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Phylogenetic relationships among biological species of *Armillaria* from China

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ABSTRACT

Fourteen Chinese Biological Species (CBS) of *Armillaria* were previously identified in a collection of Chinese isolates. CBS C, F, G, H, J, L, N and O remained unnamed, while the remaining isolates included *A. borealis*, *A. cepistipes*, *A. gallica*, *A. mellea*, *A. sinapina* and *A. tabescens*. CBS F was suggested to represent *A. singula* based on basidiocarp morphology. In this study, phylogenetic relationships between Chinese *Armillaria* isolates and those from other parts of the world were determined based on DNA sequence data. Results of this study suggest that CBS F might not represent *A. singula*, and that *A. monadelpha* (a name applied to the North American form of *A. tabescens* by some authors) and *A. tabescens* should be treated as a single species. Four main phylogenetic lineages, referred to as the *A. ostoyae*, *A. gallica*, *A. tabescens* and *A. mellea* clusters, were identified on the phylogenetic trees. The unnamed biological species grouped within the "*A. gallica* cluster" and were phylogenetically closely related. The results of this study contribute to our current understanding of the systematics of *Armillaria* from South East Asia where these fungi are relatively poorly known.

1. Introduction

Species of *Armillaria* (Fr.) Staude are well-known in China and other parts of the world where some are important pathogens mainly of woody plants (Shaw and Kile 1991; Baumgartner et al. 2011). Some *Armillaria* species are primary pathogens, causing the disease generally referred to as Armillaria root rot, which is considered amongst the most serious diseases of trees in boreal and temperate forests and various species damage high-value crops. Other species are important components of woody ecosystems by virtue of their saprophytic life strategy, where they contribute significantly to wood degradation (Gregory et al. 1991; Kile et al. 1991). *Armillaria* species also have an important role in the traditions of various Asian cultures as a source of nutrients or linked to traditional medicine (Hobbs 1986). For example, the mushroom fruiting structures of some edible species are utilized as a food source or used in the treatment of hypertension, neurasthenia and epilepsy (Hobbs 1986).

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The taxonomy of *Armillaria* is largely based on the morphological and biological species concepts (Baumgartner et al. 2011). As additional species have been described, basidiocarp morphology has provided increasingly limited value and the biological species concept, reliant on reproductive isolation (Mayr 1942), has been increasingly useful (e.g., Morrison et al. 1985; Proffer et al. 1987; Dumas 1988; Coetzee et al. 2003b). This approach gained popularity in the late 1970's with the introduction of mating tests to differentiate *Armillaria* species (Korhonen 1978; Anderson and Ullrich 1979) and it remains a useful method in taxonomic studies. The morphological and biological species concepts have thus been applied to describe various taxa, including *A. mellea* subsp. *nipponica*, *A. sinapina*, *A. gallica*, *A. ostoyae*, *A. cepistipes*, *A. ectypa*, *A. jezoensis*, *A. singula*, *A. nabsnona* and various unnamed biological species from South East Asia (Sung et al. 1989, 1992; Mohammed et al. 1994; Cha and Igarashi 1995; Sung et al. 1995; Ota et al. 1998, 2009).

In a relatively recent study, Qin et al. (2007) expanded current knowledge regarding the *Armillaria* species diversity in China. Using mating studies, fourteen Chinese Biological Species (CBS A to D and F to O) of *Armillaria* were identified among isolates that were collected from 15 provinces of northern and southern China. Eight CBS (C, F, G, H, J, L, N and O) were unnamed, while the remainder included *A. sinapina* (CBS A), *A. gallica* (CBS B), *A. solidipes* (CBS D), formerly treated as *A. ostoyae* (Burdsall and Volk 2008) and pending nomenclatural conservation (Redhead et al. 2011), *A. tabescens* (CBS I), heterothallic *A. mellea* (CBS K), homothallic *A. mellea* (CBS G, suggested to represent *A. mellea* subsp. *nipponica*) and *A. borealis* (CBS M). Based on morphological characteristics, Qin et al. (2007) suggested that CBS F could be *A. singula*, a species that has been reported from Japan (Cha et al. 1994). However, mating tests were not performed to support this assertion.

Mating studies done by Qin et al. (2007) showed that CBS I is compatible with tester strains of *A. tabescens* from Europe. The taxonomy of *A. tabescens* from Asia, Europe and North America is, however, controversial mainly because sexual compatibility studies have provided inconclusive results. Preliminary results of Darmono et al. (1992), based on sexual compatibility tests between North American strains of *A. tabescens* and one strain identified as *A. tabescens* from Italy, suggested that *A. tabescens* from the two continents represent the same taxon. In contrast, Guillaumin et al. (1993) found that strains from Europe identified as *A. tabescens* are intersterile with North American strains of this species. Kile et al. (1994) subsequently proposed that *A. tabescens* from North America should be treated as a distinct species and referred to as *A. monadelpha*, a name that is considered illegitimate by Volk and Burdsall (1995). Ota et al. (1998) reported that Japanese isolates were interfertile with European isolates but intersterile with one North American isolate of this species. Although this would resolve some of these discrepancies, a phylogenetic study of these species has not been undertaken.

On the basis of their basidiocarp and culture morphology, the Chinese biological species were assigned to one of the species clusters introduced by Korhonen (1995). These clusters comprise species that share morphological characteristics and that are phylogenetically closely related. The clusters were referred to by Korhonen (1995) as the "*A. ectypa* cluster", "*A. gallica* cluster" (including *A. altimontana*, *A. calvescens*, *A. cepistipes*, *A. gallica*, *A. nabsnona*, *A. sinapina*, *A. singula* and *A. jezoensis*), "*A. mellea* cluster", "*A. ostoyae* cluster" (*A. ostoyae*, *A. borealis* and *A. gemina*) and "*A. tabescens* cluster" (*A. tabescens* and *A. monadelpha*). Based on their morphological characteristics, the unnamed biological species from China (C, F, H, J and L) were suggested to reside in the "*A. gallica* cluster", while CBS N and CBS O were not placed in any of the clusters (Qin et al. 2007). Despite the availability of techniques to resolve such questions, nothing is known regarding the phylogenetic relationships of the unnamed Chinese biological species with those of *Armillaria* spp. from other parts of the world.

Phylogenetic methods utilising DNA sequence data have been widely employed to elucidate the identity of field isolates of *Armillaria* (Coetzee et al. 2003a, b, 2005b; Keča et al. 2006; Sekizaki et al. 2008; Kikuchi and Yamaji 2010; Elías-Román et al. 2013) and to resolve the phylogenetic relationships of *Armillaria* species from various parts of the world (Maphosa et al. 2006; Coetzee et al. 2011). For phylogenetic inference, the internally transcribed spacer regions (ITS) and intergenic spacer region one (IGS-1) have been useful in studies focused on the relationships of taxa from Africa (Coetzee et al. 2005a), South America (Pildain et al. 2009), Australasia (Coetzee et al. 2001), North America (Anderson and Stasovski 1992), Europe (Chillali et al. 1998) and Asia (Terashima et al. 1998; Coetzee et al. 2000). In addition, sequences for part of the transcription elongation factor one alpha (TEF-1α) gene has been used to determine the phylogenetic relationships of taxa from Japan (Hasegawa et al. 2010), Europe (Tsykun et al. 2013) and a global collection of isolates of *Armillaria* species (Maphosa et al. 2006). Despite the importance of *Armillaria* in China, there have not been studies to determine the phylogenetic relationships of Chinese biological species.

The aims of this study were to address some of the unresolved questions that emerged from the research of Qin et al. (2007). The identity of the unnamed CBS F was considered and a species recognition approach based on gene genealogical concordance was followed to assess the suggested differentiation of European and South East Asian *A. tabescens* from its North American counterpart. An additional aim was to determine the phylogenetic relationships between the Chinese biological species and *Armillaria* species from other regions of the world.

2. Materials and methods

2.1. *Fungal isolates*

Isolates included in this study that represent different Chinese Biological Species (Supplementary Table S1) were obtained from the culture collection of Dr. J. Zhao and were previously assigned to biological species in the study by Qin et al. (2007). Additional isolates from other parts of the world were also included to expand the geographical representation of *Armillaria* species in the Northern Hemisphere *Armillaria* phylogeny (Supplementary Table S2). Isolates were grown on malt yeast agar (MYA: 1.5% w/v malt extract, 0.2% w/v and yeast extract 1.5% w/v agar) medium. Isolates are maintained

in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria.

2.2. DNA sequencing

DNA was extracted from isolates representing each of the CBS and other *Armillaria* species following the methods outlined in Coetzee et al. (2005a). PCR reaction conditions and mixtures were the same as those described by Coetzee et al. (2003b) for the IGS-1 region, and Maphosa et al. (2006) for the TEF-1α gene. The IGS-1 region was amplified for all isolates using primers P-1 (Hsiau 1996) and O-1 (Duchesne and Anderson 1990). Amplicons for the partial TEF-1α gene were obtained using primer pair EF595F/EF1160R (Kauserud and Schumacher 2001). Amplicons were purified with a MSB® Spin PCRapace purification kit (Invitek) following the instructions of the manufacturer prior to DNA sequencing. DNA sequences were obtained in both directions for each PCR product with the same primers used for their amplification. Sequencing reactions were done using a BigDye Terminator v3.1 cycle sequencing kit (ABI) following the protocol outlined by the manufacturer. Sequences were determined on an ABI 3100 DNA automated sequencer. Base calling was visually inspected in CLC Main Workbench (CLC) and forward and reverse strands were assembled into contigs using the same software. The sequences obtained were used in DNA sequence similarity searches against those in GenBank using Blastn to ensure the identity of the isolates.

2.3. Phylogenetic methods

IGS-1 and TEF-1α sequences were obtained from GenBank for *Armillaria* species from other parts of the world (Supplementary Table S2). Sequences selected from GenBank were from well characterised isolates used in previously published studies (see Supplementary Table S2 for references). These sequences together with those for the isolates included in this study formed the IGS-1 and TEF-1α Northern Hemisphere *Armillaria* species matrices, respectively. All multiple sequence alignments (TreeBase Study number: S17215) were done using MAFFT (Katoh and Standley 2013) and applying the default settings. Nucleic substitution models were determined with jModelTest (Posada 2008). Phylogenetic trees were generated based on maximum likelihood and Bayesian inference or maximum likelihood and parsimony. In each analysis, the IGS-1 and TEF-1α data were analyzed separately. *Armillaria mellea* was used as the outgroup taxon in all analyses.

Maximum likelihood analyses were done using PHYML v. 3.0 (Guindon et al. 2010). The analyses incorporated substitution models that best fitted the individual data sets (Supplementary Table S3) and these were applied using a custom model setting in PHYML. The maximum likelihood trees that were obtained were rooted to *A. mellea*. Confidence levels for the nodes were obtained through a bootstrap analysis (1000 replicates) using the same settings employed to search tree-space for the fundamental maximum likelihood tree.

Bayesian inference of phylogenies was determined using MrBayes 3.2.1 (Huelsenbeck and Ronquist 2001). The likelihood and prior settings were based on the models for each dataset obtained from the analyses using jModelTest (Supplementary Table S3). For analyses of the combined data sets, a model specific to each data partition was used. Posterior probability distributions were obtained by setting the Markov Chain Monte Carlo (MCMC) function to 4×10^6 generations for each analysis with a sampling frequency of every 100th tree. Posterior probability values were calculated after excluding (burnin) 25% of the trees generated during the MCMC analysis. ESS (Estimated Sample Size) values for the parameters were subsequently assessed in Tracer v. 1.5 (http://tree.bio.ed.ac.uk/software/tracer/) as a measure of convergence. The trees generated were viewed in FigTree v. 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/) to obtain tree topologies and the posterior probability values for their nodes.

Parsimony analyses were conducted using PAUP ver. 4 (Swofford 2002) and employed a heuristic tree search algorithm with 10 replicates of random addition of sequences and TBR branch swapping. Confidence levels at the nodes were determined using bootstrap analyses (1000 replicates) with the same settings but with the addition of sequences set to "closest". Missing, ambiguous and uninformative characters were excluded in all parsimony-based analyses.

2.4. Assessing the conspecificity of CBS F and A. singula

IGS-1 sequences generated for isolates belonging to CBS F were compared with sequences of *Armillaria* in GenBank using Blastn searches. The available IGS-1 sequence (D89926) for *A. singula* in GenBank was downloaded and aligned with sequences of CBS F. Percentage similarity, converted from p-distances, was then determined in MEGA 6 (Tamura et al. 2013) and compared against those obtained from Blastn searches for other *Armillaria* species. In addition, the phylogenetic placement of CBS F and *A. singula* was assessed in a phylogenetic tree generated for the Northern Hemisphere *Armillaria* species.

2.5. Phylogeny of isolates from Europe, North America and South East Asia identified as A. tabescens

In addition to the *A. tabescens* sequences included in Northern Hemisphere *Armillaria* species matrices, other available IGS-1 and TEF-1α DNA sequences for this species were downloaded from GenBank. These were aligned with sequences of *A. tabescens* and *A. mellea* generated in this study (Supplementary Table S1). Phylogenetic trees were obtained separately for the IGS-1 and TEF-1α data matrices based on parsimony and maximum likelihood. The grouping of isolates representing *A. tabescens* was also assessed in the context of a Northern Hemisphere *Armillaria* species phylogeny.

2.6. Phylogenetic relationships of Chinese Armillaria species with those from other parts of the world

Phylogenetic trees were constructed from the IGS-1 and TEF-1α Northern Hemisphere *Armillaria* species matrices separately based on maximum likelihood and Bayesian analyses as described above.

3. Results

3.1. *Amplification of the IGS-1 and TEF-1α regions*

The IGS-1 and EF-1α regions consistently yielded a single band after PCR. The amplicon size for the IGS-1 region varied among the CBS, ranging from 600 bp to 900 bp. Amplification of the EF-1α yielded an amplicon size of approximately 600 bp.

3.2. *Assessing the conspecificity of CBS F and* A. singula

Isolates belonging to CBS F and considered by Qin et al. (2007) to represent *A. singula* had the highest IGS-1 sequence similarity to those of *A. cepistipes*, *A. gallica* and *A. altimontana* (99% similarity) in GenBank. In contrast, sequence comparisons after aligning the IGS-1 sequence of *A. singula* from GenBank with those for the isolates representing CBS F revealed a 98% sequence similarity.

Phylogenetic trees generated from the IGS-1 data matrix separated the isolates representing CBS F and the sequence of *A. singula* in well supported monophyletic groups (Fig. 1). *Armillaria singula* grouped closest to *A. gallica* from Japan (PP = 1, bootstrap = 91%) within a monophyletic group that included *A. jezoensis* (PP = 0.96, bootstrap = 68%) (Fig. 1). Together this group formed a monophyletic group with *A. gallica* from Europe and China (PP = 1, bootstrap = 71%). Isolates belonging to CBS F resided in monophyletic group that constituted *A. calvescens*, *A. cepistipes*, *A. gallica* and *A. sinapina* (PP = 1, bootstrap = 58%) (Fig. 1).

3.3. *Phylogeny of isolates from Europe, North America and South East Asia identified as* A. tabescens

Phylogenetic trees generated for the Northern Hemisphere collection of *Armillaria* species placed all isolates of *A. tabescens* in a strongly supported monophyletic group (both IGS-1 and TEF-1α: PP = 1, bootstrap = 100%) (Figs. 1A and 2A). Phylogenetic trees generated for isolates from China and a larger collection of sequences obtained from GenBank for *A. tabescens* yielded incongruent topologies for the phylogenetic trees generated from IGS-1 and TEF-1α sequences (Supplementary Figure S1). Trees obtained from IGS-1 sequence data placed the isolates from Japan and China in a clade that included sequences for *A. tabescens* from North America. Trees based on TEF-1α sequences grouped the isolates from Asia with sequences of *A. tabescens* originating in Europe. In all phylogenetic trees, the Asian isolates formed a sub-clade with high bootstrap support (IGS-1: maximum likelihood 99%, parsimony 100%; TEF-1α: 86% for both analyses).

3.4. *Phylogenetic relationships of Chinese* Armillaria *species with those from other parts of the world*

Phylogenetic trees generated from the different data matrices differed in their resolution and grouping of *Armillaria* species (Figs. 1 and 2). Phylogenetic trees generated from the IGS-1 sequence data generally yielded high bootstrap support at the nodes (Fig. 1). In contrast, trees generated from the TEF-1α sequence data had low resolution at the deeper nodes (Fig. 2). In general, four groups emerged from the IGS-1 sequence matrix (Fig. 1). Following the *Armillaria* cluster names of Korhonen (1995), these groups are referred as the "*A. mellea*", "*A. tabescens*", "*A. ostoyae*" and "*A. gallica*" clusters, respectively.

The "*A. ostoyae* cluster" included isolates belonging to *A. borealis*, *A. ostoyae* and *A. gemina*. Isolates representing *A. gemina* clustered in a well supported group in all trees generated from the IGS-1 (Fig. 1A) and TEF-1α (Fig. 2A) sequence data. Isolates belonging to *A. borealis* were placed in a strongly supported group based on IGS-1 sequence data (Fig. 1A). However, isolates of this species from China, Belarus, Germany and Finland were placed distant to isolates belonging to the same species from Finland and Switzerland as well as *A. gemina* and *A. ostoyae* on the trees obtained from the TEF-1α sequence matrix (Fig. 2A). Trees generated from the IGS-1 sequence matrix placed isolates of *A. ostoyae* from China and Europe in two distinct groups, and together they formed a sister group to *A. borealis* (Fig. 1A). Isolates of *A. ostoyae* from Japan and the USA grouped sister to *A. gemina*, *A. borealis* and the cluster that included isolates of this species from Europe and China (Fig. 1A). Trees generated from the TEF-1α sequence matrix grouped isolates of *A. ostoyae* from China, Japan and Europe in a well supported group, while isolates from the USA formed a group with high support and were placed sister to *A. gemina* (Fig. 2A).

The "*A. gallica* cluster" included *A. calvescens*, *A. cepistipes*, *A. gallica*, *A. sinapina*, *A. nabsnona* and the unnamed biological species CBS C, CBS F, CBS H, CBS J, CBS L, CBS N and CBS O (Figs. 1B and 2B). With the exception of some species, most of the isolates could not be separated into monophyletic groups representing their respective species assignments. In this cluster, isolates representing *A. altimontana*, *A. nabsnona* and NAG E were placed in their distinctive species groups with high statistical support on trees generated from the IGS-1 (Fig. 1B) and TEF-1α (Fig. 2B) matrices, respectively. Isolates representing *A. calvescens* formed a strongly supported monophyletic group in phylogenetic trees obtained from the IGS-1 sequence matrix (Fig. 1B), but this was not the case for phylogenetic trees based on the TEF-1α matrix (Fig. 2B). With the exception of CBS J and H, all remaining isolates belonging to CBS grouped together forming their respective species groups based on TEF-1α sequence data (Fig. 2B). *Armillaria nabsnona* formed a sister group with the remaining species having strong bootstrap support and PP = 1 based on the IGS-1 sequences (Fig. 1B). Isolates residing in CBS C (only isolate CMW31123), H, J, L, N, O grouped together with NAG E, *A. cepestipes*, *A. gallica* and *A. sinapina* on the trees generated from IGS-1 sequences (Fig. 1B). The grouping of CBS C, H, J, L, N, O and *A. gallica* (CMW31087) was supported by the trees generated from TEF-1α sequences (Fig. 2B). Within this group, CBS L and CBS N were placed sister to each other with high bootstrap support and posterior probability (Fig. 2B). Chinese biological species F together with an isolate belonging to CBS C (CMW31124) clustered with isolates representing *A. calvescens*, *A. cepistipes*, *A. gallica* and *A. sinapina* based on the IGS-1 sequence matrix (Fig. 1B). CBS F clustered with isolates belonging to *A. cepistipes* (PP = 0.98, bootstrap = 98%) and together, these species were grouped sister to *A. sinapina* based on the TEF-1α sequence data (Fig. 2B).

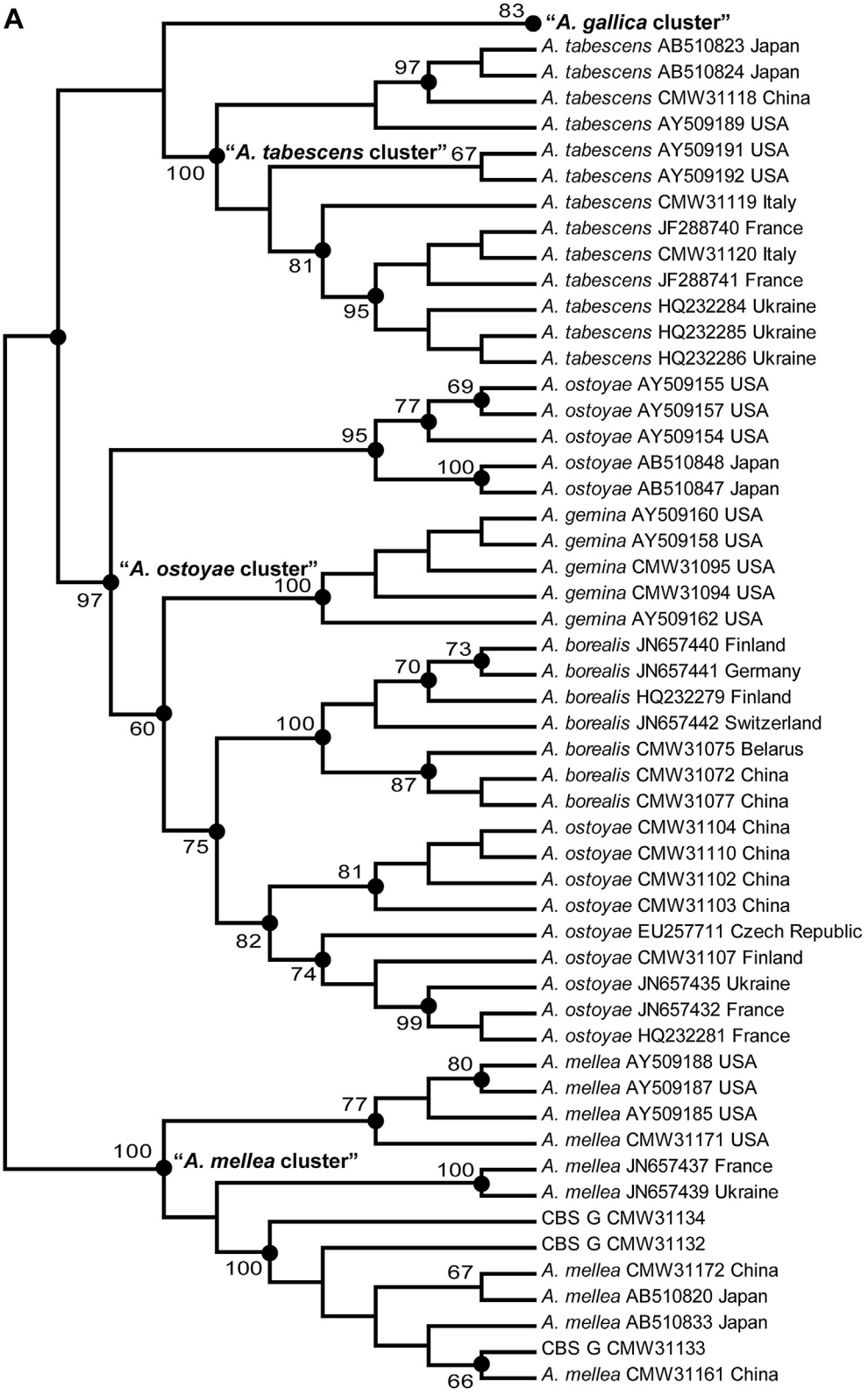

Fig. 1 – Phylogenetic tree generated from IGS-1 sequences based on maximum likelihood and converted to a cladogram for a collection of Northern Hemisphere *Armillaria* species. A: Phylogenetic relationships of species in the "*A. mellea*", "*A. ostoyae*" and "*A. tabescens*" clusters with isolates in the "*A. gallica* cluster" collapsed to a single terminal node. B: Phylogeny of species in the "*A. gallica* cluster" with isolates in the *A. mellea*, *A. ostoyae* and *A. tabescens* clusters collapsed to single terminal nodes. Bootstrap values (≥60%) based on maximum likelihood are indicated at the nodes. Posterior probability values (≥0.90) are indicated with circles at the nodes. The four main lineages are shown on the branches of the tree. (*) node shared by *A. gallica*, *A. jezoensis* and *A. singula* (PP = 1, bootstrap = 71%). (**) node shared by CBS F, CBS C (CMW31124) *A. calvescens*, *A. cepistipes*, *A. gallica* and *A. sinapina* (PP = 1, bootstrap = 58%).

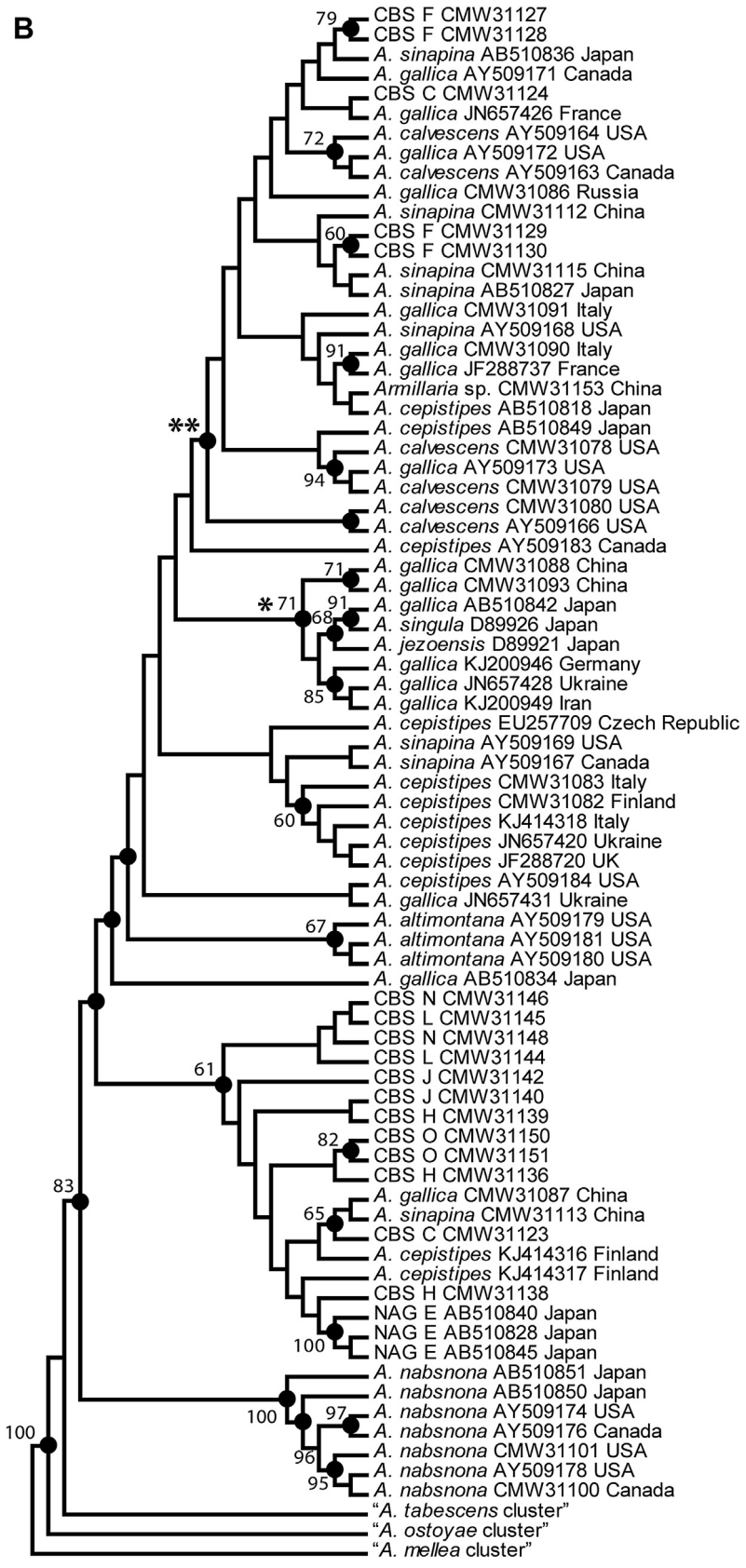

Fig. 1 – (*continued*).

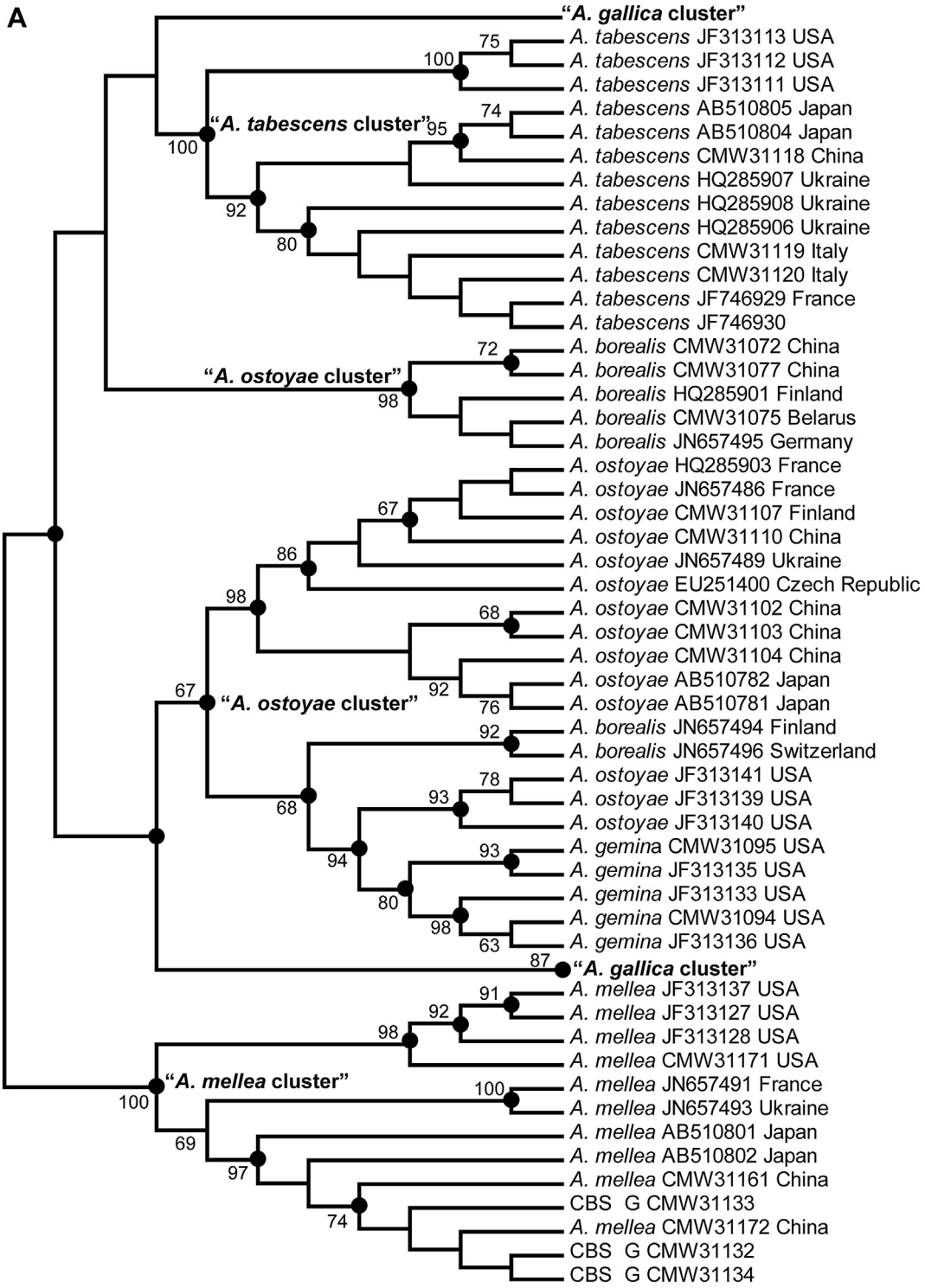

Fig. 2 – Phylogenetic tree, based on maximum likelihood analysis of TEF-1α sequences and converted to a cladogram, showing the relationships isolates for a collection of Northern Hemisphere *Armillaria* species. A: Phylogenetic relationships of species in the "*A. mellea*", "*A. ostoyae*" and "*A. tabescens*" clusters with isolates in the "*A. gallica* cluster" collapsed to a single terminal node. B: Phylogeny of species in the "*A. gallica* cluster" with isolates in the "*A. mellea*", "*A. ostoyae*" and "*A. tabescens*" clusters collapsed to single terminal nodes. Bootstrap values (≥60%) based on maximum likelihood are indicated at the nodes. Posterior probability values (≥0.90) are indicated with circles at the nodes.

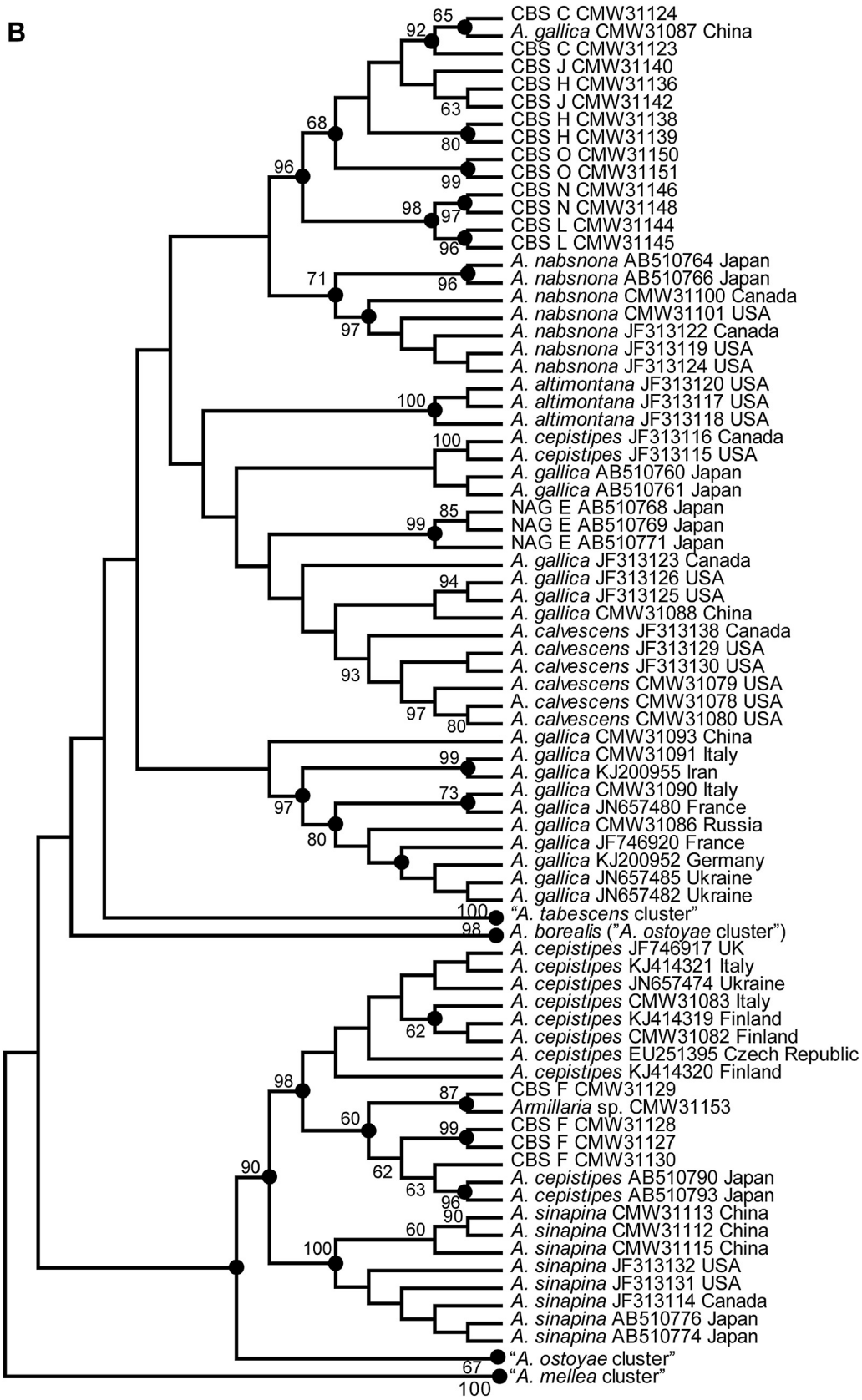

Fig. 2 – (*continued*).

4. Discussion

Although *Armillaria* spp. are common in China, very little work has been done to identify these fungi. This is the first study to apply DNA sequence analyses to consider the identity of a relatively large collection of isolates from the country, and to assess the phylogenetic relationships among these isolates and those known from other parts of the world. The specific aims of this study were to determine the identity of the unnamed CBS F, to consider the suggestion that *A. tabescens* from North America should be treated as a species different from its European and Asian counterparts and to determine the phylogenetic relationships of *Armillaria* species from China.

4.1. *Identity of CBS F*

Qin et al. (2007) suggested that CBS F and *A. singula* are conspecific on the basis of their basidiocarp morphology. *Armillaria singula* was described from Hokkaido (Cha et al. 1994) and it has not been found elsewhere (Ota et al. 1998). There is only one IGS-1 sequence for this species (Terashima et al. 1998) and no living cultures are known to exist (Ota et al. 2012).

Results of Blastn searches and phylogenetic analyses of the IGS-1 region revealed that CBS F possibly represents an undescribed *Armillaria* sp. other than *A. singula* but closely related to *A. calvescens*, *A. cepistipes*, *A. gallica* and *A. sinapina*. IGS-1 DNA sequences of isolates belonging to CBS F were most similar to those of *A. cepistipes*, *A. gallica* and *A. altimontana* on GenBank. Phylogenetic trees generated from IGS-1 sequences grouped CBS F distant from *A. singula* and together with *A. calvescens*, *A. cepistipes*, *A. gallica* and *A. sinapina* with high bootstrap support and high posterior probability. Phylogenetic trees based on TEF-1α sequences grouped representatives of CBS F and *A. cepistipes* together with high bootstrap support. Isolates of CBS F were, however, sexually incompatible with those of *A. cepistipes*, *A. gallica* and *A. sinapina* in the study of Qin et al. (2007). Although the results of the current study are not conclusive, given the fact that IGS-sequences could not resolve isolates identified as *A. gallica*, *A. cepistipes* and *A. sinapina* into their respective species groups on the phylogenetic trees, it suggests that CBS F represents a novel taxon. Future research should focus on obtaining isolates belonging to *A. singula* so that mating tests and phylogenetic studies can be conducted in order to reach a definitive identification.

4.2. *Are* Armillaria tabescens *isolates from Asia, Europe and North America conspecific?*

Results of this study suggest that *A. tabescens* from Asia, Europe and North America should be treated as a single taxon. Phylogenetic trees grouped isolates of *A. tabescens* from various parts of the world in a strongly supported monophyletic group on trees generated in this study for a large collection of isolates belonging to different *Armillaria* species. Phylogenetic trees placed isolates of *A. tabescens* from China in a monophyletic group with those from Japan. Trees generated from IGS-1 and TEF-1α sequence data were, however, incongruent in the placement of this group relative to isolates of *A. tabescens* from Europe and North America. Although only two loci were used in this study, application of genealogical concordance phylogenetic recognition (Taylor et al. 2000) indicates that these isolates are conspecific. Results of the present study thus support the view that *A. tabescens* from Asia, Europe and North America should be treated as a single taxon. However, further studies including a larger collection and a broader distribution of isolates from the Northern Hemisphere should be undertaken to confirm these results.

4.3. *Phylogeny of* Armillaria *species from China*

Phylogenetic trees generated from the IGS-1 region and partial TEF-1α gene for the collection of isolates from China used in this study generally resolved four main phylogenetic groups. These are referred to as the "*A. ostoyae*", "*A. gallica*", "*A. tabescens*" and "*A. mellea*" clusters and they are more or less consistent with those defined by Korhonen (1995). The "*A. ostoyae* cluster" included *A. ostoyae* (CBS D), *A. borealis* (CBS M) and *A. gemina*. The "*A. gallica* cluster" formed the largest group and included *A. gallica* (CBS B), *A. sinapina* (CBS A), CBS C, CBS F, CBS H, CBS J, CBS K, CBS L, CBS N and CBS O as well as *A. altimontana*, *A. calvescens*, *A. cepistipes*, *A. nabsnona* and NAG E. The "*A. mellea* cluster" was represented by isolates belonging to *A. mellea* s.s. (CBS K) and CBS G.

The unnamed *Armillaria* Chinese biological species H, J, L, N and O were suggested to be closely related to members of the *A. gallica* cluster based on the characteristics of their basidiocarps (Qin et al. 2007). This view was supported in the present study where isolates representing these biological species formed a monophyletic group that included *A. gallica* and its closest relatives in the phylogenetic trees generated from IGS-1 and TEF-1α sequences. Trees obtained from the IGS-1 sequences also showed a close relationship between these biological species and NAG E from Japan, although this only had low bootstrap support. The phylogenetic relationships among the Chinese biological species could not be resolved based on IGS-1 sequences due to poor phylogenetic resolution. In contrast, TEF-1α sequences provided better resolution for these biological species. Phylogenetic trees generated from the latter sequences revealed a close relationship between CBS L and CBS N and that they have a sister relationship with CBS C, CBS H, CBS J and CBS O.

Determining the phylogeny of *Armillaria* species from China was complicated by gene trees that differed in their topologies and phylogenetic resolution. Incongruence in the placement of isolates belonging to CBS C, *A. borealis*, *A. cepistipes*, *A. gallica*, *A. sinapina* and *A. ostoyae*, was observed on trees generated respectively from IGS-1 and TEF-1α sequences in this study. Phylogenetic trees obtained from the IGS-1 region grouped the isolates belonging to CBS C in two different clusters, while they grouped together on trees generated from TEF-1α sequences. Similarly, isolates identified as *A. sinapina* were placed at different positions on trees generated from the IGS-1 region, while they formed a monophyletic group on the tree obtained from TEF-1α sequences. Isolates belonging to *A. borealis* formed a monophyletic group in the tree generated

from the IGS-1 matrix, while they were separated into distantly related groups on the TEF-1α phylogenetic trees. Isolates belonging to *A. ostoyae* were grouped in two monophyletic groups on trees obtained from the IGS-1 and TEF-1α matrices. Isolates of *A. gallica* and *A. cepistipes* were scattered within the "*A. gallica* cluster" in trees generated for both loci, however, isolates of *A. gallica* from Europe and Iran formed a monophyletic group on the tree obtained from TEF-1α sequences. The discordance between the gene trees could be ascribed to incomplete lineage sorting as result of recent divergence (Maddison 1997). A larger sample size and additional gene regions would be required to resolve this question, but the results are congruent with those of earlier studies (Maphosa et al. 2006; Mulholland et al. 2012; Ross-Davis et al. 2012; Tsykun et al. 2013) suggesting that the TEF-1α gene will be well-suited for species identification based on sequence comparisons.

The results of this study contribute to our current understanding of the systematics of *Armillaria*, and more specifically *Armillaria* species from South East Asia. With the exception of a few phylogenetic studies that have focused on the species occurring in Japan (Terashima et al. 1998; Hasegawa et al. 2010; Ota et al. 2012), nothing was previously known regarding the phylogeny of Chinese *Armillaria* species prior to this study. This study also expanded the current IGS-1 and TEF-1α DNA sequence database for *Armillaria* species and the data can now be employed in future research to identify field isolates from China using sequence comparisons. Clearly, many questions remain regarding the identity of the genus *Armillaria* from China. In this regard, the most important challenge ahead will be to collect isolates linked to sporocarps and to study these using all available taxonomic tools for *Armillaria*. There are clearly numerous novel species in China and these deserve to be named and studied.

Disclosure

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.myc.2015.05.001.

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