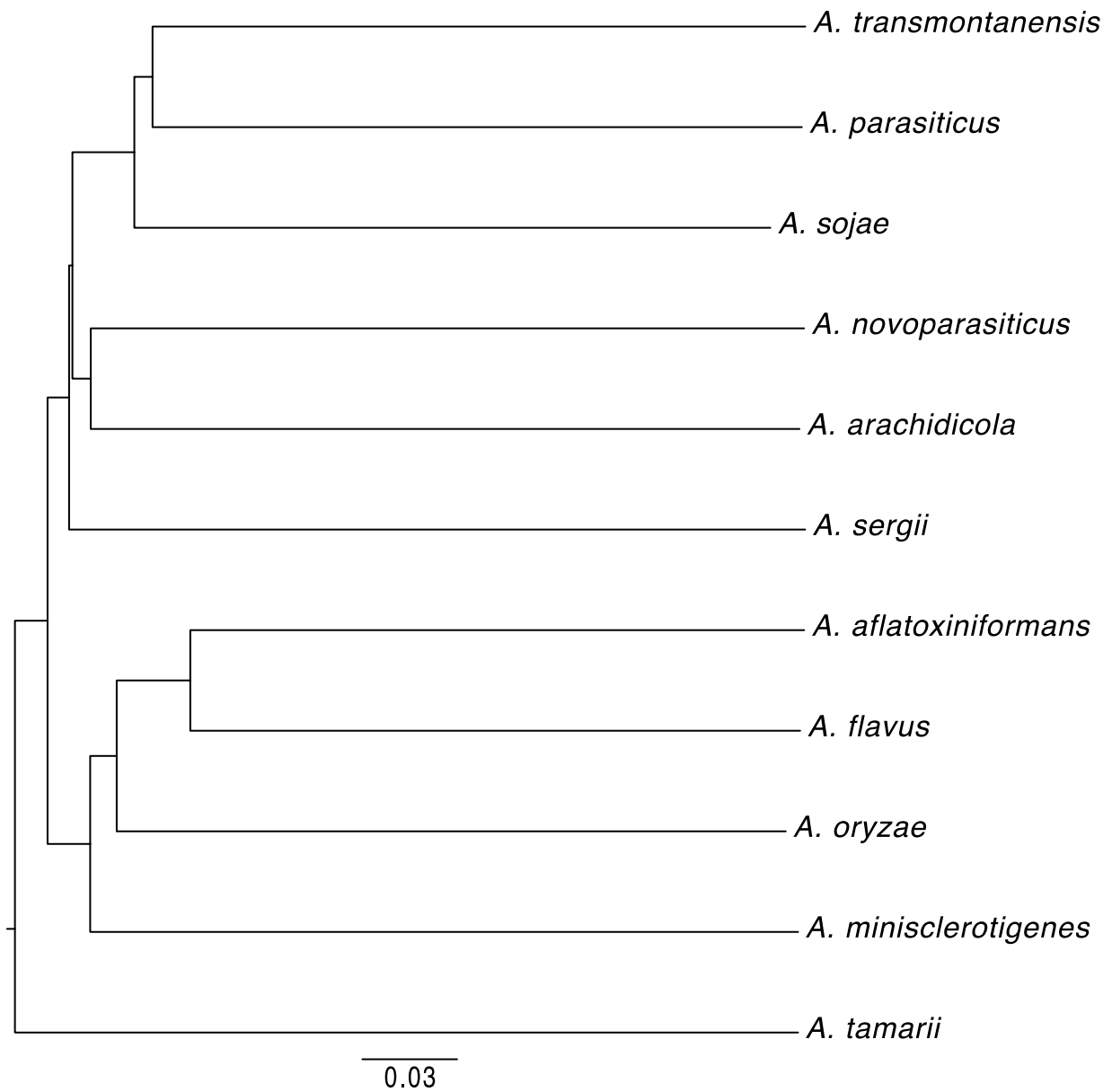


Supplementary information for article:

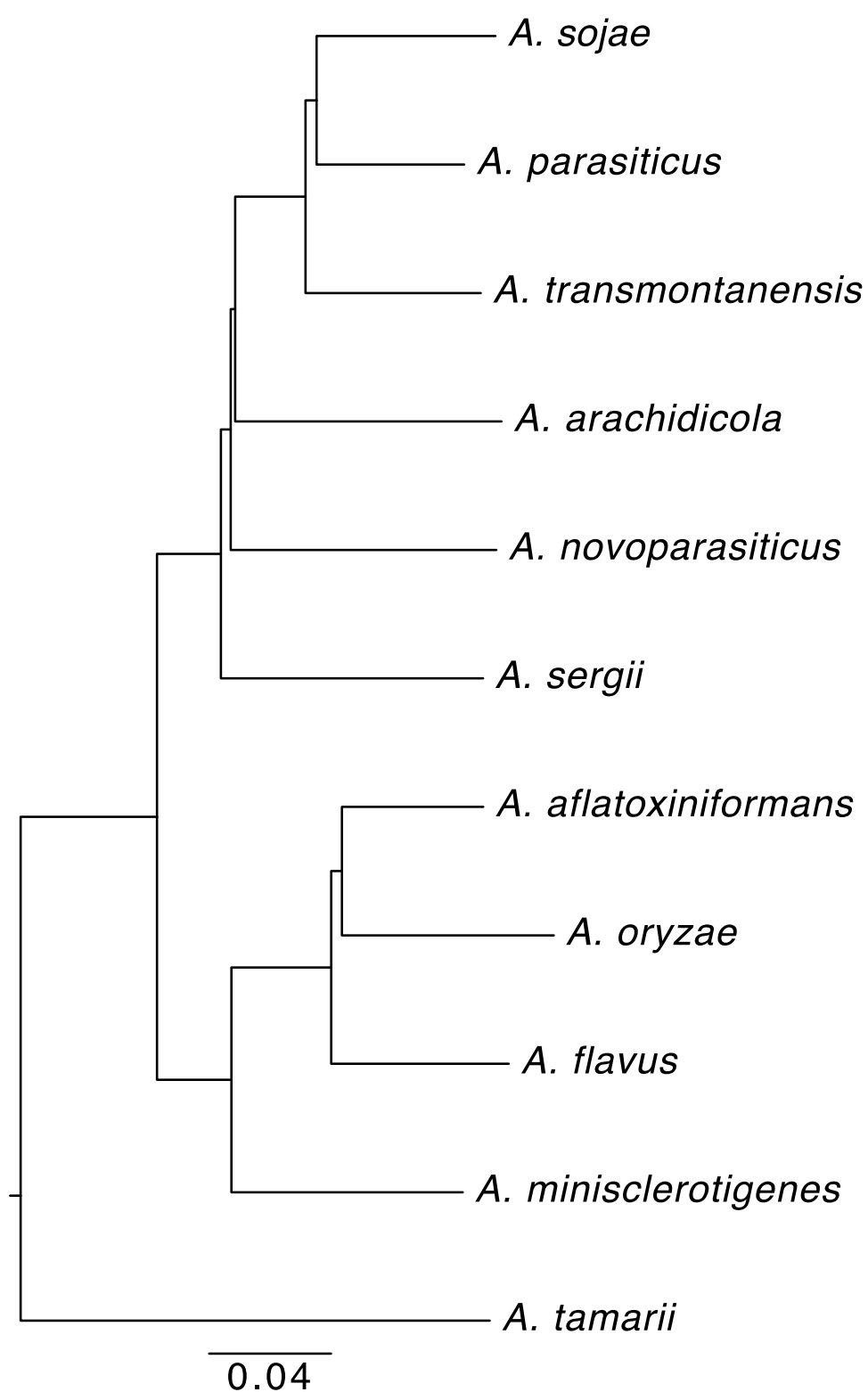
'A comparative genomics study of 23 *Aspergillus* species from section *Flavi*' by Kjærboelling et al.

Supplementary Table 1 - Overview of species and genomes used in this study ^{3,12,14,17,21,24,25,37,88–91}.

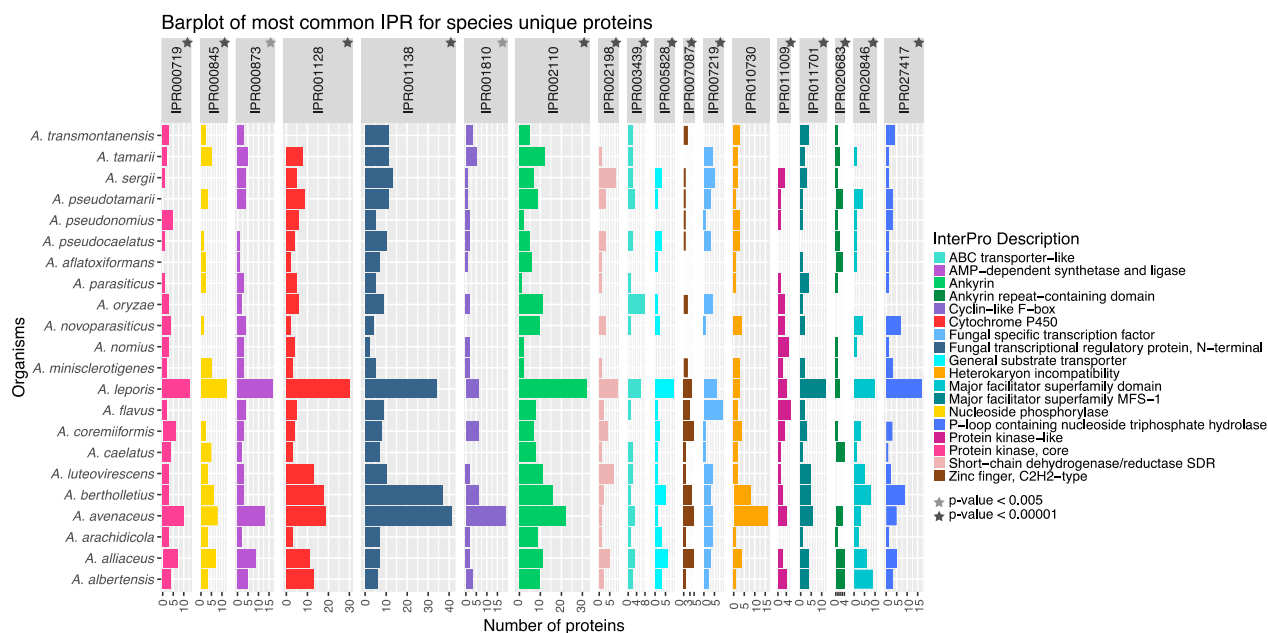
Genus	species name	Section	JGI portal	References
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<i>Aspergillus</i>	<i>steynii</i>	<i>Circumdati</i>	genome.jgi.doe.gov/Aspste1	[17]
<i>Aspergillus</i>	<i>coremiiformis</i>	<i>Flavi</i>	genome.jgi.doe.gov/Aspcor1	This study
<i>Aspergillus</i>	<i>pseudocaelatus</i>	<i>Flavi</i>	genome.jgi.doe.gov/Asppsec1	This study
<i>Aspergillus</i>	<i>pseudonomius</i>	<i>Flavi</i>	genome.jgi.doe.gov/Asppsen1	This study
<i>Aspergillus</i>	<i>avenaceus</i>	<i>Flavi</i>	genome.jgi.doe.gov/Aspave1	This study
<i>Aspergillus</i>	<i>bertholletius</i>	<i>Flavi</i>	genome.jgi.doe.gov/Aspber1	This study
<i>Aspergillus</i>	<i>caelatus</i>	<i>Flavi</i>	genome.jgi.doe.gov/Aspcae1	This study
<i>Aspergillus</i>	<i>flavus</i>	<i>Flavi</i>	genome.jgi.doe.gov/Aspfl1	[13]
<i>Aspergillus</i>	<i>fumigatus</i>	<i>Fumigati</i>	genome.jgi.doe.gov/Aspfu1	[37]
<i>Aspergillus</i>	<i>nidulans</i>	<i>Nidulantes</i>	genome.jgi.doe.gov/Aspnid1	[21]
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<i>Aspergillus</i>	<i>oryzae</i>	<i>Flavi</i>	genome.jgi.doe.gov/Aspor1	[12]
<i>Aspergillus</i>	<i>terreus</i>	<i>Terrei</i>	genome.jgi.doe.gov/Aspte1	[88]
<i>Penicillium</i>	<i>digitatum</i>	<i>Penicillium</i>	genome.jgi.doe.gov/Pendi1	[90]
<i>Neurospora</i>	<i>crassa</i>	<i>Neurospora</i>	genome.jgi.doe.gov/Neucr2	[91]
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<i>Aspergillus</i>	<i>arachidicola</i>	<i>Flavi</i>	genome.jgi.doe.gov/Aspara19utr	This study
<i>Aspergillus</i>	<i>parasiticus</i>	<i>Flavi</i>	genome.jgi.doe.gov/Asppar1	This study
<i>Aspergillus</i>	<i>novoparasiticus</i>	<i>Flavi</i>	genome.jgi.doe.gov/Aspnovo1	This study
<i>Aspergillus</i>	<i>minisclerotigenes</i>	<i>Flavi</i>	genome.jgi.doe.gov/Aspmin1	This study
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<i>Aspergillus</i>	<i>aflatoxiniformans</i>	<i>Flavi</i>	genome.jgi.doe.gov/Asppari1	This study
<i>Aspergillus</i>	<i>luteovirescens</i> (formerly <i>bombycis</i>)	<i>Flavi</i>	genome.jgi.doe.gov/Aspbom1	[14]
<i>Aspergillus</i>	<i>sojae</i>	<i>Flavi</i>	-	[3, 24-25]



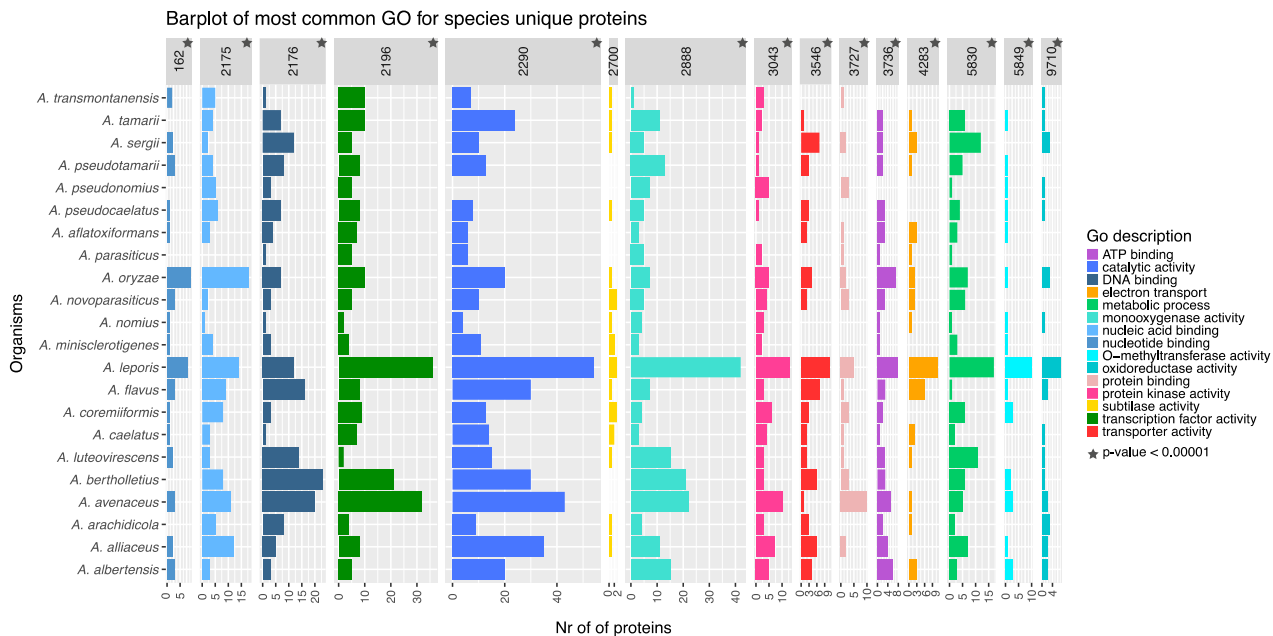
Supplementary Figure 1 - Phylogenetic tree based on composition vector approach. Phylogenetic tree constructed using CVTree²⁶-²⁷making alignment free whole genome based phylogenetic trees. This is created using the whole genome and the K'mer was set to 18.



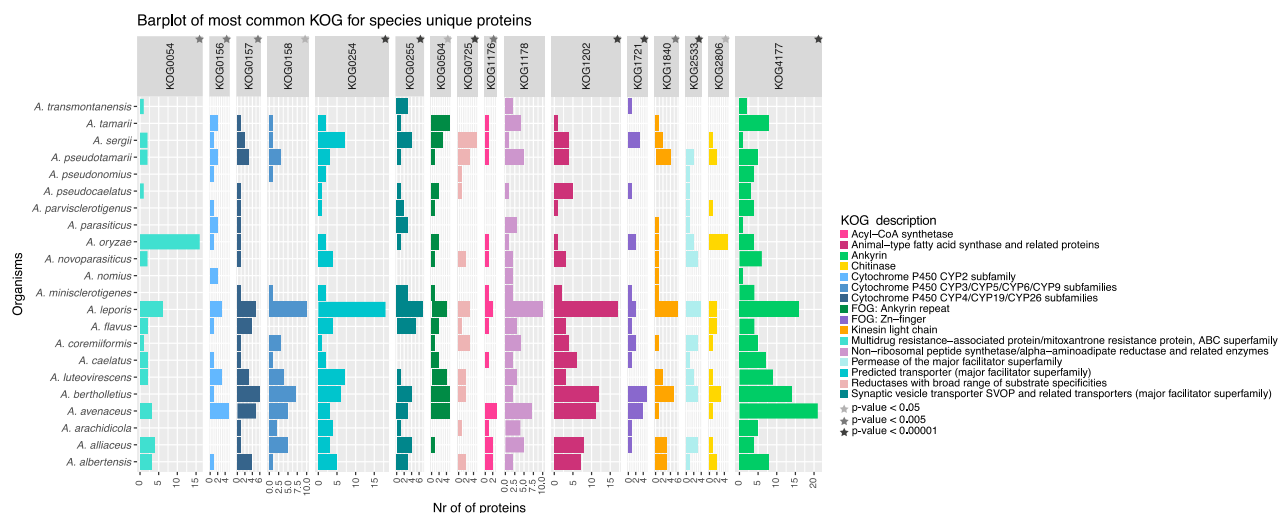
Supplementary Figure 2 Phylogenetic tree based on composition vector approach. Phylogenetic tree constructed using CVTree²⁶⁻²⁷ making alignment free whole genome based phylogenetic trees. This is created using the whole proteome and the K'mer was set to 8.



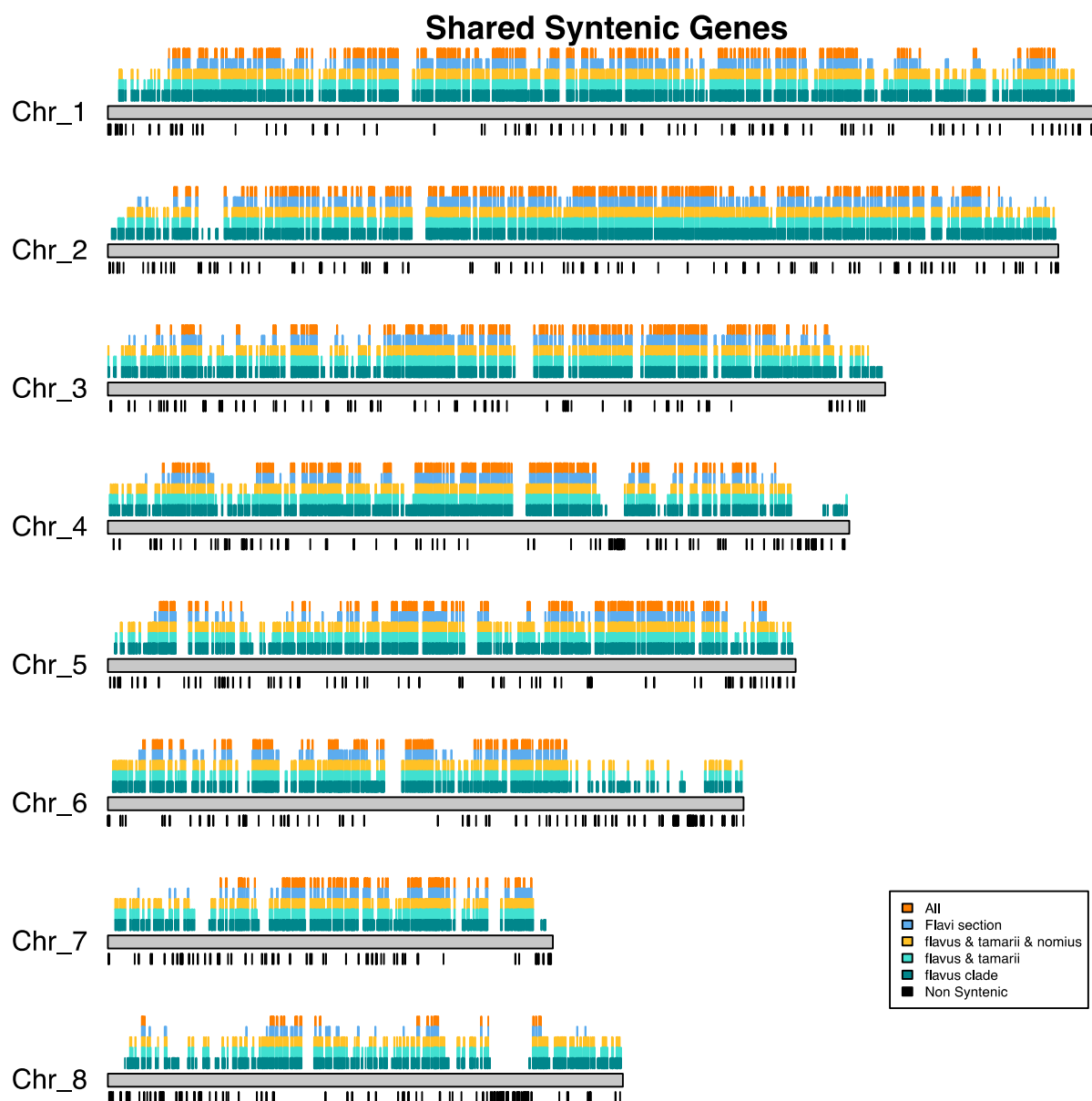
Supplementary Figure 3 - Most common InterPro domains in species unique proteins. Bar plot showing the number of species unique proteins with an InterPro domain per species, shown for the most common InterPro annotations³². Light grey star indicates p-values below 0.005 and dark grey star indicates p-value below 0.00001 of enrichment in the species unique genes for the specific functional domain (for both Fisher's exact and Chi-squared test).



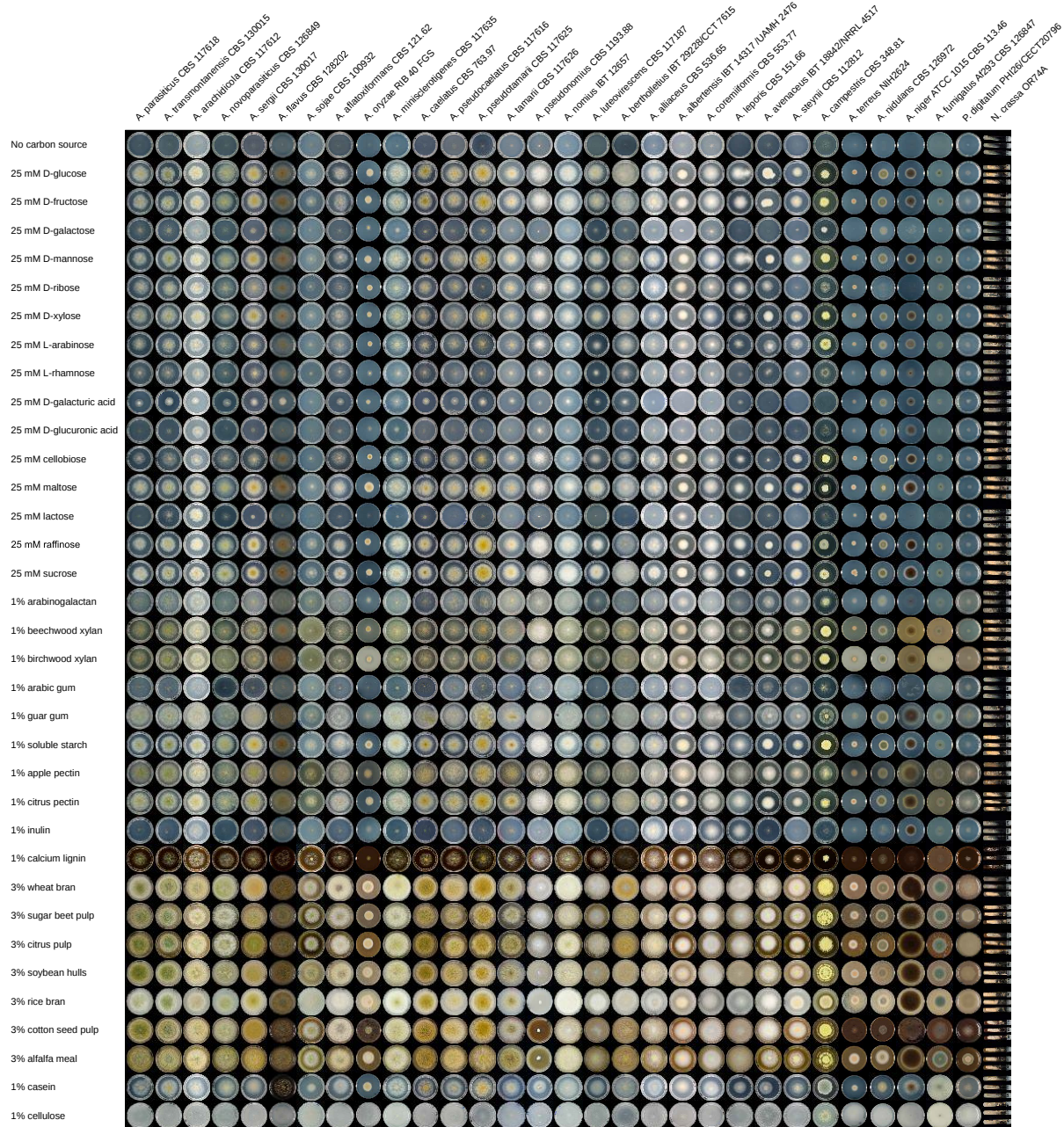
Supplementary Figure 4 - Most common GO domains in species unique proteins. Bar plot showing the number of species unique proteins with a GO domain per species, shown for the most common GO annotations³⁴. Dark grey star indicates p-value < 0.00001 of enrichment in the species unique genes for the specific functional domain (for both Fisher's exact and Chi-squared test).



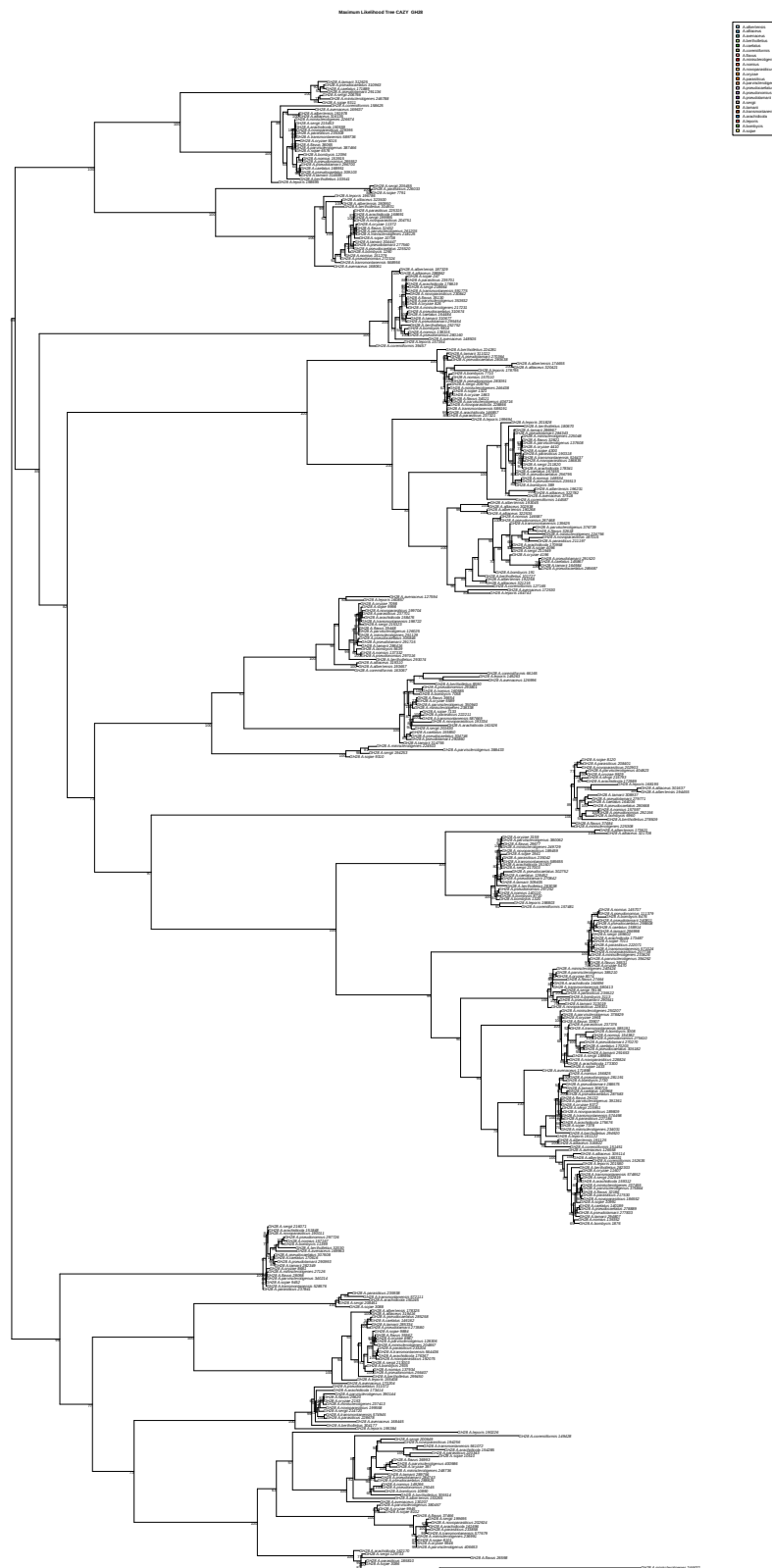
Supplementary Figure 5 - Most common KOG domains in species unique proteins. Bar plot showing the number of species unique proteins with an KOG domain per species, shown for the most common KOG annotations³⁵. P-values indicates enrichment of a certain KOG term in the species unique genes for the specific functional domain. The p-values are indicated by stars of light to dark grey; the lightest star indicates p-values < 0.05, followed by p-values < 0.005 and the darkest stars indicates p-value < 0.00001 (for both Fisher's exact and Chi-squared test).



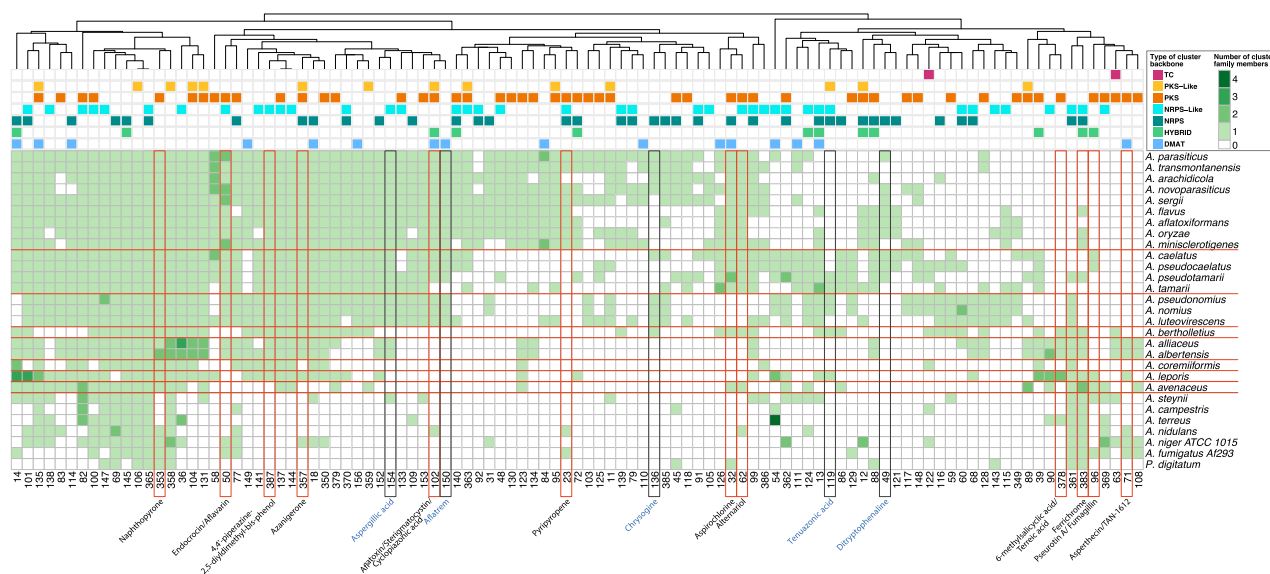
Supplementary Figure 6 - Syntenic genes mapped to the *A. oryzae* genome. The grey bars represent the *A. oryzae* genome. Syntenic genes were found using *A. oryzae* as reference comparing all the other species to this genome. Above the chromosomes syntenic genes shared with all species in a certain group is shown. Dark turquoise - *A. flavus* clade; light turquoise - *A. flavus* and *A. tamarai* clade; yellow - *A. flavus*, *A. tamarai* and *A. nomius* clade; blue - Flavi section; orange - Flavi section, *A. nidulans* and *A. fumigatus*. Below the chromosome the non-syntenic genes, found to be non-syntenic in all the species, are shown in black.



Supplementary Figure 7 - Growth analysis of 23 Flavi species plus 8 additional species on 35 different growth media.

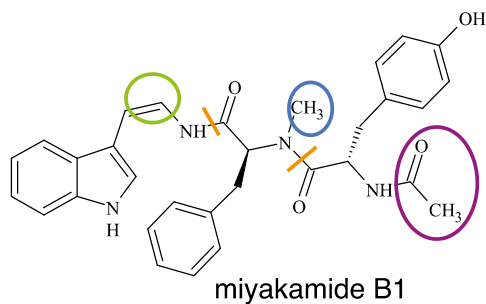


Supplementary Figure 8 - Phylogenetic tree of GH28. Phylogenetic tree of all proteins assigned to the GH28 CAZyme family. The GH28 family consists of polygalacturonase. Alignment of the members of GH28 CAZyme family found in all section *Flavi* species was created using clustalo. The ML phylogenetic tree was created using the ape package in R⁹².

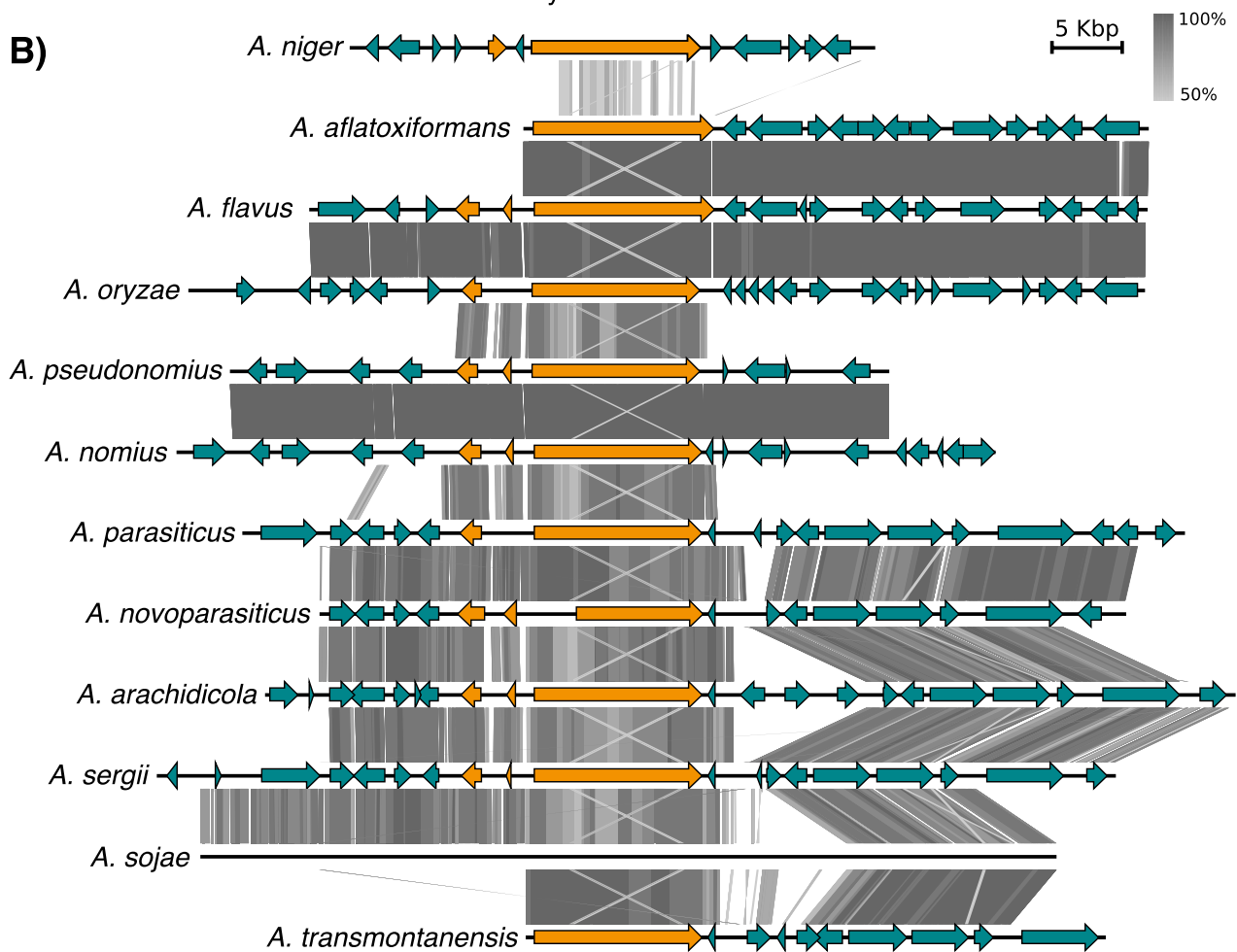


Supplementary Figure 9 - Heatmap of secondary metabolite cluster families. SM cluster families with members in at least five species are illustrated by a heatmap. The top rows indicate the backbone enzymes found within the SM cluster family. Compounds with similar SM clusters are added from the dereplication using MIBiG marked by orange boxes and black text in addition to manually curated compounds (marked by black boxes and blue text). Aspergillilic acid⁹³, aflatrem⁹⁴, chrysogine⁹⁵, tenuazonic acid⁹⁶, ditryptophenaline⁹⁷.

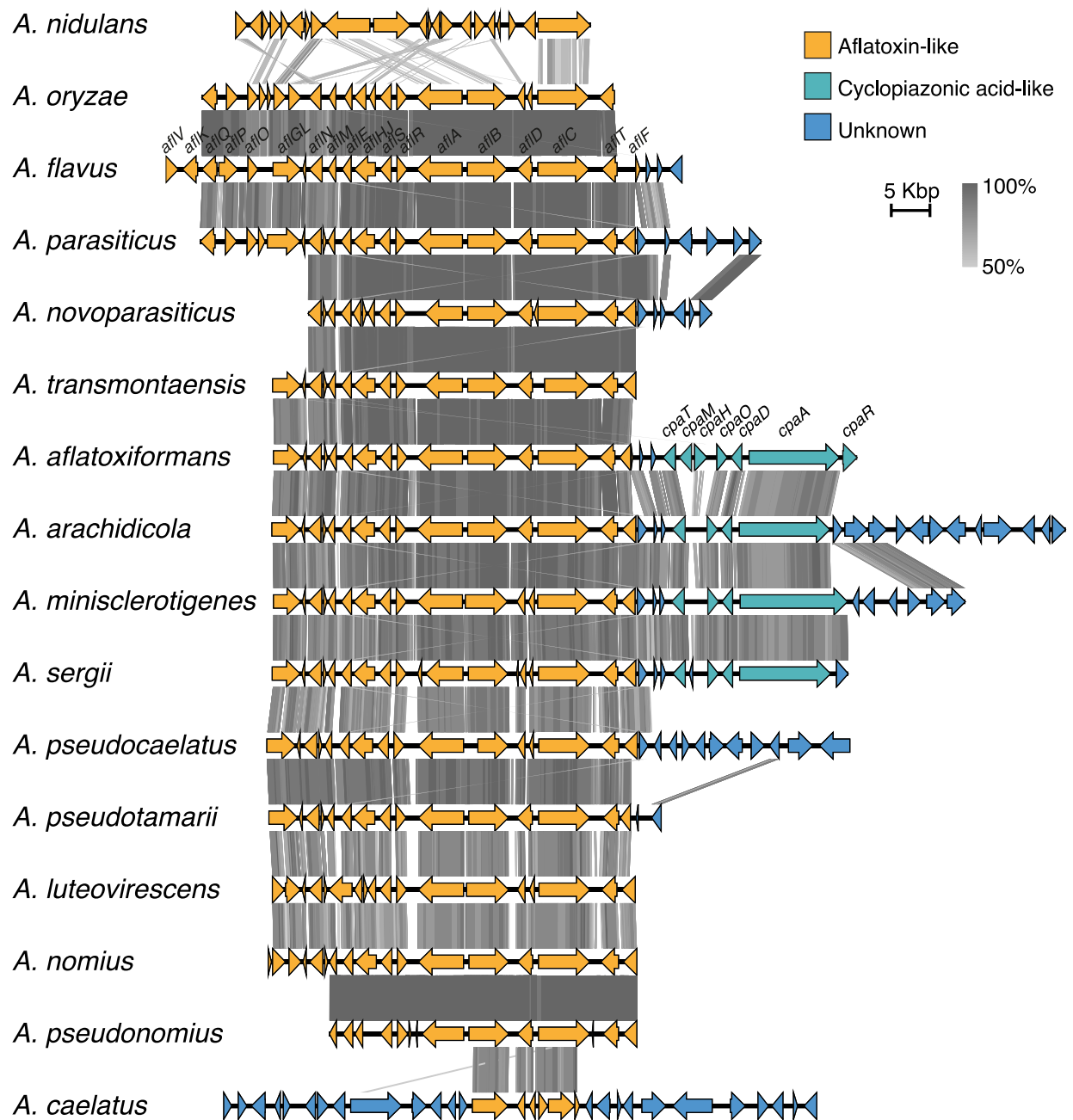
A)



B)



Supplementary Figure 10 - Miyakamide and putative clusters. A) Miyakamide B1 showing the three amino acid parts (orange line), the acetylation (purple circle), the decarboxylation (green circle) and the N-methylation (blue circle). B) Syntenic plot of the putative miyakamide cluster family plus surrounding genes. The syntenic plot was generated using EasyFig⁸⁶ with minimum length set to 50 bp and minimum identity to 50%. The genes potentially involved in miyakamide production are marked by orange.



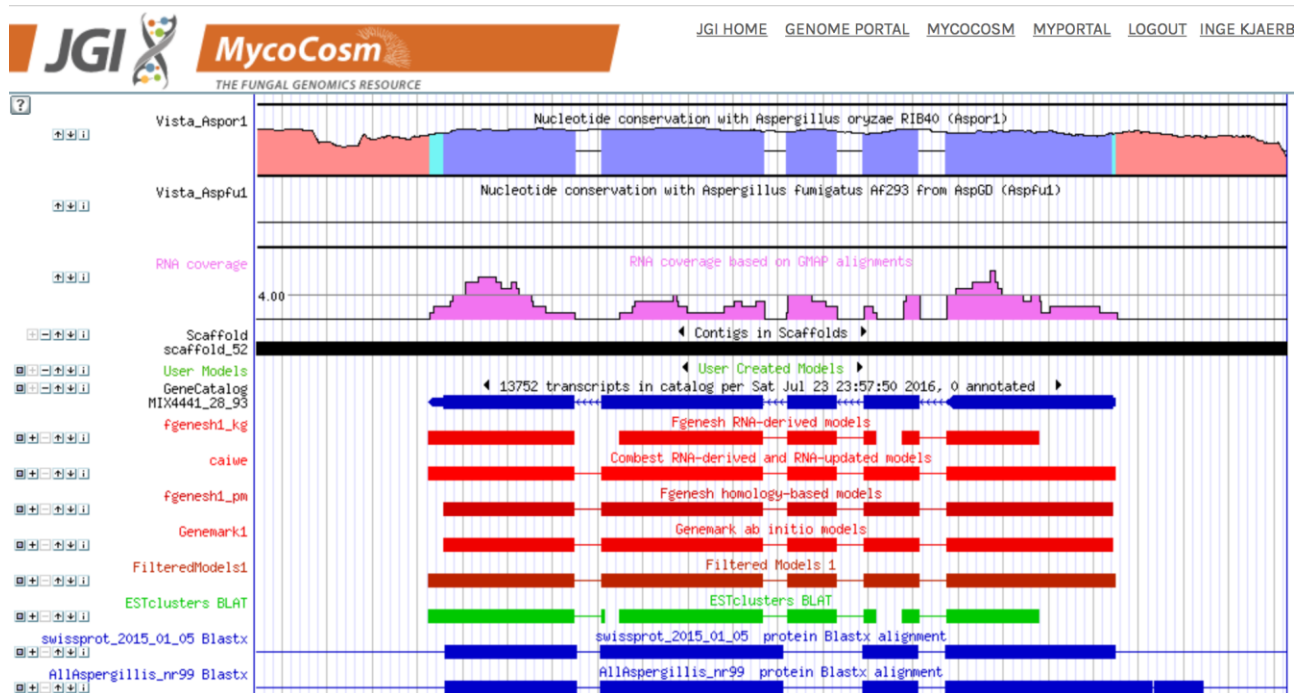
Supplementary Figure 11- Synteny of aflatoxin cluster family. Syntenic plot of the predicted clusters belonging to the 'sterigmatocystin - aflatoxin - cyclopiazonic-acid' cluster family. The synteny plot was generated using EasyFig⁸⁶ with minimum length set to 50 bp and minimum identity to 50%. The genes are color coded based on known pathways; aflatoxin (orange), cyclopiazonic-acid (turquoise) and unknown (blue).

CLUSTAL O(1.2.4) multiple sequence alignment

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Aspara19utr_161418	ihgvedtdkvtmmehdirrnpvrgadvyllrsilhdypdaacveilsnvtamdpsksr	349
Asppar1_236245	ihgvedtdkvtmmehdirrnpvrgadvyllrsilhdypdaacveilsnvtamdpsksr	341
Aspnovo1_191948	ihgvedtdkvtmmehdirrnpvrgadvyllrsilhdypdaacveilsnvtamdpsksr	350
	* **::*:***:*****: **::*****:*****:*****:*****	
Aspf11_36747	illdemimpdllaqdsqrfmngidmtvvltlngkerstkewnslitvmdnrletekiwwr	393
Aspbom1_5312	illdemvmpdllaqdsqrfmngidmtvvltlngkerstkewdsliatveggkletekiwwr	405
Asppsec1_279719	illdemvvpdllaqdsqrfmngidmtvvltlngkerstkewdsliatvegggleiektwwr	410
Asppset1_276010	illdemvvpdllaqdsqrfmngidmtvvltlngkerstkewdsliatvegggleiektwwr	410
AsojaeX_7236	illdemimpdllaqdsqrfmngidmtvvltlngkers-kewnsli-tvdgrletekiwwr	397
Aspnom13137_1_6258	illdemimpdllaqdsqrfmngidmtvvltlngkersvkewdsliatveggkletekiwwr	401
Aspnom1_129446	illdemvvpdllaqdsqrfmngidmtvvltlngkersakewdsliatveggkletekiwwr	401
Asppsen1_274161	illdemvvpdllaqdsqrfmngidmtvvltlngkersakewdsliatveggkletekiwwr	401
Aspser1_195135	illdemimpdllaqdsqrfmngidmtvvltlngkerstkewdsliatveggkletekiwwr	410
Aspmin1_217119	illdemimpdllaqdsqrfmngidmtvvltlngkerstkewdsliatveggkletekiwwr	401
Asptral_544984	illdemimpdllaqdsqrfmngidmtvvltlngkerstkewdsliatveggkletekiwwr	401
Aspor1_5677	illdemimpdllaqdsqrfmngidmtvvltlngkersokewdsliatveggkletekiwwr	410

Asppar1_400163	illdemimpdllaqdsqrfrmnqidmtvltlngkerspkewnslitmvdgrletekiwvr	410
Aspara19utr_161418	illdemimpdllaqdsqrfrmnqidmtvltlngkerstkewnsnittvdgrletekiwvr	409
Asppar1_236245	illdemimpdllaqdsqrfrmnqidmtvltlngkerstkewnsnittvdgrletekiwvr	401
Aspnovo1_191948	illdemimpdllaqdsqrfrmnqidmtvltlngkerstkewnsnittvdgrletekiwvr	410
	*****: ** : ** ***** ***** ***** : ** * : ** *	
Aspf11_36747	kgeegshwgvqqlrlrk*----	410
Aspbom1_5312	qgeegshwgvqqlrlrgnsak*	426
Asppsec1_279719	kgeegshwgvqqlrlrgnsak*	431
Asppset1_276010	kgeegshwgvqqlrlrgnsak*	431
AsojaeX_7236	kgeegshwgvqqlrlrk*----	414
Aspnom13137_1_6258	kgeegshwgvqqlrlhgssad*	422
Aspnom1_129446	kgeegshwgvqqlrlhgslan*	422
Asppsen1_274161	kgeegshwgvqqlrlhgslan*	422
Aspser1_195135	kgeegshwgvqqlrlrk*----	427
Aspmin1_217119	kgeegshwgvqqlrlrk*----	418
Asptral_544984	kgeegshwgvqqlrlrk*----	418
Aspor1_5677	kgeegshwgvqqlrlrk*----	427
Asppar1_400163	kgeegshwgvqqlrlrk*----	427
Aspara19utr_161418	kgeegshwgvqqlrlrk*----	426
Asppar1_236245	kgeegshwgvqqlrlrk*----	418
Aspnovo1_191948	kgeegshwgvqqlrlrk*----	427
	:*****:	



Supplementary Figure 13 - Gene prediction of *aflP*. An overview of the gene prediction in the *aflP* gene in *A. parasiticus* and the RNA coverage from JGI at MycoCosm.

Supplementary Data

Supplementary Data 1 - Quantitative growth analysis of 23 *Flavi* species plus 8 additional species on 35 different growth media, quantitated by growth from 0-10, normalized based on growth on 1% glucose.

Supplementary Data 2 - CAZyme content in section *Flavi*. Overview of the CAZyme content and plant degradation related CAZyme content.

Supplementary Data 3 - Secondary metabolite gene clusters section *Flavi*. Long format table with all the predicted clusters in the species and the cluster family they belong to. Column 1 - Species, column 2 - JGI protein id of the predicted backbone, column 3 - type of the predicted backbone, column 4 - size of the cluster, column 5 - cluster family number.

Supplementary Data 4 - Compounds produced by *Flavi* species after growth on CYA 7 days.