

Supplementary Information for

Novel genes and evolutionary fates of the amanitin biosynthesis pathway in poisonous mushrooms

Hong Luo¹, Heather Hallen-Adams², Yunjiao Lüli², R. Michael Sgambelluri, Xuan Li, Miranda Smith, Zhu L. Yang, Francis M. Martin¹

¹To whom correspondence may be addressed. Email: francis.martin@inrae.fr or luohong@mail.kib.ac.cn

²These authors contributed equally.

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Figures S1 to S15
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Other supplementary materials for this manuscript include the following:

Datasets S1 to S3

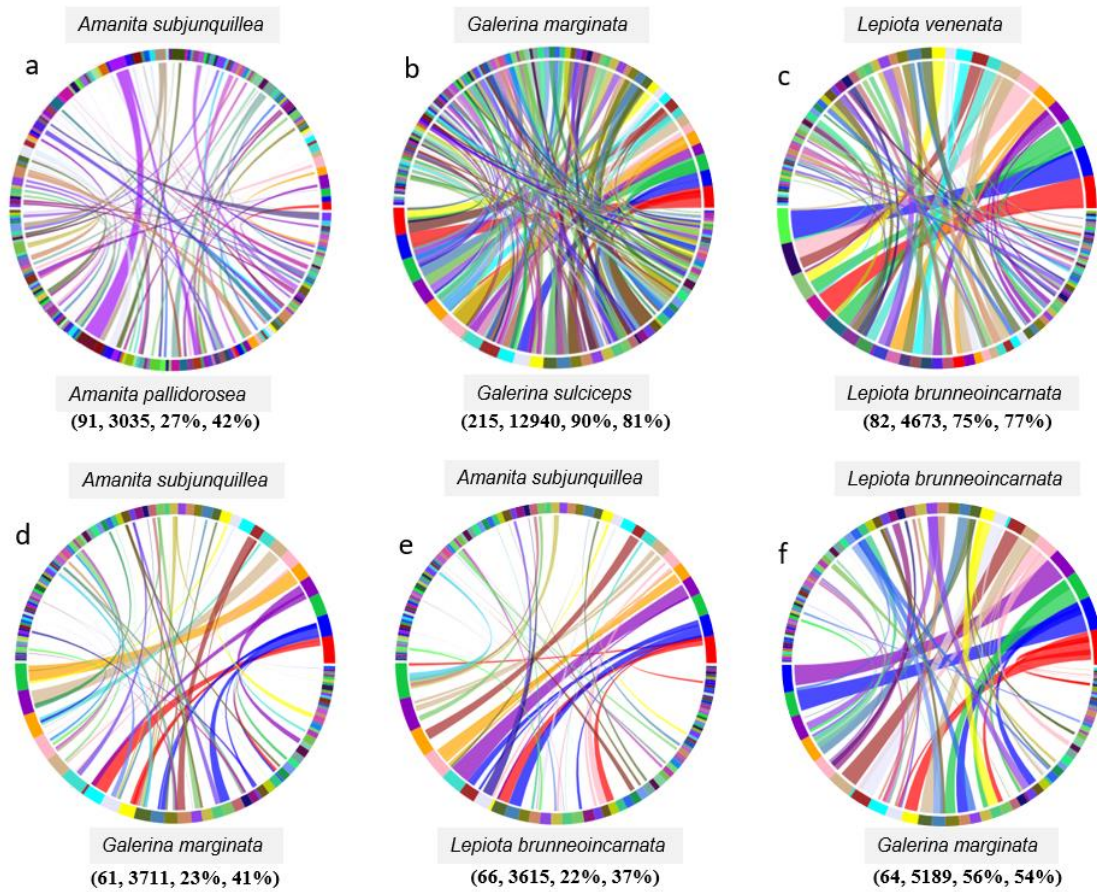


Fig. S1. Synteny analyses of the genomes of amanitin-producing agarics. Genome synteny within respective genus is indicated in the upper row, and the synteny between genera shown in the bottom row. Ribbons indicate genomic regions with significant synteny, and density of the ribbons represents syntenic levels. Statistics below each section are numbers of synteny block, anchor, block coverage of the species on the top, and block coverage of the species at the bottom.

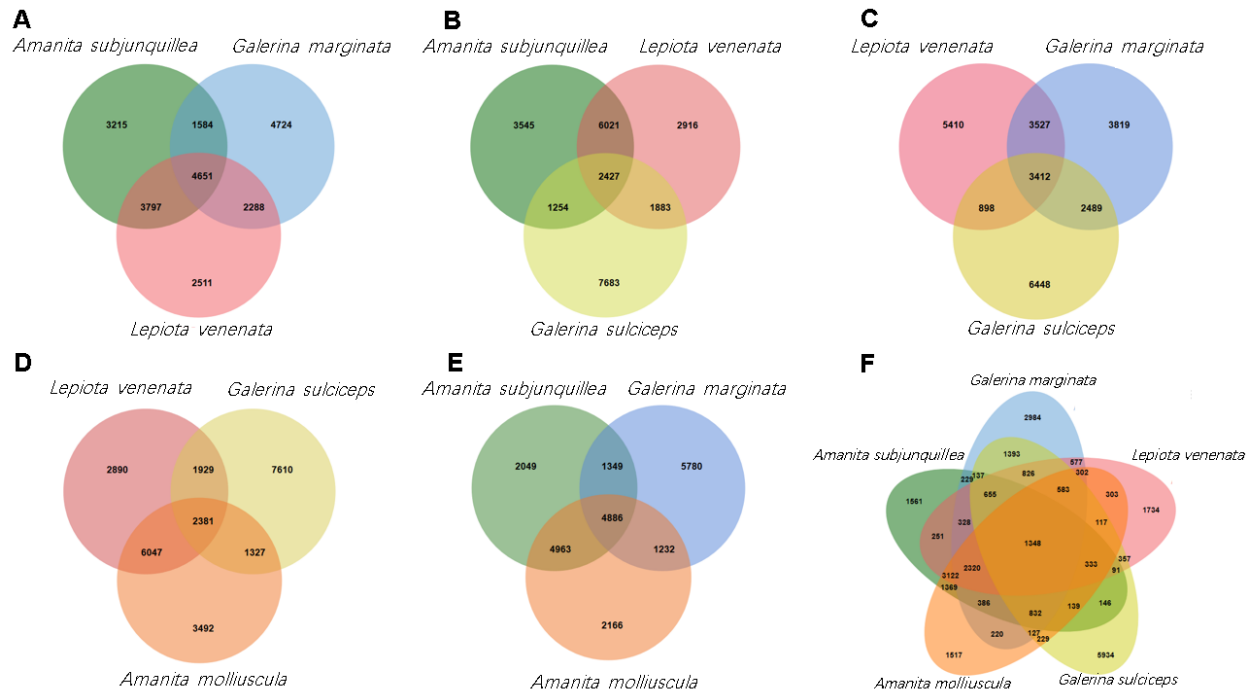


Fig. S2. Venn diagrams of predicted orthogroups in amanitin-producing *Amanita*, *Lepiota* and *Galerina* species. Species names are labeled above or beneath each circle.

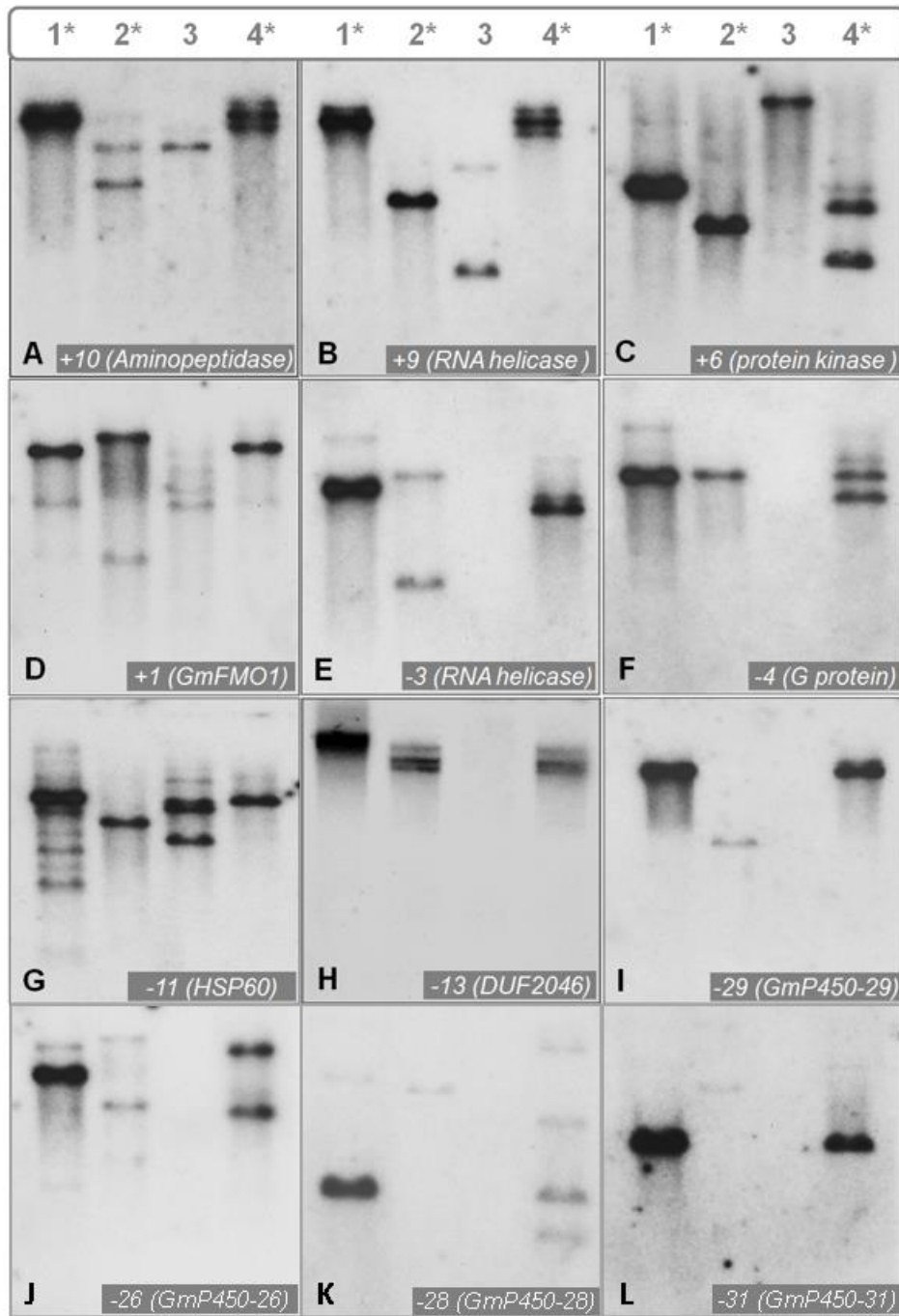


Fig. S3. DNA blotting of genes in the vicinity of *GmAMA1-1* in amanitin-producing and amanitin non-producing *Galerina* species. Lanes 1, *G. marginata*; Lanes 2, *G. badipes*; Lanes 3, *G. hybrida*; Lanes 4, *G. venenata*. Asterisks indicate toxin-producing species. Gene number: position upstream (-) or downstream (+) of *GmAMA1-1*. Four *CYP450* orthologs (I-L) are present in three amanitin-producing species and absent from the amanitin-nonproducing species.

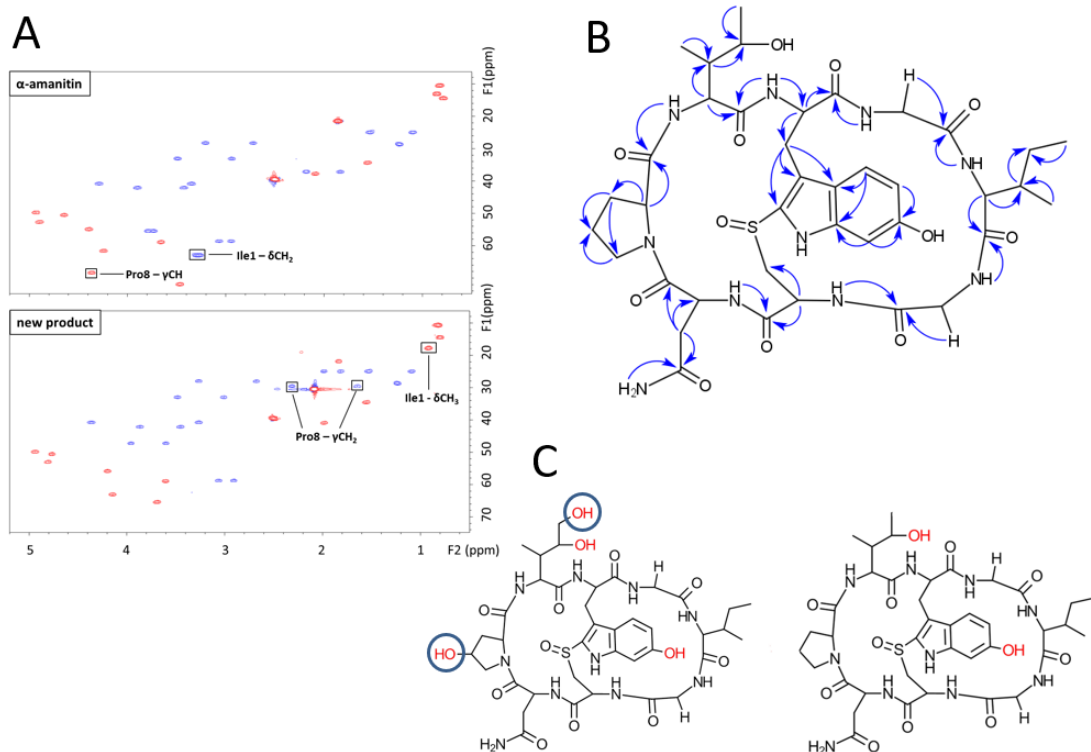


Fig. S4. Structure of θ -amanitin. (A) ^1H - ^{13}C HSQC spectra of α -amanitin and θ -amanitin with major differences indicated. (B) Key HMBC correlations in θ -amanitin. (C) Structures of α -amanitin (left) and θ -amanitin (right): two blue circles indicate the missing hydroxyl groups in θ -amanitin.

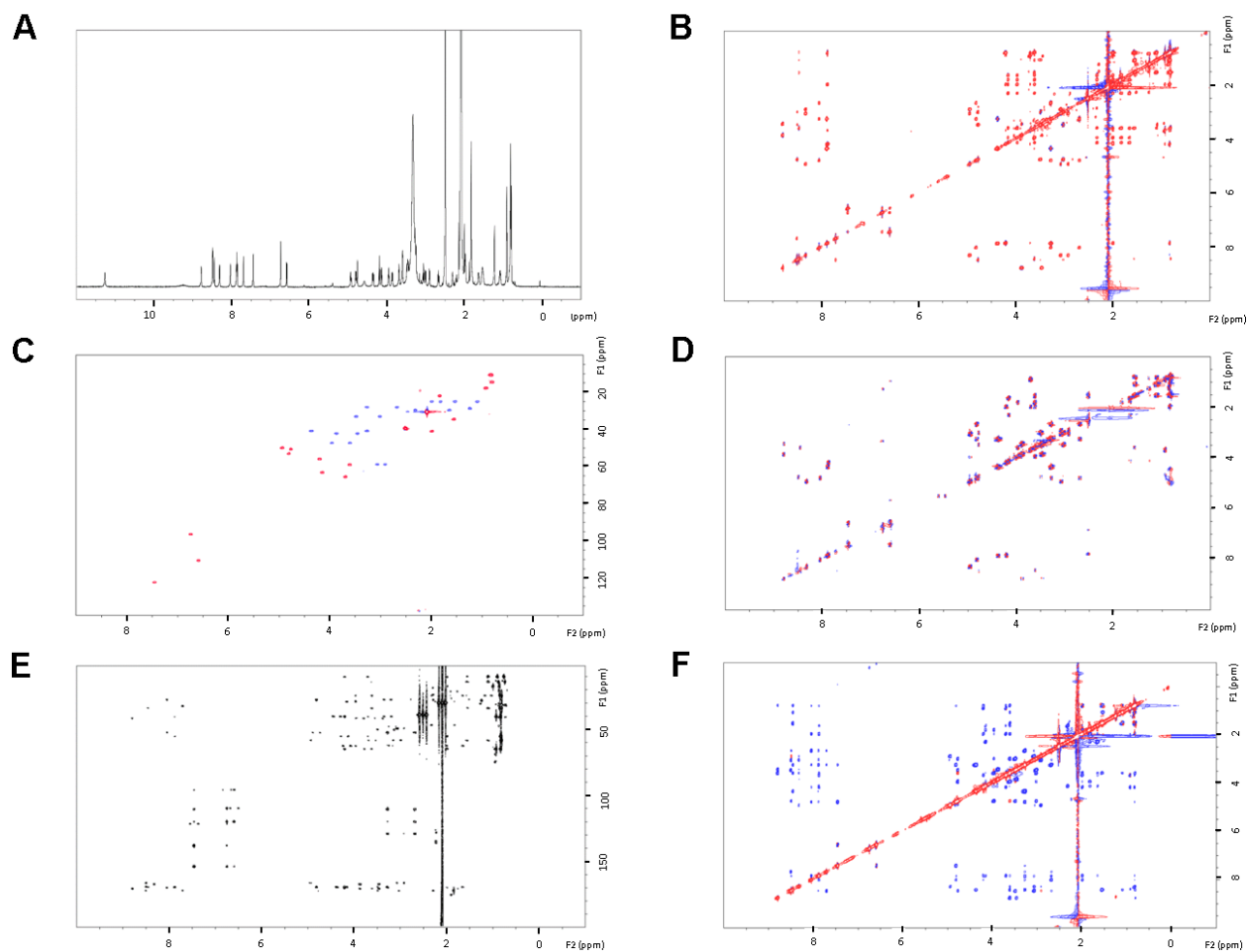


Fig. S5. 1D and 2D NMR analyses of θ -amanitin from *GmP450-29* mutant. (A) ^1H -NMR spectrum. (B) TOCSY spectrum. (C) ^1H - ^{13}C -HSQC spectrum, a section of this spectrum is shown in the main text as supplementary Fig. 5. (D) 2D DQF-COSY spectrum. (E) ^1H - ^{13}C -HMBC spectrum. (F) 2D ROZY spectrum.

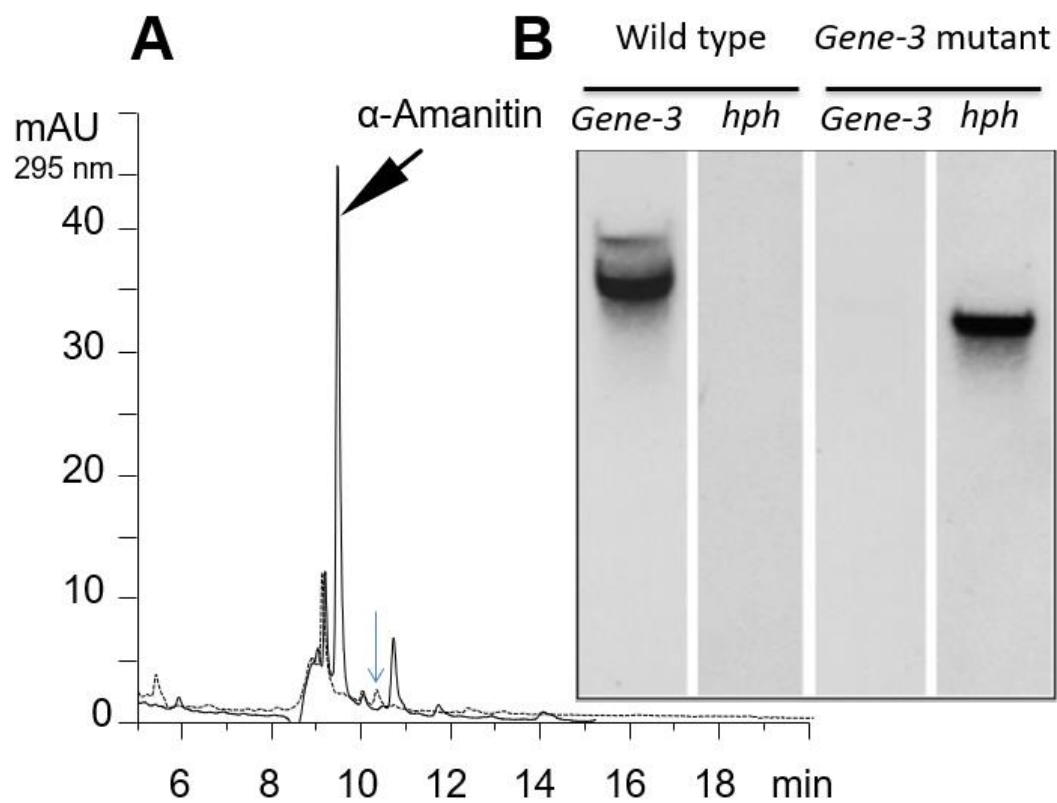


Fig. S6. Deletion of *Gene-3* in *Galerina marginata* results in loss of α -amanitin production and accumulation of a new product. (A) LC-MS analysis. Solid line, OD₂₉₅ for wild type; dashed line, OD₂₉₅ for *Gene-3* mutant. (B) DNA blot analysis for wild type and mutant; *hph* for hygromycin B marker.

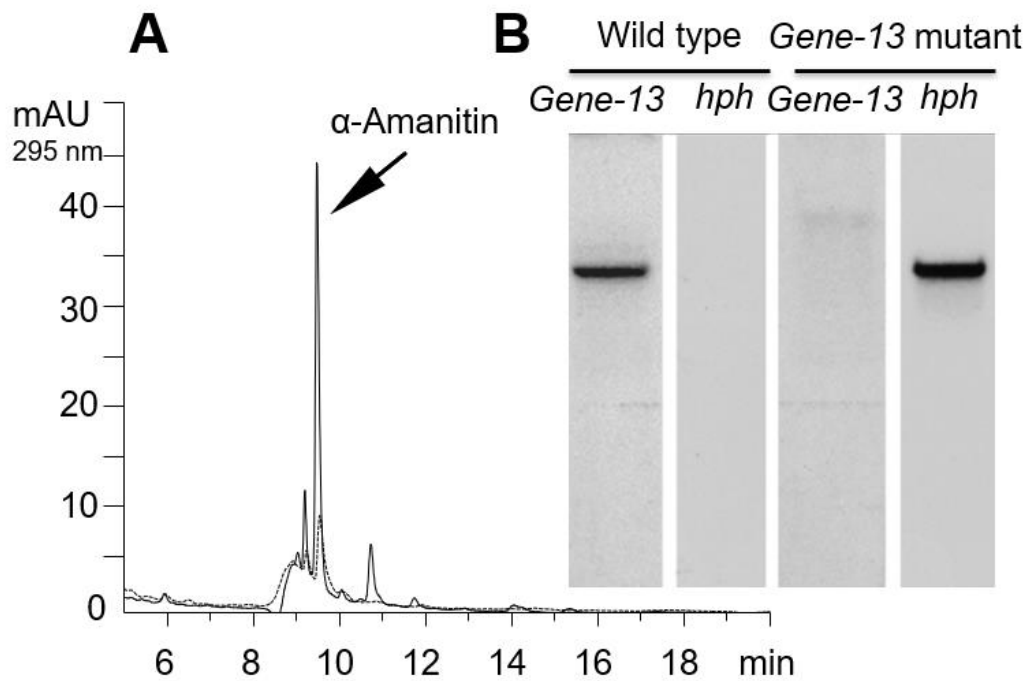


Fig. S7. Deletion of *Gene-13* in *Galerina marginata* results in drastic reduction of α -amanitin. (A) LC-MS analysis. Solid line, OD₂₉₅ for wild type; dashed line, OD₂₉₅ for *Gene-13* mutant. (B) DNA blot analysis for wild type and mutant; *hph* for hygromycin B marker.

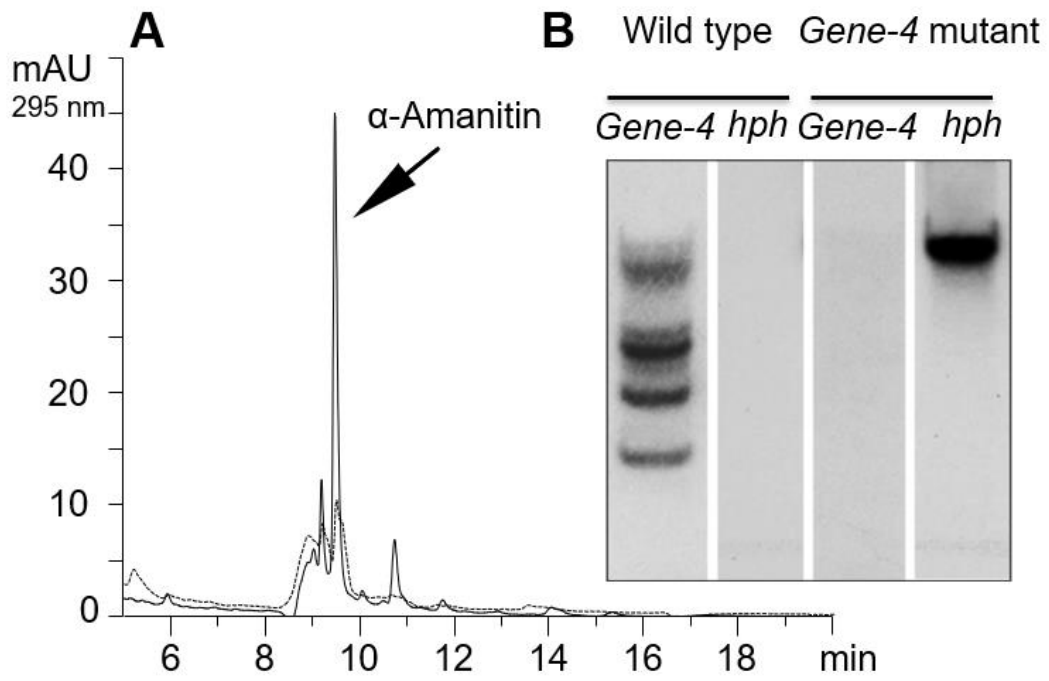


Fig. S8. Deletion of *Gene-4* in *Galerina marginata* results in drastic reduction of α -amanitin. (A) LC-MS analysis. Solid line, OD₂₉₅ for wild type; dashed line, OD₂₉₅ for *CYP450-29* mutant. (B) DNA blot analysis for wild type and mutant; *hph* for hygromycin B marker.

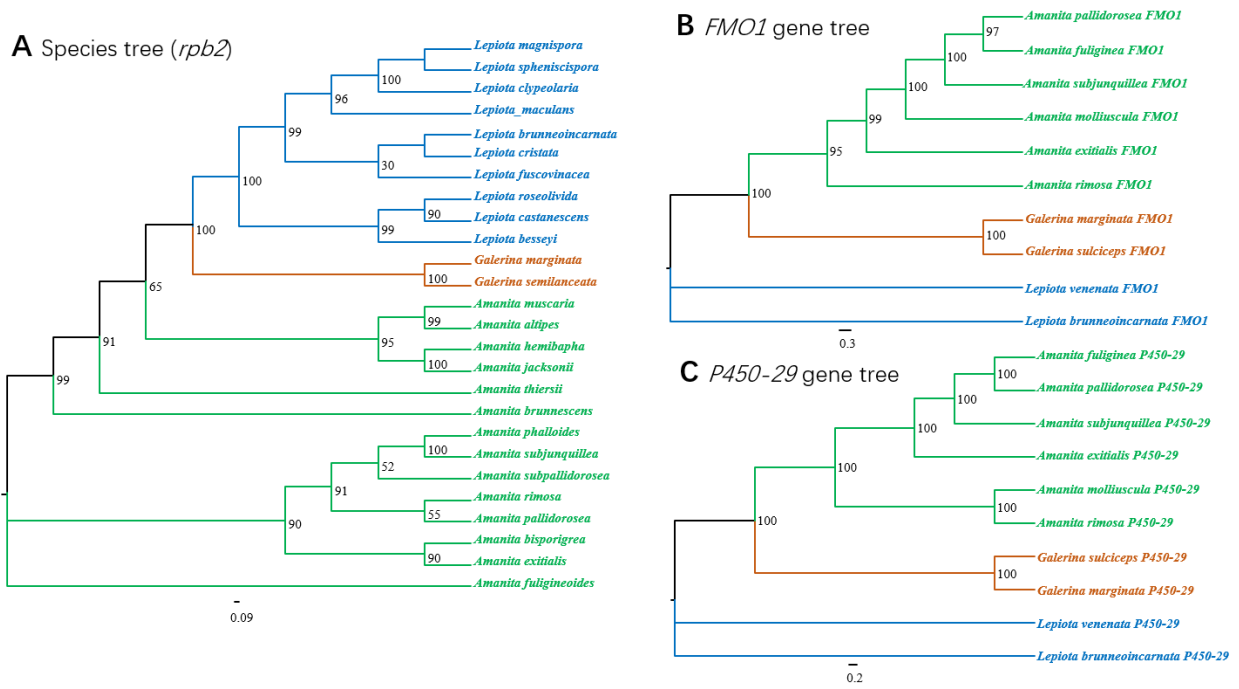


Fig. S9. Comparison of *FMO1* and *P450-29* with species tree (*rpb2*). *Amanita* was indicated in green, *Lepiota* in blue, and *Galerina* in brown (Maximum likelihood bootstraps over 50% are shown on the branches)

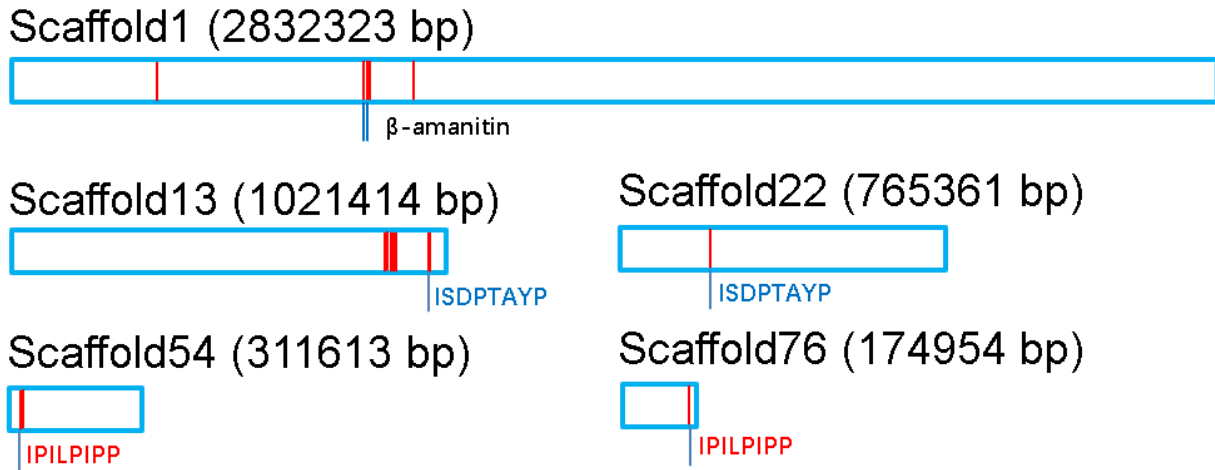
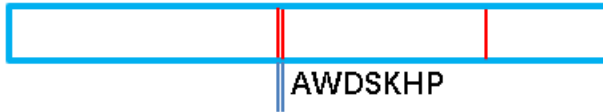


Fig. S10. Distribution of *MSDIN* genes present in two copies in the genome of *Amanita subjunquillea*. Blue lines under the scaffolds (blue boxes) represent the locations of the *MSDIN*s (red lines) in two copies. Amino acid sequences of the cyclic peptides were indicated on the right of the blue lines, with the same color for the same peptide. β -Amanitin-encoding *MSDIN* has two tightly-linked copies.

Contig 1 (1811650 bp)



Contig 8 (1101286 bp)



Contig 31 (543168 bp)



Contig 70 (170775 bp)



Fig. S11. The distribution of *MSDIN* genes present in in two copies in the genome of *Amanita rimosa*. Blue lines under the contigs (blue boxes) represent the locations of the *MSDIN*s (red lines) in two copies. Amino acid sequences of the cyclic peptides were indicated on the right of the blue lines. All denoted *MSDIN* genes in this figure have two tightly linked copies.

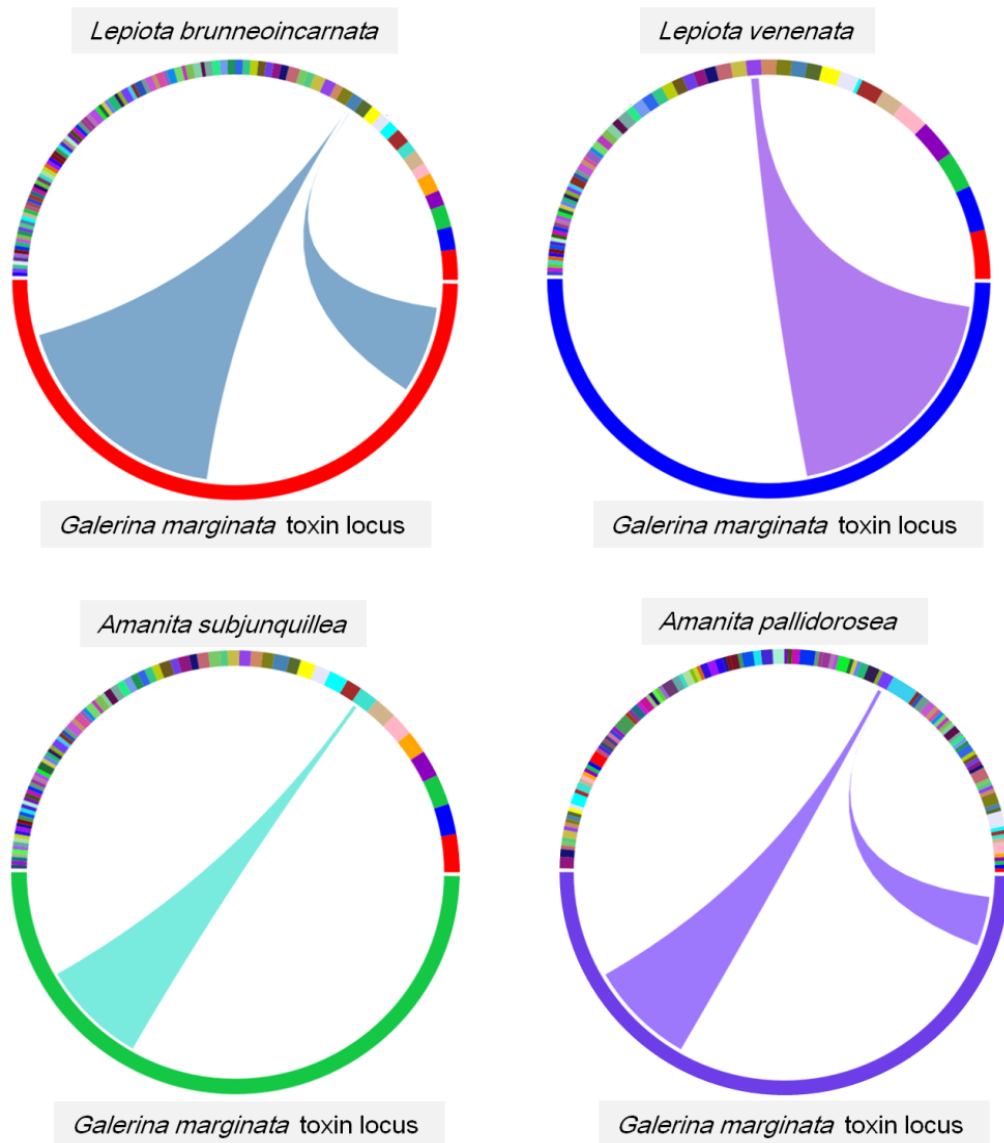


Fig. S12. Synteny analyses of the amanitin biosynthetic locus in *Galerina marginata* vs. those of *Amanita* and *Lepiota* species. The upper row shows two *Lepiota* loci vs the *Galerina* locus, while the bottom row represents two *Amanita* loci vs. the *Galerina* one. The *G. marginata* biosynthetic locus is placed as the lower half circles for all comparisons, and *Amanita* and *Lepiota* genomes as the upper half circles. Syntenic regions containing amanitin biosynthetic genes are connected by colored ribbons.

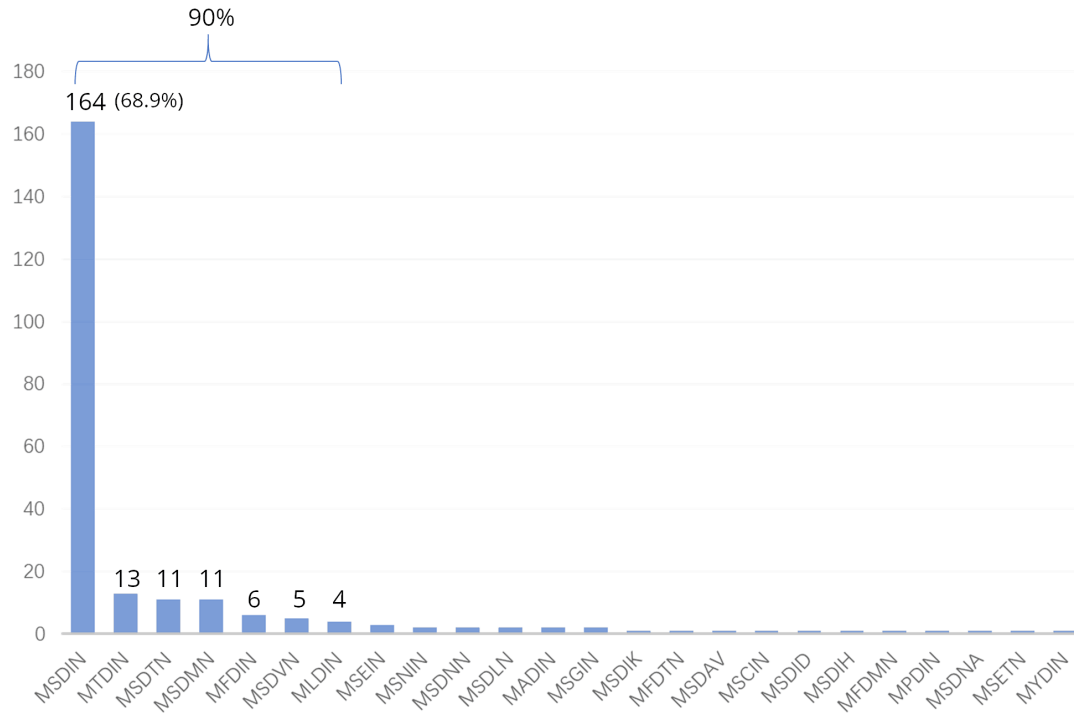


Fig. S13. N-terminals of *MSDIN* genes in 8 *Amanita* species. The species include *A. molliuscula*, *A. exitialis*, *A. phalloides*, *A. subjunquillea*, *A. fuliginea*, *A. rimosa*, *A. pallidorosea*, and *A. bisporigera*

Gene structure of *AbP450-1* from *A. bisporigera*



Gene structure of *AbP450-2* from *A. bisporigera*



Gene structure of *AbP450-3* from *A. bisporigera*



>AbP450-1 deduced amino acid sequence

```
MVDLHAISYSALVTFRLIFQFLKLSAAALTIYGLYRVTRVIYVELTSPIRHLPGPANANIFLGNLQQLWTDITYH
WHSQYGP MIRLNGFLGLSHLYVTD PQALNHILTNGYVYTKPSFTRRQIGKLWGPGLPFVEGDQHKKQRKI
LNPAFGPVRIREFTDCFVKKSKRLQDSWATECSKQGGTCRLDIMVGLGKVVMDIISSTGFRYELDSLDR
SDFSRVGYNFISIEPDSLATPKIHPTSMVHTCIPVETQLDDIKQTLRITSRLLNESKGSVRTNNDNSGSRD
LLSLLVRTNMSPDVPEHRRLSDDDEVKAQVISFVIAGRESPINVMAWALFSLAKKPEIQAKLRRELLTVDTC
QPTTDQLNALS YLDMVIRETLRLYPSSKANSRVCAKDDILPLAKPITDRRG NLFSSISIKRGQVVIIPISAIHK
DKSIWGEDALDFRPERWECLPEGVNTIPGVVSHLLSFWGGPRSCIGFRFAIAEMKALLFTLVRALFDLA
VPAEQISVESGLSNRPILTTNPGRYQLPLLIKPYKARS
```

>AbP450-2 deduced amino acid sequence

```
MLNLNFNGLWPDVAEYFKGNSIRIVTSVFTLLVVISIYRRRRGIRTPRLQGPRSGSFIFGNTKEIFPSANLSV
VYRDWERMYGPPVYEIPTGIGSSHVALSDPKALTHIYSKDTTTYCRLAGTTALTRKVF GDVVSISEGETH KR
LRRGLSSPLSVSAIRNLT PVCLDSAYQLKAAWDSCSPSSERSNNPVIIDVVKWMNSVTLDTIGKAILSHDF
GTLRGRTSLMMAAFDSIHTVKPSPFIRLIHFLSPIFYALFKVTLMSVREEKLAQSV AHLNRLTTNRLNKAHK
EPEDTVNGSVL GILVKSENAIPTAVCHSPRSRPRPVSF SWLHMKQQQSPYTWSLIELARRPEVQESLRAE
LSECLAKGERPTYDQLTKDLKYLD AFIAEILRLHAPEMQSIRV RLLHCFILSLHPP ILAGSRRRCGTVTNPI
RIASGATIDSLFLKKGTVVRIPLGGVNMSEALWGPDA GMFDP SRWLDAEGHKKGNKGELAGYGV S
```

>AbP450-3 deduced amino acid sequence

```
MLNLNFSSLWPYVAEYLKVN SMRIIASGISLLVVVISIYRSRRGPRTPRLQGPHMESFILGNARKIFPSANLS
LVYQGLEQTYGPPVYEIASGFGSNHVVLNDPKAL THLFSKDTVTSQPARQKDMGRKLF GDILVLTEGETH
KRIRRVLSSPLSVSAIRNFT PMCLDSAYQLKASWDSCFQLSNNSNRAIVLDAEKWMNCYTMDNIGKAVLS
YDFGNMRGHTCSILADLDAFHAVSPSGLYIRFIVFTREILYNL FKITLPNAKEKQFEELAAHFV LATGFLRE
ALEAPEDSAVHQ SILGVMLKSKNENANVRLSLPEIT AQAGGLVLAGYETTAIAMTWSLIELARRAEIQETLR
AELKECLADGERPTYDQLTKDLKYLD AFISEILRLHPSEMVLTRVAAEDDVIPLTDPIRTASGAMIDSLFVRK
GTVFRIPLGGMNISETLWGPDAATFDPSRWLEVDGHKGRREKVPGYRNLLTFGAGQRLCPGRDLALLE
MKAALVILVLHFSFEFPNGPSTELSWQFGRPKVAGEDGPKVPMLVRRLT
```

Fig. S14. Intron/exon structures and deduced amino acid sequences of *AbP450-1*, *AbP450-2* and *AbP450-3* in *Amanita bisporigera*.

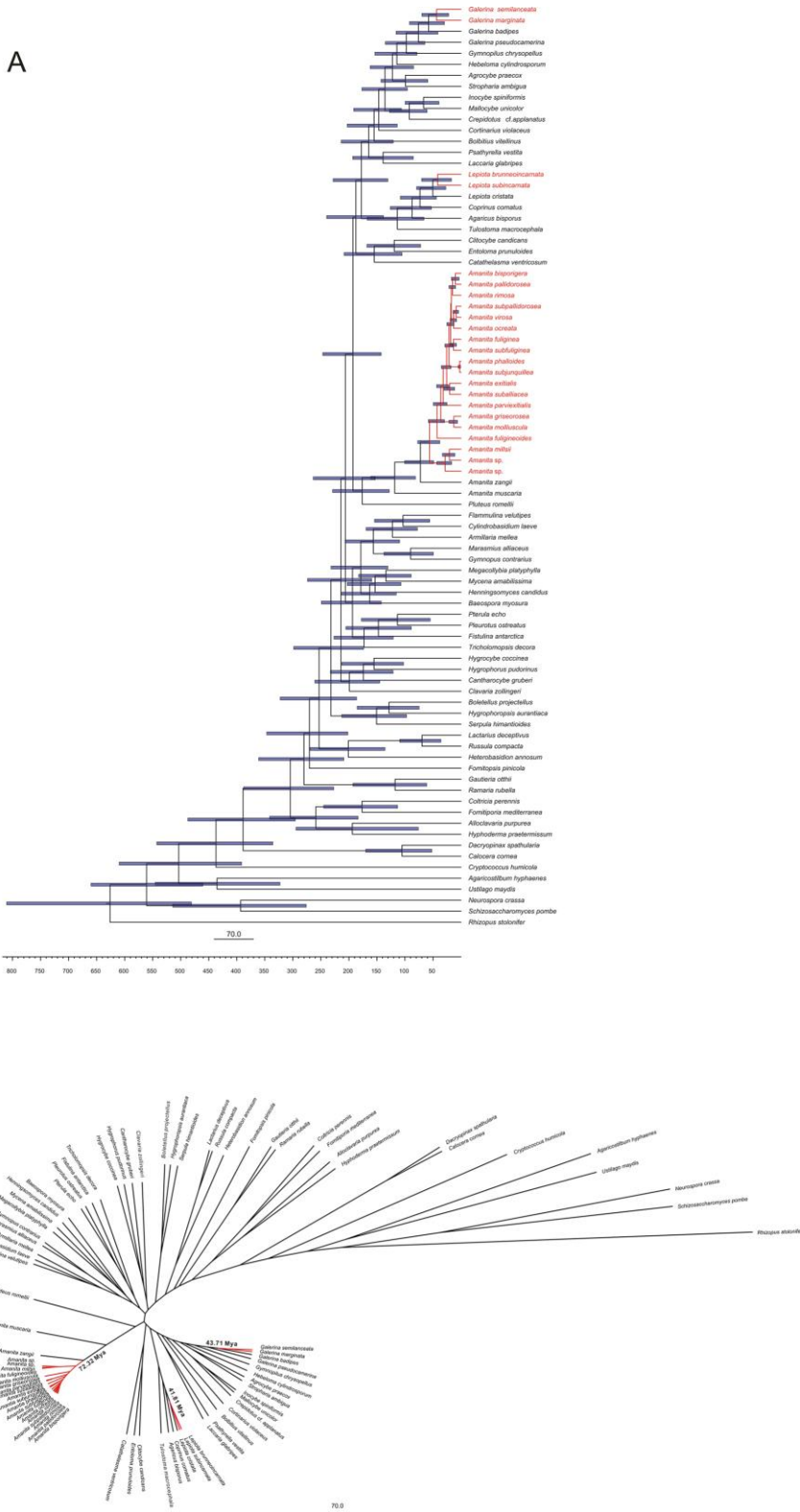


Fig. S15. Divergence estimation of the genera *Amanita*, *Galerina* and *Lepiota* using nrLSU, *rpb2* and *tef1- α* . (A) The rectangular tree layout of the Maximum clade credibility trees (MCC) trees with blue node bars representing the 95% highest posterior density intervals. (B) The radial tree layout of the MCC trees. The amanita-producing species in *Amanita*, *Galerina* and *Lepiota* are indicated in red. Bar = 70 Mya.

Table S1A. Genome features of *Amanita* species.

Species	<i>A. molliuscula</i>	<i>A. fuliginea</i>	<i>A. pallidorosea</i>	<i>A. subjunquillea</i>	<i>A. exitialis</i>	<i>A. rimosa</i>
Size of assembled genome	71.17 Mb	60.23 Mb	57.85 Mb	53.20 Mb	49.12 Mb	45.35 Mb
Number of contigs (scaffolds)	155	35	246 (scaffold)	119 (scaffold)	129	134
N50 (bp)	1053316	3461821	537462	838703	3025964	697900
N90 (bp)	188356	1258523	145062	240927	296766	162745
Maximum length (bp)	3180309	589859	2246582	2832323	4698837	1811650
GC content of assembled genome	46.50%	45.91%	46.24%	46.57%	46.60%	45.99%
Transposable elements of genome	64.78%	39.15%	55.60%	53.70%	43.78%	50.48 %
BUSCO	87.3%	95.1%	90.3%	91.4%	92%	86.5%
CEGMA*	95.16%, 93.55%	96.77%, 95.16%	96.35%, 93.28%	97.89%, 96.12%	97.18%, 95.56%	97.18%, 95.56%
Number of <i>MSDIN</i> genes	21	30	33	24	23	39

Table S1B. Genome features of *Lepiota* species.

Species	<i>L. venenata</i>	<i>L. brunneoincarnata</i>	<i>L. subincarnata</i> 1	<i>L. subincarnata</i> 2
Size of assembled genome	49.26M	55.61 Mb	38.40 Mb	37.84 Mb
Number of contigs	88	97	324 (scaffold)	576 (scaffold)
N50	1272972bp	1225926bp	920212bp	415558bp
N90	344036bp	275636bp	163783bp	52448bp
Maximum length	3318280bp	3973606bp	2859616bp	2597781bp
GC content of assembled genome	49.02%	48.85%	49.76%	49.79%
Transposable elements of genome	34.18%	34.04 %	N/A	N/A
BUSCO	87.3%	89.1%	93.1%	93.5%
CEGMA*	94.32%, 93.44%	95.97%, 91.94%	93.55%, 95.97%	81.85%, 83.47%
Number of <i>MSDIN</i> genes	4	3	6	5

Table S1C. Genome features of *Galerina* species.

Species	<i>G. marginata</i> JGI	<i>G. marginata</i>	<i>G. sulciiceps</i>
Size of assembled genome	59.42 Mb	69.36 Mb	101.89 Mb
Number of contigs (scaffolds)	414	44	137
N50	1219514 bp	2607326 bp	1836322 bp
N90	334874 bp	745800 bp	505162 bp
Maximum length	3629680 bp	4516205 bp	4131357 bp
GC content of assembled genome	47.97%	47.60%	47.81%
Transposable elements of genome	N/A	13.97%	17.56%
BUSCO	95.2%	89.7%	97.9%
CEGMA*	N/A	96.37%, 96.37%	97.18%, 95.97%
Number of <i>MSDIN</i> genes	1 in 2 copies	1 in 2 copies	1 in 2 copies

*CEGMA: the two percentages are (Complete+Partial)% and Complete%, respectively

Table S2. Genes in the vicinity of *GmAMA1* (numbered gene 0) on scaffold_42 of *Galerina marginata* genome. Predictions were based on JGI annotation, KEGG and/or Pfam motifs and COG/SwissProt/PDB. Proteins for which no reasonable function could be deduced are listed simply as “predicted protein”. N.D. not determined.

Gene number; position upstream (-) or downstream (+) of <i>GmAMA1-1</i>	JGI gene number and genomic position	Predicted function	Restricted to amanitin-producing species?	Gene deletion phenotype
+10	Galma1_257027 42:140765-144287	aminopeptidase	No	N.D.
+9	Galma1_257029 42:144355-148517	RNA helicase	No	N.D.
+8	Galma1_1042023 42:148306-149401	Zn finger	No	N.D.
+7	Galma1_161032 42:149378-152593	Splicing coactivator SRm160/300, subunit SRm300	No	N.D.
+6	Galma1_257032 42:152634-156484	Cdc2-related protein kinase	No	N.D.
+5	Galma1_78730 42:157417-157974	NUDIX hydrolase core	N.D.	N.D.
+4	Galma1_104940 42:157739-159538	Predicted protein	No	N.D.
+3	Galma1_257038 42:159684-160745	Predicted protein	N.D.	N.D.
+2	Galma1_78538 42:161453-164563	Prolyl oligopeptidase B (GmPOPB)	Yes	No amanitin
+1	Galma1_104945 42:165397-167604	Flavin monooxygenase	Yes	No amanitin; appearance of modified products
0	Galma1_1387421 42:167593-168145	<i>GmAMA1-1</i> : Core propeptide for α -amanitin	Yes	No amanitin
-1	Galma1_78564 42:169158-170505	Predicted protein	No	No phenotype
-2	Galma1_129463 42:171096-172792	26S proteasome subunit P45	N.D.	N.D.
-3	Galma1_78731 42:173298-176762	RNA helicase	Yes	No amanitin
-4	Galma1_78688 42:180479-	G protein alpha subunit	Yes	No amanitin

	183180			
-5	Galma1_231355 42:192672- 193619 [9.5 kb gap is between this one and -4]	Predicted protein	No	N.D.
-6	Galma1_257051 42:194701- 197274	Glycoside hydrolase family 5	N.D.	N.D.
-7	Galma1_215833 42:198847- 200755	Predicted protein	N.D.	N.D.
-8	Galma1_78691 42:201312- 204681	HAT repeat protein	No	N.D.
-9	Galma1_231359 42:207145- 209370	Predicted protein	N.D.	N.D.
-10	Galma1_146353 42:209732- 210893	Predicted protein	N.D.	N.D.
-11	Galma1_215837 42:211640- 213731	Chaperonin Cpn60	No	N.D.
-12	Galma1_257062 42:215343- 216248	Predicted protein	No	N.D.
-13	Galma1_1043998 42:219282- 221843	DUF2046	Yes	No amanitin
-14	Galma1_1043988 42:221415- 222437	Predicted protein	N.D.	N.D.
-15	Galma1_231366 42:222497- 225560	Predicted protein	No	N.D.
-16	Galma1_231368 42:227121- 229532	NUDIX hydrolase	N.D.	N.D.
-17	Galma1_257091 42:231164- 232545	Predicted protein	No	N.D.
-18	Galma1_1044687 42:233923- 235734	Protein kinase-like	N.D.	N.D.
-19	Galma1_257096 42:236124- 238040	Protein kinase-like	N.D.	N.D.
-20	Galma1_146362 42:238703- 240154	Protein kinase-like	N.D.	N.D.
-21	Galma1_257099 42:240649- 243248	Multidrug resistance efflux transporter (high expression); pfam05653	No	N.D.

-22	Galma1_1044278 42:243547- 244070	Predicted protein	N.D.	N.D.
-23	Galma1_1044290 42:244042- 245433	Predicted protein	N.D.	N.D.
-24	Galma1_1354407 42:247479- 252016	Kinesin light chain	No	N.D.
-25	Galma1_257105 42:254825- 256261	ankyrin-containing protein	N.D.	N.D.
-26	Galma1_231377 42:256478- 258563	CYP450 family 512	Yes	Unsuccessful
-27	Galma1_257111 42:258816- 260766	Ankyrin-containing protein	N.D.	N.D.
-28	Galma1_78764 42:261689- 263647	CYP450 family 512	Yes	Unsuccessful
-29	Galma1_1044748 42:264395- 266860	CYP450 family 512	Yes	No amanitin; appearance of modified product
-30	Galma1_215850 42:267138- 268828	Trypsin-like peptidase	N.D.	N.D.
-31	Galma1_215851 42:269431- 271514	CYP450 family 512	Yes	Unsuccessful
-32	Galma1_257133 42:272517- 273556	40S ribosomal protein S26 (high expression)	N.D.	N.D.
-33	Galma1_257135 42:273729- 275057	β -1,6-N- acetylglucosaminyl- transferase	N.D.	N.D.
-34	Galma1_257137 42:276090- 277950	Zn finger	N.D.	N.D.
-35	Galma1_78570 42:279908- 282443	Major facilitator superfamily	N.D.	N.D.
-36	Galma1_129535 42:283203- 287081	Histone transcription regulator Tup1-like	N.D.	N.D.
-37	Galma1_257141 42:287317- 289072	predicted protein	N.D.	N.D.
-38	Galma1_272650 42:289228- 292537	Zn finger	N.D.	N.D.

Table S3. Accession numbers of the sequences used in divergence time estimation for *Amanita*, *Galerina* and *Lepiota*.

Taxon	Voucher	Accession numbers		
		nrLSU	<i>rpb2</i>	<i>tef1-α</i>
<i>Agaricostilbum hyphaenes</i>	CBS7811	AY634278	AY780933	AY879114
<i>Agaricus bisporus</i>	TUB 011586	DQ071710	FJ623644	N/A
<i>Agrocybe praecox</i>	AFTOL-ID 728	AY646101	DQ385876	N/A
<i>Alloclavaria purpurea</i>	AFTOL-ID 1736	DQ457657	N/A	N/A
<i>Amanita bisporigera</i>	RET 377-9	KJ466434	N/A	KJ481936
<i>Amanita exitialis</i>	HKAS75774	JX998052	KJ466591	JX998001
<i>Amanita fuliginea</i>	HKAS75780	JX998048	KJ466595	JX997995
<i>Amanita fuligineoides</i>	HKAS83694	MH486553	MH486020	MH508824
<i>Amanita griseorosea</i>	HKAS89004	KU168387	KU168388	KU168386
<i>Amanita molliuscula</i>	HKAS77324	KJ466472	KJ466639	KJ481974
<i>Amanita muscaria</i>	GAL2810	DQ060884	N/A	EU071872
<i>Amanita ocreata</i>	HKAS79686	KJ466442	KJ466607	KJ481947
<i>Amanita pallidorosea</i>	HKAS61937	KJ466443	KJ466609	KJ481949
<i>Amanita parviexitialis</i>	HKAS79049	KT971342	KT971345	KT971346
<i>Amanita phalloides</i>	HKAS75773	JX998060	KJ466612	JX998000
<i>Amanita rimosa</i>	HKAS75777	JX998044	KJ466615	JX998005
<i>Amanita</i> sp.	HKAS77322	KJ466457	KJ466643	KJ481978
<i>Amanita</i> sp.	HKAS75150	KJ466477	KJ466641	KJ481976
<i>Amanita</i> sp.	HKAS77323	KJ466478	KJ466642	KJ481977
<i>Amanita suballiacea</i>	RET 490-1	KJ466485	KJ466601	KJ481941
<i>Amanita subfuliginea</i>	HKAS77326	KJ466467	KJ466636	KJ481971
<i>Amanita subjunquillea</i>	HKAS75770	JX998062	KJ466653	JX997999
<i>Amanita subpallidorosea</i>	HKAS77350	KJ466462	KJ466631	KJ481966
<i>Amanita virosa</i>	HKAS56694	JX998058	KJ466664	JX998007
<i>Amanita zangii</i>	GDGM29241	KJ466499	KJ466668	KJ482000
<i>Armillaria mellea</i>	AFTOL-ID 449	AY700194	AY780938	AY881023
<i>Baeospora myosura</i>	AFTOL-ID 1799	DQ457648	DQ470827	GU187762
<i>Bolbitius vitellinus</i>	AFTOL-ID 730	AY691807	DQ385878	N/A
<i>Boletellus projectellus</i>	MB03-118	AY684158	AY787218	AY879116

<i>Calocera cornea</i>	GEL5359	AY701526	AY536286	AY881019
<i>Cantharocybe gruberi</i>	AFTOL-ID 1017	DQ234540	DQ385879	DQ059045
<i>Catathelasma ventricosum</i>	AFTOL-ID 1488	DQ089012	DQ470830	N/A
<i>Clavaria zollingeri</i>	TENN58652	AY639882	AY780940	AY881024
<i>Clitocybe candicans</i>	AFTOL-ID 541	AY645055	DQ385881	DQ408149
<i>Coltricia perennis</i>	DSH93-198	AF287854	AY218526	AY885147
<i>Coprinus comatus</i>	AFTOL-ID 626	AY635772	AY780934	N/A
<i>Cortinarius violaceus</i>	AFTOL-ID 814	DQ457662	DQ470835	N/A
<i>Crepidotus cf. applanatus</i>	AFTOL-ID 817	AY380406	AY333311	DQ028581
<i>Cryptococcus humicola</i>	PYCC 3387T	DQ645514	DQ645517	DQ645519
<i>Cylindrobasidium laeve</i>	AFTOL-ID 453	DQ234541	AY536283	DQ408151
<i>Dacryopinax spathularia</i>	GEL5052	AY701525	AY786054	AY881020
<i>Entoloma prunuloides</i>	AFTOL-ID 523	AY700180	DQ385883	DQ457633
<i>Fistulina antarctica</i>	CBS 701.85	AY293181	N/A	N/A
<i>Flammulina velutipes</i>	AFTOL-ID 558	AY639883	AY786055	AY883423
<i>Fomitiporia mediterranea</i>	Michael Fischer 3/22	AY684157	AY803748	AY885149
<i>Fomitopsis pinicola</i>	MB03-036	AY684164	AY786056	AY885152
<i>Galerina badipes</i>	GLM 45922	AY207201	N/A	N/A
<i>Galerina marginata</i>	N/A	JGI	JGI	JGI
<i>Galerina pseudocamerina</i>	O 73481 PBM 1398 (WTU)	AJ871519	N/A	N/A
<i>Galerina semilanceata</i>	Washington	AY038309	AY337357	N/A
<i>Gautieria otthii</i>	REG 636	AF393058	AY218486	AY883434
<i>Gymnopilus chrysopellus</i>	PR-1187	JGI	JGI	JGI
<i>Gymnopus contrarius</i>	AFTOL-ID 1758	DQ457670	GU187700	DQ472716
<i>Hebeloma cylindrosporum</i>	h7	JGI	JGI	JGI
<i>Henningsomyces candidus</i>	AFTOL-ID 468	AY571008	AY883424	AY218513
<i>Heterobasidion annosum</i>	N/A	JGI	JGI	JGI
<i>Hygrocybe conica</i>	PBM 918	AY684167	AY803747	AY883425
<i>Hygrophoropsis aurantiaca</i>	MB03-127	AY684156	AY786059	AY883427
<i>Hygrophorus pudorinus</i>	AFTOL-ID 1723	DQ457678	DQ472725	GU187710
<i>Inocybe spiniformis</i>	PERTH: 08363277	KP171063	N/A	N/A
<i>Laccaria glabripes</i>	GMM7521	KU685849	KU685991	KU686117
<i>Lactarius deceptivus</i>	PBM2462	AY631899	AY803749	AY885158

<i>Lepiota brunneoincarnata</i>	HMAS 63488	EU416303	N/A	N/A
<i>Lepiota cristata</i>	HKAS 45053	JN940283	JN993699	JN993699
<i>Lepiota subincarnata</i>	N/A	Genome	Genome	Genome
	PBM 1481 (WTU)			
<i>Mallocybe unicolor</i>	Missouri	AY380403	AY337409	N/A
<i>Marasmius alliaceus</i>	TENN55620	AY635776	AY786060	AY883431
<i>Megacollybia platyphylla</i>	AFTOL-ID 560	AY702016	DQ385887	DQ435786
<i>Mycena amabilissima</i>	AFTOL-ID 1686	DQ457691	DQ474121	GU187727
<i>Neurospora crassa</i>	MUCL 19026	AF286411	XM_952013	XM_959775
<i>Pleurotus ostreatus</i>	AFTOL-ID 564	AY645052	AY786062	AY883432
<i>Pluteus romellii</i>	ECV 3201	AY634279	AY786063	AY883433
<i>Psathyrella vestita</i>	SZMC-NL-2346	FN430695	N/A	FN430696
<i>Pterula echo</i>	AFTOL-ID 711	AY629315	GU187805	N/A
<i>Ramaria rubella</i>	PBM 2408	AY645057	AY786064	AY883435
<i>Rhizopus stolonifer</i>	DAOM225708	DQ273817	AFTOL	AFTOL
<i>Russula compacta</i>	BPL669	JGI	JGI	JGI
<i>Schizosaccharomyces pombe</i>	Strain972	Z19136	NM_001018498	NM_001022750
<i>Serpula himantoides</i>	AFTOL-ID 1387	AF518648	AY218517	DQ059046
<i>Stropharia ambigua</i>	AFTOL-ID 726	AY646102	DQ484054	N/A
<i>Tricholomopsis decora</i>	AFTOL-ID 537	AY691888	DQ408112	DQ029195
<i>Tulostoma macrocephalum</i>	Long 10111 FH	AF518663	N/A	N/A
<i>Ustilago maydis</i>	PBM2469	AF453938	AY485636	AY885160

Table S4. 2D-NMR correlations observed in new product from the *CYP450-29* mutant (θ -amanitin).

	Residue	Nucleus	Atom	ppm	TOCSY	COSY	HSQC	HMBC	ROESY
1	Ile1	H	HN	7.86	1,2,3,4,5,6	1,2	x	83	2,4,6,13,74
2	Ile1	H	H α	4.20	1,2,3,4,5,6	1,2,3	7	8,9,10,12	1,4,6,13
3	Ile1	H	H β	1.99	1,2,3,4,5,6	2,3,5	8	7,9,10,11,12	x
4	Ile1	H	H γ	3.69	1,2,3,4,5,6	4,6	9	7,8,10,11	1,2,6,74
5	Ile1	H	γ CH3	0.82	1,2,3,4,5	3,5	10	7,8,9	x
6	Ile1	H	δ CH3	0.92	2,3,4,6	4,6	11	8,9	1,2,4
7	Ile1	C	C α	55.87	x	x	2	3,4,5	x
8	Ile1	C	C β	40.97	x	x	3	2,4,5,6	x
9	Ile1	C	C γ	65.41	x	x	4	2,3,5,6	x
10	Ile1	C	C γ'	10.73	x	x	5	2,3,4	x
11	Ile1	C	C δ	17.77	x	x	6	3,4	x
12	Ile1	C	CO	170.58	x	x	x	2,3,13,14	x
13	Trp2	H	HN	8.04	13,14,15,16	13,14	x	12,21,22	1,2,14,15,16
14	Trp2	H	H α	4.81	13,14,15,16	13,14,15,16	21	12,22,31	13,15,18,32
15	Trp2	H	H β 1	3.26	13,14,15,16	14,15,16	22	21,23,24,25	13,14,16,18,67
16	Trp2	H	H β 2	2.68	13,14,15,16	14,15,16	22	21,23,24,25	13,15,32,62,67
17	Trp2	H	1(N)H	11.27	x	x	x	x	x
18	Trp2	H	4H	7.45	18,19,20	18,19	26	24,25,28,29,30	14,15,19
19	Trp2	H	5H	6.58	18,19,20	18,19	27	25,28,29	18
20	Trp2	H	7H	6.74	18,19,20	x	29	25,27,28,30	x
21	Trp2	C	C α	53.03	x	x	14	13,15,16	x
22	Trp2	C	C β	28.04	x	x	15,16	13,14	x
23	Trp2	C	C2	129.86	x	x	x	15,16	x
24	Trp2	C	C3	111.28	x	x	x	15,16,18	x
25	Trp2	C	C3a	120.66	x	x	x	15,16,18,19,20	x
26	Trp2	C	C4	122.20	x	x	18	x	x
27	Trp2	C	C5	110.41	x	x	19	20	x
28	Trp2	C	C6	154.66	x	x	x	18,19,20	x
29	Trp2	C	C7	96.45	x	x	20	18,19	x
30	Trp2	C	C7a	138.76	x	x	x	18,20	x

31	Trp2	C	CO	170.34	x	x	x	14,32	x
32	Gly3	H	HN	7.88	32,33,34	32,33	x	31	14,16,33,34,57,62
33	Gly3	H	Ha1	4.37	32,33,34	32,33,34	35	36	32,34,37
34	Gly3	H	Ha2	3.27	32,33,34	33,34	35	36	32,33,37
35	Gly3	C	Ca	40.82	x	x	33,34	x	x
36	Gly3	C	CO	170.35	x	x	x	33,34,37	x
37	Ile4	H	HN	8.45	37,38,39,41,42	37,38	x	36,45	33,34,39,41,42
38	Ile4	H	Ha	3.60	37,38,39,41,42,43	37,38,39	44	45,47,49	x
39	Ile4	H	Hβ	1.55	37,38,39,41,42,43	38,39,40,41,42	45	44,46,47,49	37
40	Ile4	H	Hγ1	1.53	x	39,40,41,43	46	44,45,47,48	37,50
41	Ile4	H	Hγ2	1.09	38,39,41,42,43	39,40,41,43	46	44,45,47,48	37
42	Ile4	H	γ'CH3	0.80	37,38,39,41,42	39,42	47	44,45,46	37,50,51
43	Ile4	H	δCH3	0.82	39,41,43	40,41,43	48	45,46	x
44	Ile4	C	Ca	58.94	x	x	38	39,40,41,42	x
45	Ile4	C	Cβ	34.52	x	x	39	37,38,40,41,42,43	x
46	Ile4	C	Cγ	24.97	x	x	40,41	39,42,43	x
47	Ile4	C	Cγ'	14.52	x	x	42	38,39,40,41	x
48	Ile4	C	Cδ	10.73	x	x	43	40,41	x
49	Ile4	C	CO	171.50	x	x	x	38,39,50,51,52	x
50	Gly5	H	HN	8.79	50,51,52	50,51,52	x	49,53	38,40,41,42,51,52,55
51	Gly5	H	Ha1	3.87	50,51,52	50,51,52	53	49,54	42,50,52,55
52	Gly5	H	Ha2	3.45	50,51,52	50,51,52	53	49,54	50,51,55
53	Gly5	C	Ca	42.17	x	x	51,52	50	x
54	Gly5	C	CO	168.10	x	x	x	51,52,55	x
55	Cys6	H	HN	8.31	55,56,57,58	55,56	x	54	50,51,52,56,57,58,63
56	Cys6	H	Ha	4.94	55,56,57,58	55,56,57	59	60,61	55,58,62
57	Cys6	H	Hβ1	3.06	55,56,57,58	56,57,58	60	59,61	16,32,55,58,62
58	Cys6	H	Hβ2	2.91	55,56,57,58	56,57,58	60	59,61	55,56,57
59	Cys6	C	Ca	49.92	x	x	56	57,58	x
60	Cys6	C	Cβ	58.80	x	x	57,58	56	x
61	Cys6	C	CO	167.14	x	x	x	56,57,58,62	x
62	Asn7	H	HN	8.49	62,63,64,65	62,63	x	61,71	16,32,56,57,63

63	Asn7	H	H α	4.77	62,63,64,65	62,63,64,65	68	70	62,64,65,77
64	Asn7	H	H β 1	3.48	63,64,65	63,64,65	69	68,70,71	62,63,65,66
65	Asn7	H	H β 2	3.01	63,64,65	63,64,65	69	68,70	63,64,77
66	Asn7	H	δ NH2(1)	8.50	66,67	x	x	70	64,67
67	Asn7	H	δ NH2(2)	7.70	66,67	x	x	70	15,16,66
68	Asn7	C	C α	50.65	x	x	63	64,65	x
69	Asn7	C	C β	33.05	x	x	64,65	x	x
70	Asn7	C	γ CO	173.09	x	x	x	63,64,65,66,67	x
71	Asn7	C	CO	170.01	x	x	x	62,64	x
72	Pro8	H	H α	4.15	72,73,74,75,76,77,78	72,73,74	79	80,83	1,63,73,76,74
73	Pro8	H	H β 1	2.31	72,73,74,75,76,77,78	72,73,74	80	81,82	72,74,76
74	Pro8	H	H β 2	1.64	72,73,74,75,76,77,78	72,73,74,76	80	79,81,83	1,4,72,73,75
75	Pro8	H	H γ 1	1.98	72,73,74,75,76,77,78	x	81	79	x
76	Pro8	H	H γ 2	1.81	72,73,74,75,76,77,78	78	81	80,82	72,73,77
77	Pro8	H	H δ 1	3.96	72,73,74,75,76,77,78	75,76,77,78	82	79,80,81	63,65,75,76
78	Pro8	H	H δ 2	3.60	72,73,74,75,76,77,78	76,77,78	82	81	x
79	Pro8	C	C α	63.15	x	x	72	74,75,77	x
80	Pro8	C	C β	29.65	x	x	73,74	72,76,77	x
81	Pro8	C	C γ	24.96	x	x	75,76	73,74,77,78	x
82	Pro8	C	C δ	47.30	x	x	77,78	73,76	x
83	Pro8	C	CO	170.42	x	x	x	1,72,74	x

Table S5. Likelihood ratio tests of *FMO1* and *P450-29* genes among *Amanita*, *Galerina* and *Lepiota*.

Species	<i>FMO1</i>				<i>P450-29</i>				<i>rbp2</i>			
	dN	dS	dN/dS	Distance	dN	dS	dN/dS	Distance	dN	dS	dN/dS	Distance
<i>Amanita subjunquillea</i> vs. <i>Galerina marginata</i>	0.5226	1.0033	0.5209	1.9752	1.0034	0.3489	2.8762	2.497	0.0821	6.712	0.0122	5.9329
<i>A. subjunquillea</i> vs. <i>Lepiota brunneoincarnata</i>	0.9599	1.6527	0.5808	3.2686	0.9439	0.2916	3.2364	2.3514	0.1047	4.5	0.0233	3.9296
<i>L. brunneoincarnata</i> vs <i>G. marginata</i>	0.9609	1.7225	0.5578	3.1642	0.8985	0.3942	2.2791	2.3159	0.0859	5.2655	0.0163	3.681

Table S6. MSDIN genes in amanitin-producing *Lepiota* and *Galerina* species, including *L. brunneoincarnata* (*Lb*), *L. venenata* (*Lv*), *L. subincarnata1* (*Ls1*), *L. subincarnata2* (*Ls2*), *G. sulciceps* (*Gs*) and *G. marginata* (*Gm*).

Species name	No.	Leader peptide	Core Peptide	Recognition Sequence	Coding and Notes
<i>Lepiota brunneoincarnata</i>	1	MDANTTRLP	IWGIGCNP	WAPESVNDTLTRGK	α -amanitin
	2	MDANSTRLP	IWGIGCNP	WAPESVNDTLTRGK	α -amanitin
	3	TSDINSARLP	VSATAYSA	WSPDIANDPVNHG	lacking ATG
<i>Lepiota venenata</i>	1	MDANATRLP	IWGIGCNP	WTPESVNDTLTK	α -amanitin
	2	MSDANNTRLP	FFAPGLPFPP	WTGENADHILARSK	
	3	MSDANNTRLP	FFVPGLPFPP	WTGENADHILARSK	
	4	MSDLNNTLRP	VVTVLFTPPP	WSGESVDHSLTRSK	
<i>Lepiota subincarnata 1</i>	1	MDANATRLP	IWGIGCNP	WTPENVNDTLTRGK	α -amanitin
	2	MLDANNTRLP	LVAAVGIPP	WSGEDADHTLTRGK	
	3	MSDANSTRLP	FVLLLVPP	WTGEGFDNTLTRGK	2 copies in <i>Ls2</i>
	4	MSDVNSXRLP	IALVLXLFP	WTGEGFDNTLTRGK	2 copies in <i>Ls2</i>
	5	MSEANSARLP	IPILAHLGP	WTGEGFDSTLTRGK	
	6	MSDANNTRLP	LFFPLPIPP	WSGEDTDHTLTRGK	2 copies in <i>Ls2</i>
<i>Lepiota subincarnata 2</i>	1	MDANATRLP	IWGIGCNP	WTPENVNDTLTRGK	α -amanitin
	2	MSDANSTRLP	FVLLLVPP	WTGEGFDNTLTRGK	2 copies in <i>Ls1</i>

	3	MSDVNSXRLP	IALVLXLP	WTGEGFDNTLTRGK	2 copies in Ls1
	4	MSEANSARLP	IPILAHXGP	WTGEGFDSXLTRGK	
	5	MSDANNTRLP	LFFPLPIPP	WSGEDTDHTLTRGK	2 copies in Ls1
<i>Galerina sulciceps</i>	1	MFDTNATRLP	IWGIGCNP	WTAEHVDQTLASG	α -amanitin, 2 copies
<i>Galerina marginata</i>	1	MFDTNATRLP	IWGIGCNP	WTAEHVDQTLASG	α -amanitin
	2	MFDTNSTRLP	IWGIGCNP	WTAEHVDQTLVSG	α -amanitin
