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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