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RESOURCE ANNOUNCEMENT

First Draft Genome Assemblies of *Pleochaeta shiraiana* and *Phyllactinia moricola*, Two Tree-Parasitic Powdery Mildew Fungi with Hemiendophytic Mycelia

e-Xtra*

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Abstract

Powdery mildew fungi (Erysiphaceae) are widespread obligate biotrophic plant pathogens. Thus, applying genetic and omics approaches to study these fungi remains a major challenge, particularly for species with hemiendophytic mycelium. These belong to a distinct phylogenetic lineage within the family Erysiphaceae. To date, only a single draft genome assembly is available for this clade, obtained for *Leveillula taurica*. Here, we generated the first draft genome assemblies of *Pleochaeta shiraiana* and *Phyllactinia moricola*, two tree-parasitic powdery mildew species with hemiendophytic mycelium, representing two genera that have not yet been investigated with genomics tools. The *Pleochaeta shiraiana* assembly was 96,769,103 bp in length and consisted of 14,447 scaffolds, and the *Phyllactinia moricola* assembly was 180,382,532 bp in length on 45,569 scaffolds. Together with the draft genome of *L. taurica*, these resources will be pivotal for understanding the molecular basis of the lifestyle of these fungi, which is unique within the family Erysiphaceae.

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Keywords

evolution, fungal pathogens, genomic resources, genomics, host–parasite interactions, obligate biotrophs, plant pathogens

Genome Announcement

More than 10,000 dicot and monocot plant species, including important crops, are commonly infected by powdery mildew fungi (Erysiphaceae, Helotiales, Ascomycota) on a global scale (Braun and Cook 2012; Glawe 2008; Kiss et al. 2020). In total, there are approximately 900 species of powdery mildew fungi in 19 recognized genera (Braun and Cook 2012; Kiss et al. 2020). All of these ubiquitous fungi are obligate biotrophic plant pathogens, which means that nutrient uptake can happen only from their living host plant tissues and, thus, they cannot

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Author contributions: Epiphytic fungal materials were collected by S.L. (*Phyllactinia moricola*), S.T., M.Z.N., and D.S. (*Pleochaeta shiraiana*). High molecular weight DNA was isolated and DNA quality control performed by L.F., S.K., and S.L. S.K. did genome assemblies, assembly quality control, and filtering. N.V. conducted gene prediction and phylogenetic analysis of nrDNA sequences. P.C. and N.V. performed the transposable element analysis. R.P. and L.K. provided reagents, materials, and resources. S.K. and L.K. drafted the manuscript, and all authors edited and proofread the manuscript.

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grow or be cultured in the absence of their hosts (Panstruga and Schulze-Lefert 2002). The mycelia of most powdery mildew species are epiphytic, with haustoria (i.e., feeding structures) developed exclusively in the epidermal cells of their plant hosts (Kuhn et al. 2016; Micali et al. 2011, 2008). Species of the genera *Leveillula*, *Queirozia, Pleochaeta*, and *Phyllactinia* represent a distinct phylogenetic lineage within the Erysiphaceae family known as the endoparasitic group (Takamatsu 2013; Takamatsu et al. 2016). The endoparasitic group comprises, in total, approximately 85 species (Braun and Cook 2012). These powdery mildew fungi have partly endophytic (hemiendophytic) mycelia, with haustoria found exclusively in the mesophyll cells of their hosts (Zheng et al. 2013). Because their infection process, most notably the localization of haustoria, is markedly different from the rest of the members of Erysiphaceae, their effector repertoire and other molecular patterns associated with the hemiendophytic lifestyle are likely to be different from that of the epiphytically proliferating powdery mildew fungi.

Omics-based approaches can be powerful methods to understand molecular mechanisms underlying the powdery mildew lifestyle but are challenging due to the obligate biotrophic nature of these fungi (Bindschedler et al. 2016). This is particularly true for the species with hemiendophytic mycelium. So far, a single draft genome assembly is available for the entire group, that of *Leveillula taurica* collected from sweet pepper (*Capsicum annuum*) (Kusch et al. 2020). The genomes of powdery mildew fungi are generally large and gene poor compared with other ascomycetes (Barsoum et al. 2019; Mohanta and Bae 2015), coinciding with an abundance of repetitive elements (Frantzeskakis et al. 2018; Spanu et al. 2010; Wicker et al. 2013). Therefore, most powdery mildew genomes published thus far are highly fragmented (Barsoum et al. 2019; Bindschedler et al. 2016). Here, we present the first annotated draft genomes of *Pleochaeta shiraiana* infecting Chinese hackberry (*Celtis sinensis*) and *Phyllactinia moricola* infecting white mulberry (*Morus alba*), obtained by short-read next-generation DNA sequencing.

We collected powdery mildew-infected leaves of *C. sinensis* from the Mie University Campus (Tsu-shi, Mie Prefecture, Japan) in December 2017, and of *M. alba* in Yintai City (Shandong Province, China) in November 2019. We identified the powdery mildew species infecting *C. sinensis* as *Pleochaeta shiraiana* and the powdery mildew colonizing *M. alba* as *Phyllactinia moricola* based on morphology and the internal transcribed spacer (ITS) sequences of their nuclear ribosomal DNA (nrDNA) determined as described earlier (Kiss et al. 2006; Takamatsu et al. 2008). The *Pleochaeta shiraiana* specimen was deposited at Institutsbereich Geobotanik und Botanischer Garten, Martin-Luther-Universität Halle-Wittenberg, Germany, under accession number HAL3440 F, and its ITS sequence in GenBank (MZ661116). The *Phyllactinia moricola* specimen was deposited at Herbarium of Mycology of Jilin Agricultural University, China, under accession HMJAU-PM91933; its ITS sequence is available in GenBank (MZ541088). We collected the external part of the powdery mildew mycelium, including many fruiting bodies (chasmothecia) of both species, from leaves of their respective host plants with cellulose acetate peelings, as described before (Frantzeskakis et al. 2019). The peelings were collected in 2-ml reaction tubes and ground to a fine powder in liquid nitrogen using metal balls (3 mm in diameter) and a bead mill at 30 m/s for 30 s. We followed a modified protocol from Feehan et al. (2017) to isolate and purify high molecular weight DNA, as described previously (Frantzeskakis et al. 2019; Kusch et al. 2020). We assessed the quality of the DNA by agarose gel electrophoresis (0.8% agarose, 60 V) and measured the DNA amount using the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, U.S.A.). Short-read DNA sequencing was conducted on a NovaSeq6000 machine by CeGaT (Tübingen, Germany) for *Pleochaeta shiraiana* and by GENEWIZ (Leipzig, Germany) for *Phyllactinia moricola*. The reads were quality and adapter trimmed using Trimmomatic v0.36 with the following settings: ILLUMINACLIP:TruSeq3-PE.fa:5:30:10 SLIDINGWINDOW:3:18 LEADING:6 TRAILING:6 MINLEN:90 (Bolger et al. 2014). We assessed the quality of the DNA sequencing data before and after trimming with FastQC v0.11.5 (Babraham Bioinformatics, Cambridge, U.K.). Subsequently, we generated draft genome assemblies for *Pleochaeta shiraiana* and *Phyllactinia moricola* using SOAPdenovo2 (Luo et al. 2012) and filtered the scaffolds based on BLASTN against the nucleotide database (updated February 2021), DNA sequencing remapping coverage, and GC content via BlobTools (Laetsch and Blaxter 2017), following the same procedure as described by Kusch et al. (2020). This pipeline filtered out viral, bacterial, and other nonfungal contaminating contigs.

The assembly of *Pleochaeta shiraiana* contained 14,447 scaffolds with a total length of 96,769,103 bp after filtering (Table 1). We used Benchmarking Universal Single-Copy Ortholog (BUSCO) v5 with the ascomycota_odb10 (Simão et al. 2015), and identified 1,296 complete

Table 1. Genome assembly statistics for *Phyllactinia moricola* and *Pleochaeta shiraiana* compared with the genomic features of *Leveillula taurica*

Feature	*Pleochaeta shiraiana*	*Phyllactinia moricola*	*L. taurica*[a]
Genome assembly (bp)	96,769,103	180,382,532	187,262,869
N count	1,048,934	3,727,173	2,326,588
Estimated size (k-mer) (bp)	138,417,801	242,574,339	192,740,367
Scaffold/contigs	14,447	45,569	23,599
Largest contig (bp)	111,755	40,802	99,596
Average length (bp)	6,698.21	3,958.45	7,935.2
GC content (%)	38.2	39.6	39.2
N_{50}	14,429	5,366	13,899
N_{90}	3,186	1,852	3,522
Rfam small nucleolar RNAs including U3	16	15	13
Rfam 5S RNA loci	9	2	3
Transfer RNAscan nuclear transfer RNAs	91	163	191
BUSCO[b]			
Complete single-copy (%)	75.7	66.5	87.5
Duplicated (%)	0.2	0.0	0.0
Fragmented (%)	1.7	6.6	0.6
Missing (%)	22.4	26.9	11.9
Interspersed repeats (%)[c]	49.2	75.1	80.1
LINE retroelements (%)	11.5	3.1	5.2
LTR elements (%)	16.3	21.2	32.8
DNA transposons (%)	11.9	16.0	20.8
Unclassified (%)	9.5	34.9	21.3
Genes (MAKER)	6,180	7,497	8,268
Putative secreted proteins[d]	310	221	248
Putative effectors	219	112	149

[a] Kusch et al. 2020.
[b] Using the database for ascomycete core genes (ascomycota_odb10) for Benchmarking Universal Single-Copy Orthologs (BUSCOs).
[c] LINE = long interspersed nuclear element and LTR = long terminal repeat.
[d] Identified using SignalP5.0; transmembrane domain proteins were filtered by TMHMM-2.0c.

and 29 fragmented of the 1,706 core ascomycete genes (77.6%). Thus, the assembly is approximately 8% less complete than the assembly of *L. taurica* (Kusch et al. 2020), which is the only genomic resource available thus far for powdery mildew fungi with hemiendophytic mycelium. Genome size calculation via jellyfish 30-mers (Marçais and Kingsford 2011) estimated the *P. shiraiana* genome size to be approximately 138.4 Mbp. The *Phyllactinia moricola* assembly consisted of 45,569 scaffolds totaling 180,382,532 bp in length and had 1,135 complete and 113 fragmented (73.1%) core ascomycete genes (Table 1). The genome size was estimated to be 242.6 Mbp, representing one of the largest known powdery mildew genomes. By comparison, the two largest genome assemblies to date are from *Erysiphe alphitoides* at 220 Mbp and *L. taurica* with 187 Mbp (Dutech et al. 2020; Kusch et al. 2020). The new genome assemblies are publicly available at NCBI GenBank under accessions JAHYSP000000000 (*Pleochaeta shiraiana*) and JAHYSQ000000000 (*Phyllactinia moricola*). We identified 15 small nucleolar RNAs (snoRNAs) in both fungi: the fungal snoRNA U3 in *Pleochaeta shiraiana* but not *Phyllactinia moricola* and the spliceosomal RNAs U1, U2, U4, and U6 by Rfam searches (Table 1). The U5 spliceosomal RNA was not detectable in the assemblies even at an E value threshold of 0.01. We further identified the large and small subunits of the nrDNA on contig C475265 of *Pleochaeta shiraiana* and on contig C733007 of *Phyllactinia moricola*, nine 5S ribosomal RNA loci in *Pleochaeta shiraiana*, and two in *Phyllactinia moricola*. *Pleochaeta shiraiana* had 91 transfer RNAs (tRNAs) and *Phyllactinia moricola* had 163 tRNAs decoding all 20 standard amino acids based on tRNAscan-SE (Table 1) (Chan et al. 2021). In the absence of expressed sequence tags, whole-transcriptome shotgun sequencing (RNA sequencing) data, protein evidence for *Pleochaeta shiraiana* and *Phyllactinia moricola*, ab initio genome annotations were conducted in MAKER pipeline v3.01 (Holt and Yandell 2011) using AUGUSTUS v3.3.3 (Stanke and Waack 2003) trained for *L. taurica* (Kusch et al. 2020), which is the closest relative of *Pleochaeta shiraiana* and *Phyllactinia moricola* with available transcriptome data. This MAKER pipeline identified 7,497 and 6,180 coding sequences in the *Phyllactinia moricola* and *Pleochaeta shiraiana* genomes, respectively. These annotations likely underestimate the number of coding genes due to lower recovery of the total genomic sequence (73 to 76% calculated completeness) in these assemblies. Nonetheless, these values are comparable with other powdery mildews, which carry fewer genes than other ascomycetes (Frantzeskakis et al. 2018; Jones et al. 2014; Liang et al.

2018). Next, we employed SignalP5.0 (Almagro Armenteros et al. 2019) and filtered out transmembrane proteins via TMHMM v2.0c to predict secreted proteins in *Phyllactinia moricola* (*n* = 221), *Pleochaeta shiraiana* (*n* = 310), and *L. taurica* (*n* = 248) (Table 1). EffectorP3.0 (Sperschneider and Dodds 2021) predicted 112 effectors in *Phyllactinia moricola*, 219 in *Pleochaeta shiraiana*, and 149 in *L. taurica*, which is similar to other dicot-infecting powdery mildew species (Jones et al. 2014; Liang et al. 2018). We used RepeatModeler to discover repetitive sequences in the genomes of *Phyllactinia moricola*, *Pleochaeta shiraiana*, and *L. taurica*, and RepeatMasker v4.0.9 (Smit et al. 2016) to map repeats to their respective genomes. In all, 47,137,141 bp (49.2%) in *Pleochaeta shiraiana*, 133,135,259 bp (75.1%) in *Phyllactinia moricola*, and 148,336,729 bp (80.1%) in *L. taurica* were covered by repeats and transposons (Table 1). The majority of these elements were long terminal repeat (LTR) retroelements and DNA transposons (almost exclusively Tc1-IS630-Pogo); however, >15% were unclassified repeats. This architecture is similar to the genomes of different formae speciales of the cereal powdery mildew pathogen *Blumeria graminis*, which consist of >70% repetitive elements (Frantzeskakis et al. 2018; Müller et al. 2019, 2021). Interestingly, DNA transposons, particularly of the type Tc1-IS630-Pogo, were highly abundant in the two new draft genome assemblies and the draft genome of *L. taurica* (11.9 to 20.8%) (Table 1). This is markedly different from *B. graminis*, whose genomes are dominated by long interspersed nuclear element and LTR retrotransposons (Frantzeskakis et al. 2018; Spanu et al. 2010; Wicker et al. 2013). The characteristics and the fragmentary nature of the short-read-based assemblies of the highly repetitive *Pleochaeta shiraiana* and *Phyllactinia moricola* genomes are largely consistent with the genomic features of epiphytically proliferating powdery mildew fungi (Jones et al. 2014; Liang et al. 2018; Wu et al. 2018).

Then, we retrieved nucleotide sequences of the ITS, 18S, and 28S regions of the nrDNA from 18 reference powdery mildew specimens from NCBI GenBank (Supplementary Table S1) to include representative species of all of the genera with hemiendophytic mycelium, and representatives of epiphytic species, which were phylogenetically closely related to the hemiendophytic lineage in previous analyses (Bradshaw and Tobin 2020; Jin et al. 2021; Kiss et al. 2020; Marmolejo et al. 2018). The 18S and 28S sequences of the nrDNA region in *Pleochaeta shiraiana* strain HAL 3440 F and 18S sequence of *Phyllactinia moricola* strain HMJAU-PM91933 sequenced in this study as well as ITS, 18S, and 28S sequences of *L. taurica* strain HNHM-MYC-006405 sequenced by Kusch et al. (2020) were extracted from their respective genome assemblies (Supplementary Table S1). We excluded the sequences of the ITS1 and ITS2 regions from the analysis because they were too variable to allow an unambiguous alignment. Multisequence alignments were constructed in MAFFT v7.450 (Katoh and Standley 2013). The concatenated alignment included a total of 1,089 nucleotide sequences (5.8S, 154 bp; 18S, 589 bp; and 28S, 587 bp). Maximum-likelihood analysis of the concatenated alignment was conducted using RAxML v8 (Stamatakis 2014) in Geneious Prime v2021.1.1 (https://www.geneious.com) based on the GTR substitution model applied to individual partitions and 1,000 bootstrap replicates. The tree was rooted to *Parauncinula polyspora* MUMH 197. Bayesian analysis of the same alignment was conducted in MrBayes v3.2.4 (Ronquist and Huelsenbeck 2003) based on SYM, K80+I, and SYM+I+G, nucleotide substitutions models selected by MrModeltest v2.3 (Nylander 2004) for 5.8S, 18S, and 28S loci, respectively. Two Markov Chain Monte Carlo chains were run and one tree was saved per 100 generations. The run was ended when the standard deviation of split frequencies reached <0.01. The 50% majority-rule consensus tree was generated after 25% burn-in of the saved trees. The phylogenetic analysis based on the sequences of the 5.8S, 18S, and 28S regions of the nrDNA confirmed the identity of the sequenced powdery mildew fungi as *Pleochaeta shiraiana* and *Phyllactinia moricola*. Both specimens belonged to strongly supported clades with reference specimens of their respective species (Fig. 1). In line with previous phylogenetic studies of the Erysiphaceae family (Bradshaw and Tobin 2020; Kiss et al. 2020; Liberato et al. 2006; Marmolejo et al. 2018; Takamatsu et al. 2016), powdery mildew species with hemiendophytic mycelium were clustered together in a clade with moderate phylogenetic support, with *Pleochaeta shiraiana* placed closer to *Queirozia turbinata* and *Phyllactinia moricola* showing a closer phylogenetic affinity to *L. taurica*.

In this announcement, we present the first draft genome resources for two powdery mildew fungi, *Pleochaeta shiraiana* and *Phyllactinia moricola*. Both powdery mildew species are little-studied pathogens of diverse tree species, with *Pleochaeta shiraiana* being common on *Celtis* spp. (Kiss et al. 2006) and *Phyllactinia moricola* on *Morus* spp. (Takamatsu et al. 2008). These represent two genera with hemiendophytic mycelium that have not yet been investigated with genomics tools. The evolutionary history of

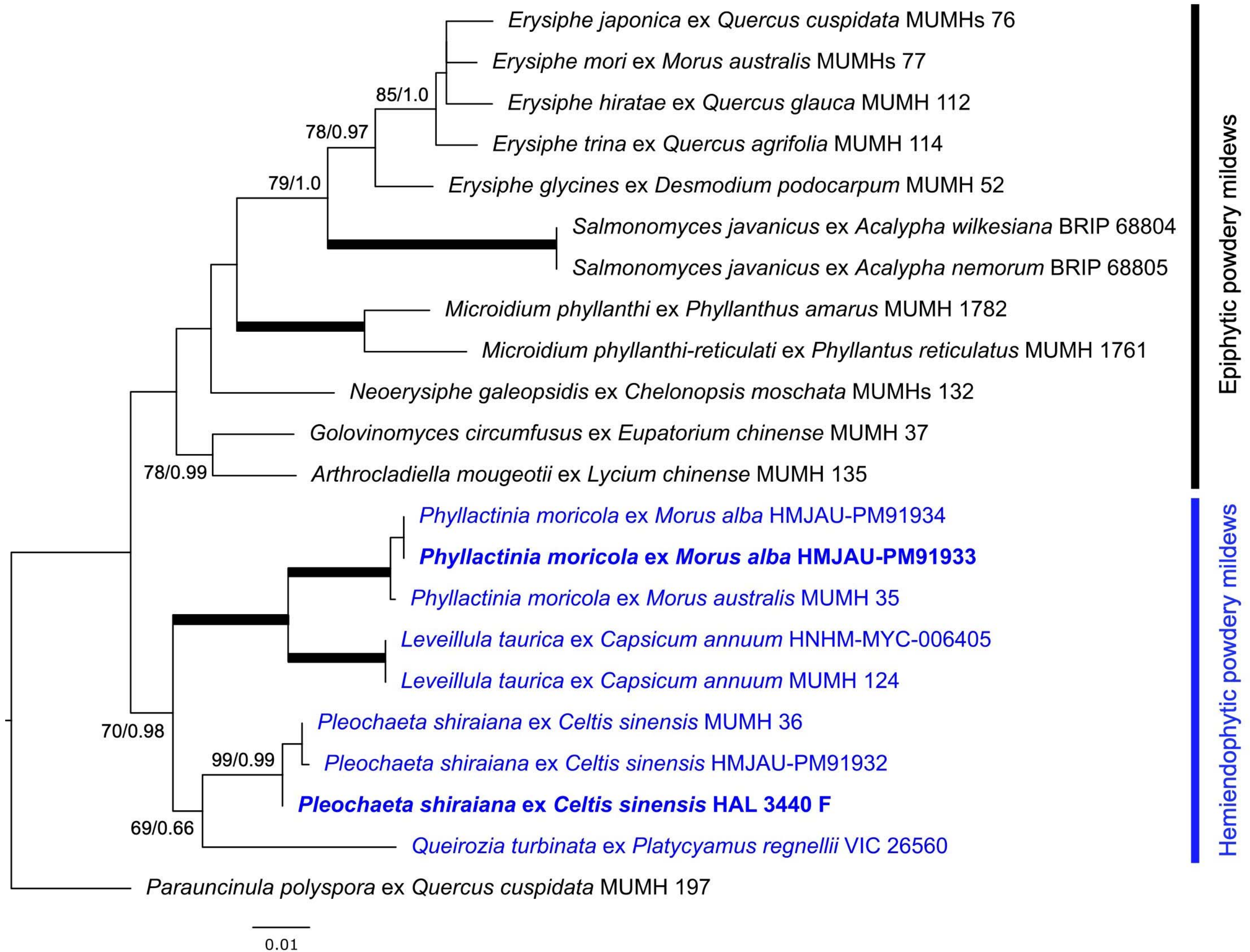

Fig. 1. Maximum-likelihood phylogram based on the concatenated sequences of the 5.8S, 18S, and 28S regions of the nuclear ribosomal DNA of representative powdery mildew taxa. Maximum-likelihood bootstrap values (BS) and Bayesian Posterior Probability (PP) values are shown above or below the branches, separated by a slash. Thickened branches represent BS and PP values of 100% and 1.0, respectively. Tip labels in bold represent specimens sequenced in the current study. The tree is rooted to *Parauncinula polyspora* MUMH 197, an early-diverged powdery mildew species (Frantzeskakis et al. 2019). The scale bar represents nucleotide substitutions per site.

tree-parasitic powdery mildew fungi is markedly different from herb-parasitic species (Takamatsu 2013). Therefore, the new genomic resources reported here will be highly valuable for future analyses toward understanding the differences between tree-parasitic and herb-parasitic species, as well as for deciphering the molecular basis of the unique lifestyle of powdery mildew fungi with hemiendophytic mycelium.

Data Availability

This Whole Genome Shotgun project has been deposited at NCBI GenBank under the accession PRJNA749662, and Sequence Read Archive accessions SRR15245358 (*Pleochaeta shiraiana*) and SRR15245359 (*Phyllactinia moricola*). The genome assemblies have been deposited at DNA Data Bank of Japan/European Nucleotide Archive/GenBank under the accessions JAHYSP000000000 (*Pleochaeta shiraiana*) and JAHYSQ000000000 (*Phyllactinia moricola*). The versions described in this article are versions JAHYSP010000000 and JAHYSQ010000000, respectively. The alignment and phylogenetic tree have been deposited in TreeBASE (number 28611).

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