**Phyllachora** species infecting maize and other grass species in the Americas represents a complex of closely related species

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**Abstract**
The genus *Phyllachora* contains numerous obligate fungal parasites that produce raised, melanized structures called stromata on their plant hosts referred to as tar spot. Members of this genus are known to infect many grass species but generally do not cause significant damage or defoliation, with the exception of *P. maydis* which has emerged as an important pathogen of maize throughout the Americas, but the origin of this pathogen remains unknown. To date, species designations for *Phyllachora* have been based on host associations and morphology, and most species are assumed to be host specific. We assessed the sequence diversity of 186 single stroma isolates collected from 16 hosts representing 15 countries. Samples included both herbarium and contemporary strains that covered a temporal range from 1905 to 2019. These 186 isolates...
were grouped into five distinct species with strong bootstrap support. We found three closely related, but genetically distinct groups of Phyllachora are capable of infecting maize in the United States, we refer to these as the P. maydis species complex. Based on herbarium specimens, we hypothesize that these three groups in the P. maydis species complex originated from Central America, Mexico, and the Caribbean. Although two of these groups were only found on maize, the third and largest group contained contemporary strains found on maize and other grass hosts, as well as herbarium specimens from maize and other grasses that include 10 species of Phyllachora. The herbarium specimens were previously identified based on morphology and host association. This work represents the first attempt at molecular characterization of Phyllachora species infecting grass hosts and indicates some Phyllachora species can infect a broad range of host species and there may be significant synonymy in the Phyllachora genus.

**KEYWORDS**
biotrophs, pathogen diversity, phyllachorales, phylogeny, sympatric speciation, tar spot

**TAXONOMY CLASSIFICATION**
Disease ecology; Microbial ecology

1 | INTRODUCTION

Phyllachorales is a monophyletic order of biotrophic fungi comprised of approximately 1,226 recognized species (Maharachchikumbura et al., 2016; Mardones et al., 2017), but global estimates of species within this order approach 160,000 (Cannon, 1997). The Phyllachorales largely contain plant parasitic fungi and are commonly associated with monocotyledonous plants across a range of habitats. These fungi are often referred to as "tar spot" fungi due to the production of stromata on plant hosts that resemble black flecks of tar (Figure 1) (Mardones et al., 2017).

Tar spot of maize (Figure 1a–c), caused by the fungus Phyllachora maydis, emerged in the United States in 2015, with the disease expanding each year since the initial report and continuing to have a significant economic impact on maize across many production regions in the United States (Kleczewski et al., 2020; Valle-Torres et al., 2020). Since first identified in North America in 2015, P. maydis has spread rapidly throughout the United States and Canada (Kleczewski & Bowman, 2020; Kleczewski et al., 2020), and resulted in yield losses exceeding $US 658 million in 2018 (Mueller et al., 2020). Although tar spot symptoms caused by members of the genus Phyllachora have been commonly observed on a number of grasses (Figure 1d–f) and shrub species throughout North, Central, and South America, historically the fungus has rarely been known to cause significant plant damage. However, tar spot has been occasionally reported to cause severe damage to maize in Mexico, Central America, and several Caribbean Islands (Valle-Torres et al., 2020).

The origin of P. maydis within the United States is not currently known, although the presence of two distinct epicenters of maize tar spot in the Midwest and Southeast indicates at least two separate emergence events. While tar spot is a new disease on maize in the United States and Canada, it has been present in Mexico, several Caribbean islands including Puerto Rico, Cuba, and the Dominican Republic as well as Central American Countries, such as Guatemala, Honduras, Nicaragua, and Costa Rica for the last century but only caused limited damage. In addition, tar spot signs and symptoms caused by Phyllachora species are common on several native and weedy grass species in North America (Figure 1d–f) (Orton, 1944). The monographic work by Orton (1944) was completed solely by morphological identification and host affinity. Given our understanding of phenotypic plasticity of many fungi and the ability of biotrophic pathogens to infect multiple hosts (Morris & Moury, 2019), it is possible that cryptic species or species complexes may be present.

Species definitions within the Phyllachorales have historically been based largely on morphological characteristics and assumption of high host specificity due to their presumed biotrophic nature. However, there are examples in the genus where this assumption of host specificity does not hold true (Cannon, 1991, 1997). Furthermore, species designations based on host specificity are highly dependent on accurate identification of the host species, which may be difficult or impossible in some instances. For example, P. graminis (Pers.) Fuckel is considered a "dustbin" species where many specimens of isolates infecting grasses are deposited with the host not often identified to species (Parbery, 1967). Furthermore, factors such as nutrients available to the fungus, temperature, light quality, light cycles, substrate type, host, and epigenetic factors may also result in alterations in fungal morphology that may result in inaccurate species designations (Francisco et al., 2019; Money, 2013; Slepecky & Starmer, 2009; Stockinger et al., 2009). Thus, our current understanding of the genetic diversity, host range, and species delimitation within the genus Phyllachora is relatively limited and requires reevaluation.
The recent emergence of *P. maydis* in the United States and Canada may also be associated with the ability of the fungus to better persist and spread than previously thought. Once established, the fungus can survive at least one winter at subzero temperatures on corn residue as ascospores within stromata, which are believed to be the main inoculum source the following season (Groves et al., 2020; Kleczewski et al., 2019). Under periods of moderate temperatures and wet weather, it is believed that ascospores are dispersed by wind and rain splash where they land on the foliage, stalks, and husks of corn. After spore germination and infection of the host, the fungus remains dormant for at least 2 weeks after which stromata, and associated spermatia and ascospores, are produced. Data from Central America indicated a relatively steep dispersal curve of *P. maydis* ascospores from a source (Hock et al., 1995). However, the rapid spread of this fungus throughout the Midwest, coupled with observations of “top down” infestations in fields with no history of disease and observations of infestations of isolated plots located 1,200 m from potential inoculum sources, indicate that the pathogen can travel much further across local/regional topographies than estimated previously (Kleczewski et al., 2020).

Based on this information, the emergence of *P. maydis* on corn in the United States and Canada could have been the result of many factors including the introduction of the fungus on infected plant material, natural northern dispersal through wind, establishment in the United States favored by climate change, changes in hybrid genetics, a host jump from a grass species, or a combination of any of these four. This study represents the first attempt to extract and sequence DNA from *Phyllachora* stroma on fresh and herbarium-infected grass specimens. The goal of this study is to understand the genetic diversity of *Phyllachora* species causing tar spot disease in contemporary corn production regions in the United States, and compare this to historical specimens of *Phyllachora* species from herbarium samples of maize and other grasses from Mexico, Central, and South America, the Caribbean, and Europe, as well as contemporary and herbarium species of *Phyllachora* species associated with grass hosts in the United States. This represents the first attempt at genetic characterization of *Phyllachora maydis*, *Phyllachora graminis*, and other grass infecting *Phyllachora* species. The objectives of this study are to: (1) determine if a single species of *Phyllachora* is responsible for tar spot disease of maize throughout its range in the Americas over the last century or if distinct genetic groups are responsible for these symptoms; and (2) determine if *Phyllachora* species infecting native and weedy grasses in close proximity to maize production fields are the same species as those infecting maize.

Understanding the phylogenetic diversity and the potential host and geographic range of *Phyllachora* species associated with maize and other grasses in the Americas will also help to infer the potential evolutionary origins and speciation patterns in this genus.

### 2 | MATERIAL AND METHODS

#### 2.1 | Sample collection

Samples of maize and wild grasses with characteristic stromata of *Phyllachora* spp. (Figure 1a–f) were collected from across North America and Mexico in 2018 and 2019 (Table 1). Field specimens of infested maize and other grasses were collected by numerous individuals from the agricultural community as described in Kleczewski et al. (2020). Samples were pressed, dried at room temperature, and stored at 20°C in manila envelopes until processed. Herbarium
specimens were obtained from the U.S. National Fungus Collection (BPI, Beltsville, Maryland) and the University of Illinois Herbarium (Urbana, Illinois), which included specimens on maize and other grasses from additional hosts, countries, and years (Table 1). A total of 186 samples from 16 hosts and 15 countries, collected from 1905 to 2019, were included in the analyses.

2.2 | DNA extraction, PCR amplification, and sequencing of stroma from leaf tissue

The DNA of individual stroma not surrounded by a necrotic halo were extracted using the X-Tract-N-AMP kit following manufacturer protocols (Sigma). The complete internal transcribed spacer region of ribosomal DNA (ITS1-5.8S-ITS2) with primers ITS1f and ITS4 (White et al., 1990). Stroma without necrotic halos was selected to reduce the potential for contamination by saprophytic fungi that may be present on necrotic tissue within these lesions.

The ITS gene region was amplified from DNA extracted from each stroma using the primer pair ITS1f and ITS4 (Bruns & Gardes, 1993; White et al., 1990) with 35 cycles of the following: 95°C 5 min, 94°C 30s, 52°C 30s, 72°C 1 min, followed by 72°C for 8 min, and a final hold at 4°C in a Thermo Fisher SimpliAmp thermocycler (Thermo Fisher Scientific, Waltham, WA). Individual PCR products from corresponding DNA extractions were loaded into 2% agarose gels and gels were visualized using an Axygen gel imaging station (Axygen, Union City, CA). Stroma of Phyllachora spp. can be colonized by or associated with several other fungal species (Hock et al., 1992, 1995; McCoy et al., 2019). Consequently, samples returning a single band between 300 and 500 bp were considered free of additional fungal contaminants and used in subsequent analyses.

DNA from samples returning a single ITS band were subject to amplification of the large ribosomal subunit (LSU) region using the primer pair LR0R and LR5 (Dayaratne et al., 2017) using the aforementioned thermocycler conditions. All PCR products were purified using QIAquick PCR kits (Qiagen, Inc., Hilden, Germany), and the ITS and LSU amplicons for all samples were sequenced in the forward and reverse directions at the University of Illinois Core DNA Sequencing Facility (Urbana, Illinois).

2.3 | Sequence alignment, phylogenetic analysis, and molecular identification

Sequences generated from this study were combined with sequences obtained from GenBank. Exserohilum turcicum and Coccoicola californica were selected as the outgroups. Sequence data were aligned and concatenated using MAFFT v.7 (www.mafft.cbrc.jp/alignment/server/) using the G-INS-I model and manually inspected. The best fit partitioning schemes were determined using PartitionFinder (Lanfear et al., 2017) and used to build the phylogenies. Both single gene and concatenated gene sets were analyzed using a maximum likelihood (ML) analysis using RaxML and Bayesian inference with MrBayes. The ML phylogenies were generated by RaxML (Stamatakis, 2014) under GTR model with gamma distributed rate heterogeneity with 1000 bootstrap replicates. For the Bayesian inference, we used MrBayes v. 3.2.6 (Ronquist et al., 2012) using the general time reversible (GTR) model selected for the entire unpartitioned alignment, with the likelihood parameters setting (iset) number of substitution types (nst) = 6, with a proportion of sites invariable and the rest drawn from the gamma distribution (rate = invgamma). Four independent analyses, each starting from a random tree, were run under the same conditions for the combined gene alignment. Three hot and one cold chains of Markov Chain Monte Carlo iterations were performed. Analyses were run with 1,000,000 generations with sampling every 100 generations. The first 250,000 generations were discarded as the chains were converging (burnin period). Resulting trees were visualized with iTOL (Interactive Tree of Life) v.6 (https://itol.embl.de/) or MEGA. Sequences generated in this study were deposited in GenBank (Table 1).

3 | RESULTS

3.1 | DNA extraction and PCR amplification from herbarium and contemporary samples

A total of 186 samples from 12 states in the United States (n = 130), 4 states in Mexico (n = 13), 3 Central American countries (n = 13), 4 South American countries (n = 6), 4 Caribbean Islands (n = 16), Germany (n = 3), India (n = 3), and the Philippines (n = 2) were sequenced and analyzed as noted above. There were varying levels of success for the amplification of each genetic locus among the samples. This was particularly the case for many of the herbarium samples, some of which were more than 100 years old. The ITS region was the most successfully amplified and sequenced, with 168 sequences generated. Whereas 91 sequences were generated for the LSU locus (Table 1).

3.2 | Phylogenetic diversity of Phyllachora isolates infecting maize and grasses

Based on both ITS + LSU (Figure 2) and ITS (Figure S1) phylogenies, we observed five genetically distinct groups that represent individual species of Phyllachora with strong bootstrap and posterior probability support (>70%). The results suggest that tar spot on maize in the United States is caused by three closely related species of Phyllachora (Figure 2). In all, four species were found on maize but only Phyllachora sp. 1, Phyllachora sp. 2, and Phyllachora sp. 3 were recovered from contemporary maize in the United States, while Phyllachora sp. 4 was recovered from herbarium samples collected in Guatemala and Venezuela (Table 1).

Samples of Phyllachora sp. 3 represent the broadest geographic and host range and was also the most frequently recovered species...
| Sample ID          | Genetic cluster | Species      | State     | Country | Host       | Year collected | Source                | GenBank accessions |
|--------------------|-----------------|--------------|-----------|---------|------------|----------------|-----------------------|-------------------|
| BP1893226_2        | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342800           |
| BP1893227_2        | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342801           |
| BP1893228_2        | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342802           |
| BP1893229_2        | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342803           |
| BP1893230_2        | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342804           |
| BP1893231_2        | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342805           |
| C18001-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2018           | Field collection      | O342781           |
| C18004-1           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2018           | Field collection      | O342783           |
| C18001-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342784           |
| C18002-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342785           |
| C18004-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342786           |
| C18004-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342787           |
| C18001-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342788           |
| C18004-1           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342789           |
| C18001-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342790           |
| C18004-1           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342791           |
| C18001-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342792           |
| C18004-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342793           |
| C18004-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342794           |
| C18001-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342795           |
| C18004-1           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342796           |
| C18001-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342797           |
| C18004-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342798           |
| C18004-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342799           |
| C18001-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342800           |
| C18004-1           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342801           |
| C18001-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342802           |
| C18004-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342803           |
| C18004-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342804           |
| C18001-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342805           |
| C18004-1           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342806           |
| C18001-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342807           |
| C18004-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342808           |
| C18004-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342809           |
| C18001-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342810           |
| C18004-1           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342811           |
| C18001-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342812           |
| C18004-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342813           |
| C18004-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342814           |
| C18001-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342815           |
| C18004-1           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342816           |
| C18001-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342817           |
| C18004-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342818           |
| C18004-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342819           |
| C18001-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342820           |
| C18004-1           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342821           |
| C18001-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342822           |
| C18004-2           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342823           |
| C18004-3           | 1               | P. maydis    | Indiana   | USA     | Zea mays   | 2015           | Field collection      | O342824           |

(Continued)
| Sample ID | Species | State | Country | Host | Year collected | Source |
|-----------|---------|-------|---------|------|----------------|--------|
| BP10562_1 | P. maydis | Michigan | USA | Zea mays | 2017 | USDA Herbarium OL342827 |
| C18036-3  | P. maydis | Puebla | Mexico | Zea mays | 2018 | Field collection OL342806 |
| C18036-2  | P. maydis | Guerrero | Mexico | Zea mays | 2018 | Field collection OL342807 |
| C18036-1  | P. maydis | Mexico | Mexico | Zea mays | 2018 | Field collection OL342808 |
| C18033-1  | P. maydis | Veracruz | Mexico | Zea mays | 2018 | Field collection OL342810, OL342811 |
| C18033-2  | P. maydis | Queretaro | Mexico | Zea mays | 2018 | Field collection OL342813, OL342814 |
| C18033-3  | P. maydis | Mexico | Mexico | Zea mays | 2018 | Field collection OL342815, OL342816 |
| C18038-1  | P. maydis | Veracruz | Mexico | Zea mays | 2018 | Field collection OL342817, OL342818 |
| C18040-1  | P. maydis | Florida | USA | Zea mays | 2018 | Field collection OL342819, OL342820 |
| C18040-2  | P. maydis | Florida | USA | Zea mays | 2018 | Field collection OL342821, OL342822 |
| C18040-3  | P. maydis | Florida | USA | Zea mays | 2018 | Field collection OL342823, OL342824 |
| C18069-1  | P. maydis | Illinois | USA | Zea mays | 2018 | Field collection OL342825, OL342826 |
| C19001-1  | P. maydis | Florida | USA | Zea mays | 2019 | Field collection OL342827, OL342828 |
| C19001-2  | P. maydis | Santiago | Dominican Republic | Zea mays | 1931 | UIUC Herbarium OL342829, OL342830 |
| C19001-3  | P. maydis | Santiago | Dominican Republic | Zea mays | 1931 | UIUC Herbarium OL342831, OL342832 |
| 92794-1   | P. maydis | Santiago | Chile | Setaria sp. | 1931 | UIUC Herbarium OL342833, OL342834 |
| 92794-2   | P. maydis | Santiago | Chile | Setaria sp. | 1931 | UIUC Herbarium OL342835, OL342836 |
| 92825-1   | P. maydis | California | USA | Distichlis spicata | 1942 | UIUC Herbarium OL342837, OL342838 |
| 92825-2   | P. maydis | Arizona | USA | Muhlenbergia emersleyi | 1948 | UIUC Herbarium OL342839, OL342840 |
| 92825-3   | P. maydis | Arizona | USA | Muhlenbergia emersleyi | 1948 | UIUC Herbarium OL342841, OL342842 |
| 92825-4   | P. maydis | Arizona | USA | Euphorbia sp. | 1948 | UIUC Herbarium OL342843, OL342844 |
| 92825-5   | P. maydis | India | India | Euphorbia sp. | 1948 | UIUC Herbarium OL342845, OL342846 |
| 92825-6   | P. maydis | Maine | USA | Euphorbia sp. | 1948 | UIUC Herbarium OL342847, OL342848 |
| 92825-7   | P. maydis | Maine | USA | Euphorbia sp. | 1948 | UIUC Herbarium OL342849, OL342850 |
| 92825-8   | P. maydis | Maine | USA | Euphorbia sp. | 1948 | UIUC Herbarium OL342851, OL342852 |

| Sample ID | Genetic cluster | Species | GenBank accessions | ITS | LSU | Year collected | Source |
|-----------|-----------------|---------|-------------------|-----|-----|----------------|--------|
| 92825-3   | 3               | P. euphorbiaceae | 92825-1 | 92825-2 | 92825-3 | 1932 | Mumbai, India | Euphorbia sp. | 1932 | UIUC Herbarium OL342869 |
| 92845-3   | 3               | P. graminis | 92845-1 | 92845-2 | 92845-3 | 1946 | Mittelfrank, Germany | Agropyron repens | 1946 | UIUC Herbarium OL342871 |
| 92892-3   | 3               | P. heraclei | 92892-1 | 92892-2 | 92892-3 | 1977 | Hessen, Germany | Heracleum spondylium | 1977 | UIUC Herbarium OL342874 |
| 92925-3   | 3               | P. junci | 92925-1 | 92925-2 | 92925-3 | 1946 | Holstein, Germany | Juncus effusus | 1946 | UIUC Herbarium OL342877 |
| 92938-3   | 3               | P. maydis | 92938-1 | 92938-2 | 92938-3 | 1917 | Arecibo, Puerto Rico | Zea mays | 1917 | UIUC Herbarium OL342873 |
| 92940-3   | 3               | P. maydis | 92940-1 | 92940-2 | 92940-3 | 1917 | Arecibo, Puerto Rico | Zea mays | 1917 | UIUC Herbarium OL342874 |
| 93013-2   | 3               | P. rottboelliae | 93013-1 | 93013-2 | 93013-3 | 1931 | Mittelfrank, Germany | Zea mays | 1931 | UIUC Herbarium OL342878 |
| 93064-3   | 3               | P. sylvatica | 93064-1 | 93064-2 | 93064-3 | 1941 | California, USA | Festuca idahoensis | 1941 | UIUC Herbarium OL342880 |
| 93126-3   | 3               | P. vulgata | 93126-1 | 93126-2 | 93126-3 | 1948 | Arizona, USA | Festuca idahoensis | 1948 | USDA Herbarium OL342883 |
| BPI638546_1 | 3             | P. maydis | 93126-1 | 93126-2 | 93126-3 | 1948 | Arizona, USA | Zea mays | 1948 | USDA Herbarium OL342884 |
| BPI638553_1 | 3             | P. maydis | 93126-1 | 93126-2 | 93126-3 | 1948 | Arizona, USA | Zea mays | 1948 | USDA Herbarium OL342885 |
| BPI638557_1 | 3             | P. maydis | 93126-1 | 93126-2 | 93126-3 | 1948 | Arizona, USA | Zea mays | 1948 | USDA Herbarium OL342886 |
| BPI638561_1 | 3             | P. maydis | 93126-1 | 93126-2 | 93126-3 | 1948 | Arizona, USA | Zea mays | 1948 | USDA Herbarium OL342887 |
| BPI638564_1 | 3             | P. maydis | 93126-1 | 93126-2 | 93126-3 | 1948 | Arizona, USA | Zea mays | 1948 | USDA Herbarium OL342888 |
| BPI638570_1 | 3             | P. maydis | 93126-1 | 93126-2 | 93126-3 | 1948 | Arizona, USA | Zea mays | 1948 | USDA Herbarium OL342889 |
| BPI638572_1 | 3             | P. maydis | 93126-1 | 93126-2 | 93126-3 | 1948 | Arizona, USA | Zea mays | 1948 | USDA Herbarium OL342890 |
| BPI638574_1 | 3             | P. maydis | 93126-1 | 93126-2 | 93126-3 | 1948 | Arizona, USA | Zea mays | 1948 | USDA Herbarium OL342891 |
| BPI638576_1 | 3             | P. maydis | 93126-1 | 93126-2 | 93126-3 | 1948 | Arizona, USA | Zea mays | 1948 | USDA Herbarium OL342892 |
| BPI638577_1 | 3             | P. maydis | 93126-1 | 93126-2 | 93126-3 | 1948 | Arizona, USA | Zea mays | 1948 | USDA Herbarium OL342893 |

(Continued)
| Sample ID | Species | Genetic cluster | State | Country | Host | Year collected | Source |
|-----------|---------|-----------------|-------|---------|------|----------------|--------|
| BPI638579_1 | P. maydis | 3 | Guatemala | Guatemala | Zea mays | 1941 | USDA Herbarium OL342906 |
| BPI638580_1 | P. maydis | 3 | Santander | Colombia | Zea mays | 1936 | USDA Herbarium OL342907 |
| BPI638581_1 | P. maydis | 3 | Vega Baja | Puerto Rico | Zea mays | 1905 | USDA Herbarium OL342909 |
| BPI638582_1 | P. maydis | 3 | Antigua | Guatemala | Zea mays | 1906 | USDA Herbarium OL342904 |
| BPI638583_1 | P. maydis | 3 | Guatemala | Guatemala | Zea mays | 1906 | USDA Herbarium OL342905 |
| BPI638584_1 | P. maydis | 3 | Antigua | Guatemala | Zea mays | 1905 | USDA Herbarium OL342901 |
| BPI638585_1 | P. maydis | 3 | Guatemala | Guatemala | Zea mays | 1906 | USDA Herbarium OL342906 |
| BPI638586_1 | P. maydis | 3 | Guatemala | Guatemala | Zea mays | 1906 | USDA Herbarium OL342907 |
| BPI638587_1 | P. maydis | 3 | Guatemala | Guatemala | Zea mays | 1906 | USDA Herbarium OL342908 |
| BPI893226_1 | P. maydis | 3 | Indiana | USA | Zea mays | 1941 | USDA Herbarium OL342909 |
| BPI893227_1 | P. maydis | 3 | Indiana | USA | Zea mays | 1941 | USDA Herbarium OL342901 |
| BPI893228_1 | P. maydis | 3 | Indiana | USA | Zea mays | 1941 | USDA Herbarium OL342902 |
| BPI893229_1 | P. maydis | 3 | Indiana | USA | Zea mays | 1941 | USDA Herbarium OL342903 |
| BPI893230_1 | P. maydis | 3 | Indiana | USA | Zea mays | 1941 | USDA Herbarium OL342904 |
| BPI893231_1 | P. maydis | 3 | Indiana | USA | Zea mays | 1941 | USDA Herbarium OL342905 |
| BPI893232_1 | P. maydis | 3 | Indiana | USA | Zea mays | 1941 | USDA Herbarium OL342906 |
| BPI893233_1 | P. maydis | 3 | Indiana | USA | Zea mays | 1941 | USDA Herbarium OL342907 |
| BPI893234_1 | P. maydis | 3 | Indiana | USA | Zea mays | 1941 | USDA Herbarium OL342908 |
| BPI910560_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342909 |
| BPI910561_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342901 |
| BPI910562_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342902 |
| BPI910563_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342903 |
| BPI910564_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342904 |
| BPI910565_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342905 |
| BPI910566_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342906 |
| BPI910567_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342907 |
| BPI910568_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342908 |
| BPI910569_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342909 |
| BPI910570_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342901 |
| BPI910571_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342902 |
| BPI910572_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342903 |
| BPI910573_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342904 |
| BPI910574_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342905 |
| BPI910575_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342906 |
| BPI910576_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342907 |
| BPI910577_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342908 |
| BPI910578_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342909 |
| BPI910579_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342901 |
| BPI910580_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342902 |
| BPI910581_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342903 |
| BPI910582_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342904 |
| BPI910583_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342905 |
| BPI910584_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342906 |
| BPI910585_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342907 |
| BPI910586_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342908 |
| BPI910587_1 | P. maydis | 3 | Wisconsin | USA | Zea mays | 1941 | USDA Herbarium OL342909 |
| Sample ID   | Genetic cluster | Species | State    | Country | Host   | Year collected | Source            | GenBank accessions |
|-------------|-----------------|---------|----------|---------|--------|----------------|--------------------|--------------------|
| C18136-1    | 3               | P. maydis | Wisconsin | USA     | Zea mays | 2018           | Field collection   | OL314432           |
| C18148-1    | 3               | P. maydis | Iowa      | USA     | Zea mays | 2018           | Field collection   | OL342833 OL314433 |
| C18148-2    | 3               | P. maydis | Iowa      | USA     | Zea mays | 2018           | Field collection   | OL342834 OL314434 |
| C18148-3    | 3               | P. maydis | Iowa      | USA     | Zea mays | 2018           | Field collection   | OL342835 OL314435 |
| C18149-1    | 3               | P. maydis | Iowa      | USA     | Zea mays | 2018           | Field collection   | OL342836 OL314436 |
| C18149-2    | 3               | P. maydis | Iowa      | USA     | Zea mays | 2018           | Field collection   | OL342837 OL314437 |
| C18153-1    | 3               | P. maydis | Wisconsin | USA     | Zea mays | 2018           | Field collection   | OL342838 OL314438 |
| C18153-2    | 3               | P. maydis | Wisconsin | USA     | Zea mays | 2018           | Field collection   | OL314439           |
| C19007-1    | 3               | P. maydis | Iowa      | USA     | Zea mays | 2019           | Field collection   | OL342839 OL314440 |
| C19007-2    | 3               | P. maydis | Iowa      | USA     | Zea mays | 2019           | Field collection   | OL342840 OL314441 |
| C19007-3    | 3               | P. maydis | Iowa      | USA     | Zea mays | 2019           | Field collection   | OL342841 OL314441 |
| C19008-1    | 3               | P. maydis | Illinois  | USA     | Zea mays | 2019           | Field collection   | OL342842 OL314442 |
| C19008-2    | 3               | P. maydis | Illinois  | USA     | Zea mays | 2019           | Field collection   | OL342843 OL314443 |
| C19008-3    | 3               | P. maydis | Illinois  | USA     | Zea mays | 2019           | Field collection   | OL342844           |
| C19009-2    | 3               | P. maydis | Minnesota | USA     | Zea mays | 2019           | Field collection   | OL342845 OL314444 |
| C19012-2    | 3               | P. maydis | Minnesota | USA     | Zea mays | 2019           | Field collection   | OL342846 OL314445 |
| C19012-3    | 3               | P. maydis | Minnesota | USA     | Zea mays | 2019           | Field collection   | OL342847 OL314446 |
| C19022-2    | 3               | P. maydis | Illinois  | USA     | Zea mays | 2019           | Field collection   | OL342848           |
| C19022-3    | 3               | P. maydis | Illinois  | USA     | Zea mays | 2019           | Field collection   | OL314443           |
| C19025-1    | 3               | P. maydis | Illinois  | USA     | Zea mays | 2019           | Field collection   | OL342849           |
| C19025-2    | 3               | P. maydis | Illinois  | USA     | Zea mays | 2019           | Field collection   | OL342850           |
| C19040-1    | 3               | P. maydis | Michigan  | USA     | Zea mays | 2019           | Field collection   | OL342851 OL314447 |
| C19040-2    | 3               | P. maydis | Michigan  | USA     | Zea mays | 2019           | Field collection   | OL342852 OL314456 |
| C19043-2    | 3               | P. maydis | Michigan  | USA     | Zea mays | 2019           | Field collection   | OL342853 OL314448 |
| C19072-1    | 3               | P. maydis | Wisconsin | USA     | Zea mays | 2019           | Field collection   | OL342854 OL314449 |
| C19072-2    | 3               | P. maydis | Wisconsin | USA     | Zea mays | 2019           | Field collection   | OL342855 OL314450 |
| C19072-3    | 3               | P. maydis | Wisconsin | USA     | Zea mays | 2019           | Field collection   | OL342856 OL314451 |
| C19106-1    | 3               | P. maydis | Iowa      | USA     | Zea mays | 2019           | Field collection   | OL342857 OL314457 |
| C19106-2    | 3               | P. maydis | Iowa      | USA     | Zea mays | 2019           | Field collection   | OL342858 OL314458 |
| C19106-3    | 3               | P. maydis | Iowa      | USA     | Zea mays | 2019           | Field collection   | OL342859 OL314462 |
| Sample ID | Genetic cluster | Species | State | Country | Host | Year collected | Source | GenBank accessions |
|-----------|----------------|---------|-------|---------|------|----------------|--------|------------------|
| BPI638565 | 4              | *P. maydis* | Venezuela | Zea mays | 1957 | USDA Herbarium | OL342920 |
| BPI638576 | 4              | *P. maydis* | Mazatenango | Guatemala | Zea mays | 1906 | USDA Herbarium | OL342921 |
| BPI638583 | 4              | *P. maydis* | Guatemala | Zea mays | 1907 | USDA Herbarium | OL342922 |
| NC19004-1 | 4              | *Phyllachora* sp. | New York | USA | Thinopyrum intermedium | 2019 | Field collection | OL342923 OL314463 |
| NC19004-2 | 4              | *Phyllachora* sp. | New York | USA | Thinopyrum intermedium | 2019 | Field collection | OL314464 |
| NC19004-3 | 4              | *Phyllachora* sp. | New York | USA | Thinopyrum intermedium | 2019 | Field collection | OL342924 OL314465 |
| NC19026-1 | 4              | *Phyllachora* sp. | Illinois | USA | Triticale | 2019 | Field collection | OL342917 OL314466 |
| NC19026-2 | 4              | *Phyllachora* sp. | Illinois | USA | Triticale | 2019 | Field collection | OL342918 OL314467 |
| NC19026-3 | 4              | *Phyllachora* sp. | Illinois | USA | Triticale | 2019 | Field collection | OL342919 |
| NC19029-1 | 4              | *Phyllachora* sp. | Illinois | USA | Unknown | 2019 | Field collection | OL342925 OL314468 |
| NC19029-2 | 4              | *Phyllachora* sp. | Illinois | USA | Unknown | 2019 | Field collection | OL342926 OL314469 |
| NC19029-3 | 4              | *Phyllachora* sp. | Illinois | USA | Unknown | 2019 | Field collection | OL342927 OL314470 |
| NC19030-1 | 4              | *Phyllachora* sp. | Illinois | USA | Unknown | 2019 | Field collection | OL342928 OL314471 |
| NC19030-2 | 4              | *Phyllachora* sp. | Illinois | USA | Unknown | 2019 | Field collection | OL342929 OL314472 |
| NC19030-3 | 4              | *Phyllachora* sp. | Illinois | USA | Unknown | 2019 | Field collection | OL342930 OL314473 |
| NC19032-1 | 4              | *Phyllachora* sp. | Illinois | USA | Fall panicum | 2019 | Field collection | OL342931 OL314474 |
| NC19032-2 | 4              | *Phyllachora* sp. | Illinois | USA | Fall panicum | 2019 | Field collection | OL342932 OL314475 |
| NC19032-3 | 4              | *Phyllachora* sp. | Illinois | USA | Fall panicum | 2019 | Field collection | OL342933 OL314476 |
| NC19034-1 | 4              | *Phyllachora* sp. | Illinois | USA | Fescue | 2019 | Field collection | OL342934 OL314477 |
| NC19034-2 | 4              | *Phyllachora* sp. | Illinois | USA | Fescue | 2019 | Field collection | OL342935 OL314478 |
| NC19034-3 | 4              | *Phyllachora* sp. | Illinois | USA | Fescue | 2019 | Field collection | OL342936 OL314479 |
| NC19035-1 | 4              | *Phyllachora* sp. | Illinois | USA | Rye | 2019 | Field collection | OL342937 OL314480 |
| NC19035-2 | 4              | *Phyllachora* sp. | Illinois | USA | Rye | 2019 | Field collection | OL342938 OL314481 |
| NC19035-3 | 4              | *Phyllachora* sp. | Illinois | USA | Rye | 2019 | Field collection | OL342939 OL314482 |
| NC19111-1 | 4              | *Phyllachora* sp. | South Dakota | USA | Brome grass | 2019 | Field collection | OL342940 OL314483 |
| NC19111-2 | 4              | *Phyllachora* sp. | South Dakota | USA | Brome grass | 2019 | Field collection | OL342941 OL314484 |
| NC19111-3 | 4              | *Phyllachora* sp. | South Dakota | USA | Brome grass | 2019 | Field collection | OL342942 OL314485 |
| NC19027-1 | 5              | *Phyllachora* sp. | Illinois | USA | Triticale | 2019 | Field collection | OL342943 OL314486 |
| NC19027-3 | 5              | *Phyllachora* sp. | Illinois | USA | Triticale | 2019 | Field collection | OL342944 OL314487 |
| Sample ID  | Species a | Host | State | Country | GenBank accessions | Source | Year collected |
|------------|-----------|------|-------|---------|--------------------|--------|----------------|
| NC19028-1  | Phyllachora sp. | Illinois | USA   | Unknown | O134488            | Field collection | 2019 |
| NC19028-2  | Phyllachora sp. | Illinois | USA   | Unknown | O134489            | Field collection | 2019 |
| NC19028-3  | Phyllachora sp. | Illinois | USA   | Full panicum | O134495 | Field collection | 2019 |
| NC19037-1  | Phyllachora sp. | Illinois | USA   | Ry | O134492            | Field collection | 2019 |
| NC19037-2  | Phyllachora sp. | Illinois | USA   | Ry | O134493            | Field collection | 2019 |
| NC19037-3  | Phyllachora sp. | Illinois | USA   | Ry | O134494            | Field collection | 2019 |

a The genetic cluster was determined as a result of the phylogenetic analysis of the combined DNA sequences from the ITS and LSU regions. These are displayed in Figures 2, 3, and Figure S1.

b The isosyntype specimen of *P. maydis* (BPI 638553) collected in Mexico in 1904 and the *P. maydis* isolate (BPI 8993226) used in the first report of tar spot in the United States are both part of *Phyllachora* sp. 3 and isolates of this species have since been recorded in Illinois, Indiana, Iowa, Michigan, Minnesota, and Wisconsin. This represents the widest geographic range of the maize-infecting *Phyllachora* species in the United States among the samples included in this study. However, isolates of *Phyllachora* sp. 3 were also recovered from another 10 host species including monocots and dicots, with a global distribution including 12 countries across South, Central, and North America and the Caribbean, as well as Germany, India, and the Philippines (Table 1; Figure 3). The herbarium samples associated with each of the 10 host species represented morphologically recognized species of *Phyllachora* including *P. graminis*, *P. heraclei*, *P. junco*, *P. chaetochloae*, *P. diplocarpa*, *P. epicampis*, *P. euphorbiaceae*, *P. rottboelliae*, *P. sylvatica*, and *P. vulgata*.

The other two contemporary maize-infecting species, *Phyllachora* sp. 1 and *Phyllachora* sp. 2, have a more limited observed host and geographic range. Both species were only recovered on maize. *Phyllachora* sp. 1 was only recovered from contemporary maize samples from Indiana and Ohio, whereas *Phyllachora* sp. 2 was found on herbarium specimens from Colombia and Puerto Rico and contemporary specimens from Puerto Rico, Mexico (Guerrero, Oaxaca, Puebla, and Veracruz), and the United States (Florida, Illinois, and Michigan).

The other species recovered from maize was *Phyllachora* sp. 4. However, samples only included herbarium specimens from Guatemala and Venezuela and did not include any contemporary maize specimens. However, *Phyllachora* sp. 4 was commonly found among grasses in the United States that are found in proximity to maize production fields in Illinois, South Dakota, and New York (Table 1). Isolates of *Phyllachora* sp. 4 were recovered from six grass species in four tribes in the United States representing a broad host range across a breadth of genetically diverse grass species. *Phyllachora* sp. 5 was the only species not recovered from maize but was found on many of the same grass species as *Phyllachora* sp. 4, including rye, triticale, and fall panicum (Table 1).

While there was limited *Phyllachora* sequence data in GenBank, we were able to include the ITS sequence of 19 isolates representing six recognized species of *Phyllachora* to determine any relationship between the isolates used in this study and those submitted previously to GenBank (Figure 3). In the case of *Phyllachora* sp. 4, two isolates referred to as *P. graminis* one from *Hordelymus eur- opeus* in Germany, and one of unknown origin, as well as isolates of *Phyllachora* on *Elymus kamoji* and *Roegneria in* China grouped together with strong Bootstrap support (90%) and posterior
probability (0.99). There was also an isolate of *P. graminis* from an unknown grass in Canada that grouped together with *Phyllachora* sp. 5, and the herbarium specimen of *P. graminis* from Agropyron repens in Germany from this study grouped in *Phyllachora* sp. 3 (Figure 3). Our results support the findings of previous observations that *P. graminis* is a poorly defined polyphyletic species that has often been assigned to tar spot symptom on a variety of grass hosts.

4 | DISCUSSION

Since *P. graminis* was described by Persoon in 1785 as *Sphaeria graminis* and then transferred to the genus *Phyllachora* by Fuckel (1870), over 300 species have been recorded on graminaceous hosts, and many more on non-grass hosts. However, Parbery (1967) recognized that there are fewer species associated with grasses and established that there were 95 valid graminicolous *Phyllachora* species world-wide based on morphological characteristics. In the most complete study of *Phyllachora* species in North America, Orton (1944) identified 45 morphological species from more than 100 host species (Orton, 1944). While this likely represents a significant overestimation of the true number of species in North and Central America, it does demonstrate the vast number of hosts on which *Phyllachora* species have been reported. Our results based on both herbarium and contemporary samples of infected hosts indicate that there are far fewer species of *Phyllachora* in the Americas than indicated by Orton (1944) and Parbery (1967), and the species that are present have a greater host range than previously thought. The predominant species in this study, *Phyllachora* sp. 3, has a broad geographic and host range with the capacity to infect maize throughout South, Central, and North America as well as seven grass species and two dicot species. This phylogenetic species also includes isolates of 11 morphologically determined species of *Phyllachora* (*P. chae-tochloae, P. diplocarpa, P. epicampsis, P. euphorbiaceae, P. graminis,
FIGURE 3  Maximum likelihood phylogenetic tree based on ITS sequence data from geographically representative isolates of the five genetic groups of maize and grass-infecting Phyllachora from this study and Phyllachora species available from GenBank. Values of Bayesian PP > 0.70 or ML BS > 70% are given at nodes at the first and second positions, respectively. Isolates sequenced in this project are denoted in bold. Information on host plant is indicated in blue text and geographic origin in red text.
P. heraclei, P. junct, P. maydis, P. rottboelliae, P. sylvatica, and P. vulgata) from herbarium samples collected in the Dominican Republic, Germany, India, Mexico, the Philippines, Trinidad and Tobago, and the United States, indicating global distribution of this species. This expanded host range also now complicates the taxonomic status and the name to be retained by this genetic group. An isolate of *P. graminis* collected from *Agropyron repens* from Germany was designated as the lectotype specimen for the genus (Clements & Shear, 1931), and the isolate of *P. graminis* examined in this study, while not the lectotype was collected from *A. repens* in Germany, indicating that *P. graminis* may have precedence for the species name of *Phyllachora* sp. 3. This would have ramifications for *P. maydis* as well as several other *Phyllachora* species in *Phyllachora* sp. 3 (Table 1; Figure 3) that appear to be synonyms of *P. graminis*. This is based on sequence data from the ITS and/or LSU region and further multi-gene phylogenetic studies of a larger representation of type material from herbaria and contemporary *Phyllachora* samples from additional hosts is needed for a thorough taxonomic assessment of this genus.

The three maize-infecting species, *Phyllachora* sp. 1, *Phyllachora* sp. 2, and *Phyllachora* sp. 3, have overlapping geographic and host ranges, providing the opportunity for co-infection and genetic exchange. Co-infection on the same leaf tissue by *Phyllachora* sp. 3 and *Phyllachora* sp. 1 was observed on four occasions with herbarium samples (BPI893231_1, BPI893232_2, BPI893231_1, and BPI893232_2) from three counties in Indiana. A recent fungal community analysis of tar spot lesions on maize found a similar trend with two distinct OTUs occurring on 21 of 22 maize leaf samples from Michigan (McCoy et al., 2019). A similar phenomenon has also been observed in *Albugo candida*, another biotrophic pathogen with a broad host range (McMullan et al., 2015). Races of *A. candida* were not able to infect a host on their own but were able to co-infect with a race-specific isolate that suppressed host immunity in that host. The offspring of any genetic introgression or recombination resulted in a race with an expanded host range able to infect both plants infected by the parental strains of *A. candida*. A whole-genome comparison of these *A. candida* races found a mosaic-like genome structure with large portions conserved between races, as well as regions with only 89% sequence similarity. This scenario may explain the wide host range and variation in morphology between hosts in *Phyllachora* species. Sexual reproduction in *P. maydis* followed by discharge of infective ascospores commonly occurs on corn leaves annually in maize-producing regions of the United States (Groves et al., 2020; Kleczewski et al., 2019). The presence of multiple maize-infecting species in the mid-western United States, and even on a single infected leaf, combined with frequent sexual recombination, ascospore release and infection, could result in novel populations and/or species of *Phyllachora* that are more virulent on maize or that have an expanded host range. This may also explain why *Phyllachora* sp. 3 has such a broad host range, whereas *Phyllachora* sp. 1 and *Phyllachora* sp. 2 were only found on maize. Individual populations may gain the ability to infect a new host but are still able to sexually recombine with the rest of the population on the original host species. Given the geographic overlap of many grass species in Central, South, and North America, small populations of *Phyllachora* sp. 3 may have adapted to infect a novel grass species, while maintaining the ability to recombine with the larger *Phyllachora* sp. 3 complex, resulting in the expansion of the host range without specialization and speciation.

Speciation has likely occurred in instances where geographic isolation of a new host prevented further introgression with the original population. As maize is commonly grown from Argentina to Canada, it represents a common host for which distinct *Phyllachora* populations may infect and recombine resulting in potentially new and more virulent populations that are still part of the same species. It is unclear if geographic or genetic barriers lead to speciation between the closely related *Phyllachora* sp. 1, *Phyllachora* sp. 2, and *Phyllachora* sp. 3, but the significant overlap in host and geography would indicate a genetic barrier. While *Phyllachora* sp. 1 and *Phyllachora* sp. 2 were only recovered from maize, our sampling scheme was strongly biased toward maize. It is possible that *Phyllachora* sp. 1 and *Phyllachora* sp. 2 were present on other grass and non-grass hosts in Central and North America and were not sampled in this study. These non-sampled hosts, if only infected by one of the *Phyllachora* species, may represent the isolation that led to adaptation and speciation.

For now the name *Phyllachora maydis* will be retained by *Phyllachora* sp. 3 as the *P. maydis* isosynotype material (BP1638553) clustered with this group. However, the presence of three maize-infecting species, the lack of type material of *P. graminis*, and the potential taxonomic synonymy with *P. graminis* and several other *Phyllachora* species makes it difficult to determine which of the maize-infecting species will retain the name *P. maydis*. Therefore, we recommend referring to *Phyllachora* sp. 1, *Phyllachora* sp. 2, and *Phyllachora* sp. 3 as the *Phyllachora maydis* species complex until further morphological and multi-gene phylogenetic studies can properly delineate these species.

In this work, we conducted the most comprehensive assessment of *Phyllachora maydis* reported to date and provided evidence that our understanding of this species and genera is limited and requires significant attention. The reasons for the emergence of tar spot caused by three species of *Phyllachora* that have been present in Central America, Mexico, and the Caribbean for over 75 years are still unclear. Several scenarios may explain the recent emergence and severity of tar spot caused by *Phyllachora* species in the upper Midwest of the United States. While *Phyllachora* sp. 2 and *Phyllachora* sp. 3 have been present in both Mexico and Puerto Rico for the last century, it is possible that when the fungus was able to be dispersed via wind and rain to the United States, it could not overwinter in colder climates and the disease could not become established. In fact, according to the herbarium specimens, *Phyllachora* sp. 3 has been present in the United States since the 1940s in California and Arizona on native grasses but not maize. However, recent studies have demonstrated that *Phyllachora* spp. can overwinter in Illinois (Kleczewski et al., 2019). Shorter and warmer winters due to climate change could be playing a role in the ability of *Phyllachora* species to survive further north in the United States. Changes in climate patterns during the growing season may also have an impact on this disease as increased
temperature and precipitation may promote epidemics of this disease. Finally, a change in maize genetics may also play a role in the increased severity of tar spot. Since maize breeding programs were not selecting for resistance to tar spot, any partial resistance that may have been present in U.S. germplasm may have been lost through genetic drift. The loss of this resistance may not have been noticed until *Phyllachora* species arrived in the primary maize-growing region of the United States. The disease remains of minor importance in Mexico and Central American maize production, as resistance to this disease would be selected for in-breeding programs. The most likely scenario for the emergence of tar spot in the United States includes a combination of these factors: (1) introduction of multiple species of *Phyllachora* from Mexico, Puerto Rico, or other Central American countries through movement of infected plant tissue or possible long-distance movement via wind, rain, hurricane/tropical storm system, etc.; (2) change in climate in the Midwestern maize growing region more hospitable to the growth, reproduction, and survival of *Phyllachora* spp.; and (3) lack of resistance in maize germplasm grown in the Midwestern United States.

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CONFLICT OF INTEREST

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Kirk Broders: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Methodology (equal); Project administration (equal); Visualization (equal); Writing – original draft (lead); Writing – review & editing (equal). Gloria Iriarte-Broders: Data curation (equal); Formal analysis (equal); Methodology (equal); Visualization (equal); Writing – original draft (equal); Writing – review & editing (equal). Gary C. Bergstrom: Resources (equal); Writing – review & editing (equal). Emmanuel Byamukama: Resources (equal); Writing – review & editing (equal). Christian Cruz: Resources (equal); Writing – review & editing (equal). Martin Chilvers: Resources (equal); Writing – review & editing (equal). Felice Dalla-Lana: Resources (equal); Writing – review & editing (equal). Zachary Duray: Data curation (equal); Formal analysis (supporting); Investigation (supporting). Dean Malvick: Resources (equal); Writing – review & editing (equal). Dareen Mueller: Resources (equal); Writing – review & editing (equal). Pierce Paul: Resources (equal); Writing – review & editing (equal). Diana Plewa: Resources (equal); Writing – review & editing (equal). Richard Raat: Resources (equal); Writing – review & editing (equal). Alison E. Robertson: Resources (equal); Writing – review & editing (equal). Catalina Salgado-Salazar: Formal analysis (supporting); Methodology (supporting); Resources (equal); Writing – original draft (supporting); Writing – review & editing (equal). Damon Smith: Resources (equal); Writing – review & editing (equal). Darcy Telenko: Resources (equal); Writing – review & editing (equal). Katherine VanEtten: Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Resources (equal). Nathan M. Kleczewski: Conceptualization (lead); Data curation (supporting); Formal analysis (supporting); Funding acquisition (lead); Methodology (equal); Project administration (equal); Resources (lead); Supervision (equal); Writing – original draft (equal); Writing – review & editing (equal).

DATA AVAILABILITY STATEMENT

All DNA sequence data generated by this project were deposited in GenBank as accessions OL314402-OL314494 and OL342781-OL342949.

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