

Insights into the phylogeny of Northern Hemisphere *Armillaria*: Neighbor-net and Bayesian analyses of translation elongation factor 1- α gene sequences

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ABSTRACT

Armillaria possesses several intriguing characteristics that have inspired wide interest in understanding phylogenetic relationships within and among species of this genus. Nuclear ribosomal DNA sequence-based analyses of *Armillaria* provide only limited information for phylogenetic studies among widely divergent taxa. More recent studies have shown that translation elongation factor 1- α (*tef1*) sequences are highly informative for phylogenetic analysis of *Armillaria* species within diverse global regions. This study used Neighbor-net and coalescence-based Bayesian analyses to examine phylogenetic relationships of newly determined and existing *tef1* sequences derived from diverse *Armillaria* species from across the Northern Hemisphere, with Southern Hemisphere *Armillaria* species included for reference. Based on the Bayesian analysis of *tef1* sequences, *Armillaria* species from the Northern Hemisphere are generally contained within the following four superclades, which are named according to the specific epithet of the most frequently cited species within the superclade: (i) *Socialis/Tabescens* (exannulate) superclade including Eurasian *A. ectypa*, North American *A. socialis* (*A. tabescens*), and Eurasian *A. socialis* (*A. tabescens*) clades; (ii) *Mellea* superclade including undescribed annulate North American *Armillaria* sp. (Mexico) and four separate clades of *A. mellea* (Europe and Iran, eastern Asia, and two groups from North America); (iii) *Gallica* superclade including *Armillaria* Nag E (Japan), multiple clades of *A. gallica* (Asia and Europe), *A. calvescens* (eastern North America), *A. cepistipes* (North America), *A. altimontana* (western USA), *A. nabsnana* (North America and Japan), and at least two *A. gallica* clades (North America); and (iv) *Solidipes/Ostoyae* superclade including two *A. solidipes/ostoyae* clades (North America), *A. gemina* (eastern USA), *A. solidipes/ostoyae* (Eurasia), *A. cepistipes* (Europe and Japan), *A. sinapina* (North America and Japan), and *A. borealis* (Eurasia) clade 2. Of note is that *A. borealis* (Eurasia) clade 1 appears basal to the *Solidipes/Ostoyae* and *Gallica* superclades. The Neighbor-net analysis showed similar phylogenetic relationships. This study further demonstrates the utility of *tef1* for global phylogenetic studies of *Armillaria* species and provides critical insights into multiple taxonomic issues that warrant further study.

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INTRODUCTION

Armillaria possesses several unique and intriguing characteristics that have inspired wide interest in understanding phylogenetic relationships within and among species of this genus. *Armillaria* species are extremely common across forest ecosystems, and they exhibit diverse ecological behaviors, ranging from virulent root and butt pathogens of diverse woody hosts (Baumgartner et al. 2011) to beneficial saprophytes (Fox 2000), mycorrhizal associates of orchids (Cha and Igarashi 1995), or associates of other forest fungi (Kikuchi and Yamaji 2010). Furthermore, genets of *Armillaria* species are recognized as being among the oldest and longest lived organisms on the earth, where a single genet has been found to occupy up to 965 ha with an estimated age of 1900–8650 years (Smith et al. 1992; Ferguson et al. 2003). Successful mating of haploid mycelia of *Armillaria* can result in a transient dikaryotic state (Larsen et al. 1992); however, the vegetative mycelia of *Armillaria* species are typically diploid (Korhonen and Hintikka 1974; Anderson and Ullrich 1982; Kim et al. 2000). Although the *Armillaria* genus has a worldwide distribution, the Northern and Southern Hemispheres typically do not share *Armillaria* species (Volk and Burdsall 1995). Several *Armillaria* species appear to have circumboreal or circumaustral distributions. The vicariance between circumboreal and circumaustral *Armillaria* species may indicate that these species have origins that date back as far as the separation of Laurasia and Gondwana 180 million years bp during the breakup of Pangea, as has been hypothesized for other forest fungi (e.g., Halling 2001; Martin et al. 2002). Alternatively, the radiation of the genus *Armillaria* may have occurred ca. 54 million years bp, well after the tectonic breakup of Laurasia and Gondwana (Coetzee et al. 2011).

On the basis of the morphological and biological species concepts, several *Armillaria* species, such as *A. mellea*, *A. solidipes* (= *A. ostoyae*; Burdsall and Volk 2008; hereafter referred to as *A. solidipes/ostoyae*), *A. socialis* (= *A. tabescens*; Antonín et al. 2006; hereafter referred to as *A. socialis/tabescens*), *A. gallica*, and *A. cepistipes*, have been shown to occur naturally in Europe, Asia, and North America (Volk and Burdsall 1995). Interestingly, *A. sinapina* and *A. nabsnona* are known to occur in North America and Asia (Japan), but not in Europe. In contrast, *A. gemina*, *A. calvescens*, *A. altimontana* (North American Biological Species X [NABS X]; Brazee et al. 2012a), and an undescribed *Armillaria* sp. from Mexico (Elias-Roman et al. 2013) have been reported only in North America, whereas *A. borealis* and *A. ectypa* have been confirmed only in Europe and Asia. [Note added at the proof stage:

Armillaria tabescens and *A. ectypa* have recently been assigned to the genus *Desarmillaria* (Koch et al. 2017)].

Previous DNA sequence-based analyses of *Armillaria* phylogeny have largely focused on nuclear ribosomal DNA (rDNA), such as internal transcribed spacer-1 (ITS1), 5.8S, internal transcribed spacer-2 (ITS2), 26S (large subunit; LSU), intergenic spacer-1 (IGS1), and 5S (e.g., Anderson and Stasovski 1992; Chillali et al. 1998; Coetzee et al. 2001, 2003, 2005a, 2015; Mueller et al. 2001; Dunne et al. 2002; Keča et al. 2006; Kim et al. 2006; Lima et al. 2008; Hasegawa et al. 2010; Keča and Solheim 2010). Although LSU, ITS, and/or IGS rDNA sequences provided useful information for phylogenetic studies among widely divergent taxa, these sequences were not reliable for resolving closely related Eurasian species, such as *A. solidipes/ostoyae* and *A. borealis* (Pérez-Sierra et al. 2004; Keča and Solheim 2010), and other Northern Hemisphere species, such as *A. gallica*, *A. calvescens*, *A. cepistipes*, and *A. sinapina* (Kim et al. 2006; Brazee et al. 2011; Tsykun et al. 2013). Surprisingly, rDNA sequences have been informative to assess diversity within currently recognized *Armillaria* species, such as *A. mellea* and *A. solidipes/ostoyae* (as *A. ostoyae*) (Coetzee et al. 2000; Hanna et al. 2007b). RNA polymerase II (*rpb2*) gene sequences were useful for separating some eastern North American *Armillaria* species, but the closely related *A. gallica* and *A. calvescens* could not be resolved by *rpb2* sequences (Brazee et al. 2011). Other studies based on anonymous nucleotide sequences (Piercey-Normore et al. 1998), amplified fragment length polymorphisms (AFLPs) (Pérez-Sierra et al. 2004; Kim et al. 2006; Terashima et al. 2006; Brazee et al. 2012b), and microsatellites/random amplified microsatellites (RAMS) (Qin et al. 2001) have contributed information useful to understanding genetic relationships within and among *Armillaria* species; however, these methods are used infrequently and are difficult to apply reliably on a wide scale.

Previous studies have demonstrated the utility of translation elongation factor 1- α (*tef1*) for understanding the phylogeny of basidiomycetous mushrooms (Matheny et al. 2007). On the basis of partial *tef1* sequences, Maphosa et al. (2006) examined phylogenetic relationships among 15 described *Armillaria* species and other unknown *Armillaria* species from diverse global regions. On the basis of that analysis, *Armillaria* species from Africa formed a separate basal clade, another major clade comprised six described *Armillaria* species from Australasia and South America, and a third major clade comprised eight described *Armillaria* species from Eurasia and North America. In subsequent studies, Coetzee et al. (2011)

used *tef1*, ITS1-5.8S-ITS2, and LSU sequences to obtain phylogenetic support for three major geographically defined clades (Holarctic, South American–Australasian, and African). Furthermore, work by Baumgartner et al. (2010a) showed that *A. mellea* contains a single copy of *tef1*, and sequences of this gene were used to show phylogenetic separation of *A. mellea* isolates derived from different geographic regions (Baumgartner et al. 2012).

The utility of *tef1* has also been demonstrated for examining differences in *Armillaria* species of different geographical regions. For European *Armillaria* species, Antonín et al. (2009) demonstrated that partial *tef1* sequences were effective at distinguishing between closely related *A. gallica* and *A. cepistipes* from the Czech Republic and Slovakia, and Mulholland et al. (2012) used a similar approach to distinguish among several *Armillaria* species from northern Europe. As part of a multilocus study, Tsykun et al. (2013) showed that six Eurasian *Armillaria* species were phylogenetically separated with partial *tef1* sequences, but isolates from Japan and isolates from Europe were generally contained within separate sister clades. Partial *tef1* sequences were used to distinguish eight described, one undescribed (biological species Nagasawa E [Nag E]), and one unknown *Armillaria* species in Japan, while allowing some comparisons with *Armillaria* species from other global sources (Hasegawa et al. 2010; Ota et al. 2011). Tsykun et al. (2013) used the *tef1* gene to distinguish *A. cepistipes* and *A. gallica* from the Ukrainian Carpathians, whereas Brazee et al. (2011) used partial *tef1* sequences to distinguish between closely related *A. calvescens* and *A. gallica* from north-eastern North America. For North America, Ross-Davis et al. (2012) showed that *tef1* sequences are effective for delineating all 10 recognized *Armillaria* species in North America, and Elías-Román et al. (2013) used *tef1* sequences to help confirm the existence of an undescribed *Armillaria* sp. in Mexico. Recently, Coetzee et al. (2015) used partial *tef1* sequences to examine phylogenetic position of 14 Chinese biological species. Thus, on the basis of multiple phylogenetic studies, *tef1* is the only examined DNA region that has consistently demonstrated utility for phylogenetic analyses that differentiate among closely related *Armillaria* species in Europe, Asia, and North America.

Although *tef1* sequences have been used successfully to examine phylogenies of *Armillaria* species from different continents within the Northern Hemisphere, no published analysis has examined phylogenetic relationships of diverse *Armillaria* species across the Northern Hemisphere. The objective of this study is to obtain

further insights into phylogenetic relationships among Northern Hemisphere *Armillaria* species on the basis of newly determined and existing *tef1* sequences from well-characterized isolates.

MATERIALS AND METHODS

***Armillaria* isolates and sequences.**—A total of 242 partial *tef1* sequences were included in this study, which included 88 newly obtained sequences of *Armillaria* isolates for this study and 154 sequences derived from GenBank, where *Armillaria* species identification had been verified (SUPPLEMENTARY TABLE 1). These *tef1* sequences were obtained from isolates representing ca. 14 currently recognized *Armillaria* species from the Northern Hemisphere and 9 *Armillaria* species from the Southern Hemisphere (for reference and comparison). Subsets of these GenBank-derived sequences were previously reported by Maphosa et al. (2006), Antonín et al. (2009), Hasegawa et al. (2010; 2011), Brazee et al. (2011), Ota et al. (2011), Mulholland et al. (2012), Ross-Davis et al. (2012), Tsykun et al. (2012, 2013), Elías-Román (2013), and Keča et al. (2015), in addition to other selected *tef1* sequences that were also used for phylogenetic analyses. The 88 *tef1* sequences that were newly obtained for this study included 48 *Armillaria* isolates from North America (31 USA, 9 Canada, and 8 Mexico), 31 from Europe (4 Poland, 7 Spain, 2 Belarus, 1 Russia, 2 Finland, 3 Switzerland, 1 Luxembourg, 2 Greece, 7 France, and 2 Norway), and 9 isolates from Asia (4 China and 5 South Korea) (SUPPLEMENTARY TABLE 1). We followed the protocols of Ota et al. (2011), Ross-Davis et al. (2012), and Elías-Román et al. (2013) for fungal culture and polymerase chain reaction (PCR) amplification/DNA sequencing of the *tef1* gene. The 88 newly determined *tef1* sequences from North America (48), Europe (31), and Asia (9) for this study are deposited in GenBank (JQ898309–JQ898317; KR061313–KR061315; KX151878–KX151953) (SUPPLEMENTARY TABLE 1).

Phylogenetic analysis and recombination analyses.—

A Neighbor-net phylogenetic approach using SplitsTree v4.1 (Huson and Bryant 2006) and a coalescence-based Bayesian approach using Bayesian Evolutionary Analysis by Sampling Trees (BEAST) (Drummond et al. 2012) were used in the phylogenetic analyses. The complete data set containing 242 *tef1* sequences was analyzed in SplitsTree with the default parameters (except transversion ratio was set to 1) using K2P-derived distances and constructed with the Neighbor-

net algorithm (Bryant and Moulton 2004). Prior to Bayesian analysis, intragenic recombination was tested with Recombination Detection Program (Martin and Rybicki 2000). Sequences were considered linear (polymorphic nucleotides at a single site were included and coded with the IUPAC [International Union of Pure and Applied Chemistry] codes), the *P* value cutoff was set to 0.05, the Bonferroni correction was applied, and all possible recombination events were visualized. For Bayesian analysis, DT ModSel (Minin et al. 2003) was used to select TN93 as the best-fit substitution model. Bayesian Evolutionary Analysis Utility (BEAUti) version 1.7.5 (Drummond et al. 2012) was used to create XML-formatted input files for BEAST v1.7.5. In BEAST, a Markov chain Monte Carlo algorithm was used to sample the posterior distribution of trees by conducting five independent runs of 100 million generations each using a constant size tree prior, strict molecular clock, and uniform priors. Trees were sampled every 1000 generations, and the first 20% was discarded as burn-in. Post burn-in trees were combined with the program LogCombiner (BEAST v1.7.5), and chains were assumed to converge when the average standard deviation of split frequencies was <0.01. The maximum clade credibility tree with posterior probability of each node was computed with the program TreeAnnotator (BEAST v1.7.5). Log files and tree files were visualized in Tracer v1.5 (<http://tree.bio.ed.ac.uk/software/tracer/>) and FigTree v1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree/>), respectively. Initial Bayesian analysis of the complete data set failed to converge and had low effective sample size (ESS) values (3.049–12.51). To reduce the complexity and likely phylogenetic conflict in the data set, we created a reduced data set of 43 groups (G1–G43) guided by genetic distances created in SplitsTree (TABLE 1). Taxa were grouped if separated by a genetic distance ≤ 0.015 . Simple consensus sequences (50% strict) were created in BioEdit (Hall 1999) for each of the 43 taxa groups (G1–G43), and these reduced data sets were subjected to SplitsTree and BEAST analyses using the same procedures described above. All phylogenetic trees generated from *tef1* sequences have been deposited into TreeBASE (submission ID 19226).

RESULTS

General phylogenetic relationships.—Of the 440 nucleotides used for phylogenetic analysis, 178 were variable. The total 242 partial *tef1* sequences were grouped into 43 consensus sequences (G1–G43) based

on a genetic distance of ≤ 0.015 (TABLE 1). Intragenetic recombination analyses on this smaller, grouped data set showed no evidence of recombination.

Both the Neighbor-net (SplitsTree) and Bayesian (BEAST) phylogenetic analyses of *tef1* sequences produced similar results that revealed four superclades of Northern Hemisphere *Armillaria* species, which were named according to the specific epithet of the most frequently cited species within the superclade (FIGS. 1–3). The four *Armillaria* superclades from the Northern Hemisphere are referred to as (i) *Socialis/Tabescens* (exannulate); (ii) *Mellea*; (iii) *Gallica*; and (iv) *Solidipes/Ostoyae*. All four of the Northern Hemisphere superclades were well separated from the two Southern Hemisphere superclades: (i) *A. heimii* (G43—Réunion, Africa–Indian Ocean), *A. fuscipes* (G42—Réunion), and an unknown *Armillaria* sp. (G42—Zimbabwe, Africa); and (ii) *A. pallidula/A. fumosa* (G36—Australia), *A. hinnulea* (G35—Australia), *A. luteobubalina* (G39—Chile, South America), *A. limonea* (G40—New Zealand), *A. puiggarii* (G31—Guadeloupe, Caribbean region [Northern Hemisphere]), *A. novae-zelandiae* (G32—Papua New Guinea, G33—Indonesia, G34—Australia), an unknown *Armillaria* species (G37, G38—Kenya, Africa), and isolates of an undetermined *Armillaria* sp. (G41—Amami-Oshima Island, Japan [Northern Hemisphere]). Thus, two *Armillaria* isolates from Amami-Oshima Island (G41) (Ota et al. 2011) and one isolate of *A. puiggarii* (G31) were the only Northern Hemisphere isolates contained within the Southern Hemisphere group. The Neighbor-net analysis showed similar phylogenetic relationships (FIGS. 1–2).

DISCUSSION

***Socialis/Tabescens* (exannulate) superclade.**—Based on phylogenetic analyses of the available *tef1* sequences, the *Socialis/Tabescens* (exannulate) superclade comprises *A. ectypa* (G1—Eurasia) within one basal monophyletic lineage, with *A. socialis/tabescens* (G3—Eurasia) and *A. socialis/tabescens* (G2—southeastern USA and Mexico) contained within paraphyletic sister clades (FIG. 3). The phylogenetic analysis showed that *A. ectypa* (G1—Eurasia) resides in a distinct monophyletic lineage that appears basal to all other *Armillaria* species, supporting current taxonomy.

In the *tef1*-based Bayesian phylogenetic tree, the basal position of the *Socialis/Tabescens* (exannulate) *Armillaria* superclade provides evidence that *A. ectypa* and *A. socialis/tabescens* are ancestral among all global *Armillaria* lineages. Furthermore, *A. socialis/tabescens* isolates are contained within two

Table 1. *Armillaria* species, isolates, and translation elongation factor 1- α (*tef1*) sequences used for phylogenetic analyses^a

<i>Armillaria</i> species	Isolate	GenBank no.	Reference/Source/Collector(s)	Origin	Consensus group
Socialis/Tabescens (exannulate) superclade					
<i>ectypa</i>	BRNM 704974	EU251403	Antonin et al. (2009)	Austria	G1 <i>A. ectypa</i> (Eurasia)
<i>socialis/tabescens</i>	O01210	JF313111	Ross-Davis et al. (2012)	USA	G2 <i>A. socialis/tabescens</i> (southeastern USA, Mexico)
<i>socialis/tabescens</i>	PT89.94	FJ618674	Stefani et al. (GenBank)	Italy	G3 <i>A. socialis/tabescens</i> (Eurasia)
Mellea superclade					
sp.	MEX1F	KX151879	D. Alvarado-Rosales, N.B. Klopfenstein, and J.W. Hanna	Mexico	G4 Undescribed <i>Armillaria</i> sp. (Mexico)
<i>mellea</i>	ST21	JF313127	Ross-Davis et al. (2012)	USA	G5 <i>A. mellea</i> (USA, Mexico)
<i>mellea</i>	V7030	FJ160490	Baumgartner et al. (GenBank)	USA	G6 <i>A. mellea</i> (western USA)
<i>mellea</i>	CMW 11265	DQ435636	Maphosa et al. (2006)	Italy	G7 <i>A. mellea</i> (Europe, Iran)
<i>mellea</i>	97-6	AB510797	Hasegawa et al. (2010)	Japan	G8 <i>A. mellea</i> (eastern Asia)
Borealis clade 1					
<i>borealis</i>	KK-03-354/1	KX151892	K. Korhonen/J. Zhao	China	G9 <i>A. borealis</i> (Eurasia 1)
Solidipes/Ostoyae superclade					
<i>borealis</i>	A618	JN657496	Tsykun et al. (2013)	Switzerland	G10 <i>A. borealis</i> (Eurasia 2)
<i>solidipes</i>	CO102 (80-R121)	KX151892	J.J. Worrall	USA	G11 <i>A. solidipes/ostoyae</i> (North America 1)
<i>solidipes</i>	MS2-11	JF895852	Brazee et al. (2011)	USA	G12 <i>A. solidipes/ostoyae</i> (North America 2)
<i>ostoyae</i>	BRNM 695627	EU251401	Antonin et al. (2009)	Czech Republic	G13 <i>A. solidipes/ostoyae</i> (Eurasia)
<i>gemina</i>	ST11A	JF313133	Ross-Davis et al. (2012)	USA	G14 <i>A. gemina</i> (eastern USA)
<i>sinapina</i>	V SP82-23	FJ618655	Stefani et al. (GenBank)	Canada	G15 <i>A. sinapina</i> (North America, Japan)
<i>cepistipes</i>	1990-25/16	KX151931	N. Keča/O. Olsen	Norway	G16 <i>A. cepistipes</i> (Europe)
<i>cepistipes</i>	94-33-01	AB510789	Hasegawa et al. (2010)	Japan	G17 <i>A. cepistipes</i> (Japan)
Gallica superclade					
<i>cepistipes</i>	W113	JF313115	Ross-Davis et al. (2012)	USA	G18 <i>A. cepistipes</i> (North America)
<i>gallica</i>	B2-6	JF895838	Brazee et al. (2011)	USA	G19 <i>A. gallica</i> (North America 1)
<i>gallica</i>	H113	KX151940	Kim et al. (2010a)	USA	G20 <i>A. gallica</i> (USA - Hawaii)
<i>gallica</i>	AZ32F (COC-02-01)	KC525954	Nelson et al. (2013)	USA	G21 <i>A. gallica</i> (North America 2)
<i>gallica</i>	BRNM 706835	EU251390	Antonin et al. (2009)	Czech Republic	G22 <i>A. gallica</i> (Europe)
sp.	2005-893 (Qp FFG)	KJ960232	Keča et al. (2015)	Serbia	G23 <i>A. gallica</i> (Serbia)
<i>gallica</i>	NA4	AB510761	Hasegawa et al. (2010)	Japan	G24 <i>A. gallica</i> (Japan)
<i>gallica</i>	NA17	AB510759	Hasegawa et al. (2010)	Japan	G25 <i>A. gallica</i> (Japan, Korea)
<i>gallica</i>	HC-5R	KX151949	M.-S. Kim	South Korea	G26 <i>A. gallica</i> (Korea)
<i>nabsona</i>	C21	JF313119	Ross-Davis et al. (2012)	USA	G27 <i>A. nabsona</i> (North America, Japan)
<i>altimontana</i>	X140.9	FJ618678	Stefani et al. (GenBank)	USA	G28 <i>A. altimontana</i> (western USA)
<i>calvescens</i>	ST18	JF313129	Ross-Davis et al. (2012)	USA	G29 <i>A. calvescens</i> (eastern North America)
Nag E	96-37-1	AB510769	Hasegawa et al. (2010)	Japan	G30 <i>Armillaria</i> Nag E (Japan)
Southern hemisphere <i>Armillaria</i> groups (including one northern hemisphere isolate from the Caribbean* and two isolates from Japan**)					
<i>puiggarii</i>	PPg85-63	FJ618636	Stefani et al. (GenBank)	Guadeloupe	G31 <i>A. puiggarii</i> (Guadeloupe*)
<i>novae-zelandiae</i>	PNZ87.54	FJ618650	Stefani et al. (GenBank)	Papua New Guinea	G32 <i>A. novae-zelandiae</i> (Papua New Guinea)
<i>novae-zelandiae</i>	CMW 4143	DQ435654	Maphosa et al. (2006)	Indonesia	G33 <i>A. novae-zelandiae</i> (Indonesia)
<i>novae-zelandiae</i>	CMW 4967	DQ435651	Maphosa et al. (2006)	Australia	G34 <i>A. novae-zelandiae</i> (Australia)
<i>hinnulea</i>	CMW 4980	DQ435648	Maphosa et al. (2006)	Australia	G35 <i>A. hinnulea</i> (Australia)
<i>hinnulea</i>	Lot2	FJ618663	Stefani et al. (GenBank)	Australia	G35
<i>pallidula</i>	3626	FJ618665	Stefani et al. (GenBank)	Australia	G36 <i>A. pallidula/fumosa</i> (Australia)
<i>fumosa</i>	CMW 4960	DQ435645	Maphosa et al. (2006)	Australia	G36
sp.	PII187.9	FJ618647	Stefani et al. (GenBank)	Kenya	G37 <i>Armillaria</i> sp. (Kenya)
sp.	PII187.42	FJ618652	Stefani et al. (GenBank)	Kenya	G38 <i>Armillaria</i> sp. (Kenya)
<i>luteobubalina</i>	CMW 8876	DQ435658	Maphosa et al. (2006)	Chile	G39 <i>A. luteobubalina</i> (Chile)
<i>limonea</i>	Plim84.66	FJ618654	Stefani et al. (GenBank)	New Zealand	G40 <i>A. limonea</i> (New Zealand)
sp.	00-13	AB558990	Ota et al. (2011)	Japan	G41 <i>Armillaria</i> sp. (Japan**)
sp.	00-14	AB558991	Ota et al. (2011)	Japan	G41
<i>fuscipes</i>	CMW 3164	DQ435621	Maphosa et al. (2006)	Réunion	G42 <i>A. fuscipes</i> (Réunion)
sp.	CMW 4456	DQ435617	Maphosa et al. (2006)	Zimbabwe	G42 <i>Armillaria</i> sp. (Zimbabwe)
sp.	CMW 9954	DQ435620	Maphosa et al. (2006)	Zimbabwe	G42
<i>heimii</i>	PH87.24	FJ618651	Stefani et al. (GenBank)	Réunion	G43 <i>A. heimii</i> (Réunion)

^a This table contains 43 *Armillaria* isolates with *tef1* sequences that correspond to the 43 consensus sequences (50%-strict), which were derived from a total of 242 isolates of *Armillaria* species. The 242 *tef1* sequences included 88 newly obtained sequences of *Armillaria* isolates for this study and 154 sequences derived from GenBank, where *Armillaria* species identification had been verified (SUPPLEMENTARY TABLE 1).

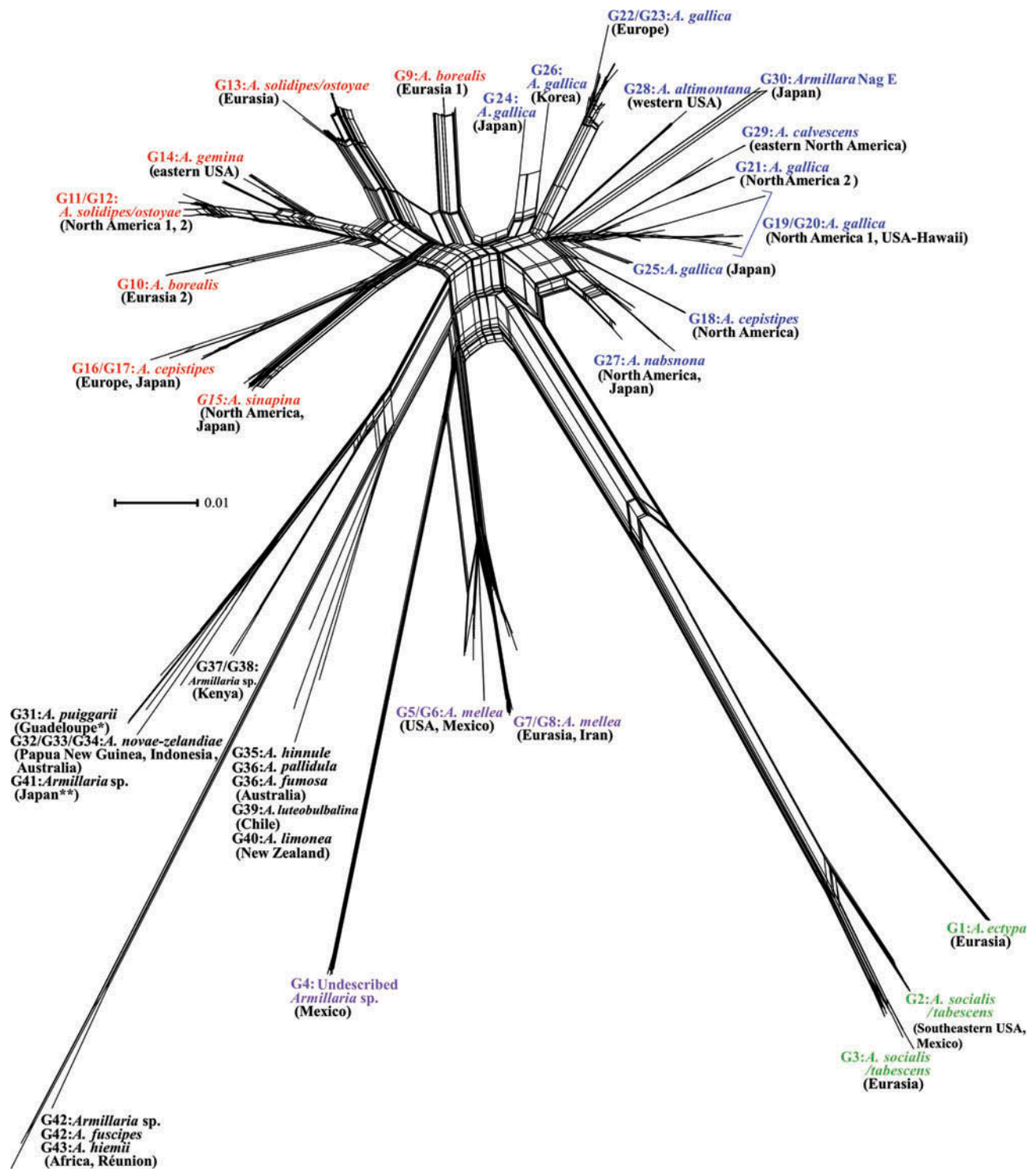


Figure 1. A Neighbor-net phylogenetic network based on partial translation elongation factor 1- α sequences from 242 *Armillaria* spp. isolates created by SplitsTree4 v4.12.8 with K2P distances. Potential reticulation between *Armillaria* species is highlighted by the circular patterns connecting terminal nodes. Southern Hemisphere *Armillaria* groups including one Northern Hemisphere isolate from the Caribbean* (G31: *A. puiggarii* from Guadeloupe in the Caribbean) and two isolates from Japan** (G41: *Armillaria* sp. from Amami-Oshima Island, Japan). Detailed information for 242 *Armillaria* spp. is described in SUPPLEMENTARY TABLE 1.

separate sister clades representing Eurasia and North America. Earlier studies on *A. socialis/tabescens* by Ota et al. (1998) determined that isolates

from Japan were compatible with isolates from Europe, but incompatible with isolates from North America; however, phylogenetic analysis of Eurasian

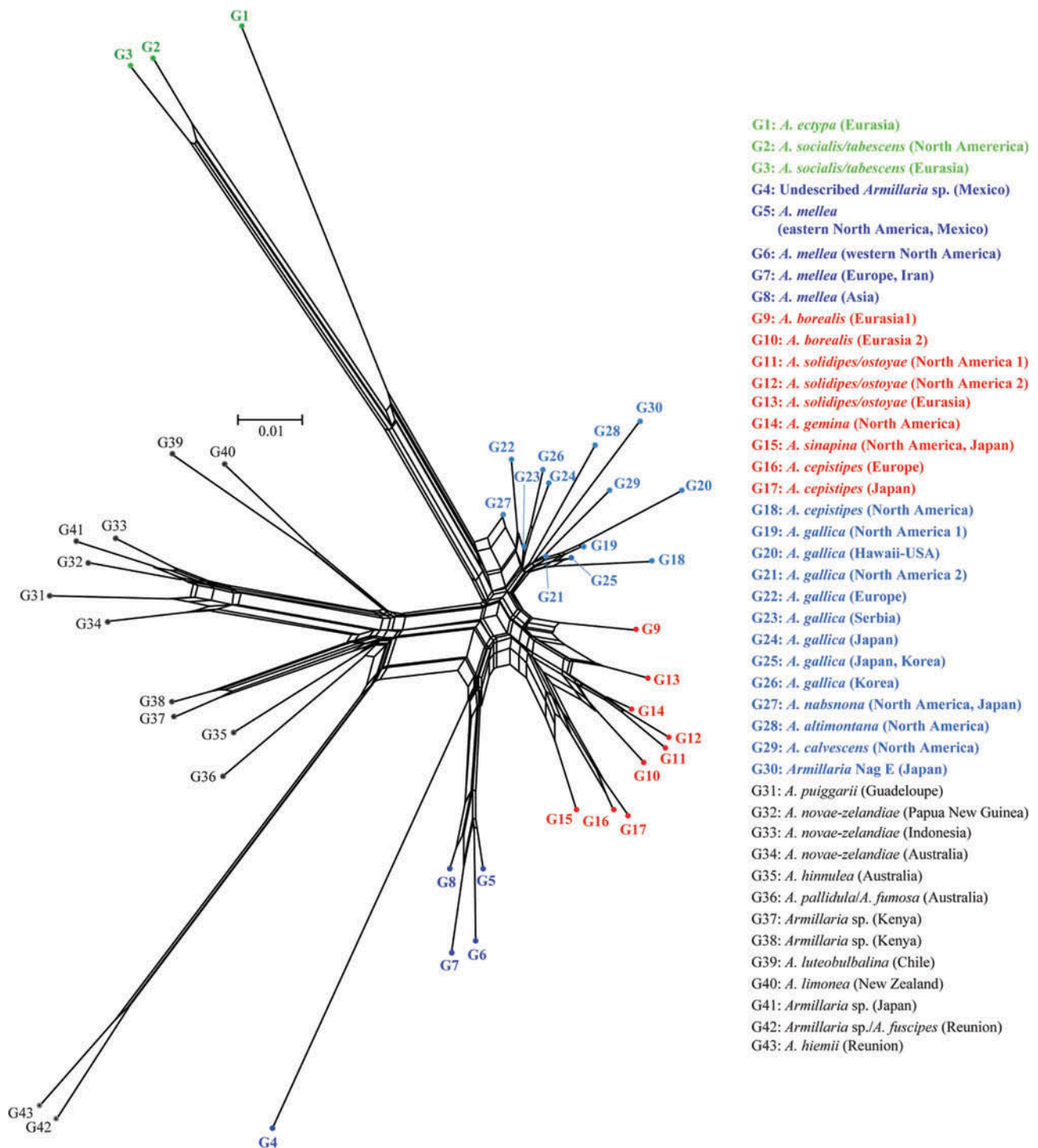


Figure 2. Neighbor-net phylogenetic network based on 43 simple consensus (50% strict) sequences of partial translation elongation factor 1- α from 43 phylogenetic groups (G1–G43) representing 242 *Armillaria* spp. isolates, which included 88 newly obtained sequences of *Armillaria* isolates for this study and 154 sequences derived from GenBank. The phylogenetic groups are described in TABLE I. The networks were constructed with Neighbor-net in SplitsTree4 v4.12.8 using K2P distances based on contigs of consensus sequences. Potential reticulation between *Armillaria* species groups is highlighted by the circular patterns connecting terminal nodes. Green, purple, red, and blue colors indicate species found in Northern Hemisphere, and black color indicates the Southern Hemisphere groups, which include some Northern Hemisphere species/isolates (G31: *A. puiggarii* from Guadeloupe in the Caribbean and G41: *Armillaria* sp. from Amami-Oshima Island, Japan).

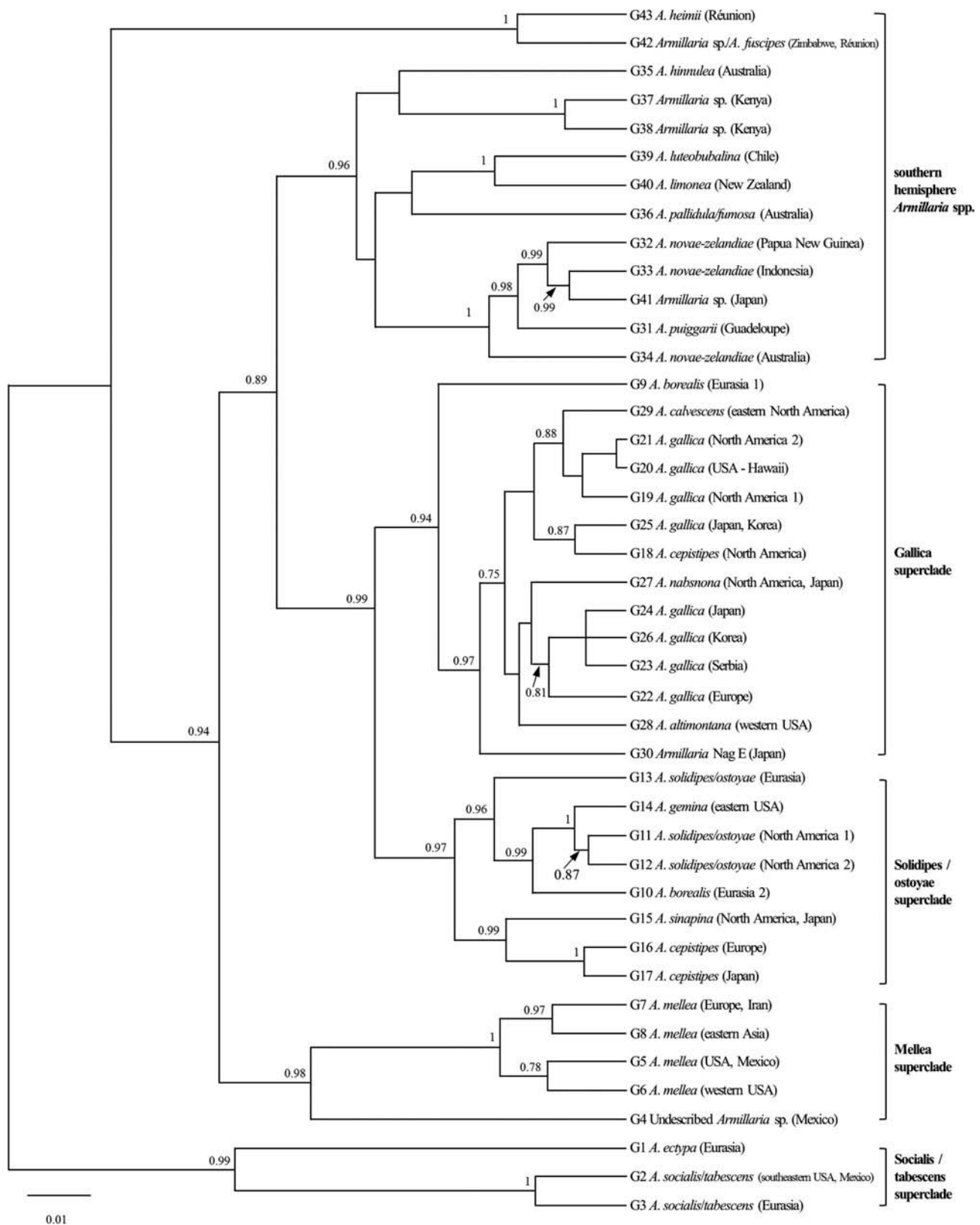


Figure 3. Consensus phylogeny of coalescence-based Bayesian analyses estimated in Evolutionary Analysis by Sampling Trees (BEAST) under the strict clock with a GTR model of substitution on partial translation elongation factor 1- α consensus (50% strict) sequences of 43 phylogenetic groups (G1–G43) representing 242 *Armillaria* spp. isolates, which included 88 newly obtained sequences of *Armillaria* isolates for this study and 154 sequences derived from GenBank. The phylogenetic groups are represented in TABLE I. Posterior support values >0.5 are indicated at the nodes.

Armillaria species by Tsykun et al. (2013) showed that *A. socialis/tabescens* from Japan and Europe reside in separate sister clades. The phylogenetic analyses presented herein suggest the potential existence of at least two distinct phylogenetic species (G2—southeastern USA and Mexico and G3—Eurasia) within what is currently considered as *A. socialis/tabescens*. Thus, a focused taxonomic study is needed to formally examine North American and Eurasian *A. socialis/tabescens* and to determine whether multiple phylogenetic species exist within the Socialis/Tabescens (exannulate) superclade. Continued taxonomic studies within this superclade must also consider a proposal to conserve the name *Agaricus tabescens* against *A. socialis* (Redhead et al. 2012), which could impact the recognized taxonomy of *A. socialis/tabescens*.

Mellea superclade.—The *tef1*-based phylogenetic analyses show the *Mellea* superclade as comprising the following clades: a basal, well-separated monophyletic lineage representing an undescribed annulate *Armillaria* sp. from central Mexico (G4), two clades of North American *A. mellea* (G5, G6), Eurasian *A. mellea* (G7), and eastern Asian *A. mellea* (G8). The undescribed annulate *Armillaria* sp. from Mexico (G4) appears distinct from, but phylogenetically adjacent to, the *A. mellea* complex clades (FIG. 3). Recently, Elías-Román et al. (2013) used sequences of *tef1*, partial 5.8S-ITS2-LSU D-domains, and partial 3' LSU-IGS1 to provide strong evidence of an undescribed annulate *Armillaria* sp. from several locations in the state of Mexico, Mexico. In the present BEAST analysis, several isolates of the undescribed *Armillaria* sp. (G4—Mexico) were contained within a monophyletic clade, which is basal within the *A. mellea* superclade and basal among the annulate *Armillaria* species from the Northern Hemisphere, an indication that this undescribed species from Mexico may have evolved earliest among known annulate *Armillaria* species of the Northern Hemisphere. Of further interest is the placement of the branch on which this undescribed species resides, which indicates that it is genetically distinct from, and further suggests that, this undescribed species from Mexico is evolutionarily ancestral to other known annulate *Armillaria* species in the Northern Hemisphere. Although this previously unrecognized species has not yet been formally described, the *tef1*-based phylogenetic separation between this undescribed *Armillaria* sp. (G4—Mexico) and all other examined *Armillaria* species supports the need to formally recognize this undescribed species from Mexico. Thus, continued studies that focus on a

formal taxonomic description of this undescribed species appear well warranted.

In these *tef1*-based phylogenies, the *A. mellea* isolates constituted four paraphyletic sister clades that represent distinct geographic lineages from (i) western USA (G6), (ii) USA and Mexico (G5), (iii) Europe and Iran (G7), and (iv) eastern Asia (G8). These *tef1*-based phylogenetic analyses support previous studies indicating that the *A. mellea* complex may comprise multiple cryptic species and support previous studies indicating multiple genetic clusters within the *A. mellea* complex. Based on RAMS, Qin and Hantula (2002) separated 18 isolates of *A. mellea* into three genetic clusters that grouped by geographic regions (Europe, North America, and China). Subsequent microsatellite-based studies revealed more recent genetic differentiation between *A. mellea* from the eastern and western USA (Baumgartner et al. 2010b). Because microsatellite data do not necessarily reflect genetic divergence at the species level, more studies (e.g., sequencing of house-keeping genes) are needed to determine whether these previously identified genetic clusters represent cryptic species. For example, in a study of natural hybridization among homothallic isolates of *A. mellea*, Baumgartner et al. (2012) used six different DNA regions (including *tef1*), which supported the existence of at least four clades within the *A. mellea* complex, and Tsykun et al. (2013) used multiple loci (including *tef1*) to show that *A. mellea* from Japan and Europe were phylogenetically distinct. The phylogenetic analyses presented herein further support the existence of at least four clades, G6 (western USA), G5 (USA and Mexico), G7 (Europe and Iran), and G8 (eastern Asia), within the *A. mellea* complex, which also support earlier studies (Coetzee et al. 2000, 2005a, 2005b, 2009, 2011; Maphosa et al. 2006). All of these findings indicate a need for taxonomic studies to better define potential species or taxa undergoing speciation (Coetzee et al. 2000) within the *A. mellea* complex.

Gallica superclade.—In the *tef1*-based phylogenies, Eurasian *A. borealis* clade 1 (G9) appears basal to both the Gallica and the Solidipes/Ostoyae superclades, but it could not be conclusively assigned to either superclade. The BEAST analyses assign *A. borealis* clade 1 (G9—Eurasia) to the Gallica superclade, whereas the Neighbor-net analyses group it with the Solidipes/Ostoyae superclade (FIGS. 2–3). The Gallica superclade displays several complex phylogenetic relationships based on the BEAST analysis (FIG. 3). Of the remaining clades within this superclade, *Armillaria* Nag E (G30—Japan) is

contained within a well-separated monophyletic lineage, which appears basal. Two major clades comprise other members of this superclade. Within the first major clade, *A. calvescens* (G29—eastern North America) appears basal in relationship to weakly supported sister clades containing North American *A. gallica* clade 1 (G19), North American *A. gallica* clade 2 (G21), and *A. gallica* from Hawaii, USA (G20). Other weakly supported sister clades comprise *A. gallica* (G25—Japan and Korea) and *A. cepistipes* (G18—North America). The second major clade comprises *A. altimontana* (G28—western USA) and appears basal to *A. nabsnona* (G27—North America and Japan) with low support. The remainder of the second major clade comprises *A. gallica* (G22—Europe) as basal to sister clades comprising *A. gallica* (G23—Serbia), *A. gallica* (G26—Korea), and *A. gallica* (G24—Japan).

General structure of the *Gallica* superclade.—In general, the complex, *tef1*-based phylogenetic structure of the *Gallica* superclade is difficult to interpret conclusively, especially given the limitations of these data. However, these results indicate that the *Gallica* superclade is a sister to the *Solidipes*/*Ostoyae* superclade. Eurasian *A. borealis* clade 1 (G9) appears as basal to both the *Gallica* and the *Solidipes*/*Ostoyae* superclades, indicating that this clade is potentially evolutionarily ancestral to both superclades on the basis of the *tef1* sequences (FIGS. 2 and 3). Of special interest is that Eurasian *A. borealis* clade 1 (G9) is widely separated from Eurasian *A. borealis* clade 2 (G10), which resides in the *Solidipes*/*Ostoyae* superclade. The phylogenetic structure of the *Gallica* superclade provides support for separation of *Armillaria* Nag E (G30—Japan) and *A. calvescens* (G29—eastern North America). However, phylogenetic relationships among other members of this superclade are difficult to definitively resolve because BEAST posterior support values are not robust. Thus, more isolates and more phylogenetic data are needed to better understand the complex phylogenetic relationships among most species within the *Gallica* superclade.

***A. gallica*.**—Within the *A. gallica* complex, species that were previously identified as *A. gallica* are polyphyletic and constitute up to eight separate clades (North America clade 1 [G19], North America clade 2 [G21], Hawaii, USA [G20], Serbia [G23], Europe [G22], Japan and Korea [G25], Korea [G26], Japan [G24]) according to the *tef1*-based phylogenetic analyses. Although microsatellite data do not necessarily reflect species divergence, previous

RAMS-based studies of Northern Hemisphere isolates delineated four clades of *A. gallica*, which were referred to as European, Chinese, North American–Chinese, and North American–European (Qin et al. 2001). As stated previously, other sequence data are needed to determine whether populations identified by microsatellites reflect species divergence. On the basis of *tef1*, previous phylogenetic studies indicated that European and North American *A. gallica* are phylogenetically distinct (Antonín et al. 2009), multiple phylogenetic groups are apparent within North American *A. gallica* (Elías-Román et al. 2013), and multiple phylogenetic groups are apparent within European *A. gallica* (Keča et al. 2015). The wide phylogenetic separation of multiple *A. gallica* clades indicates that further taxonomic studies are needed to better recognize what potentially comprises multiple cryptic species within the currently recognized *A. gallica*.

Other *Gallica* superclade species.—Within the *Gallica* superclade, the clear *tef1*-based separation of the Japanese *Armillaria* biological species Nag E (G30—Japan) further suggests that a formal taxonomic description of Nag E is warranted. Furthermore, *A. nabsnona* (G27—North American, Japan), *A. altimontana* (G28—western USA), and *A. calvescens* (G29—eastern North America) appear in distinct, monophyletic clades, which support their recognized taxonomic status; however, the *A. nabsnona* (G27—North American, Japan) and *A. altimontana* (G28—western USA) branches are not well supported in the BEAST analysis. Especially notable is that *A. cepistipes* from North America (G18) resides in the *Gallica* superclade, whereas *A. cepistipes* from Europe (G16) and *A. cepistipes* from Japan (G17) reside in the *Solidipes*/*Ostoyae* superclade. Previous phylogenetic studies, which were based on DNA regions other than *tef1*, have shown that Eurasian *A. cepistipes* and North American *A. cepistipes* tend to cluster with members of the *Gallica* superclade (Anderson and Stasovski 1992; Kim et al. 2006; Hasegawa et al. 2010; Tyskun et al. 2013). This finding suggests that a North American *A. cepistipes* (Banik and Burdsall 1998) should be subjected to additional taxonomic and phylogenetic studies to determine whether formal recognition as a distinct species is warranted, since *A. cepistipes* was first described in Europe (see Antonín et al. 2009). The apparent complexity of the *Gallica* superclade indicates that more isolates from more geographic regions and more data are needed to better understand the

phylogenetic and taxonomic relationships within this complex and polyphyletic superclade.

***Solidipes/Ostoyae* superclade.**—The *tef1*-based BEAST analysis shows that the *Solidipes/Ostoyae* superclade comprises eight distinct lineages contained within two major clades (FIG. 3). In the first major clade, *A. solidipes/ostoyae* (G13 – Eurasia) was basal and appears well separated from *A. borealis* clade 2 (G10—Eurasia), *A. gemina* (G14—eastern USA), and two sister clades of *A. solidipes/ostoyae* (G11—North America, G12—North America). Notably, the Eurasian *A. borealis* clade 2 (G10) is widely separated from Eurasian *A. borealis* clade 1 (G9), which appears basal to the Gallica and *Solidipes/Ostoyae* superclades (see above). The second major clade derived from BEAST analysis contains *A. sinapina* from North America and Japan (G15) that is distinct from two sister clades representing *A. cepistipes* from Europe (G16) and *A. cepistipes* from Japan (G17). Another notable result is that the *A. cepistipes* G16 (Europe) and G17 (Japan) are widely separated from the North American *A. cepistipes* (G18), which resides in the Gallica superclade (see below).

General structure of the *Solidipes/Ostoyae* superclade.—Evolutionary implications of *tef1*-based phylogenies derived from these limited data should be interpreted with caution; however, a few phylogenetic relationships within the *Solidipes/Ostoyae* superclade merit consideration. The BEAST analysis suggests that *A. solidipes/ostoyae* (G13—Eurasia) is basal within the major clade containing *A. borealis* clade 2 (G10—Eurasia), *A. gemina* (G14—eastern USA), and *A. solidipes/ostoyae* clades (G11—North America, G12—North America), suggesting a common evolutionary history for these species. Notably, the *A. borealis* clade 2 (G10—Eurasia) lineage is widely separated from *A. borealis* clade 1 (G9—Eurasia). In the other major clade, *A. sinapina* (G15—North America and Japan), *A. cepistipes* (G16—Europe), and *A. cepistipes* (G17—Japan) appear to share a common evolutionary ancestry.

***A. solidipes/ostoyae*.**—The results of these *tef1*-based analyses indicate that Eurasian *A. solidipes/ostoyae* clade (G13) is basal within the *Solidipes/Ostoyae* superclade and is phylogenetically distinct from two North American *A. solidipes/ostoyae* clades (G11, G12). Because these results strongly indicate that these species are not conspecific, potential taxonomic implications are raised. In 1970, Romagnesi described *A. ostoyae* (as *Armillariella ostoyae*) from Europe (Volk

and Burdsall 1995). On the basis of the biological species concept, NABS I was determined to be interfertile with European biological species (EBS) C (Anderson et al. 1980). Subsequently, Guillaumin et al. (1985) determined that EBS C was synonymous with *A. ostoyae* on the basis of morphology, and Bérubé and Dessureault (1988) concluded that NABS I was synonymous with *A. ostoyae* on the basis of morphology and the interfertility tests of Anderson et al. (1980). Recently, Burdsall and Volk (2008) used morphology of the holotype specimen collected in Colorado, USA, to conclude that *A. ostoyae* was synonymous with *A. solidipes*, described much earlier by Peck (1900), and thereby concluded that *A. solidipes* should be applied as the taxonomically appropriate name for species referred to as *A. ostoyae* in North America, Europe, and Asia. However, the resurrection of the *A. solidipes* taxon has raised considerable controversy (Hunt et al. 2011). The proposed conservation of the name “*ostoyae*” (Redhead et al. 2011) suggests that existing usage of the name be used pending formal recommendation from the general committee at the International Code of Botanical Nomenclature. The *tef1*-based phylogenetic analyses presented herein suggest that focused taxonomic studies are necessary to confirm whether Eurasian *A. solidipes/ostoyae* are indeed taxonomically and phylogenetically distinct from North American *A. solidipes/ostoyae*, and to determine whether North American *A. solidipes/ostoyae* comprises multiple phylogenetic species. Such studies are needed to determine if *A. ostoyae* is the appropriate taxon for the Eurasian species, and whether *A. solidipes* is appropriate for one or both of the North American clades.

***A. borealis* clade 2**—A striking feature of the *tef1*-based phylogenetic tree is that *A. borealis* (G9 and G10—Eurasia) isolates are contained within two quite distinct clades, which suggests that *A. borealis* might represent two distinct species. On the basis of BEAST analysis, isolate sequences of *A. borealis* clade 2 (G10—Eurasia) are contained in a separate monophyletic lineage within the *Solidipes/Ostoyae* superclade, which is phylogenetically distinct from *A. borealis* clade 1 (G9—Eurasia: basal to Gallica and *Solidipes/Ostoyae* superclades; see above). The *A. borealis* clade 2 (G10—Eurasia) belongs in the same major clade as Eurasian *A. solidipes/ostoyae* (G13—Eurasia), *A. gemina* (G14—eastern USA), and *A. solidipes/ostoyae* clades (G11 and G12—North America), which indicates a close evolutionary relationship among these species. Interestingly, no obvious geographic

associations were apparent for these two clades of *A. borealis*. A previous rDNA-based phylogenetic study noted that *A. borealis* isolates from Norway were comprised in two clades, one clade distinct from *A. solidipes/ostoyae* (reported as *A. ostoyae*) and one clade closely related to *A. solidipes/ostoyae* (Keča and Solheim 2010). Furthermore, *tef1*-based phylogenetic analyses by Antonín et al. (2009) and Mulholland et al. (2012) also demonstrated the existence of two separate *A. borealis* clades in Europe, one of these clades was closely related to *A. solidipes/ostoyae* (reported as *A. ostoyae*) isolates and one group was separate from *A. solidipes/ostoyae*. On the basis of IGS-1, ITS, and *tef1* sequences, Tsykun et al. (2013) found two phylogenetically distinct clades of *A. borealis*, for which *tef1* sequences were the primary determinant of clade separation. Previous observations by Korhonen (unpublished) suggest that *A. borealis* can vary in its observed pathogenicity, but it remains undetermined whether such differences in ecological behavior can be attributed to the different phylogenetic associations. Continued surveys are needed to determine the ecological and evolutionary bases for the two *A. borealis* clades reported herein, and focused taxonomic studies are needed to verify whether these two clades represent separate species or reflect adaptive variation. [Note added at the proof stage: Recently, *A. borealis* isolates from Switzerland were reported as having hybrid sequences for *tef1* (Heinzelmann et al. 2017)].

A. gemina.—The *tef1*-based phylogeny indicates that *A. gemina* (G14—eastern USA) is basal to closely related, but distinct, North American *A. solidipes/ostoyae* sister clades (G11, G12). This concurs with previous phylogenetic studies based on random DNA sequences (Piercey-Normore et al. 1998), IGS-1 (Kim et al. 2006), AFLPs (Kim et al. 2006), and *tef1* (Ross-Davis et al. 2012).

A. sinapina.—These *tef1*-based results support previous studies that show *A. sinapina* (G15—North America, Japan) is contained within a distinct phylogenetic clade (e.g., Piercey-Normore et al. 1998; Kim et al. 2006; Ross-Davis et al. 2012; Hasegawa 2010; Brazee et al. 2011) and that it shows no phylogenetic separation between *A. sinapina* from North America and Japan. It should be noted, however, that phylogenetic studies based on other DNA regions have typically shown that *A. sinapina* is associated with species of the Gallica superclade (e.g., Anderson and Stasovski 1992; Piercey-Normore et al. 1998; Kim et al. 2006; Hasegawa et al. 2010; Brazee et al. 2011), and mating

studies have also shown that *A. sinapina* is occasionally interfertile with species from the Gallica superclade (Banik and Burdsall 1998; Kim et al. 2001).

Eurasian A. cepistipes.—Another notable, unexpected feature of these phylogenetic analyses is that Eurasian *A. cepistipes* (G16—Europe, G17—Japan), which resides in the Solidipes/Ostoyae superclade, and North American *A. cepistipes* (G18), which resides in the Gallica superclade (see above), are widely separated on the basis of *tef1* sequence-based phylogeny. On the basis of BEAST analysis, *A. cepistipes* from Europe (G16) and *A. cepistipes* from Japan (G17) are contained in separate sister clades within a major clade that contained *A. sinapina* from North America and Japan (G15), whereas the North American *A. cepistipes* (G18) clade is contained within the Gallica superclade (see above). Phylogenetic analysis based on *tef1* by Tsykun et al. (2013) also showed that Eurasian *A. cepistipes* was grouped within a superclade containing *A. solidipes/ostoyae* (and one clade of *A. borealis*).

In contrast, previous rDNA-based phylogenetic studies indicate that Eurasian *A. cepistipes* is phylogenetically related to species of the Gallica superclade (e.g., Chillali et al. 1998; Keča et al. 2006; Hasegawa et al. 2010; Tsykun et al. 2013). Furthermore, ecological and morphological studies suggest a relationship between European *A. cepistipes* and *A. gallica* (Antonín et al. 2009; Tsykun et al. 2012), and mating studies that show some interfertility of Eurasian *A. cepistipes* with *A. altimontana* (as NABS X) from the Gallica superclade (Banik and Burdsall 1998; Kim et al. 2001). North American *A. cepistipes*, formerly known as NABS XI, was determined to be conspecific with European *A. cepistipes* on the basis of mating compatibility (Banik and Burdsall 1998). The wide phylogenetic separation of Eurasian *A. cepistipes* (G16—Europe, G17—Japan) and North American *A. cepistipes* (G18) provides evidence that these species are not conspecific; however, additional taxonomic and phylogenetic studies are needed to conclusively determine whether currently recognized *A. cepistipes* comprises multiple phylogenetic species and confirm the phylogenetic position of Eurasian and North American *A. cepistipes*.

General considerations.—Morphological and biological species concepts (Korhonen 1978; Anderson and Ullrich 1979) have served remarkably well to help define species and species relationships within the genus *Armillaria* before phylogenetic species concepts (e.g., Taylor et al. 2000, 2006) were widely applicable.

Within the genus *Armillaria*, phylogenetic studies have not indicated that any different biological species are conspecific. Trends suggested by this phylogenetic study indicate that biological species concepts appear largely applicable within a confined geographic region; however, this concept could generate misleading interpretations when comparing allopatric species on a wide geographic scale. Because fungal species can lose mechanisms that maintain reproductive isolation among species that do not co-occur in the same geographic region (e.g., Dettman et al. 2003), phylogenetic species concepts are useful when examining relationships among circumglobal species of *Armillaria*. Furthermore, phylogenetic evidence suggests that further subdivisions within *Armillaria* species are likely required to adequately reflect species diversity. Because this phylogenetic study is based on a single DNA region, it cannot provide irrefutable evidence for phylogenetic species on which to base formal taxonomic descriptions. With a few aforementioned exceptions, the topology of the *tefl*-based phylogeny reported here is generally similar to other phylogenies based on DNA sequences from other regions or on a more limited number of *Armillaria* isolates (e.g., Anderson and Stasovski 1992; Chillali et al. 1998; Pérez-Sierra et al. 2004; Keča et al. 2006; Kim et al. 2006; Wingfield et al. 2009; Hasegawa et al. 2010, 2011; Ota et al. 2011; Tsykun et al. 2013). As expected, the *tefl*-based phylogenies of Northern Hemisphere *Armillaria* species, presented herein, are generally consistent with previous results that focused on more limited geographic regions (Antonín et al. 2009; Baumgartner et al. 2010a, 2010b; Hasegawa et al. 2010; Brazee et al. 2011; Ota et al. 2011; Mulholland et al. 2012; Ross-Davis et al. 2012; Tsykun et al. 2012, 2013; Elias-Roman et al. 2013; Coetzee et al. 2015; Keča et al. 2015). However, increased insights into phylogeny of Northern Hemisphere *Armillaria* species are provided by these analyses of *tefl* from numerous isolates representing diverse global regions. The apparent higher resolution of this *tefl*-based analysis elucidates relationships among closely related species and within species that are separated by geography or other ecological features. Furthermore, these analyses can contribute to a general framework to guide future phylogenetic and taxonomic studies of global *Armillaria* species.

The results presented herein suggest that phylogenetic species concepts for *Armillaria* species should be further developed by continued sequencing and analyses of diverse, phylogenetically informative DNA regions based on more isolates and wider geographic representation. Although multigene phylogenies are quite common

in the literature, such approaches warrant caution because phylogenetic analysis of concatenated sequences can generate problematic or misleading results due to recombination, systemic biases, and hybridization (Wu et al. 2008). Furthermore, previous phylogenetic analyses of *Armillaria* species based on concatenated sequences that include *tefl* sequences suggest that *tefl* sequences are the primary determinants of phylogenetic structure in those analyses (Brazee et al. 2011; Tsykun et al. 2013). Nevertheless, additional DNA regions with phylogenetically informative sequences are needed to better understand the phylogenetic structure of *Armillaria* species. Rapidly advancing phylogenomic tools offer high-powered approaches to resolve phylogenetic issues (e.g., Binder et al. 2013; Hibbett et al. 2013), and the application of such approaches is warranted to better resolve phylogenetic relationships among *Armillaria* species.

The overall trends suggest that vicariance and distribution of circumboreal *Armillaria* species (e.g., *A. socialis/tabescens*, *A. mellea*, *A. gallica*, *A. solidipes/ostoyae*) are related to paleogeological processes that occurred since the tectonic separation of Laurasia and Gondwana during the Jurassic, ca. 145–200 million years bp (Scotese 2004). However, it is also reasonable that distribution of some *Armillaria* species shared between eastern Asia and North America (e.g., *A. nabsnona*, *A. sinapina*) could be related to more recent events. Because *A. nabsnona* and *A. sinapina* are found in North America and Japan, but not Europe, and because these species from separate continents are not phylogenetically distinct, it can be hypothesized that these species may have spread via the Bering Land Bridge, which existed intermittently from up to 100 000 000 years bp (Sanmartín et al. 2001) to ca. 11 000 years bp (Elias et al. 1996). Phylogenetic studies of higher resolution are needed to confirm potential differences between *A. nabsnona* and *A. sinapina* from Japan and North America and potentially estimate divergence times.

The *tefl*-based Neighbor-net and Bayesian analyses of *Armillaria* phylogeny provided high resolution that allows separation of recognized species, while providing additional evidence for potentially undescribed species and indicating that some currently recognized *Armillaria* species are polyphyletic and are perhaps a composite of multiple cryptic species. Furthermore, the analyses demonstrated a high potential for examining relationships among closely related taxa of *Armillaria* species. Neighbor-net analysis provides a mechanism to address conflicts in the data set that arise from common evolutionary processes that include hybridization, horizontal gene transfer, genetic recombination, genetic reassortment, homoplasy, and other mechanisms that can contribute to reticulate evolution (Makarenkov et al. 2006), although inferences from this study are

limited by the use of a single gene. Bayesian analysis provides a statistically robust evolutionary analysis of DNA sequence variation within a Bayesian framework (Drummond et al. 2012). The use of both Neighbor-net and Bayesian methods provided by SplitsTree and BEAST, respectively, allows comparisons between two distinct phylogenetic analyses that use unique algorithms to determine relationships. This provides increased insight and support for the interpretation of evolutionary relationships among the *Armillaria* taxa included here. Bayesian methods, when the appropriate evolutionary models are selected, have been shown to provide support values and tree topologies that are closer estimates of taxa relationship accuracy (Wilcox et al. 2002). Although these analyses are based solely on a relatively extensive set of partial *tefl* sequences from relatively well-characterized isolates representing diverse *Armillaria* species from across the Northern Hemisphere, several potentially important phylogenetic relationships are revealed that warrant further study. Notable phylogenetic relationships evident from these analyses include the following: (i) potential speciation between Eurasian and North American *A. socialis/tabescens*; (ii) an undescribed *Armillaria* sp. from Mexico that appears evolutionarily ancestral to annulate species from the Northern Hemisphere; (iii) potential existence of multiple cryptic species within the *A. mellea* complex; (iv) two widely separate clades of *A. borealis*; (v) potential speciation among Eurasian and North American *A. solidipes/ostoyae*; (vi) potential speciation among *A. cepistipes* from North America, Europe, and Japan; (vii) evidence that Japanese *Armillaria* Nag E is phylogenetically distinct; and (viii) potential existence of multiple cryptic species within the *A. gallica* complex. Continued studies with high-resolution genetic markers (e.g., phylogenomics) will help resolve these phylogenetic relationships.

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