

Applied Microbiology and Biotechnology

Supporting Information

for

**Evolution and Enrichment of
CYP5035 and CYP5136 in
Polyporales: Functionality of an
understudied P450 family**

Nico D. Fessner,¹ David R. Nelson² and Anton Glieder^{1,*}

1 Institute of Molecular Biotechnology, Graz University of Technology, NAWI Graz, Petersgasse 14/3, Austria

2 Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, TN 38163, USA

* corresponding author: Anton Glieder

E-mail: a.glieder@tugraz.at

phone: (+43)-316-873-4074

Fax: (+43)-316-873-9302

Table S1: Displayed are all CYP5035 expressed here and in prior literature so far together with their tested substrate tolerance, host fungus and corresponding reference.

Reference	Fungus	CYP5035...	Sequence ID	Substrate
10.1016/j.bbrc.2011.02.121	<i>Phanerochaete chrysosporium</i>	A1	7306	naproxen
		A2	138612	naproxen
		A3	8961	?
		A4	8962	?
		A5	138737	flavone
		A6	5333	?
		A7	8949	?
		B1	–	?
		B2	8912	naproxen & abietic acid
		B3	6048	?
		C1	9198	?
		D1	–	?
		E1	5317	?
10.1007/s00203-011-0753-2	<i>Postia placenta</i>	F1	112190	?
		F2	129155	?
		F3v1	89499	?
		F3v2	89499	?
This study	<i>Polyporus arcularius</i>	H2	665169	(<i>EZ</i>)-citral, <i>p</i> -cymene, indole
		N5	196845	?
		N6	521854	?
		S6	652223	(<i>E</i>)-stilbene
		S7	664247	multi-functional
		S8	665466	?
		S9	668252	(<i>E</i>)-stilbene, (<i>EZ</i>)-citral, <i>p</i> -cymene
		AU1	519317	?
		AV1	667965	?

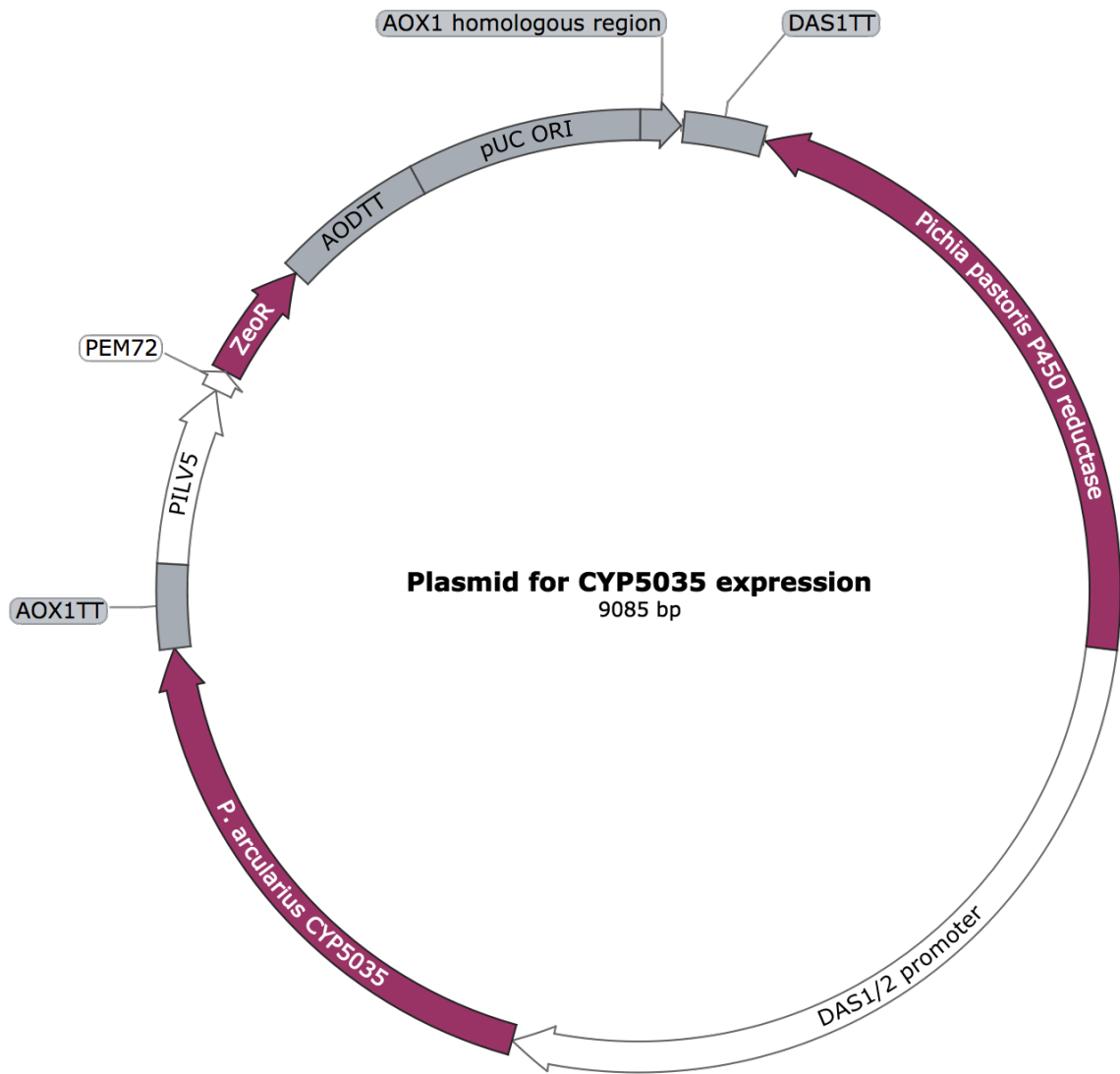


Fig. S1: Plasmid used for the coexpression of the CYP5035 enzymes and *P. pastoris*' native P450 reductase enzyme in the yeast.

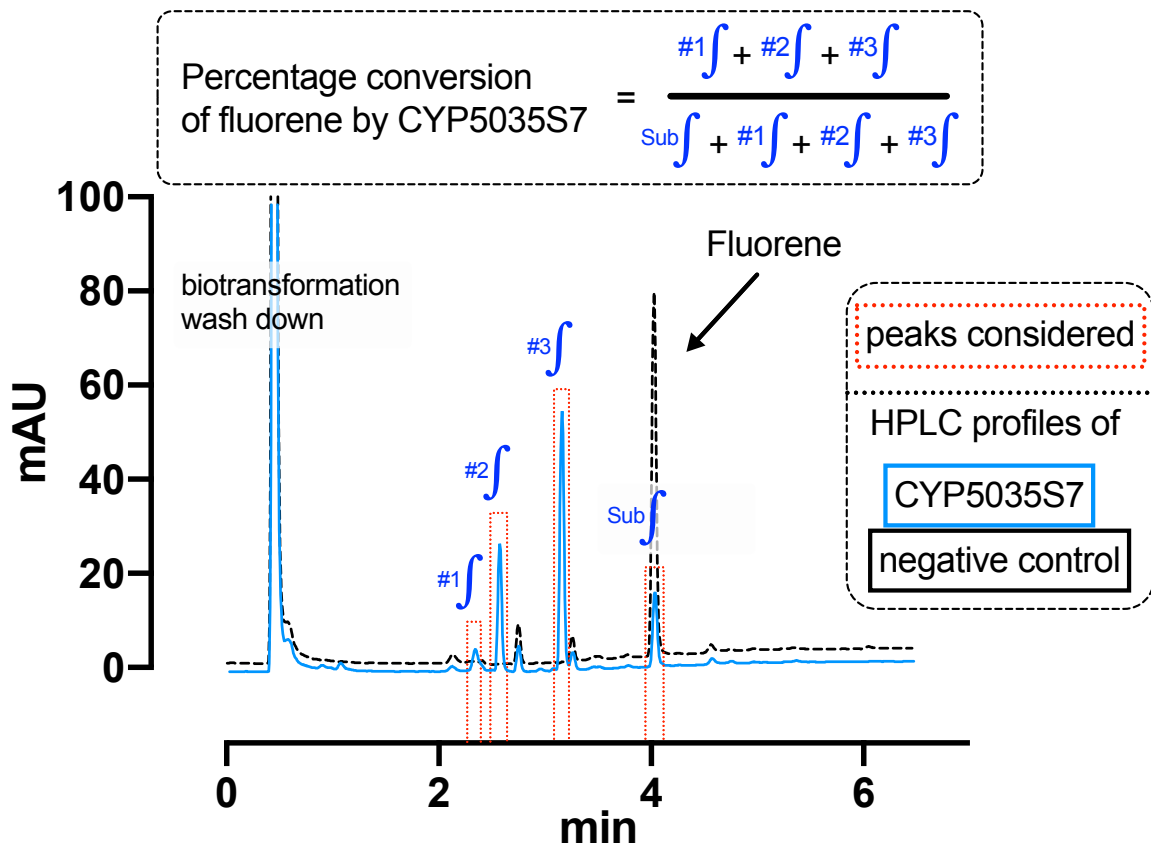


Fig. S2: Displayed are the HPLC biotransformation profiles of CYP5035S7 and the empty vector control to show the general procedure of how the percentage conversions of each compound listed in Fig. 4 were calculated. Peaks were integrated and the fraction of the combined areas of new peaks that were not present in the negative control divided by the former plus the substrate peak area.

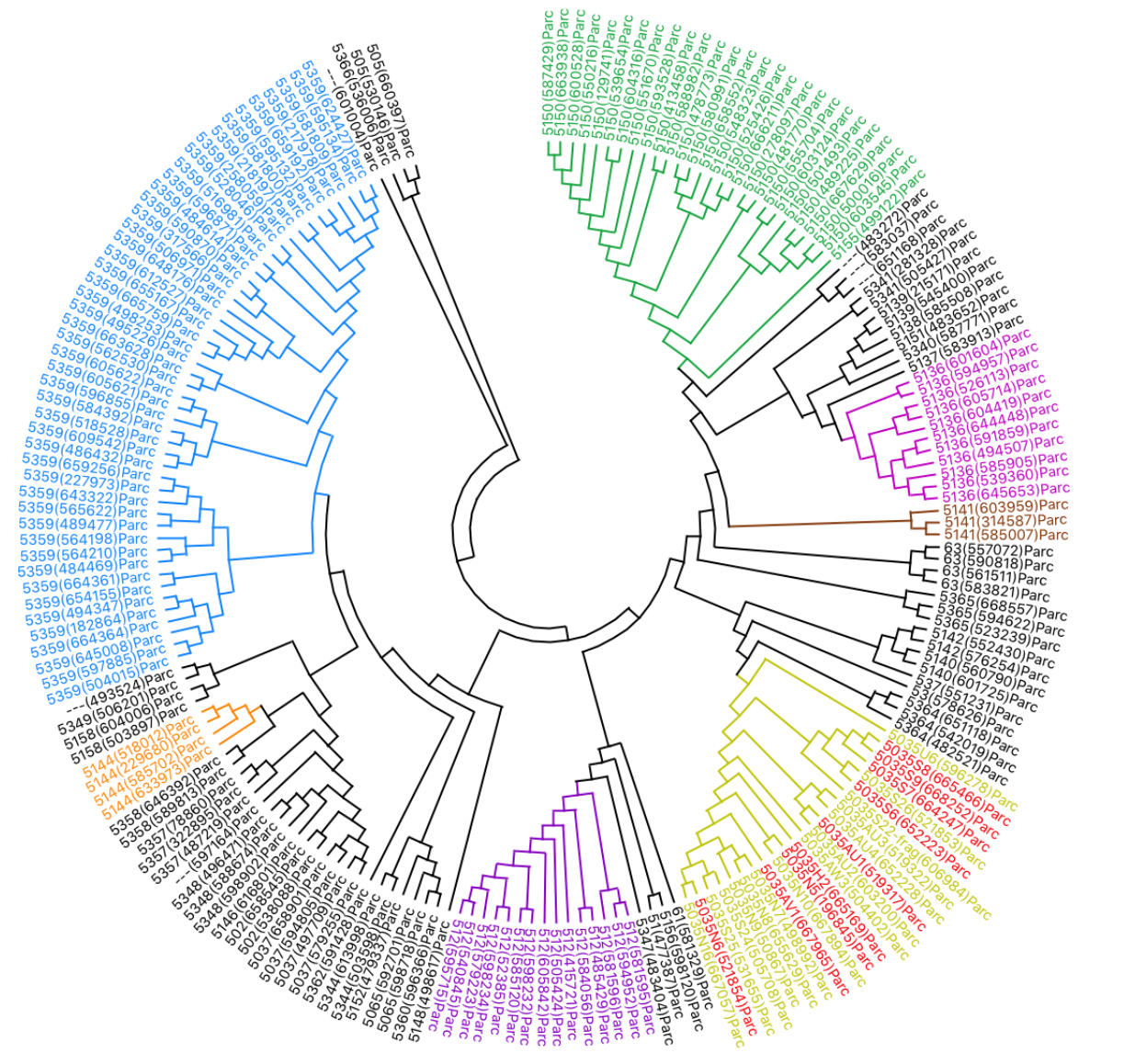


Fig. S3: A minimum evolution tree of the P450ome of *P. arcularius* involving 193 amino acid sequences. CYP512 (purple), CYP5035 (dark yellow), CYP5136 (violet), CYP5141 (brown), CYP5144 (orange), CYP5150 (green) and CYP5359 (blue) have been coloured. The P450 nomenclature of the CYP5035 selected and expressed for a functional screening is shown in red. The tree was constructed using the close-neighbour-interchange algorithm in MEGA X.

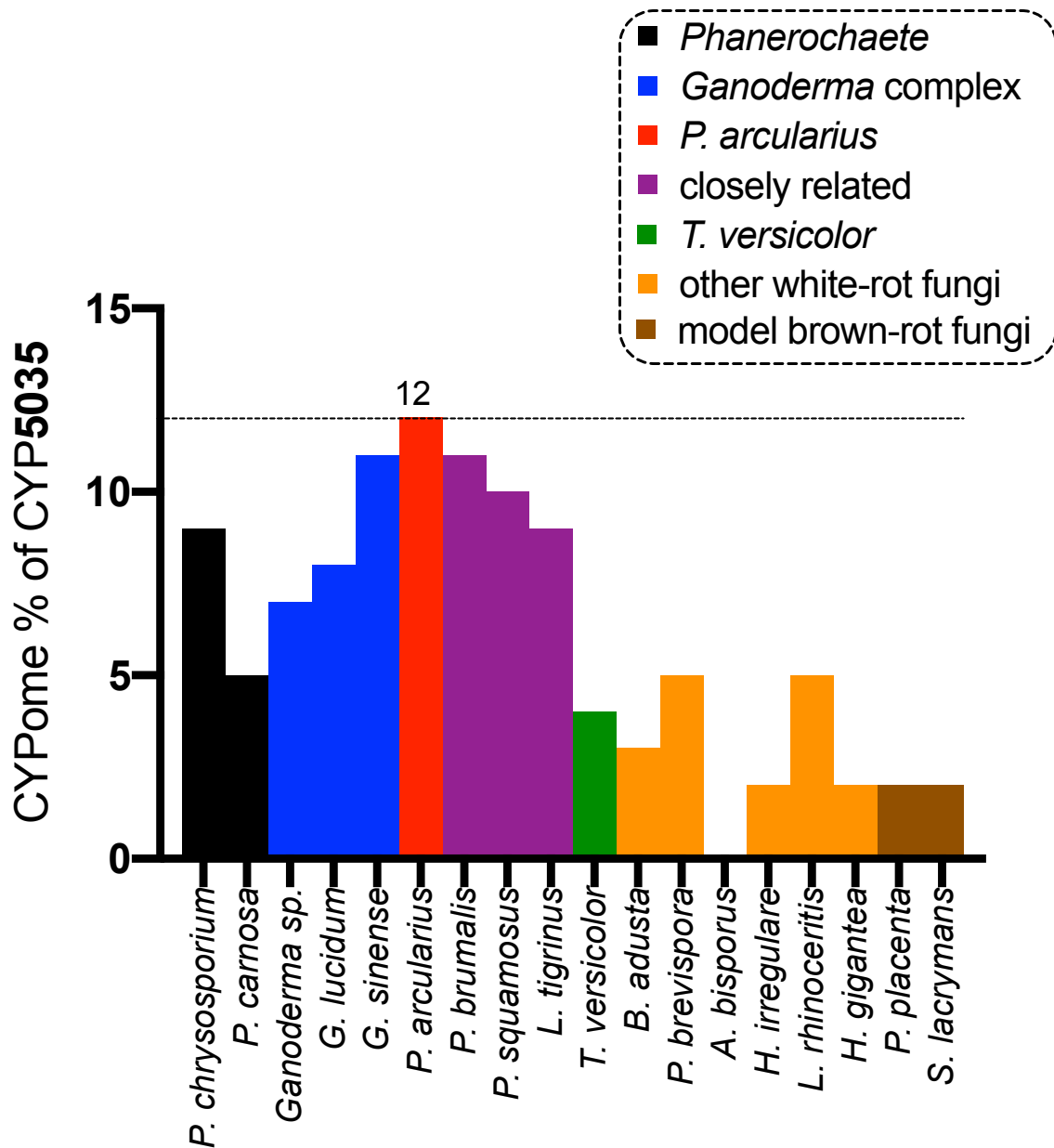


Fig. S4: Comparison of the number of CYP5035s as a percentage of the total number P450s in the genome of *P. arcularius* versus a variety of white- and brown-rot fungi.

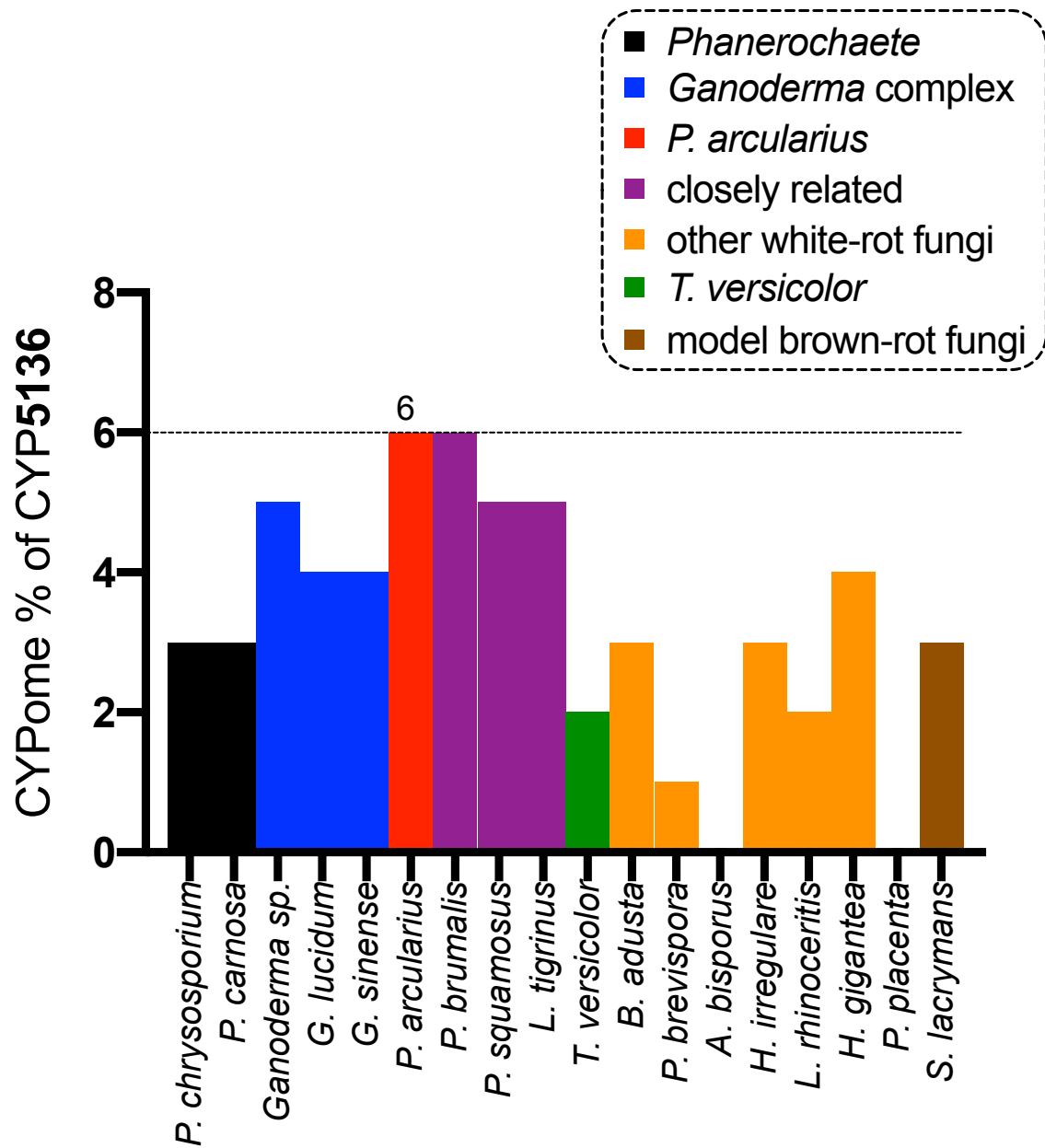


Fig. S5: Comparison of the number of CYP5136s as a percentage of the total number P450s in the genome of *P. arcularius* versus a variety of white- and brown-rot fungi.

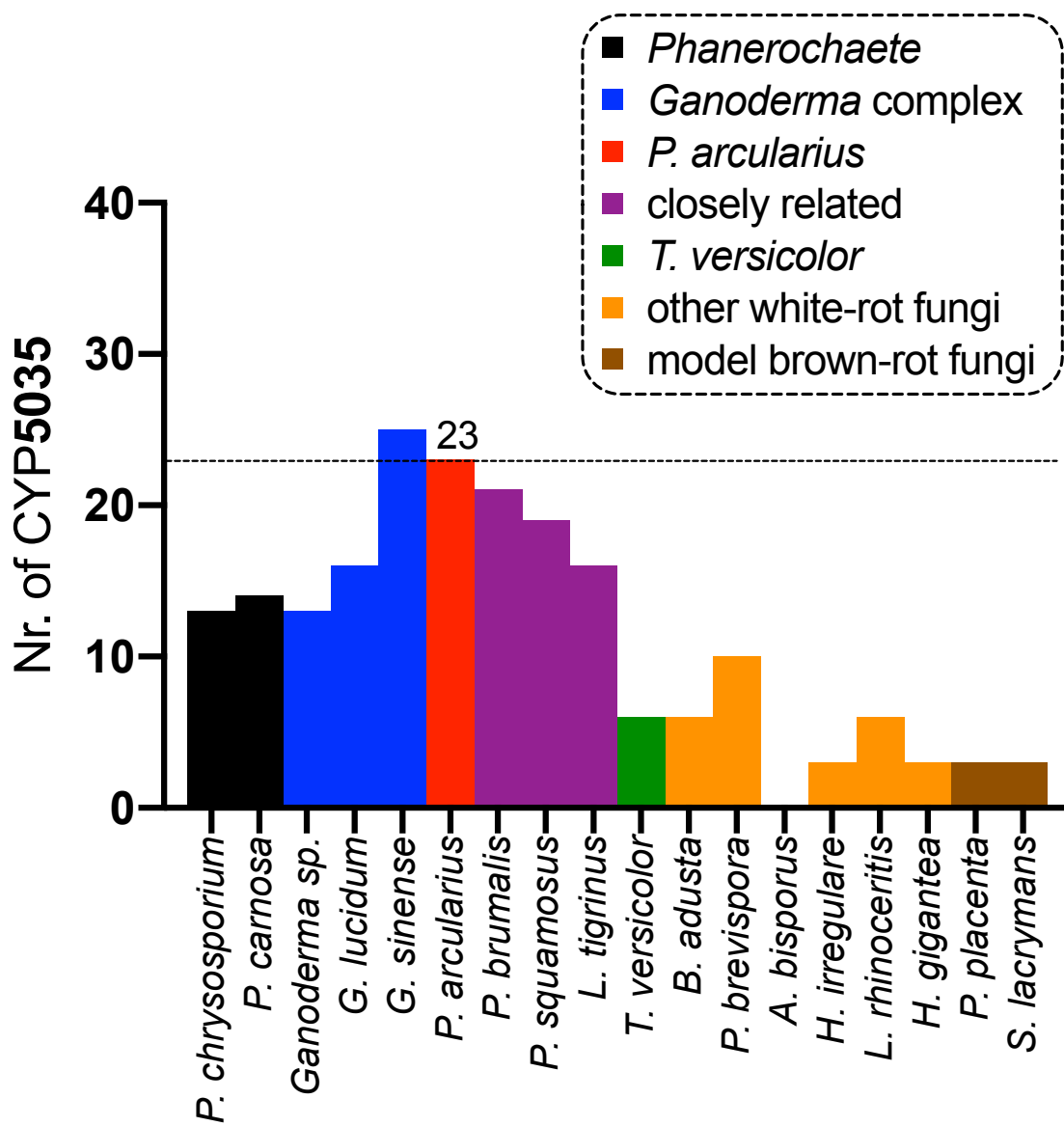


Fig. S6: Comparison of the number of CYP5035 in the genome of *P. arcularius* versus a variety of white- and brown-rot fungi.

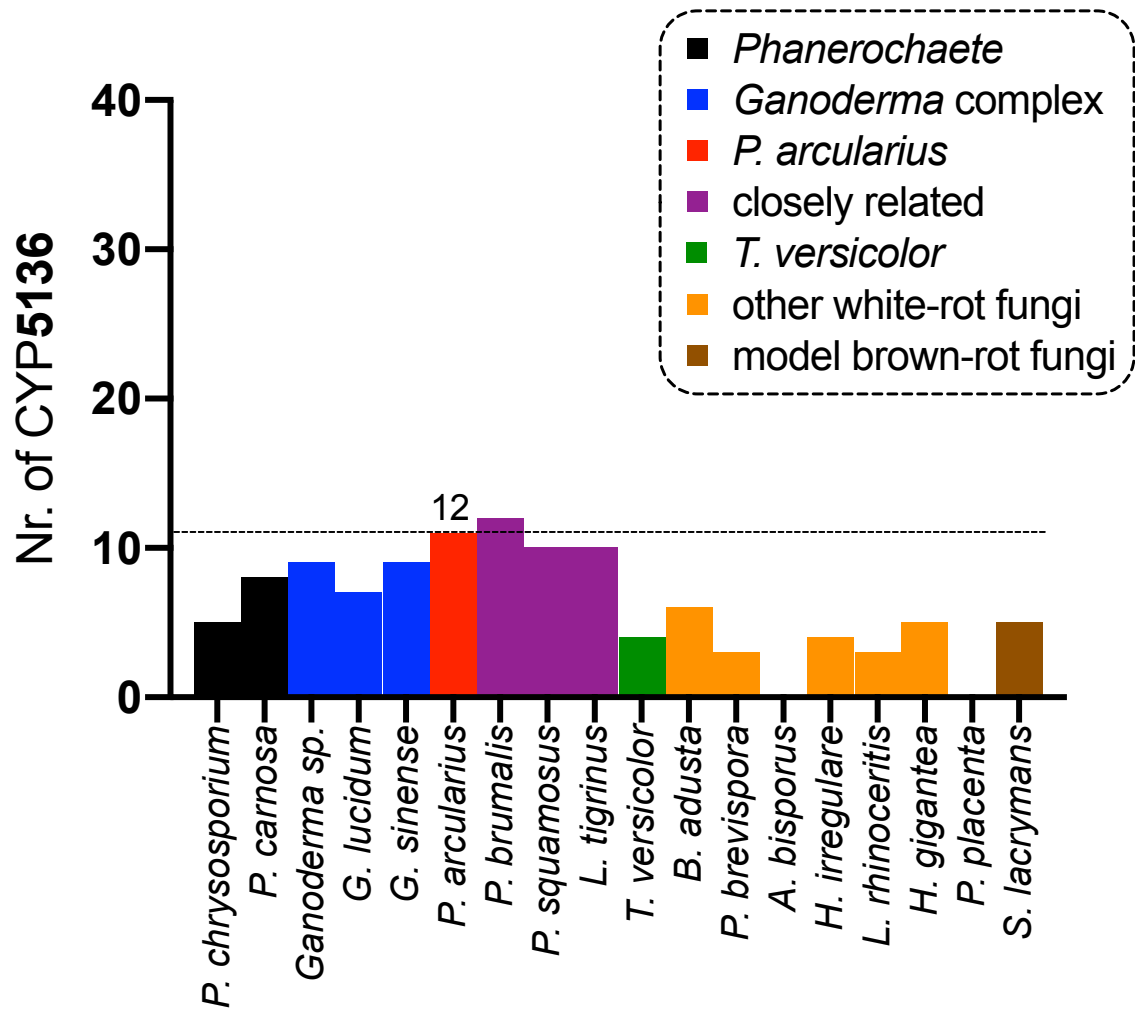


Fig. S7: Comparison of the number of CYP5136 in the genome of *P. arcularius* versus a variety of white- and brown-rot fungi.

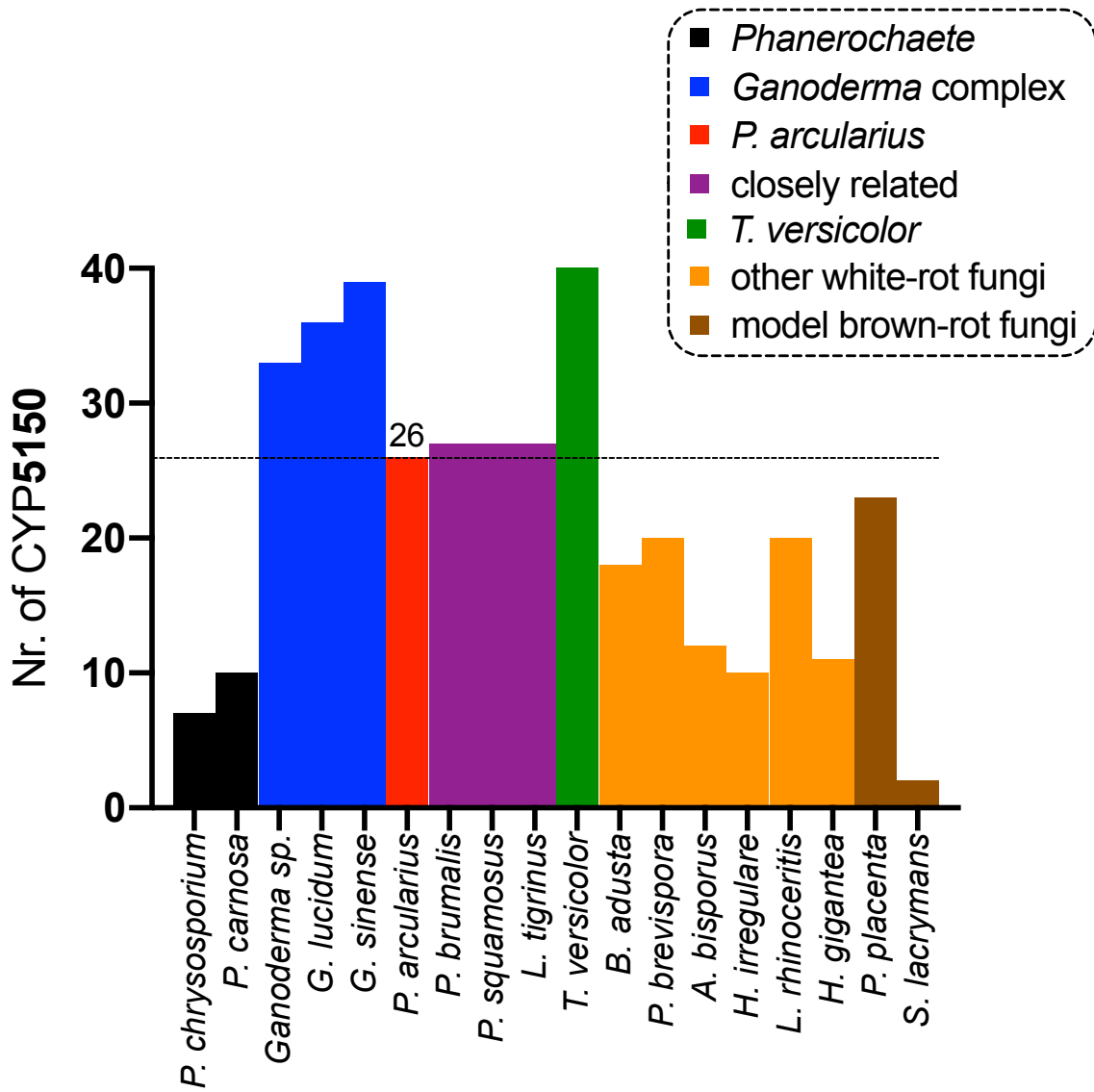


Fig. S8: Comparison of the number of CYP5150 in the genome of *P. arcularius* versus a variety of white- and brown-rot fungi.

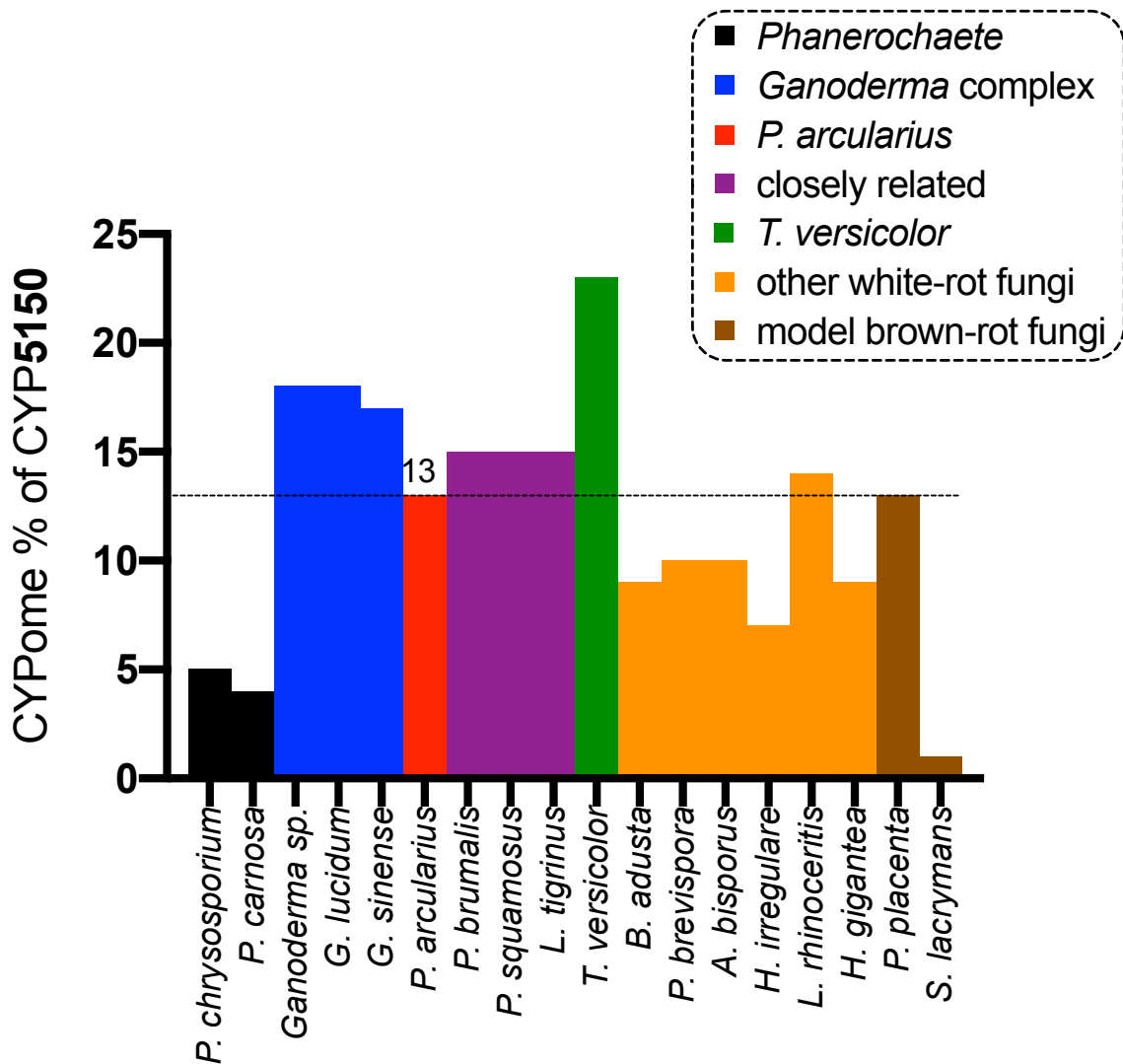


Fig. S9: Comparison of the number of CYP5150s as a percentage of the total number P450s in the genome of *P. arcularius* versus a variety of white- and brown-rot fungi.

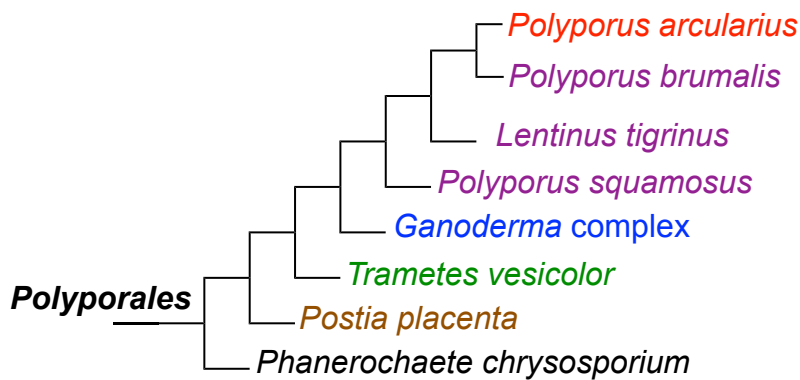


Fig. S10: Rough re-make of the phylogenetic trees drawn in previous studies illustrating the evolutionary distances of different model wood-degrading polypore fungi, and white-rot fungi selected and analysed in this study (Justo and Hibbett 2011; Floudas et al. 2012; Binder et al. 2013).

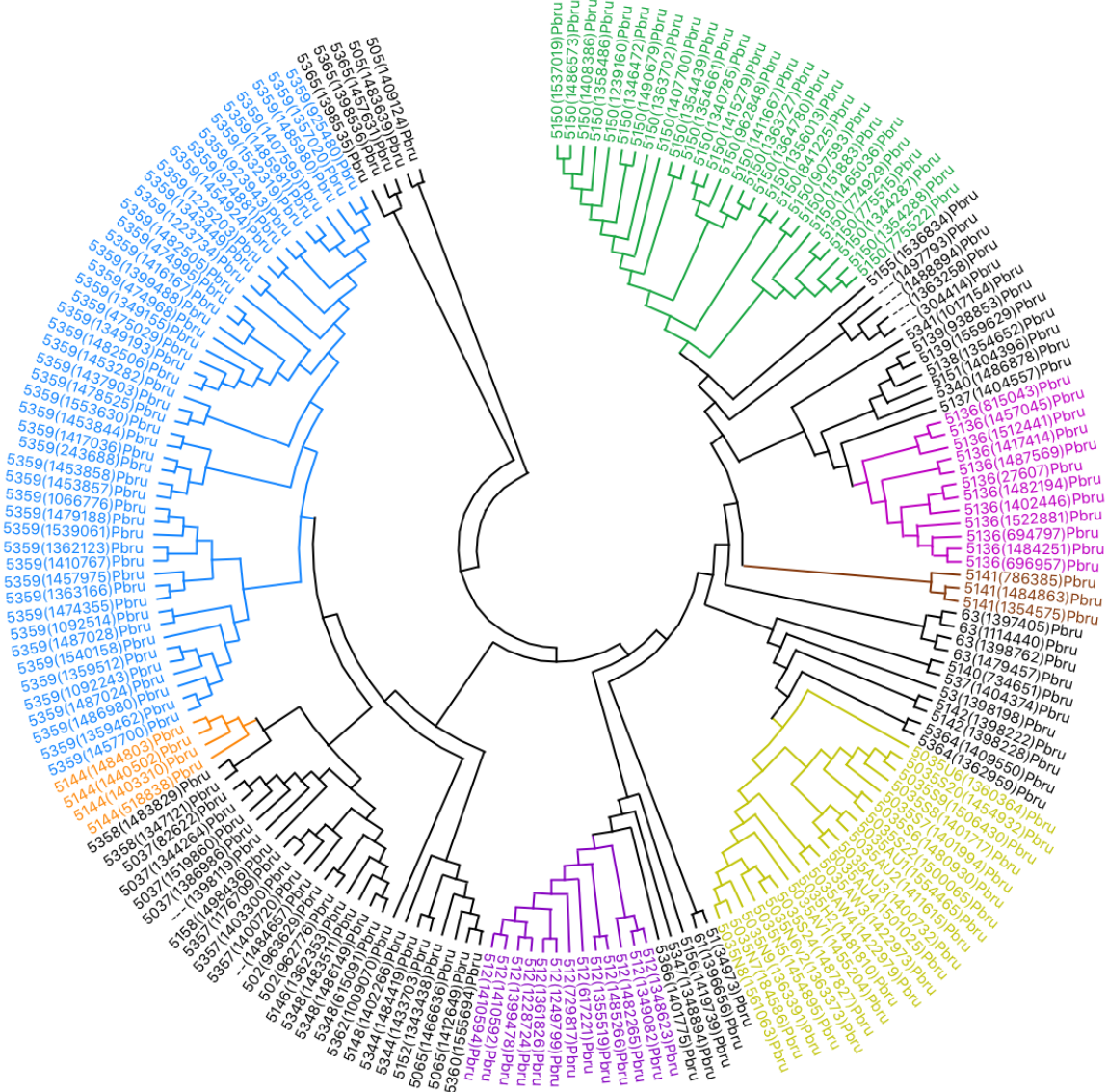


Fig. S11: A minimum evolution tree of the P450ome of *P. brumalis* involving 186 amino acid sequences. CYP512 (purple), CYP5035 (dark yellow), CYP5136 (violet), CYP5141 (brown), CYP5144 (orange), CYP5150 (green) and CYP5359 (blue) have been coloured. The tree was constructed using the close-neighbour-interchange algorithm in MEGA X.

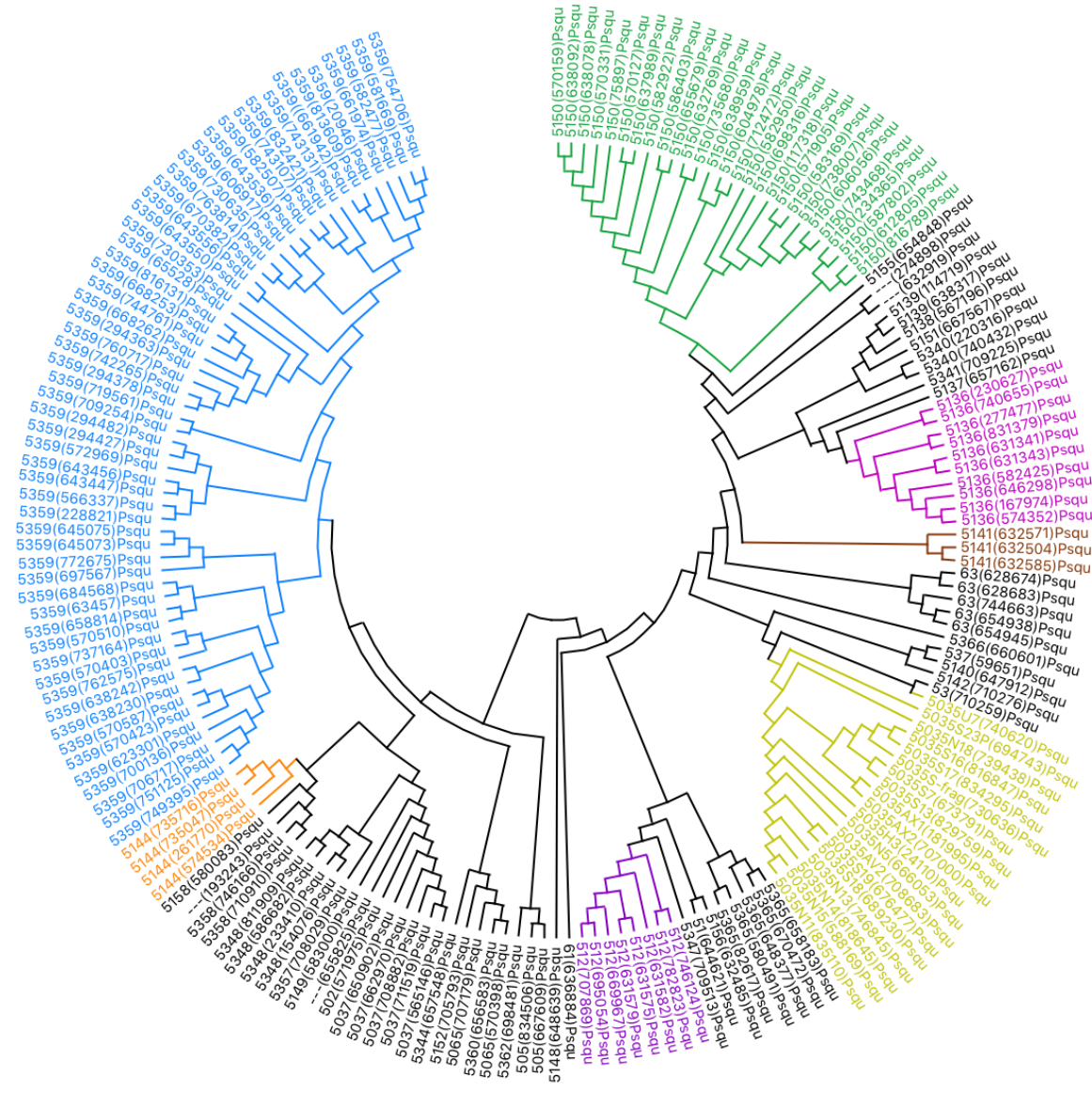


Fig. S12: A minimum evolution tree of the P450ome of *P. squamosus* involving 184 amino acid sequences. CYP512 (purple), CYP5035 (dark yellow), CYP5136 (violet), CYP5141 (brown), CYP5144 (orange), CYP5150 (green) and CYP5359 (blue) have been coloured. The P450 nomenclature of the CYP5035 selected and expressed for a functional screening is shown in red. The tree was constructed using the close-neighbour-interchange algorithm in MEGA X.

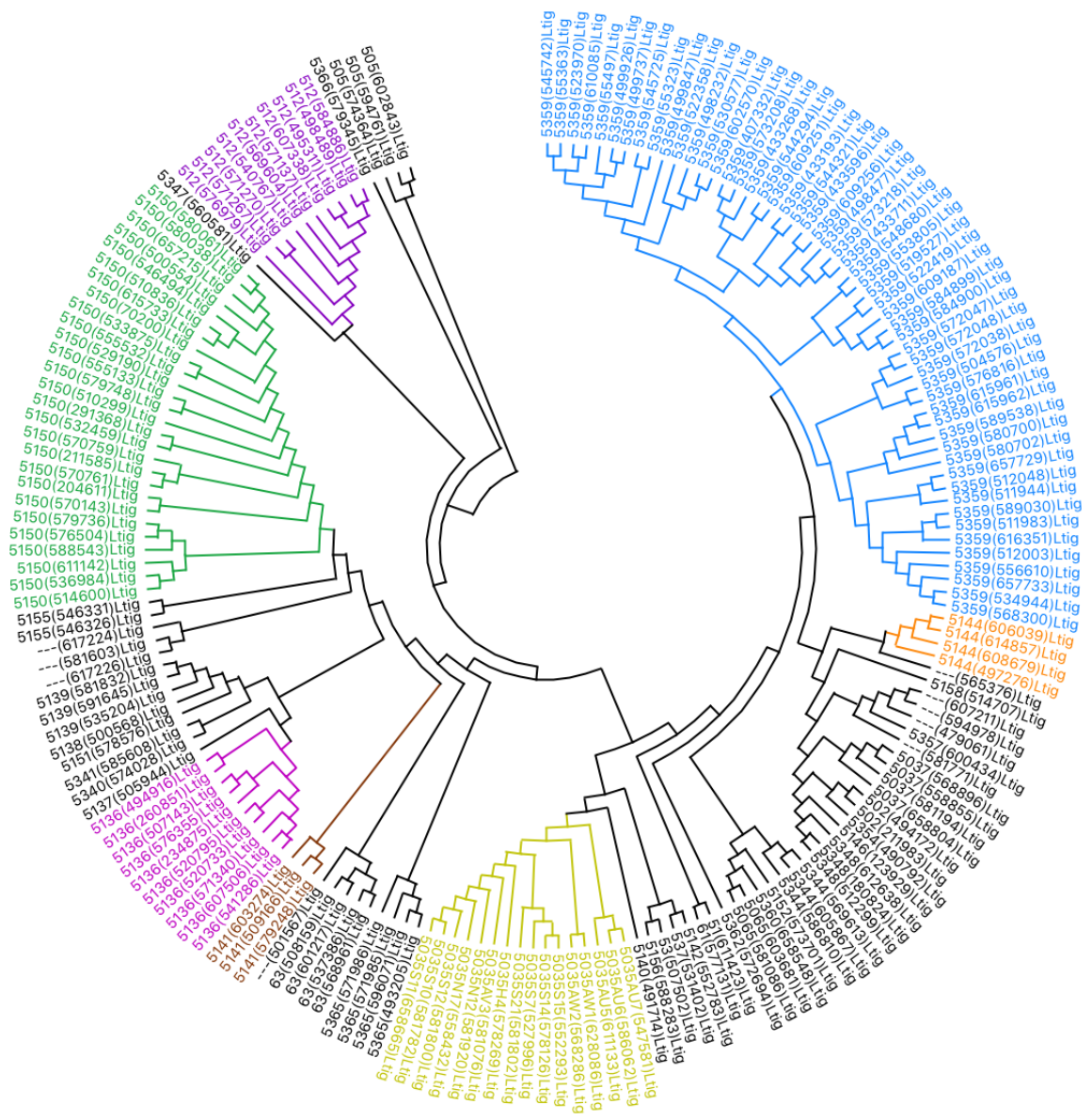


Fig. S13: A minimum evolution tree of the P450ome of *L. tigrinus* involving 184 amino acid sequences. CYP512 (purple), CYP5035 (dark yellow), CYP5136 (violet), CYP5141 (brown), CYP5144 (orange), CYP5150 (green) and CYP5359 (blue) have been coloured. The P450 nomenclature of the CYP5035 selected and expressed for a functional screening is shown in red. The tree was constructed using the close-neighbour-interchange algorithm in MEGA X.

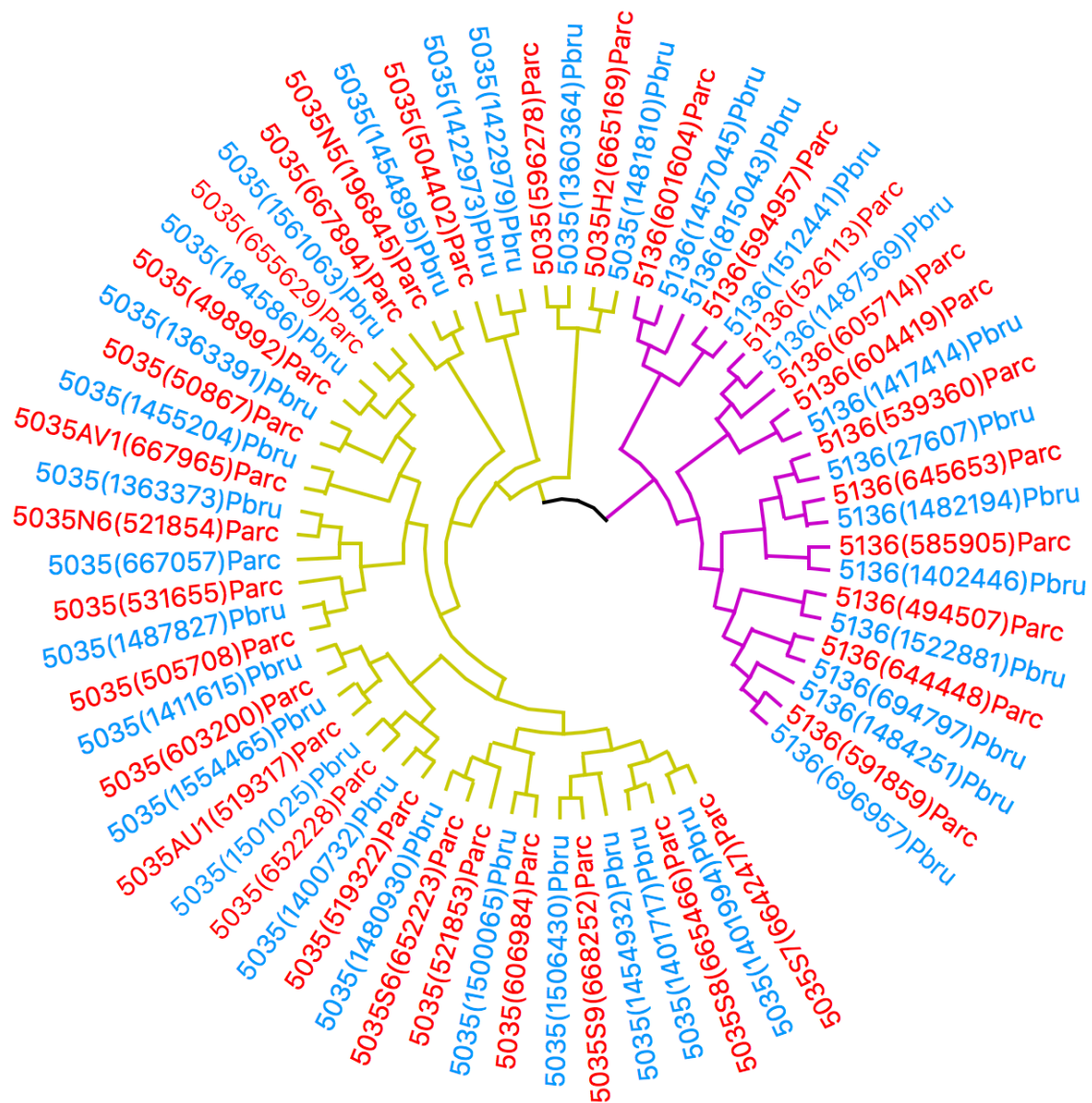


Fig. S14: Displayed is a minimum evolution tree of the CYP5035 and CYP5136 families of *P. arcularius* (Parc; red) and *P. brumalis* (Pbru; blue). Evidence for their close phylogeny is an alternating pattern of red and blue sequences almost throughout the tree. This analysis involved 67 amino acid sequences. The tree was constructed using the close-neighbour-interchange algorithm in MEGA X.

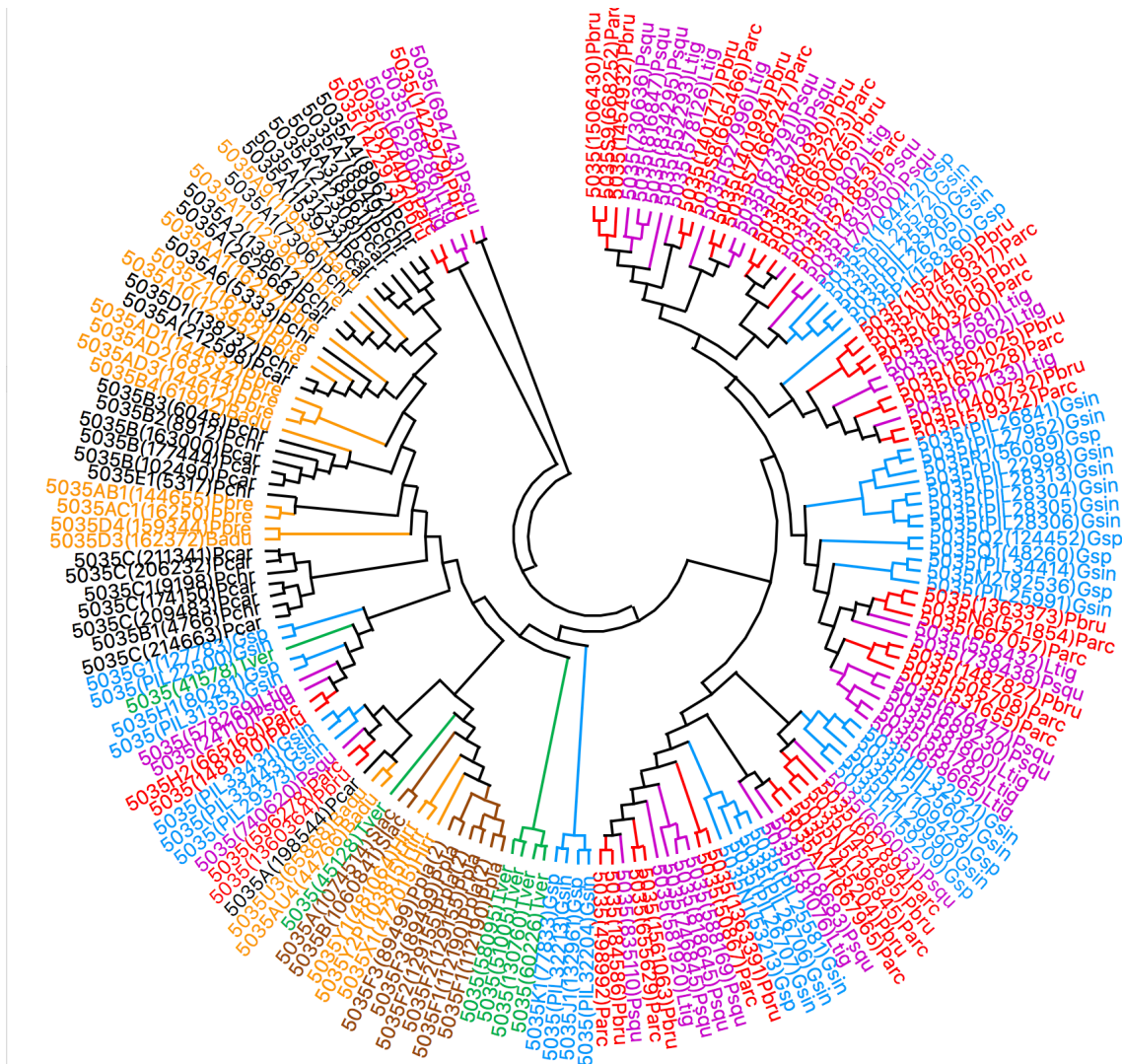


Fig. S15: Displayed is a minimum evolution tree of the CYP5035 family involving 174 amino acid sequences. Phylogeny of CYP5035 enzymes of the fungus *P. arcularius* and *P. brumalis* (Parc and Pbru; red) compared to related species *L. tigrinus* and *P. squamosus* (Ltig and Psqu; violet), and the other model white-rot fungi *Ganoderma* sp. and *G. sinense* (Gsp and Gsin; blue), *T. versicolor* (Tver; green), *P. chrysosporium* and *P. carnosa* (Pchr and Pcar; black), *B. adusta* and *P. brevispora* and *Heterobasidion irregulare* (Badu and Pbre and Hirr; orange) as well as brown-rot fungi *P. placenta* and *Serpula lacrymans* (Ppla and Slac; brown) in order to get an insight into the evolution of this P450 family. The tree was constructed using the close-neighbour-interchange algorithm in MEGA X.

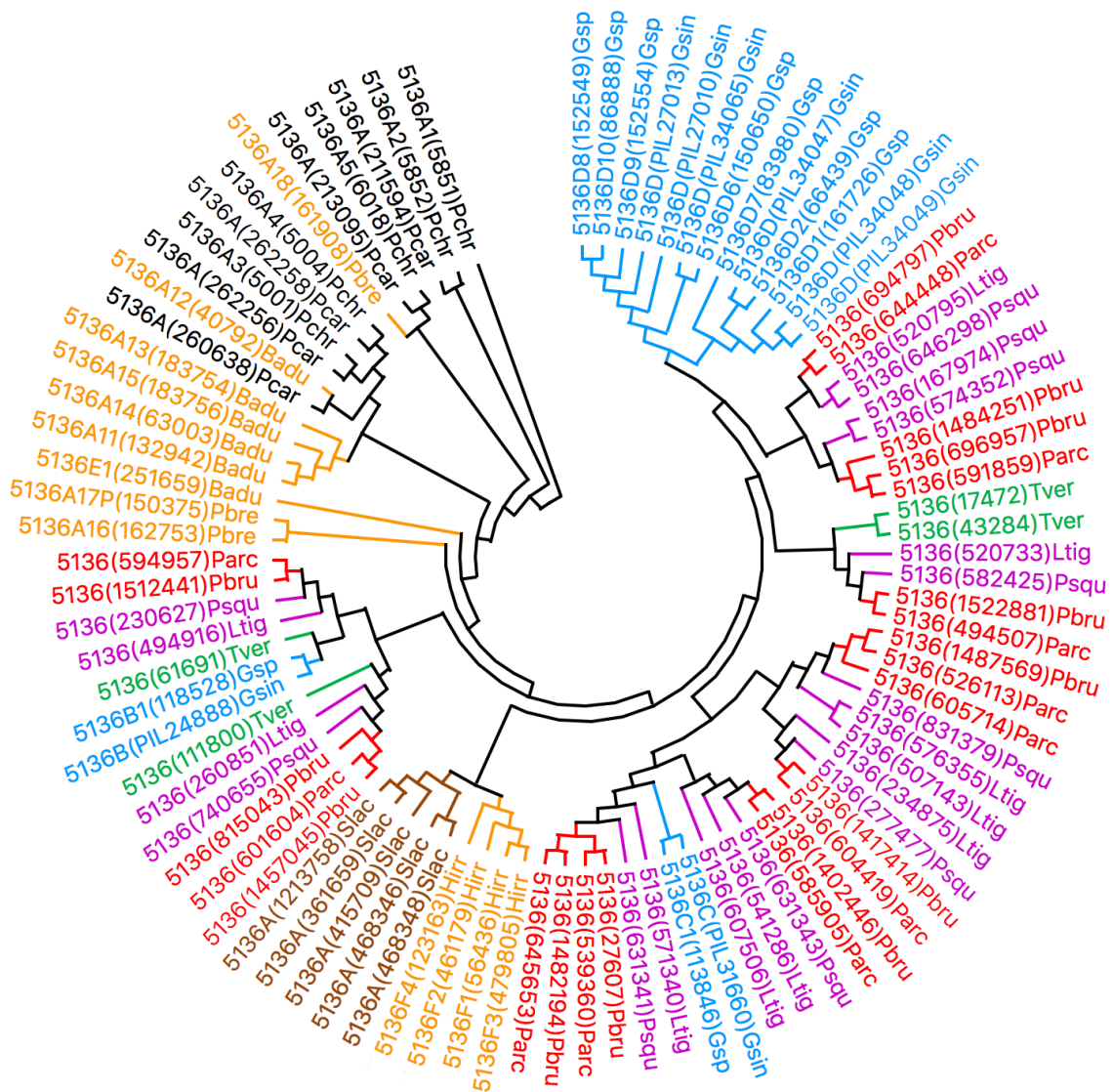


Fig. S16: Displayed is a minimum evolution tree of the CYP5136 family involving 92 amino acid sequences. Phylogeny of CYP5136 enzymes of the fungus *P. arcularius* and *P. brumalis* (Parc and Pbru; red) compared to related species *L. tigrinus* and *P. squamosus* (Ltig and Psqu; violet), and the other model white-rot fungi *Ganoderma* sp. and *G. sinense* (Gsp and Gsin; blue), *T. versicolor* (Tver; green), *P. chrysosporium* and *P. carnosa* (Pchr and Pcar; black), *B. adusta* and *P. brevispora* and *H. irregulare* (Badu and Pbre and Hirr; orange) as well as brown-rot fungus *Serpula lacrymans* (Slac; brown) in order to get an insight into the evolution of this P450 family. The tree was constructed using the close-neighbour-interchange algorithm in MEGA X.

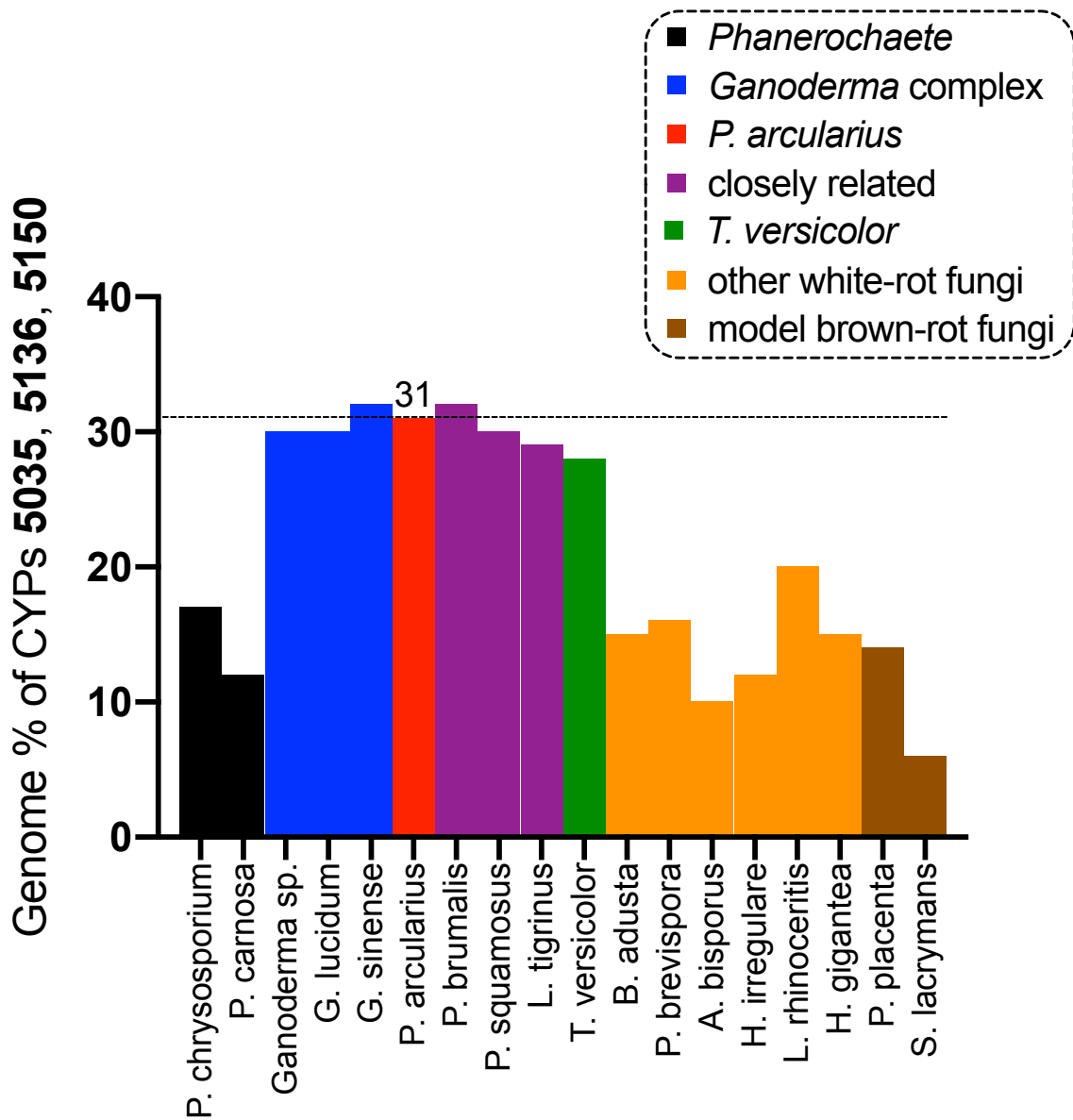


Fig. S17: Comparison of the P450 families 5035, 5136 and 5150 as a percentage of the total number P450s in the genome of *P. arcularius* versus a variety of white- and brown-rot fungi.

Sequences used in this study:

1. CYP5035N5 of *P. arcularius* (JGI: 196845; NCBI accession number: TFK85288.1)

MELDGHIVVWSPERLTATYA AVLGLLTHQVFRRHETYSIAHLTLLLAPLLIALTVSDSWQCIPKTRLLVSYAAYL
STLVLSLVSYRLSPLHLARYPGPLGCRVSKLWMAASLRAGYQHVVYRDLHKRYGSVVRIGPNELSIREPSAVMALV
GPSGLPKGPHVTGRLLTDKDLPMIGIEDLSTHMTRRRAWNRGFSAAIAEYEELVGRRAMQLVQRLEE HQKQVD
IERWFDYFSYDAMCDMTFGGSELLRDGDENNVSLSAGMTAMTFFGHVPWLG VYFGYLPAAATRIKSLAAC
KGLVEQRMQQGSRTRDLFHYLNHEDQMEQSPPPMRQLVDDGILAIVGGADTVSGALTSVIFCLLTHPETYDKLQVE
VDKYPPGEDVSSTRWHRDMKYLEAVINETLRVYSPGLGGSQRKVPADGPGVTGSLYIPPGTALWVHAFSLHRD
PSNFFPDDFWPERWLLAPHSPDLLSPEAADAKPTNFVHNEEAYMPFSHGPMNCVGNFALMEMRIVICALVQ
RFRFRLREDYDRADYDRNFKDYLIASRPNLPIIHELRE

>CYP5035N5

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CTAACACATCAAGTCTTCCGTCGGCATGAGACTTACTGCATCTCCGCTCACCTTACCCTACTGCTCGCTCCGCCCTT
ACTGATCGCACTTACAGTCTCTGACTCGTGGCAATGCATACCAAAGACAAGACTATTGGTCTCATATGCGGCGTA
TCTCTACTCTCGTACTGTCCCTCGTTTCTACCCTCTCTCCATTACATCCTCTCGCGCGCTACCCCGTCTCTC
TCGGTTGCAGGGTGTCCAAGCTGTGGATGGCGTCCCTCTCGCGGGCAGGATACCAACACGTTTACTATAGGGACCT
GCACAAGCGATACGGAAGCGTGTACGAATTGGTCCGAATGAGTCTCAATTCGCGAACCTTCGGCGGTGATGGC
CCTGGTAGGCCGAGCGGACTACCGAAAGGGCCTCATGTACCGGTAGGCTGCTCACCGACAAGGACCTCCCGATG
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TCGACATAGAGCGCTGGTTTCGACTACTTCTCGTACGACGCGATGTGCGACATGACGTTTCGGCGGCGGGTCTGA
TCTACGAGACGGAGACGAGAACAACGTCTGGTCCGTGCTCAGCGCAGGGATGACTGCGATGACGTTCTTCGGTCA
TGTTCCGTGGTTGGGAGTCTACTTTGGTTACCTTCCGGCTGCGACACGTCCGATCAAGTCACTCCTTGCTGCATGC
AAGGGTCTAGTCGAGCAGCGCATGCAGCAAGGTTTCGGGACCCGCGACTTATTCCATTATCTGAATCACGAAGAT
CAGATGGAACAATCTCCACCCCAATGCGCCAGCTCGTCGACGACGGTATCTTGGCCATTGTTGGCGGAGCGGACA
CCGTATCGGGCGCACTACAAGCGTGATCTTCTGCCTGCTCACCCACCCGAGACCTACGACAAGCTTCAAGTCCA
AGTCGACAAGTACTATCCTCCCGGCGAGGACGTGTCCAGCACCAGATGGCATCGAGACATGAAGTACCTGGAGGC
CGTCATAAACGAGACACTGCGTGTATACTCGCCCCGCTTGGCGGCTCGCAGCGCAAGGTCCTGCAGACGGTCTC
GGGGTGACAGTGGGCTCGTTGTACATCCACCCGGAACGGCGTTATGGGTGCACGCGTTCTCGCTACACCGTGACC
CGAGCAACTTCTTCCCTTCCCAGACGACTTCTGGCCGGAACGCTGGCTCCTCGCCCCCACTCACCCGACCTCCTC
TCTCCGGAAGCCGCGGATGCGAAGCCAACGAACCTTCGTGCACAACGAGGAGGCATACATGCCATTCTCGCACGGGC
CGATGAACTGCGTCGGGAAGAACTTTGCACTAATGGAAATGCGCATCGTCATCTGCGCACTCGTGCAGCGGTTCC
GCTTCCGGTTGCGGGAGGACTACGACCGCGCAGATTACGACCGCAACTTCAAGGACTACTTGATCGCTTCCGGCC
GAACCTTCCGGTCATCATTGAACTGCGGGAATGA

2. CYP5035AU1 of *P. arcularius* (JGI: 519317; NCBI accession number: TFK89225.1)

MLKRAYSSPGAVSVALALITHQVFRRYETYSWFIHGCLLFGPPTLVATFVSDTADTTRSLLQGFFRALPIHLITLSIV
ILYRLSPLHPLAGYPGLSRKVSMLVPAYLSLSGRKCQYSQALHKQYGDVVRTGPNELSIIDTAAMQHLWNLPRGP
MNVGISLSDKLVPLMGIQDPAEHARRRRPWNRGMSQAAVKEYEHVFAADVHLLVRRLEE QPGKADLAKWIEYLT
YDFMCDMAFGGGSELLREGDKESVFSVMEHGLMVAGALMLVPWLVGYMGIYIPGAALATLHQTGTRFVLERLE
RGSTTRDLFHYLNNEEDLPDTPPPRQHLVDDGILAVVAGSDTVSSTATSLIYCLITHPDAYADLKVVDKYHYPGDD
PCNPRHYQDMHYLNAVINETLRIFHPAAAGGQRRVPWDSEPVVAGPYVIPPGTIIMMPQYTIHRDARYFSYPPDD
WPERWLIASGDLRLEDARMPGKPKQLARGEVFNDAAFIPFGHGPICVKGALGVLEMRLTALVHKHFHFQAP
QGW DAGTYPEIQIKEYVTVTRPPLPVVIKPRW

>CYP5035AU1

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CGCACTGCGGATACTACTCGAAGCCTTCTGCAGGGTTTTTTCAGGGCGCTCCCAATACACCTCATCACGCTGTCC
ATCTCGGTGATACTCTATCGTTTGTCCCTTACATCCGCTCGTGGGTATCCTGGACCTCTTTCGAGGAAGGTGT
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GGGATGTGCTGCGTACAGGGCCGAACGAGTTGTGCGATCATCGACACCGCGCTATGCGACATCTGTGGAATTTGC
CTAGGGGTCCGATGAACGTTGGAATATCTCTCTCGGACAAGCTCGTCCCTTGTGATGGGCATCCAGGACCCGGCGGA
GCACGCACGCCGACGTCGGCCTTGAATCGAGGAATGAGCCAAGCAGCGGTGAAGGAGTACGAACACGTTTCCG
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CTTGACATATGACTTCATGTGCGACATGGCGTTTCGGAGGCGGCTCAGAGCTGCTGCGAGAGGGAGACAAGGAGAG
TGTCTTTTTCGGTCATGGAGCACGGTCTCATGGTGGCCGGTGCCTAATGCTCGTCCCGTGGCTTGGCGTATACATG
GGATACATCCCGGTGCTGCGAAAGCACTTGAACCTCTCCACCAGACCGGTA TAGATTCTGCTCCTCGAGCGTTTTGG

AGCGCGGGTTCGACTACGCGTGATCTCTTCCACTATCTCAACAACGAGGACCTCCCAGATACGCTCCGCCGCCACG
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CCATCCGGCAGCCGAGGTGGTCAACGCCGCTTCCCTGGGATTCAGAACCAGTCGTGGCAGGCCCTACGTGATC
CCTCCCGGAACGATCATCATGATGCCAGTACACCATCCACCGCGACGCGGATACTTCTCCTACCCGGACGATT
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GGTGGGATGCGGGGACGTATCCGGAGCAGATCAAGGAGTATGTGACGGTGCAGAGGCCGCTCTGCCGGTCTGCA
TCAAGCCGAGGTGGTAG

3. **CYP5035N6 of *P. arcularius*** (JGI: 521854;NCBI accession number: TFK87858.1)

MFRRHETYSIAIHALLLFGAPLLLVGTASTLTFGVLLSACWTYLVTLILSILLYRSLHLHPLSKYPGPICCRASKLWH
ACVVLKGRQHEYLQALHERYGDVVRIGPNDLSIRDASLIPAILGASGVPKGNFHFHGSMLTATNPPMVGIQDTRLHLA
RRRPWARGLALSALKEYHRLVGRKRNQLVHLLNRHGTVVVLGEMFDYFSYDLMCDMAFGGGAELMEEGDPKQ
VWCLLTEGLEAGASFHQMDWLVGVYFGHIPAVVKPLQTFHGHKTLALERMQRGSTRRDLFHYLNEEDLPERTSPP
LQQLVDDGILAIVGGSDTSSCLTSVFFALLTHPETVYKLAQEVKYPAGENPCATKQHRDMTYLHAVINEALRLF
PPVMTLSTRKVPARAPGVHIRSLYPPGTSISIPPYALQRDPNFSFSAFWPERWLIASGQIKLEDAPPPAAASSRT
FEFVHNEVAFMAFVSHGPMNCVKGKGFALQEI RTVVCALLQRFSFRLGEGWDPREYEATVRDYIVSSRPALPVILERR
TSS

>**CYP5035N6**

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GATCTTGCTCTACCGTCTTTCGCATCTCCATCCACTTCTAAGTATCCCGGTCCTATCTGCTGCAGGGCGTCAAG
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GTTGTTTCAATAGGGCCGAACGACTTGTCTATCCGCGACGCTCTCTCATTCTGCGATCTGGGAGCCTCAGGTG
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ACTTCGGACACATCCCCGAGTAGTGAAACCACTTCAAACGTTTCTCACGCATGGCAAGACCCTTGGCCTGGAGAG
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GCTCTTCCCGCCGTCATGACCTTGTGAGTACGCGCAAGGTCCCGCTCGTGCGCCCGGAGTGCACATCCGGTCCCTA
TACATACCCCGGGACTTTCGATTTCCATCCCGCCGTACGCACTCCAACGTGACCCACGGAATTTACGTTTCCGA
GCGGTTTCTGGCCGAACGCTGGCTCATCGCTCCGGCCAGATCAAACGAGACGCTCCTCCGCTGCCGCGG
GTCGAGTGCACCTTTCGAGTTTGTGCACAACGAAGTGCCTTTCATGGCGTTCTCGCACGGGCCATGAACTGTGTC
GGCAAGGGTTTCGCTTTCGAGGAGATCCGGACGGTGGTGTGCACTCCTGCAGCGCTTCTCATTCCGGTTGGGGG
AGGGCTGGGATCCGAGGGAGTACGAGGCTACGGTACGGGACTACATCGTCTTCTCGGCCCGGCTTCTGTGAT
CCTTGAGCGGAGGACGTCGAGTTGA

4. **CYP5035S6 of *P. arcularius*** (JGI: 652223; NCBI accession number: TFK89222.1)

MRVVGQSAVPTIILALIAHQIFRRHETYYISVHASLLFGVPAGVVLALCWGSHPANALHIGLEVFKTYLITLGISVAVY
RVSPWHPLARFPGPYLRRISHFVSACIYIPGNRSRHFALHRQYGDVVRTGPNELCIVDPSMIPHILGVPVPGKPIW
VGGSLSYKTLPLVGIADTEEHMLRRRAWNRGLAPPALREYEVVTSRAKQLVQRLQEQVEMNLGDWMNRFTYD
FMSDMAFGGGSELLNDGDKDNMWTTISDAMKIAMFLGHVPWLGVYLYIPPAVSQKRLLSRGEELAAQRLARGS
TTRDLFHLYLNEDLPDKEPAPRRQLIDDGVLAVVAGSDTTSIVLSTFYCVLTNPDAYEELQAEIDRHFPQGGDPYI
TQHHRNMPYLQAVINEALRLFPPVPGTERRVPQHGEVPIAGSLRIPPPTSIFMPPWVLRDARNFTFPTSFWPER
WLIASGQLSLEKARLPSIRSPPWAQAGDQGAHPVDFVHNESAYIPFSYGPMPNCPGKGLALMELRMVVTAVFQRFK
IRLREGWDPSEYDSGFKDYFNATRPELPTLERR

>**CYP5035S6**

ATGAGGGTCGTCCGACAATCCGCCGTGCCGACAATCATCCTAGCTCTGATCGCACACCAGATTTTCCGCCGGCAGC
AGACATACTACATCTCGGTTTCATGCTTCTTGGCTTCTCGGCGTCCCTGCAGGCGTGGTCTTGTCTCTGTTGGGG
GTCTCACCTGCAAATGCGCTCCACATTGGCCTAGAAGTCTTCAAACGATCTGATCACCTTGGGTATCTCCGTG

GCCGTCTACCGCGTCTCTCCCTGGCACCCCTCGCGCGCTTCCCTGGCCCGTATCTCCGCCGGATATCACACTTCGT
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GTCAGCTCATCGACGATGGGGTACTCGCCGTGGTAGCCGGCTCCGATACCAGTCTATCGTGCTTACATCAACCTT
CTACTGCGTGCTGACGAACCCGGACGCGTACGAAGAACTTCAAGCAGAGATTGACAGGCACTTCCCTCAGGGCGA
CGACCCGTACATCACTCAGCACCATCGCAACATGCCCTATCTGCAAGCGGTGATTAACGAAGCCCTTCGCCTCTC
CCTCCAGTTCCCGGCGGCACCGAGCGTCCGCTTCCGCAACACGGTGAACCTGTCATTGCGGGATCCTTACGCATTC
CACCCGGTACTTCCATCTTATGCCCCCATGGTCTCCATCGCGATGCGCGCAACTTACCTTCCCAACCTCGTTC
TGGCCTGAGCGTTGGCTCATTGCGTCCGGGCAACTCTCGTGGAGAAGGCTCGTCTTCCCTCTATTTCGCTCTC
CTTGGGCTCAGGCTGGAGACCAAGGGGCTATCCACCCGGTTGATTTTGTGCACAACGAGAGCGCGTACATCCCGTT
CTCGTACGGCCCGATGAACTGTCTGGGAAGGGGCTGGCCCTGATGGAGCTGCGCATGGTCTGACGGCAGTGTTC
CAGCGCTTCAAGATCCGTCTCCGTGAGGGCTGGGACCCGAGCGAGTACGACAGCGGCTTCAAGGACTACTTCAACG
CCACTCGTCCGGAGTTGCCGTTTACTGGAGCGGCGTTGA

5. **CYP5035S7 of *P. arcularius*** (JGI: 664247; NCBI accession number: TFK85799.1)

MSLREVSPLTIPLAIATHQVFRRYEIVSVHACLFLVPPALVAHISSQSYSPSSIPATFVIALVSYVAAIAASVIVYRL
SPLHPLARYPGPVWRKVMIGPAILATTGNRAWAFADMHRKYGDIVRSGPNELSIIDPSFIGPLLASGLPKGPYHV
GASVTPEHVSMAGLQDIPYHLQRRRPWNRGLNPSALKDYQPLIVERLQLLVRRLHEQSGIIDLGLWLKYFAYDFMS
DMAFGGSELLKDGDKNNIWSIIEGMVFATILHTLPWLGAYLFKIPGSVKPLLAMQQTARLAERFKRGSKTRD
LYYLSNEDLDPKPPPLRELADDGVLAVVAGSDTASLTMTSVFYLLTHPEAYTKLQEIDTSYHPGEPNAGTKR
HREMPYLHAVINEALRLFPVPLGTQRQVPHDASPVVFGSVVIPPPTSVYLPPTLALHRDPRNFTCADDFFWPERWLI
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>**CYP5035S7**

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TACTCTCCGTCGTCAATCCCAGCGACGTTCTGTAATTGCACTGGTATCCTATGTGGCTGCTATTGCTGCGTCCGTCA
TCGTGTACCGACTTTCTCCGCTCCATCCGCTTGCACGATAACCCGGTCCGGTCTGGCGCAAAGTGTCCATGATCGG
ACCTGCAATACTGGCGACCACTGGCAACCGGGCTTGGGCATTGCGAGACATGCACAGGAAGTATGGGGACATCGT
CCGCAGTGGACCGAATGAGCTGTCTATCATAGATCCCTCATTTATTGGGCCTCTGCTGGGTGCCTCGGGTCTTCCG
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ATCTGCAACGCCGACGTCCTGGAACCGAGGGCTGAACCAAGCGCTCTGAAGGACTATCAACCTCTGATCGTCA
GAGACTCCAGCTCCTTGTCCGAAGGCTCCACGAACAGTCCGGTATCATCGACCTCGGACTATGGCTCAAATACTTC
GCGTACGACTTCATGTCTGACATGGCGTTTGGCGGAGGATCAGAGTTGCTGAAAGACGGTGACAAGAACAACATC
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CCGGTCCCTCTGGGTACTCAGCGTCAGGTACCTCACGACGCTCCCGGTCGTATTCGGTCTGTGCTGATCCCTCC
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AGGCGGCAGACCTTCCCGACTTCGTTACAACGATGTGCCTTACGCGGTTCTCTGTAGGACCTATGAATTGTCC
CGGGAAGGGCTTGGCGATGCTGGAGATGCGCATGGTTCATCGTTCGAGCTGGTGAAGAATTCTGTTCAGGCTGTG
GGACGGATGGGATCCGGCGACGTACGAGAAGGAGTTCAAGGACTACTTTACTGCCGCTCGGCCCGGATTGCCTGT
CGTTCTCGAGCCGAGACAGCAACTGTAA

6. **CYP5035S8 of *P. arcularius*** (JGI: 665466; NCBI accession number: TFK83982.1)

MSLREVSLLTIPFAILAHQVLRRYEYRIYVHACLLLGPPILAAARLTSFRPTPALPPILFNSLVSYLAALVTSVIAAYRL
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DFMSDVAFGDGSDDLREGDKANIWSIHEDGMVVCTIAHSLPWLGIYLSMIPSAAGPMLAFQENGRRLARERLERGS
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TKHHREMHYLAQVINEAMRLFPPILGSQRQVPHDAASVVVGSVVIPPGTAIYLPPWVLQRDPRNFTFPDAFWPE
RWLIASGQLHYGDARLPSSAKRGHERPDFVHHEATFIPFSAGPMNCPGKGLAMMEMRSVVIALMKNFGMKLRDG
WNPATFDQEFKDYFTAARPELPPVLEPRLHVETKAYE

>CYP5035S8

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CTTACCGGCTCTCGCCATTCCACCCCTTGACAAATATCCCGGGCCTCTCTGGCGCAGAATCTCCATGTTCCGACCC
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CTCGCGGACGACGGGACCCTGGCCATCGTGGCAGGGTCCGACACCGTTTCCGGTGGCGTTAACGAGCGTTTTCTATT
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CATTCCGCTAGGATCGCAGCGTCAAGTACCTCAGATGCCGCTTCCGTGGTGGTGGATCTGTGCTCATCCCTCCG
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CGAACGTCCAGACTTCTGTCACACGAGGCTACGTTTATCCATTCTCCGCTGGTCCGATGAACTGCCCCGGGAAG
GGCCTTGGCATGATGGAGATGCGCAGCGTCTGTCATCGCACTGATGAAGAAGTTCGGGATGAAGCTGAGAGACGGG
TGGAATCCGGCGACGTTTCGATCAGGAGTTCAAGGACTACTTCACTGCGGCGGACCTGAGTTGCCTGTGGTGCTTG
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7. CYP5035AV1 of *P. arcularius* (JGI: 667965; NCBI accession number: TFK79795.1)

MSTMWYSLAVSALIAHESFKRYETYSIRAHTALLGPPSLALGFLGSTGSSRSVLHTLPLAYAAYVVGALTVYTYLRYI
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MLSDGIHLPIIGIQDPAEHLRRRRPWNRAFTVPALRGYEETIARRARQLVDALERHNGQEIVLKGWVFNDFAYDF
MCDMAFGGSELQERDDSNVWRVLDDEGMKVGTLAHVPWLGVYLSHVPLATGALDVLISHCRMLTTQRVQRG
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RGEDACDTRYHREMRWLNAVICETLRLFPVPGGSQRQVPHNSAIGVMAGDAFIPPGTSVWAHTWSIHRDPRNFS
RPDAFWPDRWLLASTTLRSPSSASSSVEADGVRDFVHNEADAWIPFAQQQMNCVGNLALLEIRMVVCALMQRF
EMRLSEGWDAREYERKFRAYLVATRPEMPVRLRVRT

>CYP5035AV1

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TTCACGAAGTGTCTCCATACGCTGCCCTCGCGTATGCTGCATACGTCGGCGCTTAAACAGTCTACACTATTCTC
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TCTCCCGCCCTGACGCATTCTGGCCCCACCGGTGGCTGCTGGCAAGTACAACATTGAGAAGCTCCCCCTCATCCGC
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TCCCAGATGCCGGTGAAGTTCGCGGGTTCGTTGTACGTGA

8. **CYP5035S9 of *P. arcularius*** (JGI: 668252; NCBI accession number: TFK79033.1)

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GSKTRDLYHYLCNEDLSRSPPIAELADDGILTVVAGSDTASMTMTNVFYCLLTHPEAYAKLQAEIDKFYPAGEP
ASETKHHRDMHYLHAVINEALRLYPVPLGSQRRVPHSGAPVVVGVSTVLPVPGTVVYLPWILHRDPRNFSFPDAF
WPERWLITSGQLRHEDAQPSSAKDATKMDISGLVHNEAFTPFISGPMNCPGKGLAMLEMRTVIVSLMKNFSFK
LRDGDWPAKFEELKDYFLVARPELPTIERRRIVT

>**CYP5035S9**

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GTCCGAATCACATCGGAGGCAATATGTGCGAGGAGACTAATCTGGTCGGCATCGTCGACATACCATACCATCTGC
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9. **CYP5035H2 of *P. arcularius*** (JGI: 665169; NCBI accession number: TFK84406.1)

MVFQLPAHHALFTVVGSFAFVHLIFKRYEPHRVAVHALLLAVPSFSLVLLDRMPAIKALSASFLTFWTALVSSVA
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IKLGNTSRKDLFYLLNEDGAEPVPPVPEVTADGTLAIVAGSDTSSVLSNVFYCLLTHPEAYARLRAEVDSSYPPG
EDALNTKHHADMPYLNVINETMRLFPVTDGSQRIVPTGSGGRIIGDSYLPETITTVHMYSIQRDARNFAPLPDS
FWPERWLHAAEGARSVIGMKLVHNPTAFFPFSYGPNCAGKGLALQEMRMVVSAMMQKLELSLAEGFDAVAYE
NEMHDYLILSRPPLPVVVKQRKVCTAEA

>**5035H2**

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10. P450 reductase of *P. pastoris* (NCBI accession number: XP_002494255.1)

MDTLDSLVLIAIALIAYFSKGLWKGEDDNSVHGVAGGFQTRDLVEILNSTNKKALVLYGSQTGTSSEYAHKYAR
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LAVWPSNANEHVESFLKVFNLTKRSSFVDFIEFLDPTVTVHFPPFTTYEAVVRHHLEISGPISRQTLKQFIPYAPDQS
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GVAPFRGFIRERCQVDNGTPNIGQSILYYGCRNSEQDFLYRDEWPTYSKKLGDKFKMYTAFSRENSHKVYVQHL
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>PpP450red

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