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Phylogeography and host range of *Armillaria gallica* in riparian forests of the northern Great Plains, USA

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Abstract

Root disease pathogens, including *Armillaria*, are a leading cause of growth loss and tree mortality in forest ecosystems of North America. *Armillaria* spp. have a wide host range and can cause significant reductions in tree growth that may lead to mortality. DNA sequence comparisons and phylogenetic studies have allowed a better understanding of *Armillaria* spp. taxonomic diversity. Genetic sequencing has facilitated the mapping of species distributions and host associations, providing insights into *Armillaria* ecology. These studies can help to inform forest management and are essential in the development of disease risk maps, leading to more effective management strategies for Armillaria root disease. *Armillaria* surveys were conducted on publicly owned lands in North Dakota, South Dakota, and Nebraska, U.S.A. Surveyed stands consisted of riparian forests ≥0.4 hectares in area. *Armillaria* was found at 78 of 101 sites. A total of 57 *Armillaria* isolates—associated with 12 host tree species—were used for DNA sequencing of the translation elongation factor-1 alpha (*tef1*) gene. *Armillaria gallica* was the only species identified within the study sites. Results suggest that *A. gallica* is a common root pathogen of hardwood trees in riparian forests of the northern Great Plains with a wider host range and geographic distribution than previously recognized.

KEYWORDS

Armillaria, host range, North America, northern Great Plains, riparian forests, root disease, species distribution

1 | INTRODUCTION

The genus *Armillaria* consists of over 40 morphological/biological species that collectively infect hundreds of tree species throughout temperate, boreal and tropical regions of the world (Baumgartner et al., 2011). *Armillaria* is primarily known as the causal agent of Armillaria root disease in forests, but it also plays a role in carbon cycling through degradation of woody tissue. Since Fries' original description of the genus *Armillaria* in 1819, the taxonomic identification of the species has been a source of controversy and confusion (Maphosa et al., 2006; Volk & Birdsall, 1995). Species within the genus differ in geographic distribution, host preference, pathogenicity and/or virulence (Wargo & Shaw, 1985), making proper identification vital for disease risk assessment and management (Ross-Davis et al., 2012). However, the basidiocarps conventionally used to differentiate among species are produced unreliably, remain viable for only a short time and in some cases are morphologically similar. This makes species differentiation difficult through morphology alone (Coetzee et al., 2003). These considerations, along with difficulties surrounding identification via sexual compatibility tests, have stimulated research into DNA-based techniques for identifying *Armillaria* species (Coetzee et al., 2003).

Advances in DNA sequencing technologies, along with reduced sequencing costs and improved bioinformatic tools, have enabled taxonomic studies that differentiate among species using DNA sequence comparisons in addition to morphological characteristics (Koch et al., 2017; Maphosa et al., 2006). Phylogenetic analyses of *Armillaria* species may provide insights into their evolutionary histories, potentially aiding in the determination of putative geographic and evolutionary origins of pathogen populations (Coetzee et al., 2001; Koch et al., 2017). Phylogenetic analyses additionally facilitate mapping of spatial distributions and host associations for insights into *Armillaria* ecology. While a substantial body of work has focused on *Armillaria* phylogenetics (Klopfenstein et al., 2017), the full range of genetic diversity within the genus has yet to be thoroughly described and the delimitation of potential cryptic species is an ongoing process. Additional surveys and assessments of *Armillaria* are needed, especially in areas where information is scarce, to further our understanding of genotypic and phenotypic variation within the genus. Such surveys and assessments will also contribute to understanding of *Armillaria* taxonomy at the global scale (Shaw & Kile, 1991). Findings from these studies can further be used to update forest management strategies and improve disease risk maps, enabling more effective management of Armillaria root disease.

Currently, 11 species from the genus *Armillaria*, and a recently elevated genus *Desarmillaria* (Koch et al., 2017) are known to occur within North America (Elías-Román et al., 2018; Kim et al., 2006; Klopfenstein et al., 2017). While *Armillaria* spp. have been well studied in many forest-dominated areas, such as the north-eastern (Blodgett & Worrall, 1992a, 1992b; Brazee & Wick, 2011) and north-western (Banik et al., 1996; Ferguson et al., 2003) USA, little information exists on *Armillaria* in the northern Great Plains, USA, where trees are generally less common on the landscape. Three species of *Armillaria* are known to occur within this region, *A. solidipes* Peck [*A. ostoyae* (Romagnesi) Herink], *A. gallica* Marxmüller & Romagnesi, and *A. sinapina* Bérubé & Dessureault. All three species have been reported in the Black Hills National Forest of western South Dakota and north-eastern Wyoming (Blodgett, 2015), where *A. solidipes* occurs primarily as a pathogen of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) (Kallas et al., 2003; Klutsch et al., 2012) and *A. gallica* and *A. sinapina* are pathogens of aspen (*Populus tremuloides* Michx.) (Blodgett, 2015). In the Niobrara Valley Preserve of north-central Nebraska, *A. gallica* has been reported as a root epiphyte of seven tree species and genera (Kim & Klopfenstein, 2011). However, the Black Hills and Niobrara Valley Reserve are unique ecosystems within the northern Great Plains, with environmental conditions and biological communities that are not representative of the entire region. Current information regarding species identities, host associations and geographic distributions of these important pathogens is limited or lacking in the northern Great Plains.

Riparian forests can be found scattered throughout much of the northern Great Plains and are the prevalent forest type in the eastern regions. These forests are characterized by diverse edaphic conditions and cover types, which makes them well suited for studying diversity of *Armillaria* and associated woody hosts. Riparian forests provide vital ecosystem services (Naiman et al., 2010; Yang et al., 2019), including mitigation of soil and air pollution, buffering against flooding and erosion, and enhancement of water quality through uptake of nutrients from agricultural runoff (Kozlowski, 2002). These forests also offer aesthetic value and opportunities for recreation (González et al., 2018), as well as habitat for woodland birds and mammals, such as the wood duck (*Aix sponsa* Linnaeus) and fisher (*Pekania pennant* Erxleben) (Davis, 2005; Triska et al., 2011). However, installation of major river impoundments in the region has interrupted natural streamflow processes affecting growth and recruitment of riparian trees, with some important species exhibiting signs of potential moisture stress in the post-dam era (Johnson et al., 1976). In recent decades, flood magnitudes have increased in the north-central USA (Peterson et al., 2013), and numerous catastrophic floods have occurred along major rivers of the region. In 2011, a 500-year-flood event on the Missouri River inundated land adjacent to the river for as long as 3 months and killed many young, native trees in the floodplain (Boever et al., 2019; Dixon et al., 2015). Novel information on facultative parasites, such as *Armillaria*, that may preferentially target stressed trees following major disturbances is important to consider when formulating management recommendations.

This study was conducted to evaluate the diversity, distribution and host associations of *Armillaria* in riparian forests of the northern Great Plains. The objectives of this study were to (a) evaluate the diversity of *Armillaria* species present in riparian forests of the northern Great Plains; and (b) determine the range of host species that are associated with *Armillaria* in these forests. A broad goal of this study was to provide data on *Armillaria* species distributions and host ranges for North American root disease risk maps (Lockman et al., 2016).

2 | MATERIALS AND METHODS

2.1 | Field Surveys

Surveys for *Armillaria* were conducted in publicly owned riparian forest stands in North Dakota, South Dakota, and Nebraska, USA. Surveyed stands covered ≥0.4 ha each, and were located on public lands, such as parks, preserves and wildlife management areas. Surveyed sites ranged from 240 to 980 m in elevation and were dominated by hardwood tree species, consistent with the regional flora. Cottonwood (*Populus deltoides*) was the most common cover type surveyed ($n = 38$), while green ash (*Fraxinus pennsylvanica* Marsh.) and box elder (*Acer negundo* L.), which frequently occurred in mixture, were the next most common types (collectively, $n = 23$). A total of 101 plots were surveyed within 2 years (Figure 1). Survey and collection methods were modified from Blodgett and Worrall (1992a, 1992b). Briefly, a forest stand exhibiting mortality (e.g. stumps/snags) and/or stress symptoms (e.g., crown dieback) was identified and searched for signs of *Armillaria* for up to 1 worker-hr. Snags, stumps and severely stressed trees (diameter at breast height or

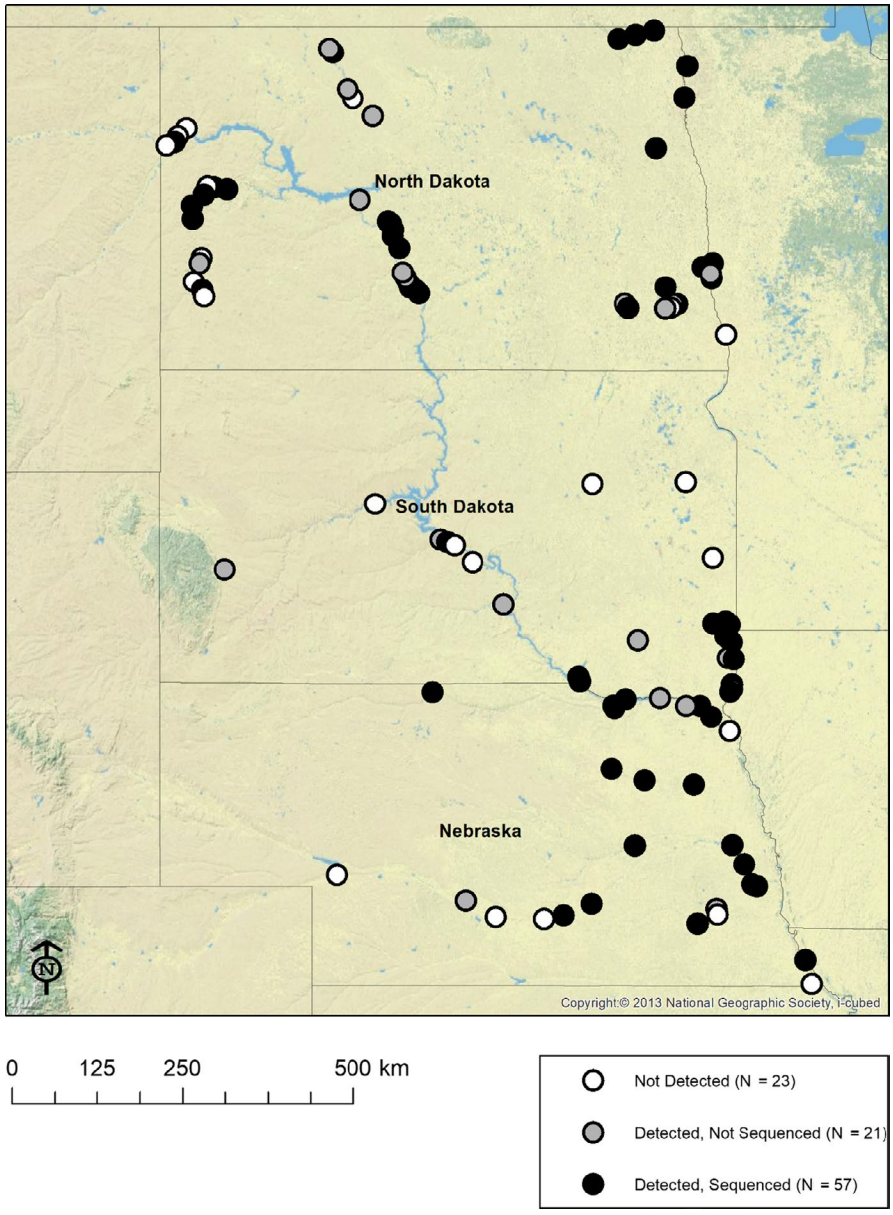

FIGURE 1 Geographic distribution of *Armillaria* in the northern Great Plains (USA) based on 2015 and 2016 surveys

DBH ≥ 8 cm) were targeted in initial surveys, which were conducted by excavating (sometimes down to ~30cm) around root collars and along major lateral roots. Excavations were accomplished with hand tools to expose rhizomorphs and mycelial fans. When *Armillaria* was first observed in a snag or stump, up to five stressed trees within the vicinity of observed signs were surveyed for evidence of Armillaria root disease and butt rot. Rhizomorphs and/or tissue samples containing mycelial fans were collected from the first live tree with root disease (killing of the cambium in a live tree) or butt rot (degrading inner wood in a live tree). At sites where *Armillaria* was found, but infections in a live tree were not observed, isolates were collected from a stump or snag, or a live tree hosting the fungus but lacking disease symptoms attributable to *Armillaria*. Isolates collected from the latter were characterized as epiphytes for simplicity. Species, DBH and condition (root disease, butt rot, stump/snag, epiphytic association) were recorded for the host collected from at each site.

2.2 | *Armillaria* isolations

Armillaria was isolated from rhizomorphs or mycelial fans collected from the host tree in each plot. Sterilized forceps were used to remove mycelial fans from host tissue. One-cm rhizomorph sections were surface-sanitized for 5 min in 1.05% NaOCl followed by 1 min in 20% ethanol. Mycelial fans and sanitized rhizomorph sections were cultured on benomyl- and streptomycin-amended malt extract agar (1.5% malt extract, 1.7% Bacto agar, 0.005% Benlate (50% benomyl; DuPont chemical company; Wilmington, DE) and 0.002% streptomycin sulphate (Sigma Chemical Co.; St. Louis, MO)) for 2–6 weeks at ca. 21°C. Pure *Armillaria* mycelium was excised and then subcultured on malt extract agar (1.5% malt extract agar, 1.5% dextrose, 0.5% peptone and 1.2% agar) overlaid with track-etch membranes (Whatman, Nucleopore, NJ, USA). Subcultures were incubated in the dark for 2 to 4 weeks.

2.3 | DNA extraction

After incubation, mycelium was scraped from the track-etch membranes, placed into microcentrifuge tubes and frozen at −80°C. The DNA extractions were performed at the Center for Genome Research and Biocomputing (CGRB) at Oregon State University (OSU), where the tissue samples were first lysed with the Qiagen TissueLyser (Qiagen Inc., Hilden, Germany) at 30 hz for 1 min. The lysate was then subjected to a magnetic particle extraction performed with the Omega Bio-tek Mag-Bind® Plant DNA DS kit (Omega Bio-tek Inc., Norcross, GA, USA). Parts of this procedure were automated with the KingFisher Flex extraction system (Thermo Fisher Scientific, Waltham, MA, USA).

2.4 | PCR amplification

A portion of the translation elongation factor-1 alpha (*tef1*) gene was amplified with primers 983F and 2218R (Rehner & Buckley, 2005), producing an amplicon approximately 1,200 bp in length. Each PCR contained 200 μM dNTPs, 400 ng bovine serum albumin, 2.5 U RedTaq DNA polymerase (Millipore-Sigma, Burlington, MA, USA), 10x RedTaq buffer containing $MgCl_2$, 0.4 μM of each primer and 10–50 ng genomic DNA in a total volume of 50 μL with sterile nuclease-free water. The PCR amplification reactions were performed on an ABI 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) with the following conditions: initial denaturation at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min and extension at 72°C for 1.5 min. A final extension was conducted at 72°C for 7 min. The PCR amplicons were electrophoresed on a 2% agarose gel and stained with Biotium GelRed® (Biotium Inc., Fremont, CA, USA) to check for specific amplification and amplicon size. Before sequencing, the PCR amplicons were enzymatically purified with ExoSAP-IT (USB Corporation, Cleveland, OH, USA). Sanger sequencing was conducted with four internal primers (ARMEF-F3A, R2, FI2 and RI2) (Elías-Román et al., 2018) on an ABI Prism ® 3730 (SeqGen, Inc., Torrance, CA, USA) capillary sequencing machine at the CGRB, OSU. The resulting sequences were visually examined and edited with Geneious Prime 2019.0.4 (https://www.geneious.com). Positions with more than one nucleic acid read resulting from heterozygosity (Hanna et al., 2007) were manually edited according to International Union of Pure and Applied Chemistry (IUPAC) ambiguity codes. For each isolate, the four sequences were aligned in Geneious Prime 2019.0.4 (https://www.geneious.com) with MAFFT v7.388 (Katoh et al., 2002; Katoh & Standley, 2013), and a consensus sequence was used to reconstruct the original 1,200 bp *tef1* amplicon. All consensus sequences produced for this study were deposited in National Center for Biotechnology Information (NCBI) GenBank with accession numbers MN477466-MN477522 (Table 1).

2.5 | Species identification

The *tef1* consensus sequence from each isolate was compared with sequences from GenBank with the Basic Local Alignment Search Tool (BLAST), implemented in Geneious Prime 2019.0.4. For each isolate, the species identity was determined based on the top BLAST hit for which the sequence coverage was highest and the pairwise identity was at least 97.9%.

2.6 | Phylogenetic analyses

Nucleotide sequences of the *tef1* gene region from the isolates were aligned with homologous sequences from 30 additional *Armillaria* isolates representing 10 distinct North American *Armillaria*/*Desarmillaria* species (Ross-Davis et al., 2012). A phylogenetic tree was constructed in Geneious Prime 2019.0.4 with RAxML 8.2.11 (Stamatakis, 2014). The GTR GAMMA nucleotide substitution model was used along with the 'rapid bootstrapping with search for best maximum-likelihood tree' algorithm. The final consensus tree was constructed from 1,000 bootstrap replicates. The tree was exported from Geneious and imported to the R statistical computing program (R Core Team, 2019). The R package GGTREE (Yu et al., 2017) was used to visualize and annotate the phylogenetic tree.

3 | RESULTS

Armillaria was found at 78 of the 101 sites surveyed (Figure 1). A total of 57 *Armillaria* isolates from 12 different tree species were used for *tef1* sequencing (Figure 2). Based on GenBank BLAST searches, all 57 isolates matched *A. gallica*. Sequence identity ranged from a minimum of 97.9% to a maximum of 99.9%. Of the sequenced isolates, 15 were from Nebraska, 14 from South Dakota, and 28 from North Dakota (Table 1); results are presented only for the subset of sequenced isolates. Among *A. gallica* isolates, 39 were associated with root disease and 10 were associated with butt rot. Root disease associated with *A. gallica* was observed in living trees ranging from 9.9 to 109.9 cm DBH. Live trees ranging from 9.9 to 63.5 cm DBH had *A. gallica*-associated butt rot. Seven of the ten host trees with butt rot also had root disease. Three *A. gallica* isolates were observed only as epiphytic rhizomorphs in the field, and 8 were collected from dead hosts. Four *A. gallica* isolates were found in association with phloem damage that had not reached the vascular cambium.

Armillaria gallica was isolated from live specimens of 10 hardwood tree species, including plains cottonwood [*Populus deltoides* Marsh. subsp. *monilifera* (Ait.) Eckenw.], box elder (*A. negundo*), green ash (*F. pennsylvanica*), silver maple (*Acer saccharinum* L.), bur oak (*Quercus macrocarpa* Michx.), American elm (*Ulmus americana* L.), red mulberry (*Morus rubra* L.), white mulberry (*Morus alba* L.), willow (*Salix* L.) and northern catalpa [*Catalpa speciosa* (Warder) Warder ex Engelm.]. All living hosts were susceptible to root disease caused by *A. gallica*, with the exception of red mulberry (Table 2(a)). However, phloem damage that had not penetrated to the cambium was observed in a red mulberry host. Butt rot associated with *A. gallica* was

TABLE 1 *Armillaria* isolates sequenced for this study including host tree species, state (USA) where isolates were collected, and NCBI GenBank accession number for each translation elongation factor-1 alpha sequence

Isolate ID	Host	State	GenBank accession no.
ARM02	*Populus deltoides*	South Dakota	MN477466
ARM03	*Fraxinus pennsylvanica*	North Dakota	MN477504
ARM04	*Quercus macrocarpa*	North Dakota	MN477467
ARM05	*Acer negundo*	North Dakota	MN477498
ARM07	*Populus deltoides*	North Dakota	MN477483
ARM08	*Populus deltoides*	North Dakota	MN477495
ARM09	*Tilia americana*	North Dakota	MN477487
ARM12	*Fraxinus pennsylvanica*	North Dakota	MN477494
ARM14	*Acer negundo*	North Dakota	MN477506
ARM15	*Fraxinus pennsylvanica*	North Dakota	MN477500
ARM16	*Fraxinus pennsylvanica*	North Dakota	MN477505
ARM17	*Acer negundo*	North Dakota	MN477509
ARM19	*Populus deltoides*	North Dakota	MN477499
ARM22	*Fraxinus pennsylvanica*	North Dakota	MN477468
ARM23	*Quercus macrocarpa*	North Dakota	MN477508
ARM24	*Acer saccharinum*	South Dakota	MN477490
ARM25	*Fraxinus pennsylvanica*	South Dakota	MN477488
ARM26	*Ulmus rubra*	South Dakota	MN477501
ARM27	*Acer negundo*	South Dakota	MN477516
ARM29	*Fraxinus pennsylvanica*	South Dakota	MN477491
ARM31	*Acer saccharinum*	South Dakota	MN477520
ARM33	*Salix* sp.	South Dakota	MN477478
ARM34	*Fraxinus pennsylvanica*	North Dakota	MN477513
ARM35	*Fraxinus pennsylvanica*	North Dakota	MN477492
ARM36	*Populus deltoides*	North Dakota	MN477489
ARM39	*Fraxinus pennsylvanica*	North Dakota	MN477496
ARM41	*Ulmus americana*	North Dakota	MN477469
ARM42	*Acer negundo*	North Dakota	MN477497
ARM43	*Ulmus americana*	Nebraska	MN477479
ARM44	*Acer saccharinum*	Nebraska	MN477507
ARM45	*Morus alba*	Nebraska	MN477514
ARM49	*Fraxinus* (unk species)	Nebraska	MN477470
ARM50	*Acer saccharinum*	Nebraska	MN477472
ARM51	*Catalpa speciosa*	Nebraska	MN477521
ARM52	*Populus deltoides*	Nebraska	MN477512
ARM57	*Morus rubra*	Nebraska	MN477502
ARM58	*Fraxinus pennsylvanica*	Nebraska	MN477473
ARM59	*Populus deltoides*	Nebraska	MN477522
ARM60	*Acer negundo*	Nebraska	MN477476
ARM61	*Salix* sp.	Nebraska	MN477475
ARM62	*Fraxinus pennsylvanica*	Nebraska	MN477517
ARM63	*Salix* sp.	Nebraska	MN477518
ARM64	*Quercus macrocarpa*	Nebraska	MN477480

(Continues)

TABLE 1 (Continued)

Isolate ID	Host	State	GenBank accession no.
ARM65	*Morus alba*	South Dakota	MN477485
ARM66	*Morus* sp.	South Dakota	MN477503
ARM68	*Morus alba*	South Dakota	MN477484
ARM69	*Acer negundo*	South Dakota	MN477511
ARM70	*Ulmus americana*	South Dakota	MN477471
ARM74	*Acer negundo*	South Dakota	MN477474
ARM84	*Acer negundo*	North Dakota	MN477493
ARM89	*Populus deltoides*	North Dakota	MN477482
ARM91	*Populus deltoides*	North Dakota	MN477486
ARM96	*Acer negundo*	North Dakota	MN477477
ARM98	*Populus deltoides*	North Dakota	MN477481
ARM99	*Fraxinus pennsylvanica*	North Dakota	MN477515
ARM100	*Populus deltoides*	North Dakota	MN477510
ARM101	*Populus deltoides*	North Dakota	MN477519

observed in white mulberry, bur oak, box elder, green ash and willow (Table 2(b)). Epiphytic rhizomorph associations of *A. gallica*, without disease, were observed only in live green ash (1 obs.) and box elder (2 obs.). *Armillaria gallica* was isolated from snags/stumps of six tree species. These included plains cottonwood, box elder, green ash, bur oak, American linden (*Tilia americana* L.) and slippery elm (*Ulmus rubra* Muhl.).

4 | DISCUSSION

While three *Armillaria* species are known to occur in the northern Great Plains, *A. gallica* was the only species we identified in riparian forests. *Armillaria gallica* is widely distributed in the Northern Hemisphere (Baumgartner et al., 2011) and occurs in the western (Banik et al., 1996; Baumgartner & Rizzo, 2001), central (Blodgett, 2015; Kim & Klopfenstein, 2011) and eastern (Blodgett & Worrall, 1992a, 1992b; Brazee & Wick, 2009, 2011) USA. This fungus incites root and butt rot in both hardwood and coniferous hosts (Blodgett & Worrall, 1992a; Brazee & Wick, 2009). *Armillaria gallica* is considered a weak pathogen, primarily of hardwoods, in the western USA, but can occur as an epiphyte (Baumgartner & Rizzo, 2001). The species is an aggressive pathogen in certain areas of the eastern USA, where it frequently causes butt rot in several hardwood species (Brazee & Wick, 2009). It also occurs as a weak pathogen of hardwoods in the Ozark Mountains of the central USA, where it appeared to reduce activity of more virulent *Armillaria* spp. (Bruhn et al., 2000). In the central USA, *A. gallica* has previously been described as an epiphyte of hardwood and conifer species (Kim & Klopfenstein, 2011) and an important root pathogen of aspen (Blodgett, 2015). The present study revealed that *A. gallica* commonly occurs as a root pathogen of hardwood trees throughout riparian forests of North Dakota, south-eastern South Dakota, and central to eastern Nebraska.

Root diseases are among the leading causes of growth loss and mortality in forest trees of the USA (Lockman et al., 2016). While *Armillaria* is among the world's most important groups of fungal root disease pathogens (Baumgartner et al., 2011), information on the ecology, incidence and geographic distributions of *Armillaria* species remains sparse in many locales (Heinzelmann et al., 2019). Prior to the present study, the diversity, distribution and host ranges of *Armillaria* in the northern Great Plains were based on few detailed observations, limiting understanding of *Armillaria* ecology at the regional scale. Additional surveys and continued applications of DNA-based identification are essential steps in determining the host ranges and geographic distributions of *Armillaria* species in regions where they have not been thoroughly surveyed.

To our knowledge, the distribution and species identification of *Armillaria* in North Dakota (Bergdahl, 2013), central through eastern South Dakota, and south-central through eastern Nebraska had never been reported prior to this study. Results of the present study show that *A. gallica* occurs in riparian forests throughout North Dakota, and extend the known distributions of the species in South Dakota and Nebraska to include the central to south-eastern and south-central to eastern portions of those states, respectively. However, further studies and surveys are needed to help predict and monitor the spread of this pathogen, especially within the context of climate change (Kim & Klopfenstein, 2011). Future studies focused on investigating the ecology and diversity of *Armillaria* species should consider upland forest types that occur throughout parts of the northern Great Plains, and explore geographic areas that were not included in this study. Information on *Armillaria* species composition and host associations can help forest managers better predict how these fungi will interact with different forests under a variety of climate and disturbance scenarios. Continued monitoring and management may be essential for maintaining the health of the riparian forests in the northern Great Plains.

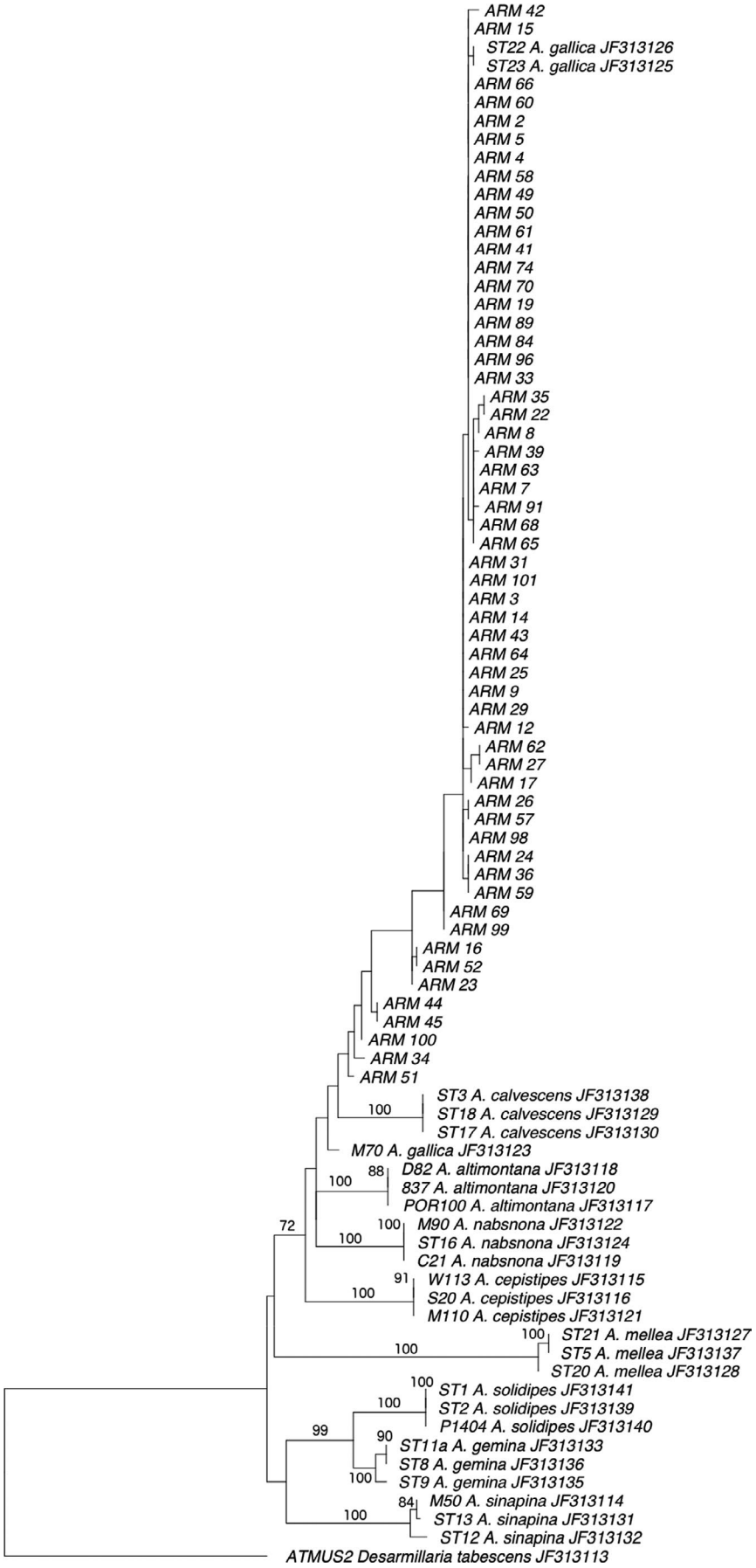

FIGURE 2 Maximum-likelihood phylogenetic tree of North American *Armillaria* species based on partial translation elongation factor-1 alpha (*tef1*) gene sequences. Branch labels represent bootstrap support calculated from 1,000 replicate trees (only values > 70% are shown). The *Armillaria* isolates sequenced for this study are described in Table 1. GenBank accession numbers are shown for *Armillaria* sequences that were sequenced for previous studies

TABLE 2 Host species found susceptible to Armillaria root disease (A) and butt rot (B) associated with *Armillaria gallica* in the northern Great Plains, USA

Host	Number of isolates	DBH (range)	States observed
(a) Armillaria root disease			
Acer negundo	5	22.5–50.9 cm	ND, SD
Acer saccharinum	4	34.1–106.9 cm	NE, SD
Catalpa speciosa	1	40.9 cm	NE
Fraxinus pennsylvanica	10	13.7–81.5 cm	NE, ND, SD
Morus alba	2	13.3–29.2 cm	NE, SD
Morus sp.	1	9.9 cm	SD
Populus deltoides	9	17.5–109.9 cm	NE, ND
Quercus macrocarpa	2	17.3–25.8 cm	NE, ND
Salix spp.	2	34.9–54.6 cm	NE, SD
Ulmus americana	3	14.2–30.8 cm	NE, ND, SD
(b) Armillaria Butt Rot			
Acer negundo	4	25.7–63.5 cm	NE, ND, SD
Fraxinus pennsylvanica	2	46.2–53.3 cm	ND
Morus alba	1	13.3 cm	NE
Morus sp.	1	9.9 cm	SD
Quercus macrocarpa	1	17.3 cm	NE
Salix sp.	1	63.0 cm	NE

Results from this study provide important baseline information on the phylogeography, host range, and ecology of *Armillaria* within riparian forest ecosystems of the northern Great Plains. Our findings expand on existing, but limited, knowledge of *Armillaria* in the region, indicating that *A. gallica* has a wider host and geographic distribution than previously recognized. Data from the present study offer a more complete picture of Armillaria root disease distribution in the northern Great Plains of the USA and will facilitate the development of risk maps for Armillaria root disease to support forest management.

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