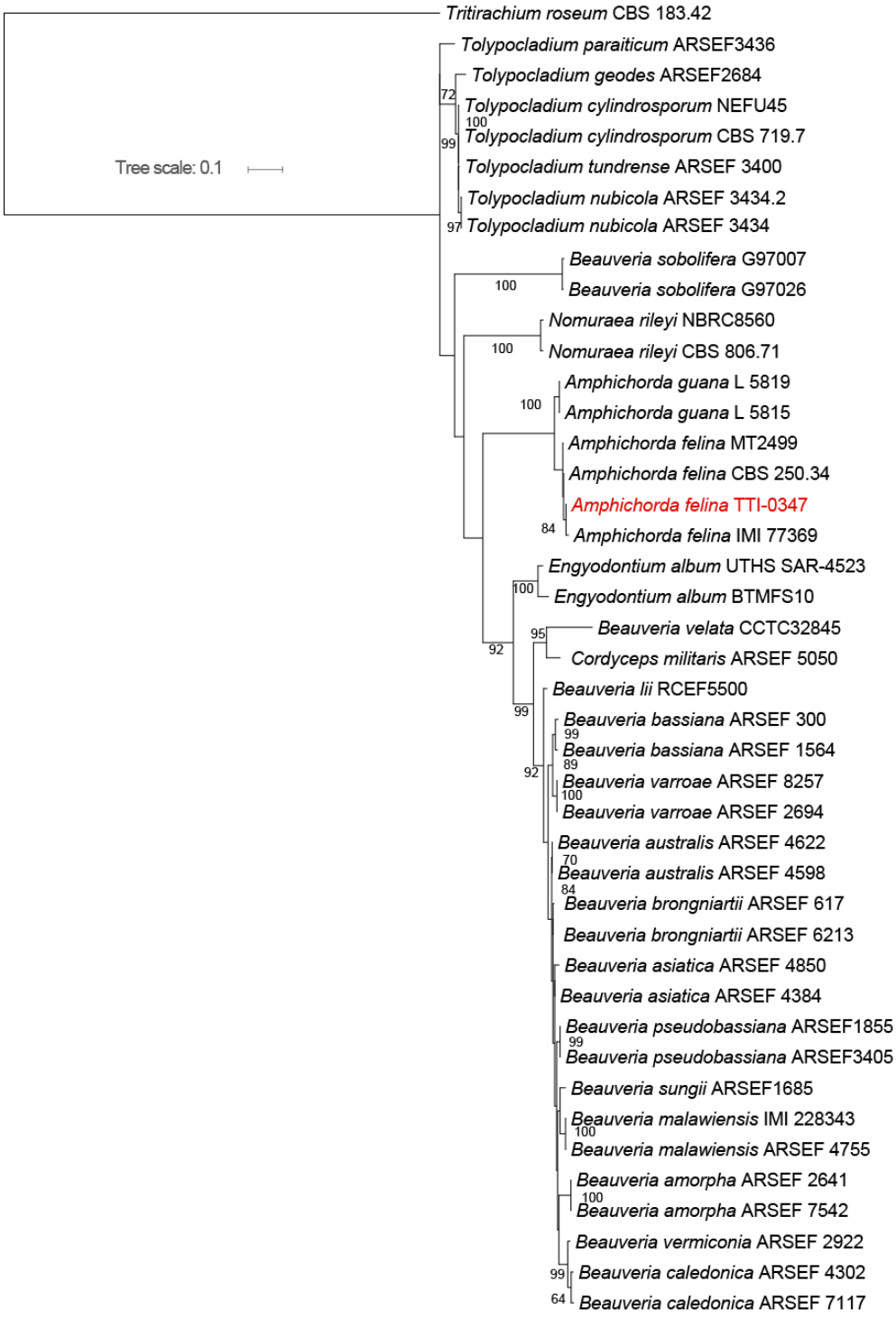


Figure S6. RAxML maximum likelihood tree for ITS rDNA sequences for *Amphichorda* species and related fungi of the Hypocreales. The sequences were aligned with MAFFT and the maximum likelihood tree was built by RAxML 8.2.10 including 1000 bootstrap replicates using a general time-reversible model. Bootstrap values greater than 50% are shown above branches. Sequences were resampled from Zhang ZF, Liu F, Zhou X, Liu XZ, Liu SJ, Cai L. 2017b. Culturable mycobiota from Karst caves in China, with descriptions of 20 new species. Persoonia in press.