

Diversity of MSDIN family members in amanitin-producing mushrooms and the phylogeny of the MSDIN and prolyl oligopeptidase genes

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Abstract

Background: Amanitin-producing mushrooms, mainly distributed in the genera *Amanita*, *Galerina* and *Lepiota*, possess MSDIN gene family for the biosynthesis of many cyclopeptides catalysed by prolyl oligopeptidase (POP). Recently, transcriptome sequencing has proven to be an efficient way to mine MSDIN and POP genes in these lethal mushrooms. Thus far, only *A. pallioides* and *A. bisporigera* from North America and *A. exitialis* and *A. rimosa* from Asia have been studied based on transcriptome analysis. However, the MSDIN and POP genes of many amanitin-producing mushrooms in China remain unstudied; hence, the transcriptomes of these species deserve to be analysed. **Results:** In this study, the MSDIN and POP genes from ten *Amanita* species, two *Galerina* species and *Lepiota venenata* were studied and the phylogenetic relationships of their MSDIN and POP genes were analysed. Through transcriptome sequencing and PCR cloning, 19 POP genes and 151 MSDIN genes were found to encode 98 non-duplicated cyclopeptides, including α -amanitin, β -amanitin, phalloidin, phalloidin and 94 unknown peptides, were found in these species. Phylogenetic analysis showed that (1) MSDIN genes generally clustered depending on the taxonomy of the genus, while *Amanita* MSDIN genes clustered depending on the chemical substance; and (2) the POPA genes of *Amanita*, *Galerina* and *Lepiota* clustered and were separated into three different groups, but the POPB genes of the three distinct genera were clustered in a highly supported monophyletic group. **Conclusions:** These results indicate that lethal *Amanita* species have the genetic capacity to produce numerous cyclopeptides, most of which are unknown, while lethal *Galerina* and *Lepiota* species seem to only have the genetic capacity to produce α -amanitin. Additionally, the POPB phylogeny of *Amanita*, *Galerina* and *Lepiota* conflicts with the taxonomic status of the three genera, suggesting that underlying horizontal gene transfer has occurred among these three genera.

Background

Amatoxins, which are lethal substances found in mushrooms, have mainly been reported to be present in species from three distinct genera classified into three different families: *Amanita* (Amanitaceae), *Galerina* (Hymenogastraceae) and *Lepiota* (Agaricaceae) [1-4]. Among these amanitin-producing mushrooms, lethal *Amanita* species are the best-known and most typical mushrooms that produce three primary groups of cyclopeptide toxins: amatoxins, phallotoxins and virotoxins, which are bicyclic octapeptides, bicyclic heptapeptides and monocyclic heptapeptides, respectively [1-4].

The precursor peptide genes of α -amanitin (α -AMA) and phalloidin (PHD) along with multiple related sequences encoding unknown cyclic peptides were first identified and predicted in *Amanita bisporigera* by genome shotgun sequencing, indicating that amatoxins and phallotoxins are encoded by the same gene family and are biosynthesized on ribosomes [5]. This gene family is referred to as MSDIN in reference to the first five conserved encoded amino acids, and the precursor peptides of its members contain 33-37 amino acids, consisting of two conserved regions, 10 upstream amino acids and 17 downstream amino acids, a highly variable core region, and a 6-10 amino acid sequence that ultimately forms the corresponding cyclopeptide [6]. *GmAMA*, which is responsible for producing α -AMA, is also found in the genome of *Galerina marginata* [7]. *Galerina marginata* is a specific amanitin-containing species in the genus *Galerina*. Unlike lethal amanitas, *G. marginata* does not harbour MSDIN-like family genes other than two copies of α -AMA genes. Additionally, α -AMA of *Lepiota brunneoincarnata*, which is an amanitin-containing mushroom of the genus *Lepiota*, has been successfully cloned [8]. The genome sequencing of *L. venenata*, another newly reported amanitin-containing species, has been completed, and it has been shown to harbour α -AMA genes [9]. Precursor

peptide sequence alignment of α -AMA sequences from *Amanita*, *Galerina* and *Lepiota* shows high divergence except in the toxin region.

It has been strongly indicated that a prolyl oligopeptidase (POP) plays an important role in the initial processing of MSDIN precursor peptides. Since the core toxin regions are flanked by two highly conserved proline (Pro) residues, this enzyme can cleave the C-terminus of Pro residues and release the peptide chain of the toxin to form a cyclopeptide [10]. It has been reported that there are two types of POP in amatoxin-producing mushrooms: POPA, which behaves like a conventional housekeeping protein that is present in all species, and POPB, which is the enzyme that actually catalyses the cutting and cyclization of precursor peptides [7, 11, 12].

Increasing numbers of MSDIN family members have been published since the first 15 MSDIN genes were found in the *A. bisporigera* genome, and four were amplified by using degenerate primers in *A. phalloides* and *A. ocreata* [5]. Twenty-four MSDIN members were obtained from 6 *Amanita* species using degenerate primers [13]. Recently, the draft genome sequences of *A. phalloides* and *A. bisporigera* showed that each species possessed approximately 30 MSDIN members, but only three of these genes were common to the two fungi [6]. Eighteen and twenty-two MSDIN genes were mined from the *A. subjunquillea* and *A. pallidorozea* genomes through PacBio and Illumina sequencing, respectively [8]. However, the MSDIN genes of many amanitin-containing *Amanita*, *Galerina* and *Lepiota* mushrooms have not been investigated in depth to date. Lethal *Amanita* species are classified in section *Phalloideae* of the genus *Amanita* [14, 15]. Approximately 50 lethal *Amanita* species have been reported worldwide, and the species diversity of lethal amanitas is strongly underestimated under the current taxonomy [15, 16]. Many new lethal *Amanita* and *Lepiota* species, including *A. rimosa*, *A. subfuliginea*, *A. subpallidorozea*, and *L. venenata*, have been discovered over the past decade [17-20]. In addition to the 22 known cyclopeptide toxins, some new cyclopeptide substances, such as cycloamanide E and cycloamanide F in *A. phalloides* and amanexitide in *A. exitialis*, have been extracted and identified [6, 21, 22]. It has been reported that *A. bisporigera* and *A. phalloides* present high potential for the biosynthesis of a variety of cyclopeptides, most of which are unknown according to predictions. Hence, considering the species diversity of amanitin-containing mushrooms and the broad genetic capacity of lethal amanitas to produce unknown cyclopeptides, there are still many new cyclopeptide genes and corresponding cyclopeptides to be discovered.

Alpha-amanitin and toxin-biosynthetic prolyl oligopeptidase B (POPB) genes have been proven to exist in some lethal *Amanita* [6, 23, 24], *Galerina* [7] and *Lepiota* [9] species. The reason that the biosynthetic pathway for α -amanitin is present in these three phylogenetically disjunct genera classified in different families has been studied in recent years. Recent studies reported that horizontal gene transfer (HGT) is the underlying cause of the distribution of MSDIN and *POPB* genes in *Amanita*, *Galerina* and *Lepiota* on the basis of phylogenetic analysis [8, 9]. The possibility of convergent evolution was negated because the MSDIN and *POPB* genes in these three genera show similarity and associations, such as a shared conserved gene structure and the encoding of precursor peptides by MSDIN genes [8].

According to previous research, whole-genome sequencing has proven to be the most comprehensive, in-depth method for identifying MSDIN genes or genes related to the cyclopeptide biosynthetic pathway in amanitin-producing mushrooms [6, 8]. Nevertheless, compared with genome sequencing, transcriptome sequencing provides an alternative efficient and low-cost method to obtain functional

gene data. To the best of our knowledge, only *A. pallioides* and *A. bisporigera* from North America and *A. exitialis* and *A. rimosa* from Asia have been studied using transcriptome sequencing [6, 25, 26].

In this study, the transcriptomes of seven amanitin-producing mushrooms (*A. exitialis*, *A. fuliginea*, *A. molliuscula*, *A. pallidorosea*, *A. rimosa*, *A. subpallidorosea* and *L. venenata*) and an *Amanita* species producing no amanitin (*A. oberwinklerana*) were sequenced. MSDIN and *POP* genes were searched and predicted from the transcriptome data. The genomic and coding sequences of the MSDIN and *POP* genes were cloned and verified. Similarly, MSDIN and *POP* sequences were cloned from two *Galerina* strains (*G. marginata* and *G. sulciceps*). In addition to the *Amanita* species mentioned above, MSDIN genes from *A. subfuliginea*, *A. subjunquillea* and *A. virosa* were cloned using specific and degenerate primers. Furthermore, phylogenetic analysis was performed on the obtained toxin and *POP* genes. Our study was aimed at (a) identifying MSDIN genes from amanitin-producing mushrooms to guide the isolation and identification of new unknown related cyclopeptides and (b) determining the evolutionary relationships of toxin MSDIN and *POP* genes in amanitin-producing mushrooms.

Results

Data filtering and assembly of transcriptomes

Transcriptome sequencing of seven amanitin-producing mushrooms was performed on the BGISEQ-500 platform using the combinational probe-anchor synthesis sequencing method. After the removal of ambiguous, adaptor-containing and low-quality sequences, clean data were obtained and *de novo* assembled using Trinity software. The main transcriptomic features and NCBI accession numbers of the transcriptome data obtained in our study are presented in Table 1.

MSDIN and *POP* genes

Through transcriptome sequencing, 110 MSDIN genes (Table 2) were manually identified in 7 lethal *Amanita* and *Lepiota* species using known MSDIN members from *A. bisporigera* as TBLASTN queries. Additionally, 70 MSDIN genes (Table 3) were obtained from 12 lethal *Amanita*, *Galerina* and *Lepiota* species by PCR cloning using degenerate and specific primers. In general, a total of 151 nonrepetitive MSDIN genes were identified at the genomic and transcriptomic levels by using these methods. All the obtained MSDIN genes were predicted to encode 98 cyclopeptides, including α -amanitin (IWGIGCNP), β -amanitin (IWGIGCDP), phalloidin (AWLVDCP), phalloidin (AWLATCP) and 94 unknown peptides. These predicted cyclopeptides were composed of 6-11 amino acids and included 5 hexapeptides, 30 heptapeptides, 73 octapeptides, 22 nonapeptides, 19 decapeptides, and 2 undecapeptides.

Among the MSDIN members found in the 9 lethal species of *Amanita* sect. *Phalloideae* included in our study, in addition to the common α -amanitin, β -amanitin, phalloidin and phalloidin (PHA) peptides, several unnamed predicted peptides overlapped among different *Amanita* species, including “FNFFRFPYP” in *A. exitialis* and *A. rimosa*; “FPWTGPFVP” in *A. fuliginea* and *A. pallidorosea*; “IIIVLGLIIP” in *A. fuliginea* and *A. rimosa*; “YFLPPIFSPP” in *A. molliuscula* and *A. subpallidorosea*; “ISDPTAYP” in *A. pallidorosea* and *A. rimosa*; “IFWFIYFP” in *A. exitialis*, *A. fuliginea*, *A. rimosa* and *A. subpallidorosea*; and “ISDPTAYP” in *A. pallidorosea*, *A. rimosa*, *A. subfuliginea* and *A. subpallidorosea*. The remaining 87 core regions were unique to their corresponding species. The MSDIN genes encoding “AWLTDCP” in *A. exitialis*; “AWLMTCP” in *A. pallidorosea*; “AWLECP” in *A. rimosa*; “AWLVTCP” in *A. fuliginea*, *A. subpallidorosea* and *A. virosa*; and “AWITDCP” and “AWLITCP”

in *A. subpallidorozea* probably produce new unknown phallotoxins because their core regions are similar to those of phallacidin (AWLVDCP) and phalloidin (AWLATCP). As expected, no MSDIN genes were found in *A. oberwinklerana*, a species belonging to *Amanita* sect. *Lepidella* [16] or sect. *Roanokenses* that does not contain cyclopeptide toxins [15].

In *G. marginata*, *G. sulciceps* and *L. venenata*, only MSDIN genes encoding α -amanitin were found, and such genes were the only genes common to the amanitin-producing genera *Amanita*, *Galerina* and *Lepiota*. Unlike the situation in lethal *Amanita* species, no MSDIN genes other than the α -amanitin gene were discovered. Interestingly, an MSDIN gene with the full amino acid sequence MFDTNSTRLPI***GIGCNP**WTAEHIDQTLVSGNDTC* (with the core region shown in bold and underlined) was found in *G. sulciceps*. Due to its similarity to the α -amanitin gene *Gs* α -AMA1 (MFDTNATRLPI**WIGICNP**WTAEHVDQTLASGNDIC*) in *G. sulciceps*, it was designated *Gs* α -AMA2.

Similarly, 19 POP genes were identified from the transcriptomes of nine *Amanita*, two *Galerina* and one *Lepiota* species using known POPA and POPB genes of *A. bisporigera* and *G. marginata*, respectively, as the TBLASTN queries and these sequences were further verified by PCR amplification. Eleven lethal *Amanita*, *Galerina* and *Lepiota* species contained both POPA and POPB genes, but *A. oberwinklerana*, an *Amanita* species producing no cyclopeptide toxins, only exhibited the POPA gene. All of the obtained POP sequences and their accession numbers are listed in Table 4.

Comparison of MSDIN precursor peptide sequences

WebLogo alignment was carried out for 145 MSDIN sequences obtained from 9 *Amanita* species (Fig. 1A). The composition and structure of these sequences and the relative degree of conservation of the amino acids at each point were analysed. As shown in Fig. 1A, the MSDIN precursor peptides of the *Amanita* species were 31-38 amino acids in length and could be divided into three regions: a highly conserved upstream leader peptide, a relatively conserved downstream recognition sequence and a highly variable core peptide. The core peptide was located between P¹⁰ and P²¹ and included the latter proline, and its ends were the leader peptide and recognition sequence of MSDIN. The leader peptide contained 10 amino acids, and the M¹S²D³I⁴N⁵R⁸L⁹P¹⁰ residues were highly conserved, with conservation rates of 100% (145/145), 91.7% (133/145), 97.2% (141/145), 93.1% (135/145), 99.3% (144/145), 99.3% (144/145), 94.5% (137/145) and 99.3% (144/145), respectively. In the leader peptide, P¹⁰ was the first cleavage site for prolyl oligopeptidase (POPB) [12]. The recognition sequence usually contained 17 amino acids, beginning with C²²V²³G²⁴D²⁵D²⁶, with conservation rates of 76.5% (111/145), 79.3% (115/145), 66.2% (96/145), 93.1% (135/145) and 83.4% (121/145), and ending with L³¹T³²R³³G³⁴E³⁵L³⁷C³⁸, with conservation rates of 86.9% (126/145), 73.8% (107/145), 82.1% (119/145), 96.6% (140/145), 93.1% (135/145), 97.9% (142/145) and 91.7% (133/145), respectively. In the recognition sequence, L³¹ and L³⁷ were conducive to the formation of an alpha helix and substrate recognition by the POPB enzyme [27]; additionally, the C-terminal Cys³⁸ (sometimes replaced with Ser) was indispensable for performing the function of POPB [12]. The core peptides were predicted to form cyclopeptides in which the last amino acid, P²¹ (the second cleavage site for POPB), was highly conserved, with a conservation rate of 94.5% [12].

The α -AMA precursor peptide sequences of the genera *Amanita*, *Galerina* and *Lepiota* were compared, as shown in Fig. 1B. The α -AMA sequences showed few differences within the same genus but presented more differences between the different genera. The α -AMA leader peptides of the three

genera showed few differences and were more conserved than the other sequences. The leader peptides of *Amanita* and *Galerina* contained 10 amino acids, while that of the genus *Lepiota* contained 9, and the sequences of the three genera started with “MSDIN”, “MFDTN”, and “MDAN”, respectively. In the recognition sequences of the three genera, with the exception of several highly conserved amino acids (specifically V, L, G, and LC or LS at the end), many differences were observed. Overall, there were large differences among the α -AMA sequences of *Amanita*, *Galerina* and *Lepiota*, but the *Galerina* and *Lepiota* α -AMA sequences were closer to each other than to those of *Amanita*.

The *Amanita* MSDIN genes encoding amatoxins (α -, β -amanitin) and phallotoxins (phalloidin and phalloidin), which are the major cyclopeptides in these mushrooms, were aligned, and the highlighted variations were compared to representative consensus sequences (Fig. 1C). In general, the precursor peptide sequences encoding the same toxin shared high identity. There were obviously more variations in the recognition sequences than in the leader peptides. The phallotoxin sequences presented more variations than the amatoxin sequences.

Structures of MSDIN and POP genes

The genomic sequences and coding sequences of the toxin MSDIN and POP genes obtained in this study (Table 2) were subjected to pairwise alignment, and the gene composition of the exons and introns was analysed. As shown in Fig. 2, the POPA genes comprised 19 exons and 18 introns, while the POPB genes comprised 18 exons and 17 introns, which was very similar to other known POP genes. The α -AMA genes of *Amanita* and *Galerina* contained three introns, while the α -AMA genes of *Lepiota* contained two or three introns. In addition to α -AMA, other MSDIN toxin genes in *Amanita* species, such as β -AMA, PHA and PHD, were also composed of three introns.

Phylogenetic analysis of MSDIN and POP genes

From the phylogenetic analysis, two maximum likelihood (ML) trees based on 46 MSDIN toxin genes from 14 amanitin-producing mushrooms and 58 POP genes from 46 agaric species were constructed. In the MSDIN toxin gene tree (Fig. 3), all MSDIN toxin gene sequences were distributed in four clades. Clade I contained 10 α -amanitin gene sequences and 10 β -amanitin gene sequences from 10 lethal *Amanita* species forming a cluster with 95% bootstrap support and a Bayesian posterior probability of 1.0. Clade II contained MSDIN genes encoding AWLVDCP (phalloidin, PHA) and the unknown related variants AWLAECP, AWITDCP and AWLTDCP forming a cluster with 95% bootstrap support and a Bayesian posterior probability of 1.0. Clade III contained MSDIN genes encoding AWLATCP (phalloidin, PHD) and the unknown related variants AWLMTCP and AWLVTCP forming a cluster with a 100% bootstrap and a 1.0 Bayesian posterior probabilities. Clade IV contained α -amanitin genes from *Galerina* and *Lepiota* species, including *G. marginata*, *G. sulciceps*, *L. subincarnata* and *L. venenata*, forming a cluster with a 100% bootstrap and a 1.0 Bayesian posterior probabilities. In the POP gene tree (Fig. 4), POPA sequences from *Amanita*, *Galerina* and *Lepiota* were separated from each other in different groups. *Amanita* POPA sequences (12) were clustered together as a single group, while *Galerina* POPA sequences (2) were clustered in a group containing *Gymnopilus dilepis* and *Gymnopilus chrysopellus*, and *Lepiota* POPA sequences (2) were clustered in a group containing *Agaricus bisporus* var. *bisporus*, *Agaricus bisporus* var. *burnettii*, *Leucoagaricus* sp. and *Macrolepiota fuliginosa*. However, POPB sequences (13) belonging to three disjunct genera (*Amanita*, *Galerina* and *Lepiota*) were clustered together forming a monophyletic group.

Discussion

It has been proven that lethal *Amanita* species are classified in section *Phalloideae* of *Amanita* and that these species contain members of the MSDIN gene family, allowing them to produce many small cyclopeptides, such as α -amanitin, on ribosomes [6, 16]. In the present study, nine lethal *Amanita* species from China, including *A. exitialis*, *A. fuliginea*, *A. mulliuscula*, *A. pallidorosea*, *A. rimosa*, *A. subfuliginea*, *A. subjunquillea*, *A. subpallidorosea* and *A. virosa*, were proven to contain MSDIN genes, as found in other lethal *Amanita* species described previously, such as *A. bisporigera*. These results further suggest that species of the *Amanita* section *Phalloideae* are genetically similar and are able to biosynthesize amatoxins, phallotoxins and some other unknown peptides.

Based on the MSDIN gene data from nine lethal *Amanita* species from China obtained in our study and some other European and North American species, such as *A. bisporigera*, *A. phalloides* and *A. ocreata* [5, 6], most MSDIN genes have not been found to be common and may even be unique among these lethal *Amanita* species. In species of *Amanita* section *Phalloideae*, the MSDIN gene encoding α -amanitin is present in all species, whereas the MSDIN genes encoding β -amanitin, phalloacidin and phalloidin are widely distributed but are not common to all species. This findings suggested that each lethal *Amanita* species exhibits its own independent MSDIN family and that few overlapping MSDIN genes occur among lethal *Amanita* species.

In addition to species of *Amanita* section *Phalloideae*, some *Galerina* and *Lepiota* species, such as *G. marginata* and *L. brunneoincarnata*, produce amatoxin [2, 28]. In our study, the MSDIN gene-mining results showed that *G. marginata* and *L. venenata* only presented two copies of the α -amanitin gene, and no additional MSDIN genes were found, consistent with the results of Luo et al. (2012) and Lüli et al. (2019) [7, 9]. Lethal *Galerina* and *Lepiota* species are considered to have two copies of the α -amanitin gene. However, the analysis of the MSDIN genes of another *Galerina* species, *G. sulciceps*, showed that *G. sulciceps* only presented a single copy of the α -amanitin gene, although it also exhibited an MSDIN gene that was extremely similar to the α -amanitin gene with an I*GIGCNP core region. This MSDIN gene seemed to represent an α -amanitin gene mutation, and we speculated that its tryptophan (W) codon, TGG, in the core region has been mutated to a termination codon, TGA, via a single-base substitution, thus inhibiting the proper expression of the gene. In general, only the α -amanitin gene is found in *Amanita*, *Galerina* and *Lepiota*, which indicates that the α -amanitin genes of the three genera might share a common origin or originate from a single genus. Additionally, MSDIN genes including β -AMA, PHA, PHD, etc., were only found in *Amanita*, which indicated that these MSDIN genes (except for α -AMA) were likely derived from lethal *Amanita* species. The higher genetic diversity of MSDIN genes in *Amanita* than in *Galernia* and *Lepiota* causes the lethal *Amanita* species to produce greater amounts of toxic compounds than *Galernia* and *Lepiota* species. For this reason, lethal *Amanita* species present a greater defence ability to prevent their consumption than *Galernia* and *Lepiota* species.

Lethal *Amanita* species contain three primary kinds of peptide toxins: amatoxins, phallotoxins and virotoxins [21]. MSDIN genes encoding amatoxin and phallotoxin were discovered in 2007 [5], but there has been no related evidence of MSDIN genes encoding virotoxins published to date. It has been reported that *A. subpallidorosea* and *A. virosa* contain virotoxins [3, 29]. In this study, toxin genes of the two lethal *Amanita* species were also identified, and no virotoxin genes were found. Nevertheless, the two species both contain MSDIN genes encoding AWLATCP (PHD) and AWLVTCP, which only

show a single amino acid difference in the composition of the virotoxins (AWLATSP or AWLVTSP). Therefore, we speculated that virotoxins might be encoded by the *PHD* gene or the phalloxin-like gene AWLVTCP and that cysteine (C) is transformed to serine (S) during posttranslational modification.

Phylogenetic analysis showed that the *Galerina* α -AMA genes and *Lepiota* α -AMA genes were homologous but were distant from the *Amanita* α -AMA gene. In the genus *Amanita*, α -AMA and β -AMA are mixed and clustered in a clade, which indicates that β -AMA might be derived from α -AMA. *PHA* genes (AWLVDCP) were clustered with MSDIN genes encoding AWLAECP, AWITDCP and AWLTDCP, and *PHD* genes (AWLATCP) were clustered with MSDIN genes encoding AWLMTCP and AWLVTCP, which indicated that the encoded products of these MSDIN genes were very likely to correspond to new unknown phallotoxins, considering the similarity of their amino acid composition with those of PHA and PHD and their capacity to contain tryptathione (Trp-Cys). These phalloxin-like genes might be variants derived from *PHA* and *PHD*. For example, we found that the *PHA* gene (AWLVDCP) sequence in *A. exitialis* was almost the same as the sequence of the MSDIN gene encoding AWLTDCP, with only a two-nucleotide difference in the core region, and the valine (V) codon GTA is likely mutated into the threonine (T) codon ACA. According to this finding, it can be inferred that the MSDIN genes in *Amanita* evolved faster than those in *Galerina* and *Lepiota*, which led to the generation of a variety of new peptide genes and might also be the reason why the *Galerina* and *Lepiota* α -AMA genes differed from the *Amanita* α -AMA genes.

Horizontal gene transfer (HGT), also known as lateral gene transfer, refers to the transmission of genetic material between distinct organisms, specifically across species boundaries [30, 31]. It has been reported that HGT is very common in prokaryotes and may be an important source of their biological evolution, and HGT also occurs in eukaryotes at a lower frequency than in prokaryotes [32-35]. The most recent reports suggest that HGT may be responsible for the α -amanitin biosynthetic pathway found in the three distantly related genera *Amanita*, *Galerina* and *Lepiota* [8, 9]. It has been reported that in amanitin-producing mushrooms, the *POPB* gene product catalyses the cleavage and cyclization of the toxin precursor peptide, while the *POPA* gene is a housekeeping gene that is unrelated to toxin biosynthesis [7, 12]. In our study, phylogenetic analysis based on the *POP* gene showed that the *POPA* genes of *Amanita*, *Galerina* and *Lepiota* were distributed in three separate groups, but the *POPB* genes of the three genera were highly homologous forming a highly monophyletic group, which apparently conflicted with the species taxonomic status and could not be explained by conserved gene inheritance. Additionally, the MSDIN and *POP* genes were proven to exhibit the same exon and intron structures. These results can be considered to represent evidence of HGT events among *Amanita*, *Galerina* and *Lepiota*. For the complete validation of HGT among amanitin-producing mushrooms in the future, the inclusion more related species and their genomic data will be required to perform a phylogenetic analysis with appropriate taxon sampling and tree-building methodologies.

Conclusions

In conclusion, the MSDIN gene family is abundant and diverse. In addition to the peptide toxins α -amanitin, β -amanitin, phalloidin, phalloidin, etc., the MSDIN family encodes a variety of unknown small cyclopeptides. The amanitin-producing species *Amanita*, *Galerina* and *Lepiota* exhibit a common

toxin biosynthetic pathway, and their α -amanitin genes and *POPB* genes may have a common origin that involving HGT among the three distant genera.

Methods

Sample collection and preparation

Samples of seven *Amanita* species and *Lepiota venenata* were collected from the wild for RNA extraction and sequencing, and their fresh basidiocarps were cleaned and placed on dry ice then transported back to the lab and stored at -80°C. The mushroom samples intended for DNA extraction were dried with silica gel and then stored at 4°C. The mycelia of two *Galerina* strains were cultivated to grow material for DNA and RNA extraction. Detailed information for the mushroom materials used in this study is given in Table 5.

Nucleic acid extraction and cDNA preparation

Total genomic DNA was extracted using the Fungal DNA Mini Kit (Omega Bio-tek, Norcross, USA). Total RNA was isolated using TRIzol Reagent (Invitrogen, Carlsbad, USA) following the TRIzol User Guide. cDNA was synthesized using TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMIX (Transgen Biotech, Beijing, China). The DNA and RNA quality and yield were detected using a SmartSpec Plus (Bio-Rad, Hercules, USA).

Transcriptome sequencing and de novo assembly

The concentration, purity and integrity of the RNA samples used for next-generation sequencing were further examined using an Agilent 2100 bioanalyzer (Agilent, Santa Clara, USA). Qualified RNA samples were used to construct circular single-stranded cDNA libraries, and the libraries were then sequenced on a BGISEQ-500 sequencer (BGI, Shenzhen, China). Clean reads were obtained using the filtering software SOAPnuke to remove reads containing adaptors, reads with more than 5% unknown bases, and low-quality reads (bases with a quality value < 15 accounting for more than 20% of the bases in a read) from the raw reads. These clean reads were *de novo* assembled using Trinity software. Finally, nonredundant unigenes were obtained using Tgicl software. All of these steps were performed by the Beijing Genomic Institute (BGI)-Wuhan in China.

Retrieval and annotation of MSDIN and POP genes

The unigene data obtained as described above were searched for MSDIN and *POP* genes (*Galerina* unigenes were provided by Professor Ping Zhang at Hunan Normal University) by using the known amino acid sequences of the MSDIN family and *POP* genes from *A. bisporigera* and *G. marginata* [5, 7] as queries for the online NCBI TBLASTN tool. Then, unigenes similar to the queries were manually annotated, and the coding sequences were predicted and translated into protein sequences using DNAMAN 7.0 software.

Cloning of MSDIN and *POP* genes

Partial MSDIN gene sequences were amplified from *Amanita* genomic DNA using the following degenerate primers: forward (5'-ATGTCNGAYATYAAYGCNACNCG-3') and reverse (5'-CCAAGCCTRAYAWRGTCMACAAC-3'), according to the method of Li et al. (2014) [13]. The PCR

mixtures contained 1× PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, each primer at 0.4 μM, 1.25 U of *Taq* polymerase (Comwin Biotech, Beijing, China), and 1 μL of DNA template in a total volume of 25 μL. PCR was performed with the following program: initial denaturation at 94°C for 4 min, 35 cycles at 94°C for 30 s, 52°C for 30 s, and 72°C for 30 s, and the reaction batches were incubated at 72°C for 2 min for terminal elongation.

Using the known MSDIN and *POP* genes from *A. bisporigera* and *G. marginata* as reference models [5, 7], specific primers (shown in Table S1) were designed to obtain target products that were close to the full lengths of the genes according to the flanking sequences of the CDS. The genomic DNA and cDNA of the *Amanita*, *Galerina* and *Lepiota* species were used as templates, and PCR was performed as follows: initial denaturation at 94°C for 4 min, followed by 32 cycles of denaturation at 94°C for 30 s, 55-60°C for 30 s (annealing temperature for each target shown in Table S1), and extension at 72°C (30 s for an MSDIN gene, 2 min for a *POP* gene), and a final extension at 72°C for 5 min.

All PCR products were detected by agarose gel electrophoresis and purified using an EasyPure Quick Gel Extraction Kit (Transgen Biotech, Beijing, China). The purified products were ligated into the *pEASY*[®]-Blunt Zero Cloning Vector (Transgen Biotech, Beijing) and transformed into competent cells. Positive clones to be sequenced were selected using Amp-resistant LB medium and were further verified by colony PCR. Finally, all of the obtained genomic and coding sequences of the genes were used to manually predict the corresponding functions and structures by using DNAMAN 7.0 software.

Phylogenetic tree construction of MSDIN and *POP* genes

Forty-six coding sequences (CDSs) of MSDIN toxin genes and fifty-eight CDSs of *POP* genes were used for phylogenetic analysis, and their source and GenBank accession numbers are presented in Table 4. These sequences were aligned by using MAFFT v7.374 [36] and then manually adjusted by using BioEdit [37]. HKY+I+G and GTR+I+G were inferred as the best-fit models for the CDSs of the MSDIN and *POP* genes selected according to the AIC in MrModeltest v2.3 [38]. Maximum likelihood (ML) trees with 1000 bootstrap replicates and Bayesian inferences were generated with RAxML v7 [39] and MrBayes v3.1.2 [40], respectively.

Abbreviations

POP: prolyl oligopeptidase; α-AMA: α-amanitin; β-AMA: β-amanitin; PHD: phalloidin; PHA: phalloidin; CDS: coding sequence; HGT: horizontal gene transfer; ML: maximum likelihood.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The mushroom species and related transcriptome data, MSDIN and *POP* genes used in this study has been deposited at GenBank and their accession numbers can be found in Table 1, 3, 4 and 5.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

ZHC and ZMH conceived and designed the experiments. ZMH and PL carried out the MSDIN and *POP* genes cloning, FF and SNL carried out the phylogenetic analysis. PZ provided some *Amanita* materials and identified the species. ZMH and ZHC wrote the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Features and accession numbers of transcriptomes.

Species	Total clean bases (Gb)	Q30 (%)	Total number of unigene	Total length of unigene (nt)	Mean length of unigene (nt)	N50	GC (%)	accession number
<i>A. exitialis</i>	6.67	86.25	24,578	48,383,999	1,968	2,891	49.75	SRR9929233
<i>A. fuliginea</i>	6.55	88.20	21,624	36,817,429	1,702	2,599	49.54	SRR9937194
<i>A. molliuscula</i>	6.32	90.65	46,471	79,566,952	1,712	3,007	49.71	SRR9937646
<i>A. oberwinklerana</i>	6.59	86.87	24,326	61,864,918	2,543	3,993	48.83	SRR9937816
<i>A. pallidrosea</i>	6.25	89.78	36,846	79,216,743	2,149	3,375	49.52	SRR9937866
<i>A. rimosa</i>	6.57	87.93	22,532	36,712,344	1,629	2,648	49.05	SRR9943992
<i>A. subpallidrosea</i>	10.24	91.21	42,803	110,323,057	2,577	3,630	49.00	SRR9943549
<i>L. venenata</i>	8.47	93.83	13,859	21,738,818	1,569	2,994	48.88	SRR9943552

Table 2 MSDIN family members searched from the transcriptomes of seven amanitin-producing mushrooms.

Name	No.	Leader peptide	Core peptide	Recognition sequence	Product
<i>A. exitialis</i>	Ae1	MTDINDTRLP	FIWLLWIWLP	SVGDDNNILNRGEDLC*	
	Ae2	MSDINATRLP	LFFPPDFRPP	CVGDADNFTLTRGENLC*	
	Ae3	MSDVNATRLP	FNFFRFPP	CIGDDSGSALRLGESLC*	
	Ae4	MSDINTARLP	IPVPPFFIP	FVGDDIDVVLRRGENLC*	
	Ae5	MSDINVTRLP	VFIFFFIPP	CVGDGTADIVRKGENLC*	
	Ae6	MSDINTARLP	VFSLPVFFP	FVSDDIQAVLTRGESLC*	
	Ae7	MSDINTTRLP	FVFFVASPP	CVGDDIAMVLTRGENLC*	
	Ae8	MSDINPTRLP	IFWFIYFP	CVSDVDSTLTLCSLS*	
	<u>Ae9</u>	MSDINTARLP	IWIIGNP	CVSDDVERILTRGESLC*	
	Ae10	MSDINATRLP	IIWAPVVP	CISDDNDSTLTRGQSLC*	
	Ae11	MSDINATRLP	IGRPQLP	CVGGDVNYILISGENLC*	
	<u>Ae12</u>	MSDINATRLP	IWGIGCDP	CVGDDVTALLTRGEALC*	β -amanitin
	<u>Ae13</u>	MSDINATRLP	IWGIGCNP	CVGDDVTSVLTRGEALC*	α -amanitin
	Ae14	MSDINVIRLP	SMLTILPP	CVSDDASNTLTRGENLC*	
	<u>Ae15</u>	MSDINATRLP	AWLTDCP	CVGDDVNRLLTRGESLC*	"phallotoxin"
	<u>Ae16</u>	MSDINATRLP	AWLVDCP	CVGDDVNRLLTRGESLC*	phalloidin
	Ae17	MSDINLTRLP	GIIAIP	CVGDDVNSTLTRGQSLC*	
	<u>Ae18</u>	MSDINATRLP	VWIGYSP	CVGDDCIALLTRGEGLC*	
	<u>Ae19</u>	MSDINATRLP	GFLFWA	YVGDDVDYILTRGESLA*	
<i>A. fuliginea</i>	Af1	MSDINATRLP	IIVLGLIIP	LCVSDIEMILTRGESLC*	
	Af2	MSDLNASRLP	ILSVLGLPVP	HVGEETNSTLARGESLC*	
	Af3	MSDINSARLP	LFFPPIFIPP	CVSDDVQVVLTRGENLC*	
	Af4	MSDINAARLP	FFPFVFIPP	CIGDDATSIVRQAENLC*	
	Af5	MSDINTIRIP	FPWTGPFVP	CVSDDVGSVLMRGESLS*	
	Af6	MSDTNATRLP	IWFIQLQIP	CAGDDVNSSLTRGESLC*	
	Af7	MSDINVTRLP	VLVFIFFPP	YISDDAVNILKQGENLC*	
	Af8	MFDINGSRLP	AFRLIPPP	CVGDDVDSTLTSGESLC*	
	Af9	MSDINATRLP	GILIVFPP	CVGDDVNSTLTRGESLC*	
	Af10	MSDINATRLP	HLFTWIPP	CISDDSTLTRGESFC*	
	Af11	MFDINSSRLP	HLYPNSRP	CVCDDACSTLTSAESLC*	
	<u>Af12</u>	MSDINATRLP	IFWFIYFP	CVGDDVDNTLTRGESLS*	
	<u>Af13</u>	MSDINATRLP	IWGIGCDP	CVGDDVAALITRGEALC*	β -amanitin
	<u>Af14</u>	MSCINATRLP	LPSRPVFP	FVSDAIEVVLGRGEDLC*	
	Af15	MSDINSLRLP	VVNSRFNP	CVGDDVSPTLTRGEGLC*	
	<u>Af16</u>	MSDINASRLP	AWLATCP	CIGDDVNPTITRGESLC*	phalloidin

	<u>Af17</u>	MSDINATRLP	AWLVDCP	CVGDDVNRLLRGENLC*	phalloidin
	Af18	MSDINATRLP	AWLVTCP	CVGDDINRLLTRGENLC*	“phallotoxin”
<i>A. molliuscula</i>	Am1	MSDINTARLP	YFLPPIFSPP	CVSDDIEMVLTRGENLC*	
	Am2	MTDINATRLP	ILFGFFLLP	CVDGVDNTLHSGENLC*	
	Am3	MSNINASRLP	IWAAFFRFP	CVGDEVDGILRSGESLC*	
	<u>Am4</u>	MSDINATRLA	IWGIGCDP	CVGDDVTALLTRGEALC*	β -amanitin
	Am5	MSDINASRLP	RLLVPRYP	CIDEDAEGATYLC*	
	Am6	MSNINAIRLP	GFFAVVP	YLATSITFSLGRGESLC*	
	Am7	MTDINATRLP	WIFFFPP	CVDDVDNTLHSGENLC*	
	Am8	MSNINALRLP	GFGFIP	YASGDVDYTLTRGESLS*	
	Am9	MSDINATRFP	GKVNPP	YVGDDVDDIIRGEKLC*	
<i>A. pallidrosea</i>	Ap1	MADINAARLP	FHGLFPFLPPP	FVDDDATSTLTRGESLC*	
	Ap2	MADINASRLP	LNILPFHLPP	CVSDDATSTLTRGESLC*	
	Ap3	MSDINATRLP	NWHAGPTRPP	CVADDVSLTLTRGESLC*	
	Ap4	MSDINTARLP	VFFMPPFIPP	CVSDDIQMVLTRGENLC*	
	Ap5	MSDINTARLP	EFIVFGIFP	CVGDDIQTVLTRGEDLC*	
	Ap6	MSDINASRLP	FFPEVGFFP	CVGDDTNPILTRGGSLC*	
	Ap7	MSDLNATRLP	FNLFRFPYP	CIGDDSGSVLTLGEGLC*	
	Ap8	MSDINTIRVP	FPWTGPFVP	CVGDDVGSVLTHGESLS*	
	Ap9	MSDINATRLP	HPFPLGLQP	CAGDVDNLTFLRGEGLC*	
	Ap10	MSDINATRLP	DPRRLIP	GSSDDVDSALTRGESLC*	
	Ap11	MSDINTTRLP	HFFNLMPP	CVGDDIETVLTRGESLC*	
	Ap12	MSDINATRLP	HQHHPFVP	GGSDDVGSTLTRGESLC*	
	Ap13	MSDMNVVRLP	ISDPTAYP	CVGDDIQAVLGRGESLC*	
	<u>Ap14</u>	MSDINATRLP	IWGIGCDP	CVGDDVTAVLTRGEALC*	β -amanitin
	<u>Ap15</u>	MSDINATRLP	IWGIGCNP	CVGDEVAALLTRGEALC*	α -amanitin
	<u>Ap16</u>	MSDINATRLP	IWGIGCNP	CVGDEVTALITRGEALC*	α -amanitin
	Ap17	MSDINATRLP	LGRPESLP	CVGDDVNYILVSGGNLS*	
	Ap18	MSDINAARLP	LVYMILFP	SVGDDIDVVLGRGENLC*	
	Ap19	MSDVNATRLP	MAFPEFLA	CVGDDVNHTLTRGERLC*	
	Ap20	MSDINTARLP	MHILAPP	CVSDDIEMVLTRGESLC*	
	Ap21	MSDINAARLP	NLFVWIPP	CISDDINSTLTRGESLC*	
	Ap22	MSDINTTRLP	YMWDHHL	CASDDIQMVFTTRGENLC*	
	<u>Ap23</u>	MSDINASRLP	AWLATCP	CAGDDVNPTLTRGESLC*	phalloidin
	<u>Ap24</u>	MSDINATRLP	AWLMTCP	CVGDDVNPTLTRGESLC*	“phallotoxin”
	<u>Ap25</u>	MSDVNATRLP	AWLVDCP	CVGDDINRLLTRGENLC*	phalloidin

<i>A. rimosa</i>	Ar1	MSDINTSRLP	FIPLGIITILP	CVSDDVNTTITRGESLC*	
	Ar2	MTDINDTRLP	FVWILWLWLA	CVGDDTSILNRGEDLC*	
	Ar3	MSDINATRLP	IIIVLGLIIP	LCVSDIEMILTRGESLC*	
	Ar4	MSDVNTTRLP	FNFFRFPYP	CICDDSEKVLLEGENLC*	
	Ar5	MSDINATRLP	HPFPLGLQP	CAGDVDNFTVVSCHSLC*	
	Ar6	MLDINATRFP	LGRPTHLP	CVGDDVNYILIGNGENLC*	
	Ar7	MSDINASCLP	LILVANGMA	YVSDDVSPTLTRGENLC*	
	Ar8	MPDINVTRLP	LLIIVLLTP	CISDDNNILNRGKDLCLC*	
	Ar9	MSDIHAARLP	FPTRPVFP	SAGDDMIEVVLGRGEDLC*	
	Ar10	MSDNNAARLP	FYFYLGIIP	SDDAHPILTRGESLC*	
	Ar11	MSDINIARLP	IFWFIYFP	CVGDDVDNTLSRGESLS*	
	Ar12	MSDINASRLP	ILKKPWAP	SVCDDVNSTLTRGEGLC*	
	Ar13	MSDINVARLP	ISDPTAYP	CVGDDIQAVVKRGESLC*	
	<u>Ar14</u>	MSDINATRLP	IWGIGCDP	CVGDDVAALTRGEALC*	β -amanitin
	<u>Ar15</u>	MSDINSTRLP	IWGIGCNP	SVGDEVTALLTRGEALC*	α -amanitin
	Ar16	MSDINATRLP	AWDSKHP	CVGDDVSRLLTRGESLC*	
	<u>Ar17</u>	MSDINATRVLP	AWLAACP	CVGDDISHLLTRGENLC*	"phallotoxin"
	Ar18	MSDINATRVLP	AWLVDCP	CVGDDISRLLTRGENLC*	phalloidin
<i>A. subpallidorosea</i>	Asp1	MTDVNDTRLP	FIWLIWLWLP	SVGDDINILNGGEDLC*	
	Asp2	MTDINYARLP	ITLFLFFFIP	CLSDDDNILNRGKDLCLC*	
	Asp3	MSDINTARLP	YFLPPIFSPP	CVSDDIEMVLTRGENLC*	
	Asp4	MSDINATRLP	HPFPLGLQP	CAGDVDNFTLTKGEDLC*	
	Asp5	MSDINATRLP	GILIVWPP	CVGDDVNFTLTRGESLC*	
	Asp6	MSDINTTRLP	IAFPEFIA	RVGDDIHRTLTRGESLC*	
	Asp7	MSDINVTRLP	IFWFIYFP	CVGDDVDNTLTRGESLS*	
	Asp8	MSDINAIRLP	IGRPENKP	CVGGDVNYILISGEKLC*	
	Asp9	MSDINATRLP	IVFLEFYS	CVGDDVNSTLTRGESLC*	
	<u>Asp10</u>	MSDINATRLP	IWGIGCDP	CVGDDVA AFLTRGEALC*	β -amanitin
	<u>Asp11</u>	MSDINATRLP	IWGIGCNP	SVGDEVTALLTRGEALC*	α -amanitin
	Asp12	MSDINASRLP	VIGLFGLP	YVSDDVQPILTRGDSLCLC*	
	Asp13	MSDINASRLP	VIPFLLPP	CVSDDVNFTLTRGESLC*	
	Asp14	MSDINATRLP	YFRPAPP	CVSDDINPILTCGESLC*	
	<u>Asp15</u>	MSDINAARLP	AWITDCP	CVGDDINRILTRGENIC*	"phallotoxin"
	<u>Asp16</u>	MSDINASRFP	AWLATCP	CVGDDVNPTIARGESLC*	phalloidin
	Asp17	MSDINATRLP	AWLVTCP	CVGDDVNFTLTRGESLC*	"phallotoxin"
	<u>Asp18</u>	MSDINATRLP	AWLVTCP	CVGDDVNPTITRGESLC*	"phallotoxin"

	Asp19	MSDINTIRIP	GPFGFA	YVGDEVENLLKRGESLS*	
<i>L. venenata</i>	<u>Lv1</u>	MDANATRLP	IWGIGCNP	WTPESVNDTLTKDLS*	α-amanitin
	<u>Lv2</u>	MDANSTRLP	IWGIGCNP	WAPESVNDTLTRGKDLG*	α-amanitin

The MSDIN members with underlined numbers were verified at the genomic level. "Phallotoxin" means a novel heptapeptide similar to the phallotoxin cyclopeptide and capable of containing tryptathione (Trp-Cys).

Table 3 MSDIN family members cloned from genomic DNA of twelve amanitin-producing mushrooms.

Name	No.	Leader peptide	Core peptide	Recognition sequence	Product	GenBank accession no.
<i>A. exitialis</i>	Ae1 ^a	MSDINATRLP	FIWVFGIP	GDIGTVLTRGENLC*		MN318165
	Ae2 ^a	MSDINATRLP	IWIIGNP	CVSDDVERILTRGESLC*		MN318166
	Ae3 ^{ab}	MSDINATRLP	IWGIGCDP	CVGDDVTALLTRGEALC*	β-amanitin	MN264225
	Ae4 ^{ab}	MSDINATRLP	IWGIGCNP	CVGDDVTSVLTRGEALC*	α-amanitin	MN264220
	Ae5 ^b	MSDINATRLP	AWLTDCP	CVGDDVNRLLTRGESLC*	“phallotoxin”	MN264235
	Ae6 ^b	MSDINATRLP	AWLVDCP	CVGDDVNRLLTRGESLC*	phalloidin	MN264231
	Ae7 ^a	MSDINATRLP	VWIGYSP	CVGDDCIALLTRGEGLC*		MN318167
	Ae8 ^a	MSDINATRLP	GFLFWA	YVGDDVDYILTRGESLA*		MN318168
	Ae9 ^a	MSDINATRLP	GFLLWA	YVGDDVDYILTRGESLA*		MN318169
<i>A. fuliginea</i>	Af1 ^a	MSDINATRLP	FPHFPPYNPP	CVSDDIHMVLTRGENLC*		MN318170
	Af2 ^a	MSDINATRLP	YLLLLILPP	CVSDDLQTVLTRGENLC*		MN318171
	Af3 ^a	MSDINATRLP	IFWFIYFP	CVGDDVDNTLARGESLS*		MN318172
	Af4 ^b	MSDINATRLP	IWGIGCDP	CVGDDVAALITRGEALC*	β-amanitin	MN264226
	Af5 ^a	MSDINATRLP	IWGIGCDP	CVGEDVAALITRGEALC*	β-amanitin	MN318173
	Af6 ^a	MSDINATRLP	IWGIGCNP	SVGDEVTALLTSGEALC*	α-amanitin	MN318174
	Af7 ^a	MSDINATRLP	LPSRPVFP	FVSDAIEVVLGRGEDLC*		MN318175
	Af8 ^b	MSDINASRLP	AWLATCP	CIGDDVNPTITRGESLC*	phalloidin	MN264249
	Af9 ^{ab}	MSDINATRLP	AWLVDCP	CVGDDVNRLLLARGENLC*	phalloidin	MN264232
<i>A. molliuscula</i>	Am1 ^b	MSDINATRLA	IWGIGCDP	CVGDDVTALLTRGEALC*	β-amanitin	MN264227
<i>A. pallidrosea</i>	Ap1 ^a	MSDINATRLP	LIFIPPFIPP	CVSDDIQMVLTRGENLC*		MN318176
	Ap2 ^a	MSDINAPRLP	LIFIPPFIPP	CVSDDIQMVLTRGEGLC*		MN318177
	Ap3 ^a	MSDINATRLP	IPFHIPAP	SVGDDIEVVLGRGENLC*		MN318178
	Ap4 ^a	MSDINATRLP	IWGIGCDP	CVGDDVTAVLTCGEALC*	β-amanitin	MN318179
	Ap5 ^b	MSDINATRLP	IWGIGCDP	CVGDDVTAVLTRGEALC*	β-amanitin	MN264228
	Ap6 ^{ab}	MSDINATRLP	IWGIGCNP	CVGDEVAALLTRGEALC*	α-amanitin	MN264222
	Ap7 ^b	MSDINATRLP	IWGIGCNP	CVGDEVTALITRGEALC*	α-amanitin	MN264221
	Ap8 ^a	MSDINATRLP	AWLATCP	CAGDDVNPTLTRGESLC*	phalloidin	MN318180

	Ap9 ^a	MSDINATRLP	AWLMTCP	CVGDDVNPILTRGESVC*	“phallotoxin”	MN318181
	Ap10 ^b	MSDINATRLP	AWLMTCP	CVGDDVNPTLTRGESLC*	“phallotoxin”	MN264236
	Ap11 ^{ab}	MSDVNATRLP	AWLVDCP	CVGDDINRLLTRGENLC*	phallacidin	MN264233
<i>A. rimosa</i>	Ar1 ^a	MSDINATRLP	IWGIGCDP	CVGDDVAALATRGEALC*	β-amanitin	MN318182
	Ar2 ^{ab}	MSDINATRLP	IWGIGCDP	CVGDDVAALTTRGEALC*	β-amanitin	MN264229
	Ar3 ^a	MSDINATRLP	IWGIGCNP	SVGDEVTALLASGEALC*	α-amanitin	MN318183
	Ar4 ^{ab}	MSDINSTRLP	IWGIGCNP	SVGDEVTALLTRGEALC*	α-amanitin	MN264223
	Ar5 ^b	MSDINATRVF	AWLAECP	CVGDDISHLLTRGENLC*	“phallotoxin”	MN264237
<i>A. subfuliginea</i>	Asf1 ^a	MSDINATRLP	HPFPLGLQP	CAGVDVNFTLTKGEGLC*		MN318184
	Asf2 ^a	MSDINATRLP	AIFLAWPP	CVGDNVNSTLTRGESLC*		MN318185
	Asf3 ^a	MSDINATRLP	IWGIGCDP	CVSDDVAALLTRGEALC*	β-amanitin	MN318186
	Asf4 ^a	MSDINATRLP	IWGIGCNP	CVGDEVAALLTRGEALC*	α-amanitin	MN318187
	Asf5 ^a	MSDINATRLP	AWLVDCP	CVGDDVNRLITRGENLC*	phallacidin	MN318188
<i>A. subjunquillea</i>	Asj1 ^a	MSDINATRLP	AYLPLFFIPP	CVSDDIEMVLTRGESLC*		MN318189
	Asj2 ^a	MSDINATRLP	AYLPLFFIPP	CVSDDIEVVLTRGESLC*		MN318190
	Asj3 ^a	MSDINATRLP	IWGIGCDP	CIGDDVTALLTRGEALC*	β-amanitin	MH142177
	Asj4 ^a	MSDINATRLP	IWGIGCDP	CVGDEVTALLTRGEALC*	β-amanitin	MH142176
	Asj5 ^a	MSDINATRLP	IWGIGCNP	CVGDEVAALLTRGEALC*	α-amanitin	MH142175
	Asj6 ^{ab}	MSDINATRLP	AWLATCP	CAGDDVNPTLTRGESLC*	phalloidin	MN264250
	Asj7 ^a	MSDINATRLP	AWLATCP	CVGDDVNPTLSRGESLC*	phalloidin	MN318191
	Asj8 ^{ab}	MSDINATRLP	AWLVDCP	CVGDDINRLLTRGENLC*	phallacidin	MN264234
<i>A. subpallidorosea</i>	Asp1 ^{ab}	MSDINATRLP	IWGIGCDP	CVGDDVAAFLTRGEALC*	β-amanitin	MN264230
	Asp2 ^{ab}	MSDINATRLP	IWGIGCNP	SVGDEVTALLTRGEALC*	α-amanitin	MN264224
	Asp3 ^{ab}	MSDINAARLP	AWITDCP	CVGDDINRILTRGENIC*	“phallotoxin”	MN272408
	Asp4 ^{ab}	MSDINASRFP	AWLATCP	CVGDDVNPTIARGESLC*	phalloidin	MN272407
	Asp5 ^a	MSDINATRLP	AWLITCP	CVGDDANPTITRGESLC*	“phallotoxin”	MN318192
	Asp6 ^{ab}	MSDINATRLP	AWLVTCP	CVGDDVNPTITRGESLC*	“phallotoxin”	MN272409
	Asp7 ^a	MSDINATRLP	AWLVTCP	CVGDDVNSTITRGESLC*	“phallotoxin”	MN318193

<i>A. virosa</i>	Av1 ^a	MSDINATRLP	FLLFIIPP	CVSDDVNSTLTRGESLC*		MN318194
	Av2 ^a	MSDINATRLP	FYFQPGFP	WSVGDDVNPTLTRGESLC*		MN318195
	Av3 ^b	MSDINATRLP	IWGIGCNP	SVGDEATAALLTRGEALC*	α-amanitin	MN272412
	Av4 ^a	MSDINATRLP	SILIVWPP	CVGDDVNSTLTRGESLC*		MN318196
	Av5 ^a	MSDINATRLP	SILVVWPP	CVSDDVNSTLTRGESLC*		MN318197
	Av6 ^a	MSDINATRLP	AWLATCP	CVGDDVNPTLARGESLC*	phalloidin	MN318198
	Av7 ^a	MSDINATRLP	AWLVDCP	CVGDDINRLLTRGENLC*	phalloidin	MN318199
	Av8 ^a	MSDINATRLP	AWLVTCP	CVGDDVNPTLTRGESLC*	“phallotoxin”	MN318200
	Av9 ^a	MSDINATRLP	GPFLFFP	FVSDDIEVILRRGEDLC*		MN318201
<i>G. marginata</i>	Gm1 ^b	MFDTNATRLP	IWGIGCNP	WTAEHVDQTLASGNDIC*	α-amanitin	MN272413
	Gm2 ^b	MFDTNSTRLP	IWGIGCNP	WTAEHVDQTLVSGNDIC*	α-amanitin	MN272414
<i>G. sulciceps</i>	Gs1 ^b	MFDTNATRLP	IWGIGCNP	WTAEHVDQTLASGNDIC*	α-amanitin	MN272417
	Gs2 ^b	MFDTNSTRLP	I*GIGCNP	WTAEHIDQTLVSGNDTC*		MN272418
<i>L. venenata</i>	Lv1 ^b	MDANATRLP	IWGIGCNP	WPESVNDTLTKDLS	α-amanitin	MN272421
	Lv2 ^b	MDANSTRLP	IWGIGCNP	WAPESVNDTLTRGKDLG	α-amanitin	MN272422

Superscripts a and b are for products cloned with degenerate and specific primers, respectively. “Phallotoxin” means a novel heptapeptide similar to phallotoxin cyclopeptide and capable of containing Tryptathione (Trp-Cys).

Table 4 Gene sequences used in the molecular phylogenetic analyses and their GenBank accession numbers.

Taxon	Gene	Source	GenBank accession no.
<i>Agaricus bisporus</i> var. <i>bisporus</i>	<i>POP</i>	NCBI	XM006459721
<i>Agaricus bisporus</i> var. <i>burnettii</i>	<i>POP</i>	NCBI	JH971409
<i>Amanita bisporigera</i>	<i>α-AMA1</i>	Pulman et al, 2016	-
	<i>α-AMA2</i>	Pulman et al, 2016	-
	<i>PHA1</i>	Pulman et al, 2016	-
	<i>PHA2</i>	Pulman et al, 2016	-
	<i>POPA</i>	Pulman et al, 2016	-
	<i>POPB</i>	Pulman et al, 2016	-
<i>Amanita exitialis</i>	<i>α-AMA</i>	Our study	MN264220
	<i>β-AMA</i>	Our study	MN264225
	<i>PHA</i>	Our study	MN264231
	"AWLTDCP"	Our study	MN264235
	<i>POPA</i>	Our study	MN264238
	<i>POPB</i>	Our study	MN264244
<i>Amanita fuliginea</i>	<i>β-AMA</i>	Our study	MN264226
	<i>PHA</i>	Our study	MN264232
	<i>PHD</i>	Our study	MN264249
	<i>POPA</i>	Our study	MN264239
	<i>POPB</i>	Our study	MN264245
<i>Amanita molliuscula</i>	<i>β-AMA</i>	Our study	MN264227
	<i>POPA</i>	Our study	MN264240
	<i>POPB</i>	Our study	MN264246
<i>Amanita muscaria</i>	<i>POPA</i>	NCBI	KN818232
<i>Amanita oberwinklerana</i>	<i>POPA</i>	Our study	MN264241
<i>Amanita pallidorosea</i>	<i>α-AMA1</i>	Our study	MN264221
	<i>α-AMA2</i>	Our study	MN264222
	<i>β-AMA</i>	Our study	MN264228
	<i>PHA</i>	Our study	MN264233
	"AWLMTCP"	Our study	MN264236
	<i>POPA</i>	Our study	MN264242
	<i>POPB</i>	Our study	MN264247
<i>Amanita phalloides</i>	<i>α-AMA</i>	Pulman. et al, 2016	-
	<i>β-AMA1</i>	Pulman. et al, 2016	-
	<i>β-AMA2</i>	Pulman et al, 2016	-
	<i>PHA</i>	Pulman et al, 2016	-
	<i>PHD1</i>	Pulman et al, 2016	-
	<i>PHD2</i>	Pulman et al, 2016	-

	<i>PHD3</i>	Pulman et al, 2016	-
	<i>POPA</i>	Pulman et al, 2016	-
	<i>POPB</i>	Pulman et al, 2016	-
<i>Amanita rimosa</i>	α -AMA	Our study	MN264223
	β -AMA	Our study	MN264229
	"AWLAECP"	Our study	MN264237
	<i>POPA</i>	Our study	MN264243
	<i>POPB</i>	Our study	MN264248
<i>Amanita subjunquillea</i>	α -AMA	Luo et al, 2018	-
	β -AMA1	Luo et al, 2018	-
	β -AMA2	Luo et al, 2018	-
	<i>PHA</i>	Our study	MN264234
	<i>PHD</i>	Our study	MN264250
	<i>POPA</i>	Luo et al, 2018	-
	<i>POPB</i>	Luo et al, 2018	-
<i>Amanita subpallidorozea</i>	α -AMA	Our study	MN264224
	β -AMA	Our study	MN264230
	<i>PHD</i>	Our study	MN272407
	"AWITDCP"	Our study	MN272408
	"AWLVTCP"	Our study	MN272409
	<i>POPA</i>	Our study	MN272410
	<i>POPB</i>	Our study	MN272411
<i>Amanita thiersii</i>	<i>POPA</i>	NCBI	KZ302001
<i>Amanita virosa</i>	α -AMA	Our study	MN272412
<i>Anomoporia bombycina</i>	<i>POP</i>	JGI	-
<i>Auriculariopsis ampla</i>	<i>POP</i>	NCBI	VDMD01000002
<i>Bolbitius vitellinus</i>	<i>POP</i>	JGI	-
<i>Conocybe apala</i>	<i>POP</i>	NCBI	FJ906819
<i>Coprinellus micaceus</i>	<i>POP</i>	NCBI	QFP01000027
<i>Coprinopsis cinerea</i>	<i>POP</i>	NCBI	XM001841192
<i>Coprinopsis marcescibilis</i>	<i>POP</i>	NCBI	ML210154
<i>Cortinarius glaucopus</i>	<i>POP</i>	JGI	-
<i>Crucibulum laeve</i>	<i>POP</i>	NCBI	ML213591
<i>Cyathus striatus</i>	<i>POP</i>	JGI	-
<i>Fistulina hepatica</i>	<i>POP</i>	NCBI	KN881639
<i>Galerina marginata</i>	α -AMA1	Our study	MN272413
	α -AMA2	Our study	MN272414
	<i>POPA</i>	Our study	MN272415

	<i>POPB</i>	Our study	MN272416
<i>Galerina sulciceps</i>	α - <i>AMA1</i>	Our study	MN272417
	" α - <i>AMA2</i> "	Our study	MN272418
	<i>POPA</i>	Our study	MN272419
	<i>POPB</i>	Our study	MN272420
<i>Gymnopilus chrysopellus</i>	<i>POP</i>	JGI	-
<i>Gymnopilus dilepis</i>	<i>POP</i>	NCBI	NHYE01005597
<i>Hebeloma cylindrosporum</i>	<i>POP</i>	NCBI	KN831777
<i>Hypholoma sublateritium</i>	<i>POP</i>	NCBI	KN817688
<i>Hypsizygus marmoreus</i>	<i>POP</i>	NCBI	LUEZ02000233
<i>Laccaria amethystina</i>	<i>POP</i>	NCBI	KN838546
<i>Laccaria bicolor</i>	<i>POP</i>	NCBI	DS547115
<i>Lepiota brunneoincarnata</i>	<i>POPA</i>	NCBI	MN912699
<i>Lepiota subincarnata</i>	α - <i>AMA1</i>	Luo et al, 2018	-
	α - <i>AMA2</i>	Luo et al, 2018	-
	<i>POPB</i>	Luo et al, 2018	-
<i>Lepiota venenata</i>	α - <i>AMA1</i>	Our study	MN272421
	α - <i>AMA2</i>	Our study	MN272422
	<i>POPA</i>	Our study	MN272423
	<i>POPB</i>	Our study	MN272424
<i>Lepista nuda</i>	<i>POP</i>	JGI	-
<i>Leucoagaricus</i> sp.	<i>POP</i>	NCBI	KQ962668
<i>Macrolepiota fuliginosa</i>	<i>POP</i>	JGI	-
<i>Panaeolus cyanescens</i>	<i>POP</i>	NCBI	NHTK01005903
<i>Pleurotus ostreatus</i>	<i>POP</i>	NCBI	KL198007
<i>Plicaturopsis crispa</i>	<i>POP</i>	JGI	-
<i>Pterula gracilis</i>	<i>POP</i>	NCBI	ML178816
<i>Schizophyllum commune</i>	<i>POP</i>	NCBI	GL377318
<i>Termitomyces</i> sp.	<i>POP</i>	NCBI	KQ412502

Table 5 Information of the mushroom materials used in this study.

Species name	Locality	Collection time	Specimen no.	GenBank accession no.	Nucleic acid extract
<i>A. exitialis</i>	Guangdong, China	2017-03-27	MHHNU 30937	KR996717	DNA, RNA
<i>A. fuliginea</i>	Hunan, China	2017-06-06	MHHNU 9047	MN061271	DNA, RNA
<i>A. molliuscula</i>	Jilin, China	2017-08-07	MHHNU 9142	MN061272	DNA, RNA
<i>A. oberwinklerana</i>	Hunan, China	2017-06-09	MHHNU 9051	MN061273	DNA, RNA
<i>A. pallidorosea</i>	Shandong, China	2018-08-13	MHHNU 31203	MN061274	DNA, RNA
<i>A. rimosa</i>	Hunan, China	2017-06-09	MHHNU 9050	MN061275	DNA, RNA
<i>A. subfuliginea</i>	Chongqing, China	2015-07-01	MHHNU 30946	MN061276	DNA
<i>A. subjunquillea</i>	Hunan, China	2012-09-10	MHHNU 7751	KR996715	DNA
<i>A. subpallidorosea</i>	Hunan, China	2017-09-14	MHHNU 8617	KU601411	DNA, RNA
<i>A. virosa</i>	Hunan, China	2016-09-09	MHHNU 8621	KY472227	DNA
<i>G. marginata</i>	-	-	MHHNU 8380	MN061277	DNA, RNA
<i>G. sulciceps</i>	-	-	MHHNU 7669	KX214585	DNA, RNA
<i>L. venenata</i>	Hubei, China	2017-9-10	MHHNU 31031	MK095189	DNA, RNA

G. marginata and *G. sulciceps* samples were cultured mycelia, and the other mushroom samples were wild fruiting bodies.

Supplementary Table

Table S1 Specific PCR Primers designed for peptide toxins and *POP* genes.

Primer name	Sequence (5'→3')	Annealing temp (°C)	Target gene
UαAMA-F	GAACCCAACTCCAATACCTCTA	55	<i>Ae, Asubp, Av_α-AMA</i>
UαAMA-R	TTCCTACAACACGGATCGTCCG		
Apa-αAMA-F	ACCCAACTCCAATACCTCTCTC	55	<i>Apa_α-AMA</i>
Apa-αAMA-R	GCATATTGACTACAACGCAGAT		
Ar-αAMA-F	ACCCAACTCCAATACTTCTC	55	<i>Ar_α-AMA</i>
Ar-αAMA-R	CACATATCGTCTACGTGGGT		
Gm-αAMA1-F	CAACCGCAAACCTCACTTAA	53	<i>Gm_α-AMA1</i>
Gm-αAMA1-R	ACACTGAGGATCATCAACGT		
Gm-αAMA2-F	CTATCTCATCGGCTCTGTTC	53	<i>Gm_α-AMA2</i>
Gm-αAMA2-R	TCAAACAGTAACGATTGTCCG		
Gs-αAMA1-F	GCTACTCGTCTCCCAATCT	53	<i>Gs_α-AMA1</i>
Gs -αAMA1-R	TTACAACACTGGAATCATCG		
Gs -αAMA2-F	ATGCAGTTCATGCCAGAGGA	53	<i>Gs_α-AMA2</i>
Gs -αAMA2-R	TGGCGTCGCAACTTGATAAT		
Lb-αAMA1-F	TAAATCGACTGCTGTTCAGG	52	<i>Lb_α-AMA1</i>
Lb-αAMA1-R	CATCGCAGATAAACAACATG		
Lb-αAMA2-F	ATCACAATGGACGCAAACCTC	54	<i>Lb_α-AMA2</i>
Lb-αAMA2-R	CGAGTCCAACAAGGGAACAT		
Lv-αAMA1-F	CGAACCCCATCACTTACT	54	<i>Lv_α-AMA1</i>
Lv-αAMA1-R	CATTCAACATAACATCGCAG		
Lv-αAMA2-F	CGCACTCAGACTCATTACTC	54	<i>Lv_α-AMA2</i>
Lv-αAMA2-R	AGTAAACGAGTCCAACAAGG		
Ae-βAMA-F	CAAGACCTCTAAACCTCTCC	54	<i>Ae_β-AMA</i>
Ae-βAMA-R	ATCGACGTGGGTTTTGTATT		
Af-βAMA-F	AAACCTCCAATACCTCTAAACC	54	<i>Af_β-AMA</i>
Af-βAMA-R	CAGATCATCGACGTGGATT		
Am-βAMA-F	TCAGCACCCAACTCCCATTTC	56	<i>Am_β-AMA</i>
Am-βAMA-R	TAAATGAGACAACGGGTCCG		
Apa-βAMA-F	CTTTCAAACCTCCAAGAACC	54	<i>Apa_β-AMA</i>
Apa-βAMA-R	CTCCCGCTAACAAGAAACAC		
Ar-βAMA-F	TCTTTCGAACATCTAACACCTC	54	<i>Ar_β-AMA</i>
Ar-βAMA-R	CACTTTGACTACAACGTGGGT		
Asubp-βAMA-F	TCCAAAGACCTCTAAACCTC	54	<i>Asubp_β-AMA</i>
Asubp-βAMA-R	ATAACACTCCCGCTAACAAG		
UPHA-F	GACCTCTGCTCTAAATCACA	55	<i>Ae_PHA, Ae_“AWLTDCP”</i>
UPHA-R	TGCATTCCATAGTACAACGC		and <i>Asubj_PHA</i>
UPHD-F	AATTCTACCTGTCAGAAACCTC	54	<i>Asubj_PHD</i>

UPHD-R	AGCACAAATCATTTCATGTGG		<i>Asubp_“AWLVTCP”</i>
Af-PHA-F	CAGCTCTAAATCACAATGTCTG	54	<i>Af_PHA</i>
Af-PHA-R	AAGGACAGATCATTACGTGG		
Af-PHD-F	TCAACCAACCGATATGTCTG	54	<i>Af_PHD</i>
Af-PHD-R	CACAGTACAACAAACATCATCG		
Apa-PHA-F	GAAC TTGAACCCTCTAAATCAC	52	<i>Apa_PHA</i>
Apa-PHA-R	AAGTTTATTGCGGTCCATG		
Apa-UPT-F	CTCAGAATCCTATCAACCGC	53	<i>Apa_“AWLMTCP”</i>
Apa-UPT-R	TCATCGACGTGGGTTTTAT		
Ar-UPT-F	TATCGTCTCCGTTGTCAGG	54	<i>Ar_“AWLECP”</i>
Ar-UPT-R	GTACAGCGCAGATCATTCA		
Asubp-PHD-F	ACCTCTTTCAACCGCTATGT	53	<i>Asubp_PHD</i>
Asubp-PHD-R	AACATCATCGACGTGGGTT		
Asubp-UPT-F	CACTCAGCTTCACTAAATCACA	54	<i>Asubp_“AWITDCP”</i>
Asubp-UPT-R	GGAGATCATTACGTGGGTT		
Ae-POPA-F	GAACGCCTTCGCTCTGCTCT	58	<i>Ae_POPA</i>
Ae-POPA-R	TGTCGTAGAATGTAGCCCAAAC		
Ae-POPB-F	TCCTCCTACCCGTCGTTCTG	58	<i>Ae_POPB</i>
Ae-POPB-R	GATTGGCAGCCTAGTCAACC		
Af-POPA-F	CCGTTCCGACCACATAGACAC	58	<i>Af_POPA</i>
Af-POPA-R	TACCTGGAATCGTGAGTGTCGT		
Af-POPB-F	CACAGTTATCCTCCTACTCGTCG	58	<i>Af_POPB</i>
Af-POPB-R	CGTGTAACAGTGTTTTGGCCTAG		
Am-POPA-F	TACCCTCCTACTCGTCGTTT	55	<i>Am_POPA</i>
Am-POPA-R	TGTAGCCCAAACAGTAACAGG		
Am-POPB-F	TCCTCACAGTTATCCTCCCA	50	<i>Am_POPB</i>
Am-POPB-R	CATTCATTGACGGCATAGT		
Ao-POPA-F	GTCCCAAAGTCCAAGTTCCA	54	<i>Ao_POPA</i>
Ao-POPA-R	TCCGTTCACAAATTGGCTTG		
Apa-POPA-F	ACACCGTCTCAAATCCAAGC	55	<i>Apa_POPA</i>
Apa-POPA-R	GCACAAGTGCAGTTGCGTTA		
Apa-POPB-F	CAGTTATCCTCCTACTCGTCG	55	<i>Apa_POPB</i>
Apa-POPB-R	GATCTAACAACGTGGCTTCA		
Ar-POPA-F	TCGCCGTTCCAGACCACATTG	58	<i>Ar_POPA</i>
Ar-POPA-R	CCATTTGTCGGCAGCCTCTT		
Ar-POPB-F	GCTTCCACCCTTACCACAGA	58	<i>Ar_POPB</i>
Ar-POPB-R	CAGCCTAGTCGTCCGTTTGC		
Asubp-POPA-F	ACCCAACACGTACCCTCCTA	60	<i>Asubp_POPA</i>

Asubp-POPA-R	ACTCATCACAGATTGGCTTGT		
Asubp-POPB-F	GGCTCCTCACAGTTATCCTCC	60	<i>Asubp_POPB</i>
Asubp-POPB-R	ATTTGACCGCCTAGTCATCC		
Gm-POPA-F	AGAGGAATACACCGATGAGAC	54	<i>Gm_POPA</i>
Gm-POPA-R	TGAGCTGGATTCTACGATTAG		
Gm-POPB-F	CTCCTGGAAATTATCCCTCT	52	<i>Gm_POPB</i>
Gm-POPB-R	ATACATTCTATGCCAAGTCCTC		
Gs-POPA-F	CCGTGGCTTCCTGATGCTTA	57	<i>Gs_POPA</i>
Gs-POPA-R	TCATGCGTCGAGTCCCAGTT		
Gs-POPB-F	GACAAATGGACGACTGCTC	55	<i>Gs_POPB</i>
Gs-POPB-R	AGCCAGGATCTAGTTCAGGT		
Lv-POPA-F	TTCACGCTCATCTACAACGG	55	<i>Lv_POPA</i>
Lv-POPA-R	ATCTCCCTGCTTTCACCCAC		
Lv-POPB-F	CTCTGCTCGCCGTTCTGAT	55	<i>Lv_POPB</i>
Lv-POPB-R	TGAATCGGATGTCTGGTTAGTT		

e, *A. exitialis*; *Af*, *A. fuliginea*; *Am*, *A. molliuscula*; *Ao*, *A. oberwinklerana*; *Apa*, *A. pallidrosea*; *Ar*, *A. rimosa*; *Asubf*, *A. subfuliginea*; *Asubj*, *A. subjunquillea*; *Ab*, *A. subpallidrosea*; *Av*, *A. virosa*; *Gm*, *G. marginata*; *Gs*, *G. sulciceps*; *Lv*, *L. venter*; *α-amanitin*, *α-AMA*; *β-amanitin*, *β-AMA*; *PHA*, *phalloidin*; *PHD*, *phalloidin*; *POP*, prolyl oligopeptidase.

Figures

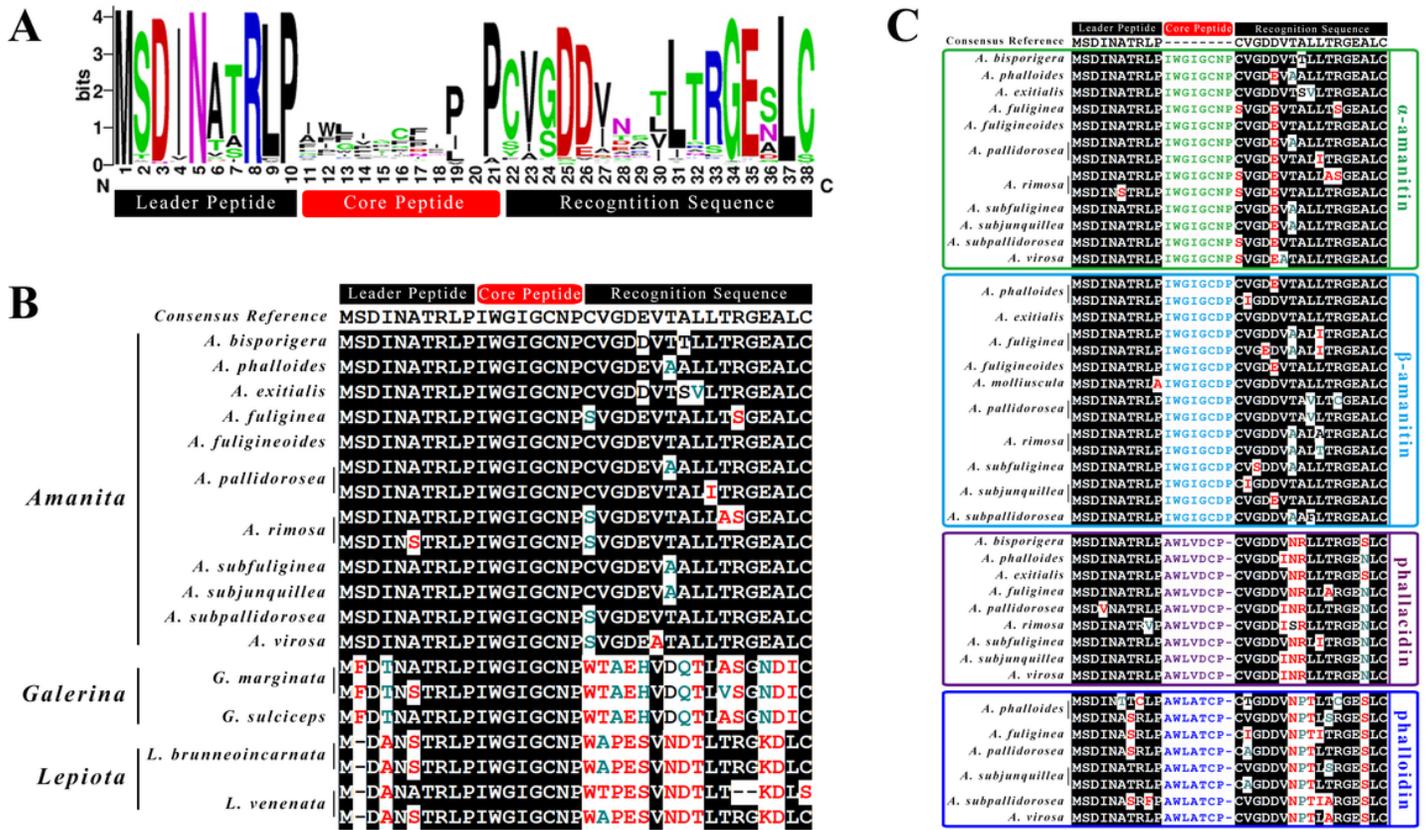


Figure 1

Alignment of MSDIN precursor peptide sequences. (A) WebLogo [41] alignment of 145 MSDIN members from 9 *Amanita* species. The letter height of each amino acid represents its conservation degree, and the higher the letter, the more conserved the site. (B) Alignment of the precursor peptide sequences of α -amanitin from 15 amanitin-producing mushrooms. (C) Alignment of the precursor peptide sequences of α -, β -amanitin, phalloidin and phalloidin from 12 *Amanita* species. Letters with white background are variations compared with the consensus sequence. The sequences of *A. bisporigera*, *A. phalloides* were from Pulman et al. (2016), the sequences of *A. fuligineoides* were from Li et al. (2014) and the sequences of *L. brunneoincarnata* were from Lüli et al. (2019).

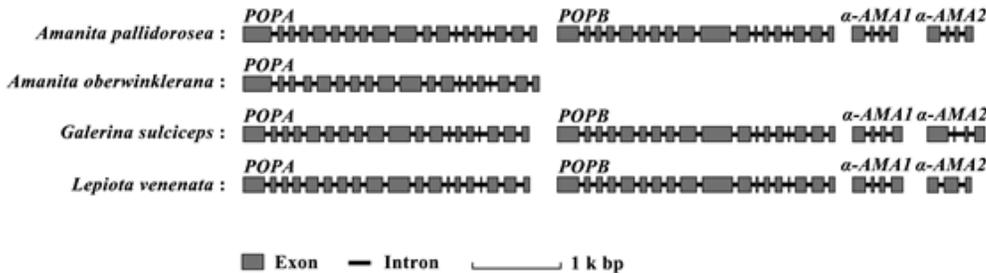


Figure 2

Structures of α -AMA and POP exemplified by four agaric species.

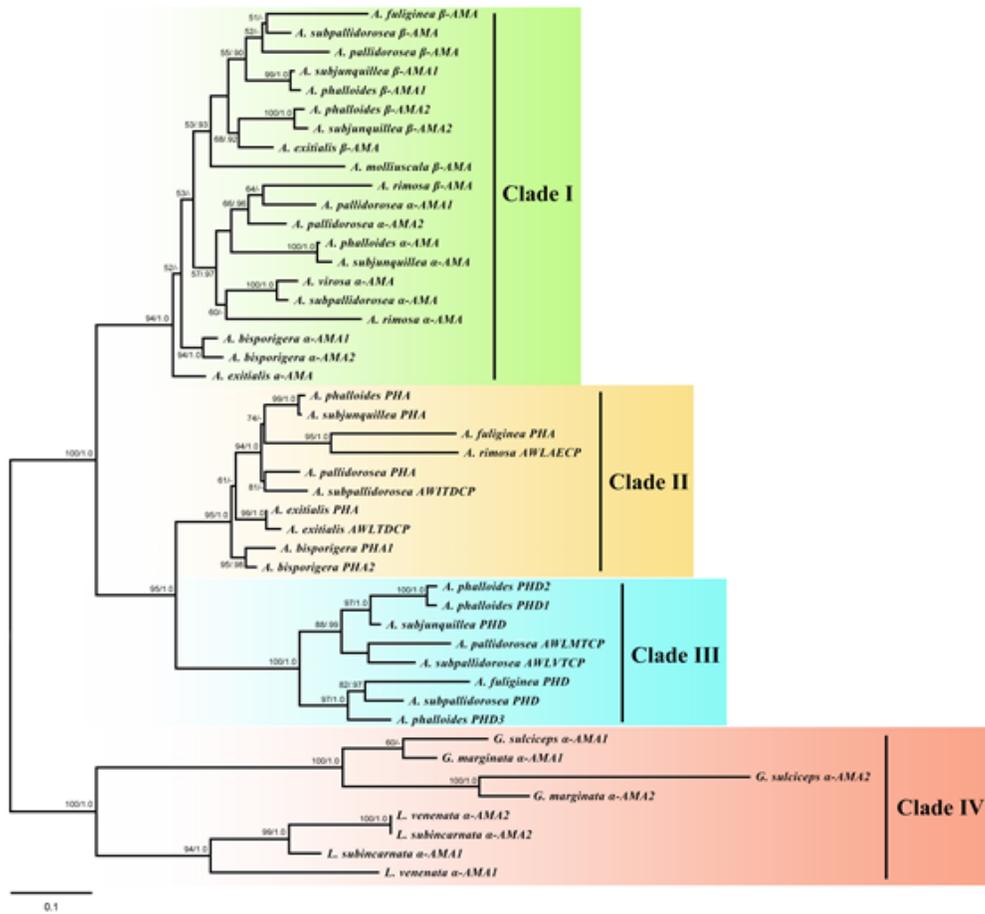


Figure 3

Phylogenetic trees generated from maximum likelihood analysis based on toxin MSDIN genes. Bootstrap percentages (>50%) based on 1,000 replications and Bayesian posterior probabilities (>0.90) are shown at nodes. Bar, a substitution per 10 nucleotides.

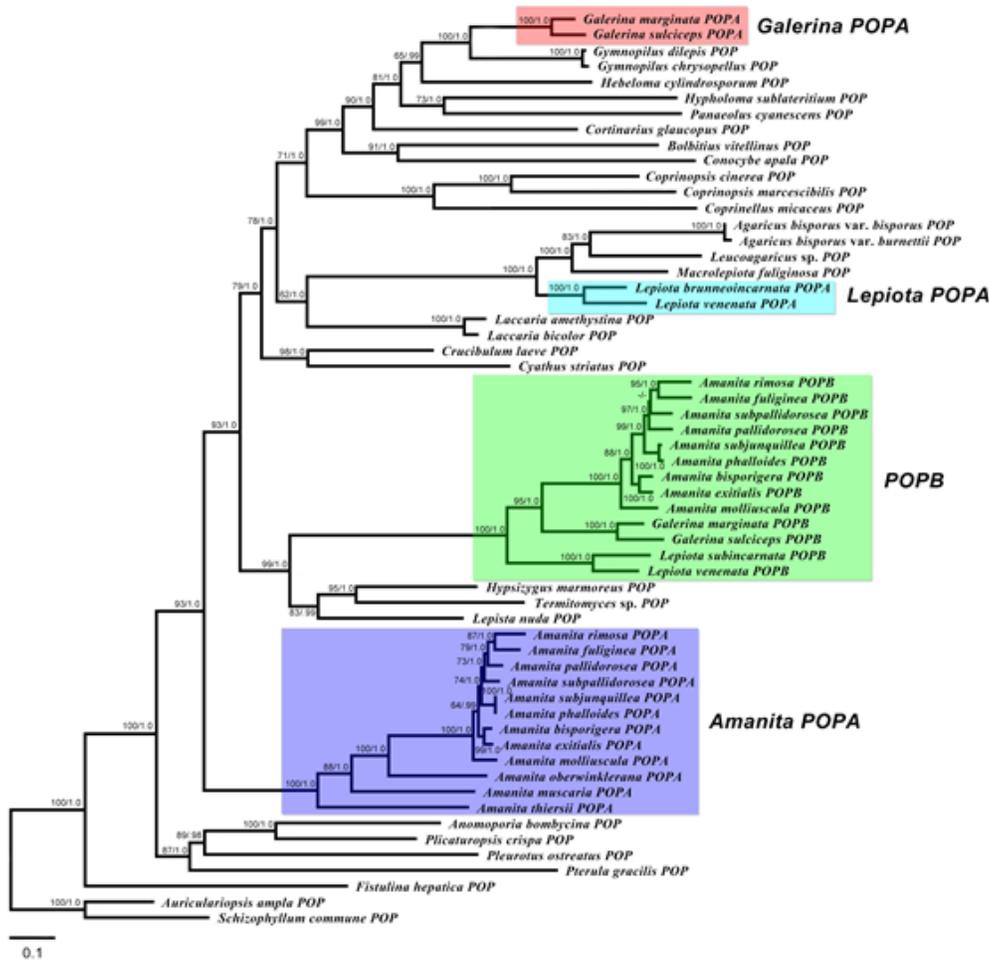


Figure 4

Phylogenetic trees generated from maximum likelihood analysis based on POP genes. Bootstrap percentages (>50%) based on 1,000 replications and Bayesian posterior probabilities (>0.90) are shown at nodes. Bar, a substitution per 10 nucleotides.