frontiers | Frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 14 June 2022
doi: 10.3389/fevo.2022.918908

Secondary DNA Barcodes (CAM, GAPDH, GS, and RpB2) to Characterize Species Complexes and Strengthen the Powdery Mildew Phylogeny

Michael J. Bradshaw[1], Guan-Xiu Guan[2], Liam Nokes[1,3], Uwe Braun[4], Shu-Yan Liu[2,5]* and Donald H. Pfister[1]*

[1] Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, United States, [2] Engineering Research Center of Edible and Medicinal Fungi, Ministry of Education, Jilin Agricultural University, Changchun, China, [3] Department of Environmental Studies, Dartmouth College, Hanover, NH, United States, [4] Department for Geobotany and Botanical Garden, Herbarium, Institute of Biology, Martin Luther University, Halle (Saale), Germany, [5] Department of Plant Pathology, College of Plant Protection, Jilin Agricultural University, Changchun, China

OPEN ACCESS

Edited by:
Danny Haelewaters, Ghent University, Belgium

Reviewed by:
Arthur Grupe, University of Florida, United States
Ning Zhang, Rutgers, The State University of New Jersey, United States

***Correspondence:**
Michael J. Bradshaw
mbradshaw@fas.harvard.edu
Shu-Yan Liu
liussyan@163.com

Specialty section:
This article was submitted to Phylogenetics, Phylogenomics, and Systematics, a section of the journal Frontiers in Ecology and Evolution

Received: *21 April 2022*
Accepted: *09 May 2022*
Published: *14 June 2022*

Citation:
Bradshaw MJ, Guan G-X, Nokes L, Braun U, Liu S-Y and Pfister DH (2022) Secondary DNA Barcodes (CAM, GAPDH, GS, and RpB2) to Characterize Species Complexes and Strengthen the Powdery Mildew Phylogeny. Front. Ecol. Evol. 10:918908. doi: 10.3389/fevo.2022.918908

Powdery mildews are a group of economically and ecologically important plant pathogens. In the past 25 years the use of ribosomal DNA (rDNA) in the powdery mildews has led to major taxonomic revisions. However, the broad scale use of rDNA has also revealed multiple species complexes that cannot be differentiated based on ITS + LSU data alone. Currently, there are only two powdery mildew taxonomic studies that took a multi-locus approach to resolve a species complex. In the present study, we introduce primers to sequence four additional regions (CAM, GAPDH, GS, and RPB2) that have the potential to improve support values in both broad and fine scale phylogenetic analyses. The primers were applied to a broad set of powdery mildew genera in China and the United States, and phylogenetic analyses included some of the common complexes. In taxa with nearly identical ITS sequences the analyses revealed a great amount of diversity. In total 154 non-rDNA sequences from 11 different powdery mildew genera were deposited in NCBI's GenBank, laying the foundation for secondary barcode databases for powdery mildews. The combined and single loci phylogenetic trees constructed generally followed the previously defined species/genus concepts for the powdery mildews. Future research can use these primers to conduct in depth phylogenetic, and taxonomic studies to elucidate the evolutionary relationships of species and genera within the powdery mildews.

Keywords: erysiphaceae, molecular phylogeny, multi-locus, powdery mildews, species complexes

INTRODUCTION

Approximately 900 species of powdery mildews (*Helotiales, Erysiphaceae*) have been described in 19 genera infecting over 10,000 plant species worldwide (Amano, 1986; Braun and Cook, 2012; Marmolejo et al., 2018; Johnston et al., 2019; Kiss et al., 2020). The taxonomy and phylogeny of these obligate plant pathogens has undergone radical change in the past 25 years as molecular methods have been widely applied (Saenz and Taylor, 1999; Braun and Takamatsu, 2000; Mori et al., 2000; Braun and Cook, 2012). The taxonomic rank of the powdery mildews (i.e. *Erysiphaceae*) has been recently resolved (Johnston et al., 2019; Haelewaters et al., 2021) using multiple loci from the full genomes of three different genera. Most of the molecular phylogenetic work conducted on this important group has focused solely on the ITS and adjacent LSU region. The biotrophic nature of powdery mildews has rendered it difficult to evaluate single copy gene regions and thus, at present, there have been only two multi-locus taxonomic studies (Qiu et al., 2020; Liu M. et al., 2021).

There are several instances of powdery mildew species complexes where morphologically distinct species cannot be delineated by ITS + LSU sequences. Examples include the *Erysiphe aquilegiae* complex (Bradshaw et al., 2021a), the *E. berberidis* complex (Liu et al., 2022)[1], the *E. elevata* complex (Tymon et al., 2022), the *E. trifoliorum* complex (Bradshaw et al., 2021b), the *Podosphaera aphanis* complex (Liu M. et al., 2021), and the *P. xanthii* complex (Shin et al., 2019). Generally, the morphological differences among species within these complexes can be difficult to discern except by experts. This has led to misidentifications that have been propagated in the literature and on public databases. Other plant-pathogen complexes have been resolved by using non-ribosomal DNA (rDNA) markers, such as, Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), calmodulin (CAL), glutamine synthetase synthase (GS), β-Tubulin (TUB2), and Actin (ACT) (*Aspergillus*-Samson et al., 2014; *Botrytis*-Saito et al., 2016; *Penicillium*-Ouhibi et al., 2018; *Colletotrichum*-Khodadadi et al., 2020). Ellingham et al. (2019) evaluated ACT, TUB2, CAL, CHS (chitin synthase), EF1- α (elongation factor 1 alpha), MCM7 (minichromosome maintenance protein 7), and TSR1 (20-S rRNA accumulation 1) to enhance identification accuracy of powdery mildews. The authors found success with MCM7 which led to a broad scale phylogenetic study by Shirouzu et al. (2020).

Markers to improve higher level support, including the powdery mildew phylogenetic backbone, have eluded this group. Recently, Shirouzu et al. (2020) evaluated MCM7, LSU, and SSU (small subunit) sequences to improve the powdery mildew phylogeny. Although their analysis improved tree resolution, low support values (less than 80 posterior values in Maximum likelihood analysis) were still present throughout their trees. Markers for higher level phylogenetic analyses in the powdery mildews are needed since support for the morphologically distinct sections, i.e., *Erysiphe* sect. *Microsphaera*, *Erysiphe* sect. *Uncinula* etc. has been elusive. Additionally, some genera are paraphyletic (*Leveillula* is nested within the *Phyllactinia* clade). With the increase of multi-locus sequences, better resolution and backbone support will be achievable.

The influx of full genome sequences of powdery mildews (there are currently 33 genomes from 14 different species on GenBank) allows the generation of specific powdery mildew primers for a range of protein and single copy genomic regions. In this manuscript, we report multiple genetic markers, and their newly designed primers, that have the potential for broad and fine scale phylogenetic evaluations of the powdery mildews.

MATERIALS AND METHODS

Sample Collection

Samples from the United States were collected at the University of Washington, Seattle, Washington, United States in 2019, the Arnold Arboretum, Boston, MA, United States, in 2021 and the Harvard University main campus, Cambridge, MA, United States in 2021 (**Table 1**). Samples from China were collected between 2017 and 2021. One herbarium specimen from the Farlow Herbarium (FH), Harvard University, was evaluated to assess the performance of the newly designed primers on an 83 year old specimen. Freshly collected specimens were deposited at the Farlow Herbarium, Harvard University (FH), and the Herbarium of Mycology of Jilin Agricultural University (HMJAU).

Primer Construction

Loci were chosen based on previous research on plant-fungal pathogen systems. For most of the regions, no powdery mildew sequences were available on GenBank. In cases where no powdery mildew sequences were available, sequences from other closely related fungi were used to blast the powdery mildew full genomes available on GenBank. There were 33 genome assemblies available on GenBank from 14 species. The blast results were downloaded into Geneious version 11.0.2[2] and aligned. From the alignment, primers were chosen and analyzed in OligoAnalyzer (integrated DNA Technologies). The following parameters were considered: primers ∼20 bps long, G/C content between 40 and 60%, double T's or double A's on the 5' or 3' end were avoided, primers ended with a GC clamp, hairpins less than ∼45°C, Delta G above ∼−9, and no more than 5°C difference in melting temperature between primer pairs.

DNA Extraction and Polymerase Chain Reaction

DNA extractions were done using the Chelex method (Walsh et al., 1991; Hirata and Takamatsu, 1996). Around 20 chasmothecia or 100 conidia were taken from the leaf surface using a sterile pipette tip for DNA extractions. Polymerase chain reaction (PCR) was carried out for the ITS and LSU region using the primer pair PM10/PM28R (Bradshaw and Tobin, 2020). When PCR was unsuccessful, a nested approach was applied

[1]Liu, L., Bradshaw, M., Braun, U., Götz, M., Khodaparast, S. A., Liu, T.-Z., et al. (2022). Phylogeny and taxonomy of *Erysiphe berberidis* (s. lat.) revisited. *Mycoscience*.

[2]https://www.geneious.com

TABLE 1 | Taxa evaluated and their associated barcodes and GenBank numbers.

Taxa evaluated	Host	Collection year	Voucher*	Locality	ITS	LSU	GAPDH	CAM	RpB2	GS
Arthrocladiella mougeotii	*Lycium chinense*	2017	HMJAU-PM92019	Changchun, Jilin, China	ON073848	ON073848		ON101636	ON119147	ON075667
Cystotheca lanestris	*Quercus rubra*	2018	FH00941225	Washington, United States	ON073849	ON073849		ON101637	ON119146	
Erysiphe aquilegiae	*Aquilegia* sp.	2021	FH00941212	Massachusetts, United States	ON073851	ON073851	ON075636	ON101639	ON119149	ON075671
Erysiphe aquilegiae	*Stylophorum diphyllum*	2021	FH00941239	Delaware, United States	ON073852	ON073852	ON075637	ON101640	ON119150	ON075672
Erysiphe aquilegiae	*Aquilegia coerulea*	2021	FH00941236	Idaho, United States	ON073850	ON073850	ON075633	ON101638	ON119148	ON075670
Erysiphe aquilegiae	*Argemone polyanthemos*	2021	FH00941255		ON073855			ON101643		ON075676
Erysiphe aquilegiae	*Clematis florida*	2020	HMJAU-PM92020	Changchun, Jilin, China				ON101642	ON119152	ON075675
Erysiphe aquilegiae	*Ranunculus repens*	2018	FH00941228	Washington, United States	ON073854	ON073854				ON075674
Erysiphe aquilegiae	*Asclepias tuberosa*	2021	FH00941240	Delaware, United States	ON073853	ON073853	ON075638	ON101641	ON119151	ON075673
Erysiphe azaleae	*Rhododendron occidentale*	2018	FH00941230	Washington, United States	ON073856	ON073856	ON075639			
Erysiphe caricae-papayae	*Carica papaya*	2021	HMJAU-PM92021	Shenzhen, Guangdong, China	ON073857	ON073857		ON101644		
Erysiphe convolvuli	*Convolvulus arvensis*	2021	FH00941244	Colorado, United States	ON073858	ON073858		ON101645	ON119153	ON075677
Erysiphe convolvuli	*Convolvus* sp.	2021	FH00941200	Massachusetts, United States	ON073859	ON073859	ON075640	ON101646	ON119154	ON075678
Erysiphe cruciferarum	*Isatis tinctoria*	2017	HMJAU-PM92022	Changchun, Jilin, China	ON073860		ON075641	ON101647		ON075679
Erysiphe digitata	*Rhododendron* sp.	2018	FH00941229	Washington, United States	ON073861	ON073861	ON075642			
Erysiphe necator	*Vitis vinifera*	2020	HMJAU-PM92023	Chengdu, Sichuan, China			ON075644	ON101649	ON119156	ON075681
Erysiphe necator	*Vitis* sp.	2021	FH00941202	Massachusetts, United States	ON073862	ON073862	ON075643	ON101648	ON119155	ON075680
Erysiphe neolycopersici	*Excoecaria cochinchinensis*	2021	HMJAU-PM92024	Shenzhen, Guangdong, China			ON075635	ON101673		ON075668
Erysiphe neolycopersici	*Solanum lycopersicum*	2021	FH00941220	Massachusetts, United States	ON073897	ON073897	ON075634	ON101674		ON075669
Erysiphe platani	*Platanus occidentalis*	2021	FH00941224	Massachusetts, United States	ON073863	ON073863		ON101650		ON075682
Erysiphe pulchra	*Cornus* sp.	2021	FH00941217	Massachusetts, United States	ON073864	ON073864		ON101651		
Erysiphe sedi	*Crassula capitella*	2017	HMJAU-PM92025	Changchun, Jilin, China	ON073865	ON073865		ON101652	ON119157	ON075683
Erysiphe syringae	*Syringa* X *hyacinthiflora*	2021	FH00941218	Massachusetts, United States	ON073866	ON073866		ON101653		ON075685
Erysiphe takamatsui	*Nelumbo nucifera*	2020	HMJAU-PM92026	Changchun, Jilin, China	ON073867	ON073867	ON075645	ON101654		ON075686
Erysiphe ulmi	*Ulmus macrocarpa*	2020	HMJAU-PM92027	China	ON073868				ON119158	
Erysiphe vaccinii	*Vaccinium vacillans*	1938	FH00112205	Tennessee, United States	ON073870	ON073870	ON075646			
Erysiphe vaccinii	*Vaccinium corymbosum*	2021	FH00941201	Massachusetts, United States	ON073869	ON073869		ON101655	ON119159	ON075687
Erysiphe vaccinii	*Vaccinium parvifolium*	2018	WTU-F-073138	Washington, United States	OK959861			ON101656		ON075688
Erysiphe vignae	*Vigna unguiculata*	2018	HMJAU-PM92028	Guangzhou, Guangdong, China	ON073844	ON073844	ON075647			
Golovinomyces ambrosiae	*Symphyotrichum patens*	2021	FH00941234	Minnesota, United States	ON073876	ON073876		ON101658	ON119165	ON075690
Golovinomyces ambrosiae	*Zinnia elegans*	2021	FH00941245	Colorado, United States	ON073878	ON073878	ON075631		ON119167	ON075691
Golovinomyces ambrosiae	*Rudbeckia fulgida*	2021	FH00941203	Massachusetts, United States			ON075630		ON119164	
Golovinomyces ambrosiae	*Asclepias syriaca*	2021	FH00941223	Massachusetts, United States	ON073873	ON073873	ON075625			

(Continued)

TABLE 1 | (Continued)

Taxa evaluated	Host	Collection year	Voucher*	Locality	ITS	LSU	GAPDH	CAM	RpB2	GS
Golovinomyces ambrosiae	*Ratibida columnifera*	2021	FH00941246	Colorado, United States	ON073842	ON073842	ON075629		ON119163	ON075689
Golovinomyces ambrosiae	*Liatris spicata*	2021	FH00941247	Colorado, United States	ON073875	ON073875	ON075628		ON119162	
Golovinomyces ambrosiae	*Eutrochium dubium*	2021	FH00941204	Massachusetts, United States	ON073874	ON073874	ON075627			
Golovinomyces ambrosiae	*Acalypha rhomboidea*	2021	FH00941205	Massachusetts, United States	ON073871	ON073871	ON075648	ON101657	ON119161	
Golovinomyces ambrosiae	*Dhalia sp.*	2021	FH00941248	Colorado, United States	ON073841	ON073841	ON075626			
Golovinomyces ambrosiae	*Verbesina alternifolia*	2021	FH00941235	Minnesota, United States	ON073877	ON073877			ON119166	
Golovinomyces ambrosiae	*Acalypha rhomboidea*	2021	FH00941206	Massachusetts, United States	ON073872	ON073872	ON075624		ON119160	
Golovinomyces asterum	*Symphyotrichum novae-angliae*	2021	FH00941249	Colorado, United States	ON073879	ON073879	ON075650	ON101659	ON119168	ON075692
Golovinomyces bolayi	*Lactuca sativa* var. *ramosa*	2017	HMJAU91770	Changchun, Jilin, China	ON073880	ON073880			ON119169	
Golovinomyces cichoracearum	*Bidens pilosa*	2021	HMJAU-PM92029	Shenzhen, Guangdong, China			ON075651			
Golovinomyces sp.	*Hydrophyllum canadense*	2021	FH00941241	Delaware, United States	ON073843	ON073843	ON075652	ON101660		
Golovinomyces latisporus	*Helianthus annuus*	2021	FH00941243	California, United States			ON075632			
Golovinomyces latisporus	*Helianthus annuus*	2021	FH00941221	Massachusetts, United States	ON073881	ON073881	ON075649			
Golovinomyces salviae	*Salvia* sp.	2021	FH00941213	Massachusetts, United States	ON073884	ON073884	ON075655	ON101663	ON119171	
Golovinomyces salviae	*Agastache scrophulariifolia*	2021	FH00941250	Colorado, United States	ON073883	ON073883	ON075654	ON101662	ON119170	ON075694
Golovinomyces sp.	*Phacecia bipinnetifida*	2021	FH00941242	Delaware, United States	ON073882	ON073882	ON075653	ON101661		ON075693
Leveillula taurica	*Capsicum annuum*	2019	HMJAU-PM92030	Chifeng, Inner Mongolia, China	ON073885	ON073885		ON101664		
Leveillula taurica	*Cleome serrulata*	2021	FH00941251	Colorado, United States	ON073886	ON073886		ON101665	ON119172	
Leveillula taurica	*Cleome serrulata*	2021	FH00941238	Colorado, United States	ON073887	ON073887		ON101666		
Neoërysiphe galeopsidis	*Lamium purpureum*	2018	FH00941231	Washington, United States	ON073888		ON075656			
Phyllactinia betulae	*Betula nigra*	2021	FH00941214	Massachusetts, United States	ON073889	ON073889		ON101667	ON119173	
Phyllactinia betulae	*Betula nigra*	2021	FH00941207	Massachusetts, United States	ON073890	ON073890		ON101668	ON119174	
Phyllactinia mali	*Crataegus* sp.	2018	FH00941226	Washington, United States	ON073891	ON073891			ON119175	
Phyllactinia moricola	*Morus alba*	2019	HMJAU-PM91933	Yantai, Shangdong, China	MZ541088	MZ540403		ON101669	ON119176	
Phyllactinia populi	*Populus simonii*	2020	HMJAU-PM92031	Changchun, Jilin, China				ON101670	ON119177	
Phyllactinia pyri-serotinae	*Pyrus ussuriensis*	2020	HMJAU-PM92032	Changchun, Jilin, China	ON073892	ON073892		ON101671	ON119178	
Phyllactinia sp.	*Oemlaria cerasiformis*	2018	FH00941232	Washington, United States				ON101672		
Podosphaera fuliginea	*Veronica spicata*	2021	FH00941252	Colorado, United States	ON073893	ON073893			ON119181	
Podosphaera leucotricha	*Malus* 'Williamette'	2021	FH00941208	Massachusetts, United States	ON073894	ON073894			ON119182	
Podosphaera sp.	*Rubus spectabilis*	2018	FH00941227	Washington, United States					ON119180	
Podosphaera sp.	*Geranium viscosissimum*	2021	FH00941237	Idaho, United States					ON119184	
Podosphaera sp.	*Geranium* 'Gerwat'	2021	FH00941253	Colorado, United States					ON119179	
Podosphaera sp.	*Rhus typhina*	2021	FH00941209	Massachusetts, United States					ON119186	
Podosphaera sp.	*Rhus glabra*	2021	FH00941210	Massachusetts, United States					ON119185	

(Continued)

TABLE 1 | (Continued)

Taxa evaluated	Host	Collection year	Voucher*	Locality	ITS	LSU	GAPDH	CAM	RpB2	GS
Podosphaera sp.	*Euphorbia alfredii*	2021	FH00941233	Georgia, United States					ON119183	
Podosphaera tridactyla	*Padus racemosa*	2019	HMJAU-PM92033	Changchun, Jilin, China	ON073895	ON073895	ON075657		ON119187	
Podosphaera xanthii	*Cucumis melo*	2019	HMJAU-PM92034	Changchun, Jilin, China			ON075658			
Podosphaera xanthii	*Cucumis sativus*	2018	HMJAU-PM92041	Changchun, Jilin, China			ON075659			
Podosphaera xanthii	*Cucurbita moschata*	2020	HMJAU-PM92035	Changchun, Jilin, China			ON075660			
Podosphaera xanthii	*Cucurbita pepo*	2020	HMJAU-PM92036	Changchun, Jilin, China			ON075661			
Podosphaera xanthii	*Impatiens balsamina*	2019	HMJAU-PM92037	Yancheng, Jiangsu, China			ON075662			
Podosphaera xanthii	*Lagenaria siceraria*	2021	FH00941254	Colorado, United States			ON075663			
Pseudoidium hortensiae	*Hydrangea macrophylla*	2021	HMJAU-PM92038	Shenzhen, Guangdong, China	ON073896	ON073896			ON119188	
Salmonomyces acalyphae	*Acalypha supera*	2019	HMJAU-PM91903	Kunming, Yunnan, China	MZ603889	MZ603889				ON075684
Salmonomyces acalyphae	*Acalypha supera*	2019	HMJAU-PM91904	Kunming, Yunnan, China				ON101675	ON119189	
Sawadaea bicornis	*Acer* sp.	2021	FH00941215	Massachusetts, United States			ON075664			
Sawadaea tulasnei	*Acer platanoides*	2021	FH00941216	Massachusetts, United States			ON075666			
Takamatsuella circinata	*Acer pycananthum*	2021	FH00941219	Massachusetts, United States			ON075665			
Ampelomyces quisqualis	*Podosphaera xanthii*	2021	HMJAU-PM92039	Shenzhen, Guangdong, China			ON101677			
Ampelomyces quisqualis	*Podosphaera xanthii*	2021	HMJAU-PM92040	Changchun, Jilin, China			ON101676			

**HMJAU, Herbarium Mycology of Jilin Agricultural University; FH, Farlow Herbarium, Harvard University, United States.*

TABLE 2 | Primers generated in the present study and the genera in which amplicons were generated.

Region	Primers	Primer sequence	Amplicon size	Genera succesfully sequenced*
GAPDH	PMGAPDH1	GGAATGGCTATGCGTGTACC	∼300 bps	*Erysiphe, Golovinomyces, Neoerysiphe, Podosphaera, Sawadaea,* and *Takamatsuella*
	PMGAPDH3R	CCCCATTCGTTGTCGTACCATG		
CAM	PMCAM1	CTTTGCATCATGAGTTGGAC	∼300 bps	*Arthrocladiella, Cystotheca, Erysiphe, Golovinomyces, Leveillula, Phyllactinia, Podosphaera,* and *Salmonomyces.*
	PMCAM4R	GGCTCGAAAAATGAAAGATACCG		
GS	GSPM2	CCAATCAGTTACTGTTTGTTCCC	∼500 bps	*Arthrocladiella, Erysiphe, Golovinomyces,* and *Salmonomyces.*
	GSPM3R	GGACTTCCTGATATTATGCC		
RpB2	PmRpb2_4	GCAAGCTCAACTGCTGGTG	∼800 bps	*Arthrocladiella, Cystotheca, Erysiphe, Golovinomyces, Leveillula, Phyllactinia, Podosphaera, Salmonomyces,* and *Sawadaea*
	PMRpb2_6R	TCCAGCGATGTGCTGTTGG		

**All the genera were not available for sequencing i.e., if a genus is not listed it does not mean the primers will not anneal to it. For some genera, new primers will need to be constructed to ensure proper annealing.*

using the primers AITS (Bradshaw and Tobin, 2020) and TW14 (Mori et al., 2000); followed by PM10 and PM28R or AITS and PM11 (Bradshaw and Tobin, 2020); followed by PM10 and PM2 (Cunnington et al., 2003). For the CAL, GAPDH, GS, and RPB2 regions the primer pairs from **Table 2** were used.

PCR specifications for all the regions were as follows for a 50 μl solution reaction: 35.7 μl molecular grade H_2O, 4 μl BSA (20 mg/ml), 1 μl of forward primer and 1 μl reverse primer at a 10 μM concentration, 2 μl of DNA, 5 μl of Buffer, 0.3 μl of TAQ DNA Polymerase (5 units per μl) and 1 μl dNTPs (10 mM). Cycling included initial denaturation at 95°C for 3 min followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 1 min, and elongation at 72°C for 2 min and then a final elongation of 72°C for 10 min.

In the United States laboratory crude PCR products were sent to Eurofins (Luxembourg) to be purified and directly sequenced in the forward and reverse directions using the primers above. Samples in China were sent to Sangon Biotech (Shanghai, China) for sequencing in both the forward and reverse direction.

Phylogenetic Analyses

To show the potential of the primers and their ability to anneal to a variety of species in different genera, phylogenetic trees were constructed with a general focus on common powdery mildew complexes. A concatenated, GAPDH-CAM-GS-ITS-LSU-RPB2, tree was generated to show their potential to improve higher level support. In the concatenated tree, sequences from different specimens of *Salmonomyces acalyphae* were spliced together. Large gaps up to ∼20 bps were manually deleted in the ITS and GAPDH alignments prior to analyses. The regions evaluated were mined from the full genomes of *Blumeria graminis* (Assembly accession: GCA_905067625.1), *Phyllactinia moricola* (GCA 019455665), *Pleochaeta shiraiana* (GCA 019455505), *Podosphaera cerasi* (GCA 018398735), *Podosphaera leucotricha* (GCA 013170925), and *Podosphaera xanthii* (GCA 010015925 and GCA 014884795) to be included in some of the analyses. The full genome of *Arachnopeziza araneosa* (GCA_00398855) was chosen as an outgroup taxon based on the analyses by Johnston et al. (2019) for the concatenated and single loci trees. An ITS + LSU tree was constructed using all the sequences obtained for comparative purposes. Sequences were aligned and edited using MUSCLE in MEGA11:Molecular Evolutionary Genetics Analysis version 11 (Tamura et al., 2021). A GTR + G + I evolutionary model was used for phylogenetic analyses as it is the most inclusive model of evolution and includes all other evolutionary models (Abadi et al., 2019). A fixed parameter-rich model (such as GTR + G + I) can be used in lieu of running a test to select the most suitable evolutionary model (Abadi et al., 2019).

For all the trees the phylogeny was inferred using Bayesian analysis using a Yule tree prior (Gernhard, 2008) and a strict molecular clock, in the program BEAST version 1.10.4 (Suchard et al., 2018). A single MCMC chain of 10^6 steps was run, with a burn-in of 10%. Posterior probabilities were calculated from the remaining 9,000 sampled trees. A maximum clade credibility tree was produced using TreeAnnotator version 1.10.4 (part of the BEAST package). Stationarity was confirmed by running the analysis multiple times, which revealed convergence between runs. The resulting tree was visualized using FigTree version 1.3.1 (Rambaut, 2009)[3]. A maximum likelihood analysis was accomplished using raxmlGUI (Silvestro and Michalak, 2012) under the default settings with a GTR + G + I evolutionary model. Bootstrap analyses were conducted using 1,000 replications (Felsenstein, 1985).

RESULTS

Primer Construction and Sequencing

Eight primers were successfully constructed and applied to 11 out of the 19 powdery mildew genera (**Table 2**). For the GAPDH region we sequenced an herbarium specimen that was

[3]Rambaut, A. (2009). *Fig Tree ver. 1.3.1.*. Available online at: http://tree.bio.ed.ac.uk/software/figtree.

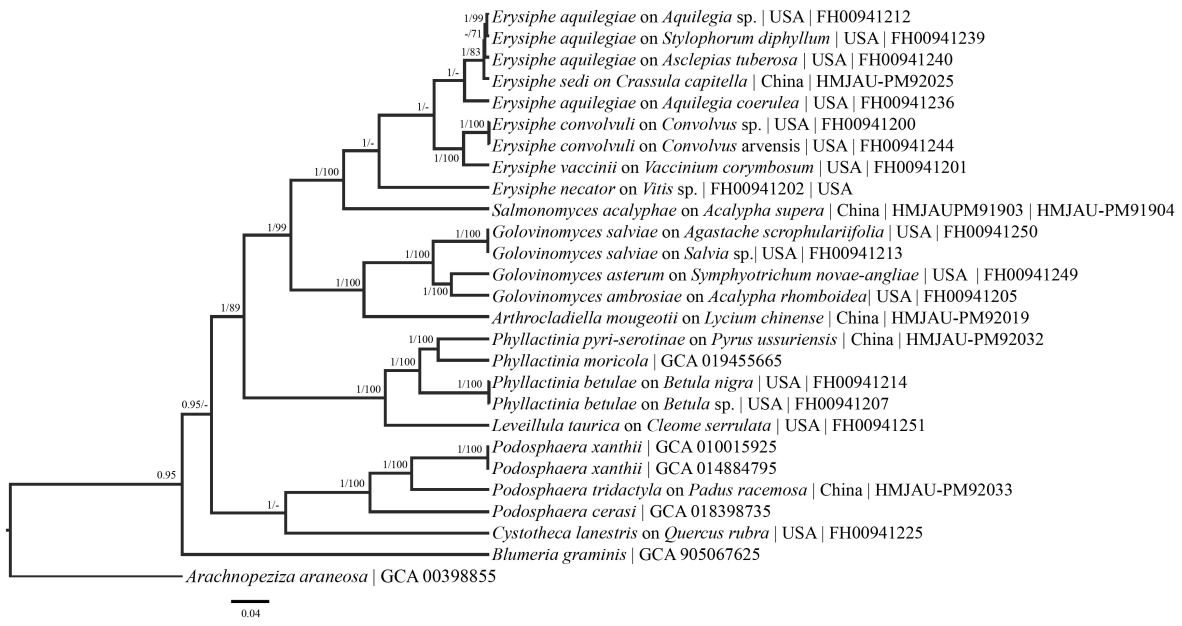

FIGURE 1 | Bayesian maximum clade credibility tree of sequences from the combined GAPDH-CAM-GS-ITS-LSU-RPB2 regions. Posterior probabilities > 90 are displayed followed by bootstrap values greater than 70% for the maximum likelihood (ML) analyses conducted. High support values are present throughout the tree. Taxa names are followed by host taxa, collection locality, and voucher/GenBank numbers.

83 years old (1938: FH00112205). We were unable to sequence the specimen from 1938 using the other regions likely due to the size of the amplicons. For the GS, RPB2, and CAM regions multiple specimens were sequenced that were up to 3 years old. In total 55 sequences were generated from the ITS/LSU loci and 310 sequences were generated from non-rDNA loci: 74 from CAM, 134 from the GAPDH, 52 from GS, and 50 from RPB2. Of these 310 sequences, 154 (43 from GAPDH, 38 from CAM, 44 from RPB2, and 29 from GS) were used for phylogenetic analysis and deposited in GenBank. In the course of the study, the GAPDH primers were found to anneal to other fungi. In particular, multiple *Ampelomyces* sequences were generated that aligned with both GCA 018398575 and GCA 010094095. We deposited 3 GAPDH sequences from *Ampelomyces* spp. in GenBank that could potentially assist future researchers evaluating GAPDH of *Ampelomyces* spp.

Phylogenetic Analyses

Amplicons for the specimens obtained were deposited in GenBank (**Table 1**). Sequences from the GAPDH, GS, CAM, and RPB2 regions were evaluated individually and in a concatenated tree that included ITS + LSU sequences. A separate ITS + LSU tree from the sequences obtained in this study is presented in **Supplementary Figure 1**. Six phylogenetic trees were constructed and presented (**Figures 1–6**). For the majority of these regions, no additional sequences were available on GenBank and could not be evaluated for phylogenetic purposes. A maximum clade credibility tree was constructed using Bayesian analyses from the single loci and combined sequences. Posterior probabilities > 90 are displayed followed by bootstrap values greater than 70% for the maximum likelihood (ML) analyses conducted. The representative maximum clade credibility tree is illustrated in **Figure 1**.

The phylogenetic analyses revealed that the different regions have great potential for splitting up the ITS + LSU complexes and increasing the backbone support of the powdery mildews. In the ITS + LSU tree (**Supplementary Figure 1**) there is no support seen within the *E. aquilegiae* or *G. ambrosiae* clade whereas support is seen within the *E. aquilegiae* clade in the concatenated (**Figure 1**) and RPB2 tree (**Figure 2**) and the *G. ambrosiae* clade in the GAPDH tree (**Figure 6**). There is also much better higher level support throughout the concatenated tree than in the ITS + LSU tree alone (**Supplementary Figure 1**). For example, in the concatenated tree (**Figure 1**), there is high support that *E. convolvuli* and *E. vaccinii* form a clade yet no

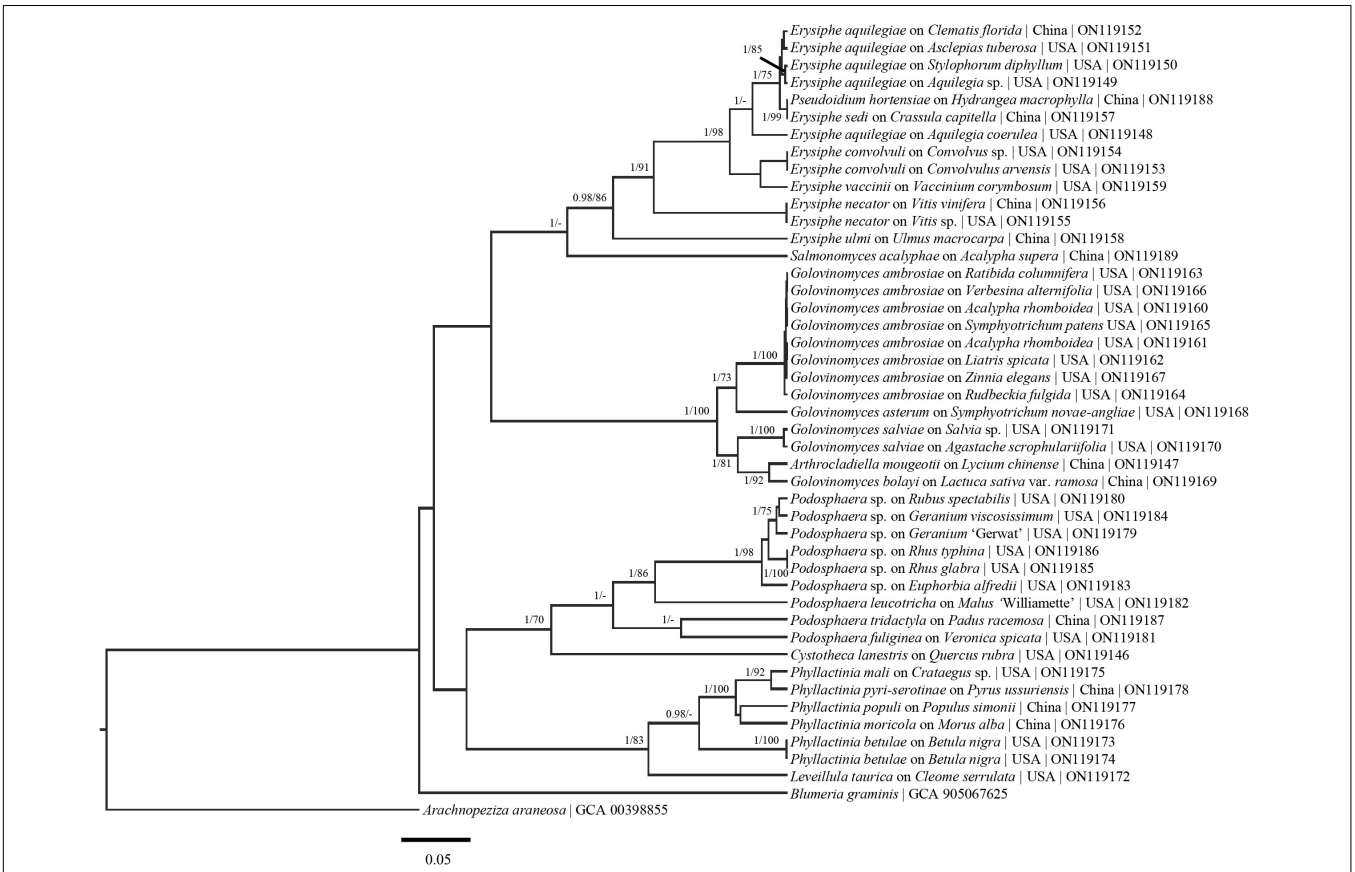

FIGURE 2 | Bayesian maximum clade credibility tree of sequences from the RPB2 region. Posterior probabilities > 90 are displayed followed by bootstrap values greater than 70% for the maximum likelihood (ML) analyses conducted. Generally, previously defined genus/species concepts are seen as well as some broad and fine scale support (*E. aquilegiae* complex). Taxa names are followed by host taxa, collection locality and GenBank numbers.

support for this grouping in the ITS + LSU tree. Additionally, in the ITS + LSU, unlike the concatenated tree, there is no support for the placement of *Blumeria* and the *Phyllactinia-Leveillula* clade.

DISCUSSION

The evaluation of protein-coding genes for phylogenetic analyses has largely been understudied in the powdery mildews. Relying solely on rDNA in analyses has led to the recognition of species complexes that group morphologically dissimilar taxa that are genetically similar in ITS and LSU profiles. In the present study, we designed primers for four protein coding genes. We have shown that these genes have potential for refining taxonomic/phylogenetic studies of the powdery mildews. Additionally, the divergent nature of these genes shows their potential in phylogenetic/taxonomic studies.

Secondary Barcodes

In recent years the ITS region has come under scrutiny due to its potential for intragenomic variation and its inability to differentiate cryptic species (Seifert et al., 1995; Simon and Weiss, 2008; Kovács et al., 2011; Kiss, 2012). Studies are finding that the ITS and adjacent LSU regions are unable to identify a large percentage of species, emphasizing the importance of secondary markers for certain fungal lineages (Badotti et al., 2017; Vu et al., 2019). Secondary barcodes can be important for understanding the species and evolutionary relationships in the Kingdom Fungi and in particular, the *Ascomycota* (Tanabe et al., 2005; Stielow et al., 2015; Ellingham et al., 2019; Johnston et al., 2019; Meyer et al., 2019). Recently, secondary markers including TEF1α, TOP1, and PGK have been established for species identification in fungi (Stielow et al., 2015; Meyer et al., 2019).

There have been two powdery mildew phylogenetic/taxonomic publications that evaluated secondary barcodes to resolve complexes. Qiu et al. (2020) used five genes (ITS, LSU, IGS, TUB2, and CHS1) to increase resolution in the *Golovinomyces ambrosiae* complex. Using solely GAPDH (**Figure 6**) we were able to delimit taxa of this complex, consistent with Qiu et al. (2020). Liu S. Y. et al. (2021) used four genes (ITS, CHS1, and fragments of two unnamed orthologous genes) to split the *Blumeria graminis* complex into eight separate species. One limitation of Liu S. Y. et al. (2021) is that using unnamed orthologous genes and their associated primers can be difficult to apply broadly to other powdery mildew taxa.

In the present research we have shown that the genes evaluated have the potential to resolve multiple powdery mildew complexes. For example, the *E. aquilegiae* complex forms well supported groups in the concatenated tree, as well as in some of the single loci (RpB2, RPB2, and GAPDH) trees (**Figures 1**, **2**, **4**, **5**). Additionally, groups, such as the *Podosphaera xanthii* complex, will likely be able to be clarified by taking a multi-locus approach on a range of hosts from throughout the world. GAPDH is especially promising for the *P. xanthii* complex where in phylogenetic analysis two separate groups formed (**Figure 5**). The divergent nature of some of these sequences of species with close ITS affinity, provides evidence that the ITS

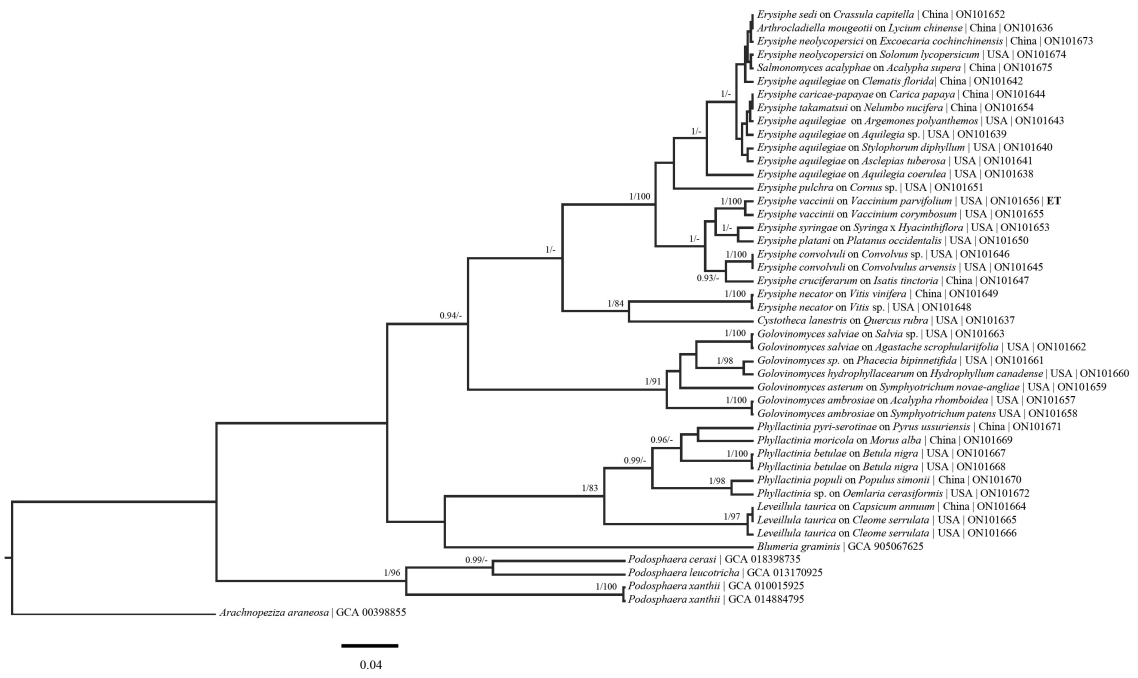

FIGURE 3 | Bayesian maximum clade credibility tree of sequences from the CAM region. Posterior probabilities > 90 are displayed followed by bootstrap values greater than 70% for the maximum likelihood (ML) analyses conducted. Generally, previously defined genus/species concepts are seen as well as some broad scale support. ET, ex epitype. Taxa names are followed by host taxa, collection locality, and GenBank numbers.

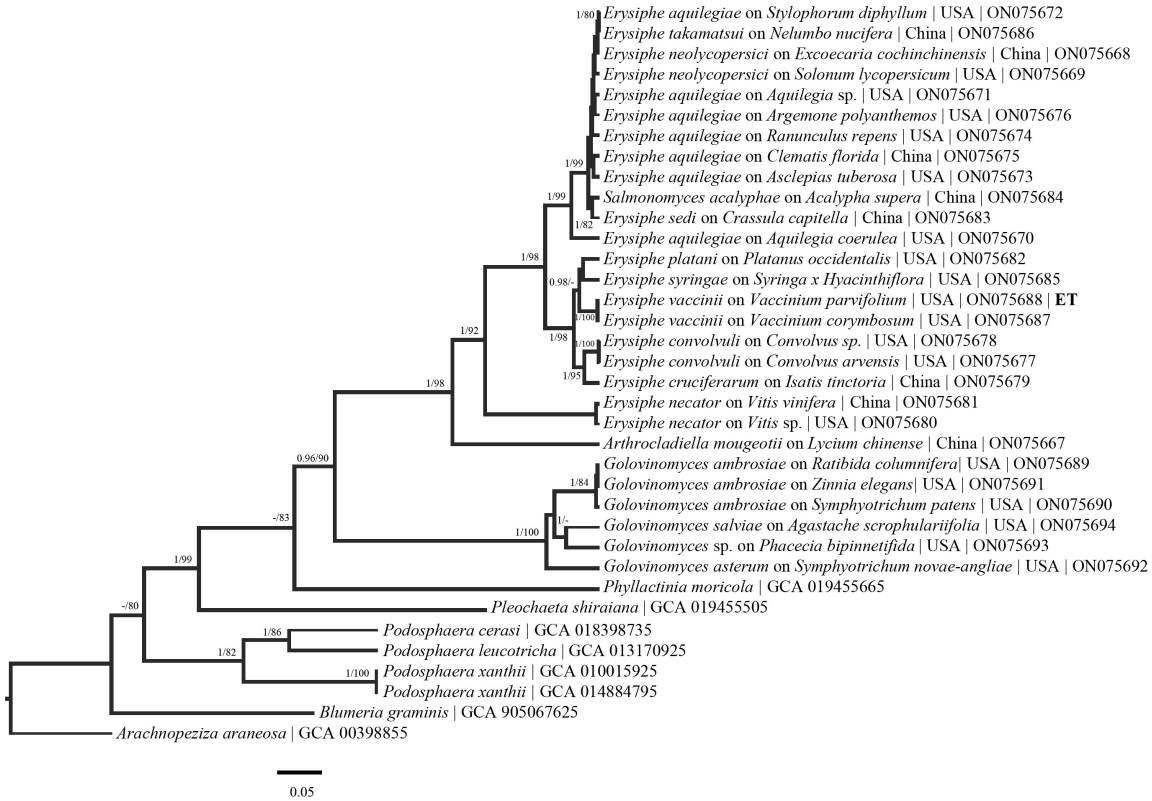

FIGURE 4 | Bayesian maximum clade credibility tree of sequences from the GS region. Posterior probabilities > 90 are displayed followed by bootstrap values greater than 70% for the maximum likelihood (ML) analyses conducted. Generally, previously defined genus/species concepts are seen as well as some broad scale support. ET, ex epitype. Taxa names are followed by host taxa, collection locality and GenBank numbers.

could not be accounting for evolutionary relationships within the powdery mildews. GAPDH is phylogenetically informative for cryptic species (Matsuda et al., 2015; Vélez et al., 2021) in other fungal systems and should be applied broadly to the powdery mildews. We suggest that GAPDH be used in conjunction with ITS for species identification due to the high variation between GAPDH sequences and the GAPDH primers ability to anneal to herbarium specimens.

Building a Better Backbone

Higher level support, not observed in previous powdery mildew studies (Bradshaw and Tobin, 2020; Shirouzu et al., 2020), can be seen in the concatenated tree (**Figure 1**). Additionally, each region evaluated generally follows the species/genus concepts established for the powdery mildews (**Figures 1**– **5**). The phylogenetic analyses presented using these underexplored loci demonstrate their potential to resolve relationships in major clades and to determine the powdery mildew sister group. The exploration of additional loci will also likely lead to major genus level taxonomic changes. For example, in the RPB2 tree (**Figure 2**) *Arthrocladiella* is nested in the *Golovinomyces* clade. This situation is also seen in the CAM and GS tree where *Salmonomyces* is nested within the *Erysiphe* clade (**Figures 3**, **4**). When evaluating "6" gene phylogenies, limitations, including single genes having a comparatively large evolutionary pull, need to be considered before any conclusions are made. For example, in the present manuscript the placement of *Blumeria* (**Figure 2**) does not align with previous studies (Bradshaw and Tobin, 2020; Shirouzu et al., 2020) that placed *Blumeria* sister to *Podosphaera*. It is likely that the RPB2 and GS loci are driving the evolution of *Blumeria*. Additional taxa, genomic regions and above all, full genome sequences need to be acquired and analyzed to solidify the taxonomy and phylogeny of this group.

CONCLUSION

The presented sequences now located in GenBank can serve as reference sequences to help future researchers determine the genus and species present in their collections. The primers evaluated were able to anneal to a broad range of powdery mildew species in multiple genera (**Table 2**). Future research can employ these primers to assist in phylogenetic and taxonomic studies of species complexes to elucidate the evolutionary relationships of the species in question. Furthermore, the sequences evaluated revealed limited contamination showing the primers specificity to powdery mildews except for the GAPDH primers which readily annealed to *Ampelomyces quisqualis* s. lat. (the mycopathogen of powdery mildew). The secondary barcode sequences provided

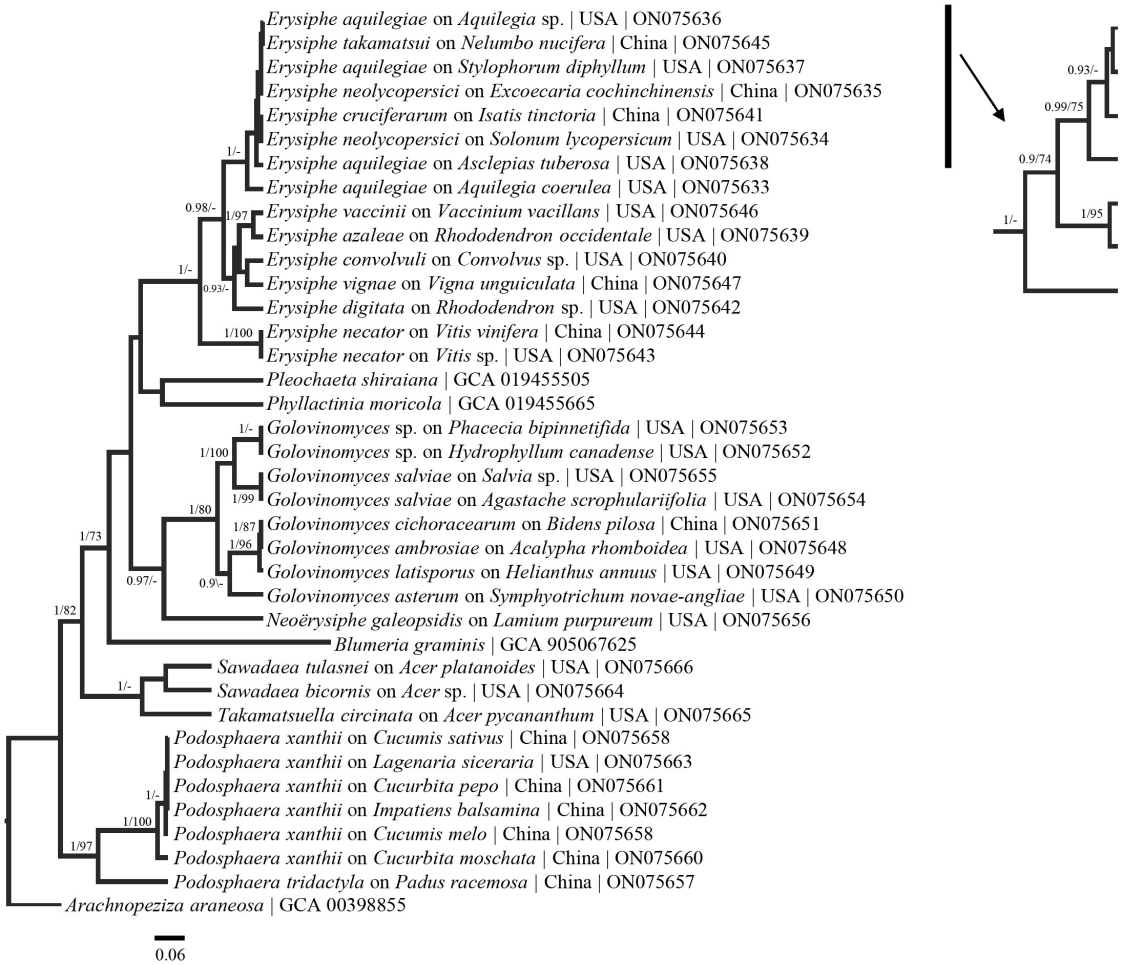

FIGURE 5 | Bayesian maximum clade credibility tree of sequences from the GAPDH region. Posterior probabilities > 90 are displayed followed by bootstrap values greater than 70% for the maximum likelihood (ML) analyses conducted. Generally, previously defined genus/species concepts are seen as well as some broad and fine scale support (*E. aquilegiae* and *P. xanthii* complexes). Taxa names are followed by host taxa, collection locality and GenBank numbers.

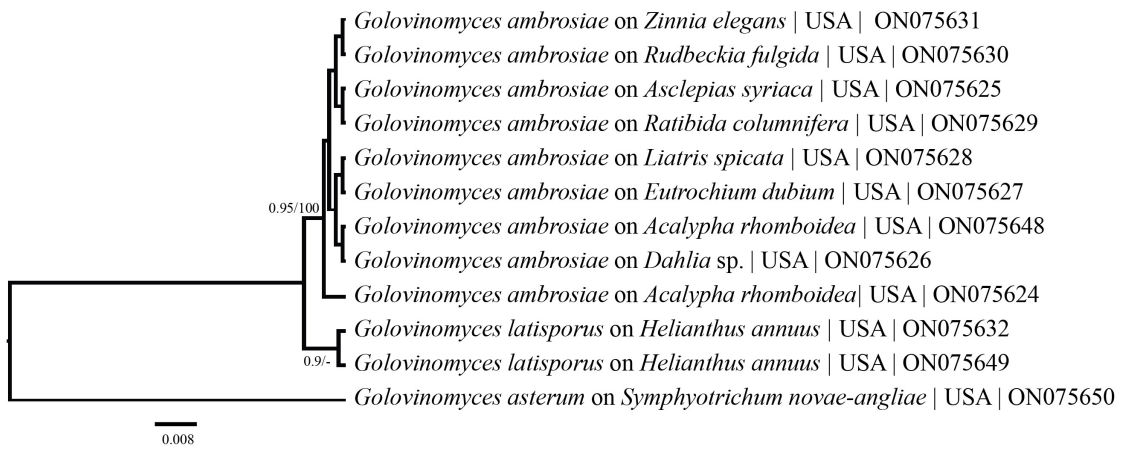

FIGURE 6 | Bayesian maximum clade credibility tree of GAPDH sequences from species in the *Golovinomyces ambrosiae* complex. Posterior probabilities > 90 are displayed followed by bootstrap values greater than 70% for the maximum likelihood (ML) analyses conducted. Support exists in the tree for the separation of *G. ambrosiae* and *G. latisporus* as defined by Qiu et al. (2020). Taxa names are followed by host taxa, collection locality and GenBank numbers.

can be further mined to generate species/genus specific primers and to improve success with herbarium specimens. Interspecies variation in virulence and fungicide resistance as well as the host specific nature of powdery mildews emphasizes the importance of species identification for these economically important plant pathogens.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

MB designed the experiment, wrote the manuscript, conducted the phylogenetic analysis, designed some of the primers, and sequenced the specimens from the United States. G-XG sequenced the specimens from China and submitted all the sequences to GenBank. LN designed some of the primers. UB helped design the experiment. S-YL helped fund the manuscript and assisted with sequencing the specimens from China. DP helped design the experiment and obtain funding for the analyses. All authors assisted with editing the manuscript.

FUNDING

We would like to thank the National Natural Science Foundation of China (31970019 and U21A20177), the Daniel E. Stuntz Memorial Foundation, the Puget Sound Mycological Society, and the Sonoma County Mycological Association for funding this research.

ACKNOWLEDGMENTS

We would like to thank the curatorial team at FH for helping with the specimens and the staff of the Arnold Arboretum for allowing access to collect fresh specimens. We would also like to thank Daniel Murphy at the Idaho Botanical Garden, Tom G. Potterfield and Elan Alford at the Mt. Cuba Center, Cindy Newlander at the Denver Botanical Garden, Erin Buchholz at the Minnesota Landscape Arboretum, Anna Bower at Lotus land, and Wade Enos at the Atlanta Botanica Garden for helping with specimen collections.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.918908/full#supplementary-material

Supplementary Figure 1 | Bayesian maximum clade credibility tree of sequences generated for the current study for the ITS + LSU region.

REFERENCES

Abadi, S., Azouri, D., Mayrose, I., and Pupko, T. (2019). Model selection may not be a mandatory step for phylogeny reconstruction. *Nat. Commun.* 10:934. doi: 10.1038/s41467-019-08822-w

Amano, K. (1986). *Host Range and Geographical Distribution of the Powdery Mildew Fungi*, 2nd Edn. Tokyo: Scientific Societies Press.

Badotti, F., de Oliveira, F. S., Garcia, C. F., Vaz, A. B. M., Fonseca, P. L. C., Nahum, L. A., et al. (2017). Effectiveness of ITS and sub-regions as DNA barcode markers for the identification of Basidiomycota (Fungi). *BMC Microbiol.* 17:42. doi: 10.1186/s12866-017-0958-x

Bradshaw, M., Braun, U., Götz, M., Takamatsu, S., Brand, T., Cabrera, M. G., et al. (2021a). Contributions to the phylogeny and taxonomy of the Erysiphaceae (powdery mildews)—part 1. *Sydowia* 73, 89–112.

Bradshaw, M., Braun, U., Götz, M., and Jurick, W. II (2021b). Phylogeny and taxonomy of powdery mildew caused by *Erysiphe* species on *Lupinus* hosts. *Mycologia* 114, 76–88. doi: 10.1080/00275514.2021.1973287

Bradshaw, M., and Tobin, P. (2020). Sequencing herbarium specimens of a common detrimental plant pathogen (powdery mildew). *Phytopathology* 110, 1248–1254. doi: 10.1094/PHYTO-04-20-0139-PER

Braun, U., and Cook, R. T. A. (2012). *Taxonomic Manual of the Erysiphales (Powdery Mildews). CBS Biodiversity Series No. 11*. Utrecht: CBS-KNAW Fungal Biodiversity Centre.

Braun, U., and Takamatsu, S. (2000). Phylogeny of *Erysiphe, Microsphaera, Uncinula* (Erysipheae) and *Cystotheca, Podosphaera, Sphaerotheca* (Cystotheceae) inferred from rDNA ITS sequences–Some taxonomic consequences. *Schlechtendalia* 4, 1–33.

Cunnington, J., Takamatsu, H., Lawrie, S., and Pascoe, A. (2003). Molecular identification of anamorphic powdery mildews (Erysiphales). *Australas. Plant Pathol.* 32, 421–428.

Ellingham, O., David, J., and Culham, A. (2019). Enhancing identification accuracy for powdery mildews using previously underexploited DNA loci. *Mycologia* 111, 798–812. doi: 10.1080/00275514.2019.1643644

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783e–791e. doi: 10.1111/j.1558-5646.1985.tb00420.x

Gernhard, T. (2008). The conditioned reconstructed process. *J. Theor. Biol.* 253, 769–778. doi: 10.1016/j.jtbi.2008.04.005

Haelewaters, D., Park, D., and Johnston, P. R. (2021). Multilocus phylogenetic analysis reveals that Cyttariales is a synonym of Helotiales. *Mycol. Prog.* 20, 1323–1330. doi: 10.1007/s11557-021-01736-2

Hirata, T., and Takamatsu, S. (1996). Nucleotide sequence diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. *Mycoscience* 37, 283–288.

Johnston, P. R., Quijada, L., Smith, C., Baral, H. O., Hosoyo, T., Baschien, C., et al. (2019). A multigene phylogeny toward a new phylogenetic classification of Leotiomycetes. *IMA fungus* 10:1. doi: 10.1186/s43008-019-0002-x

Khodadadi, F., Gonzalez, J. B., Martin, P. L., Giroux, E., Bilodeau, G. J., Peter, K. A., et al. (2020). Identification and characterization of *Colletotrichum* species causing apple bitter rot in New York and description of *C. noveboracense* sp. nov. *Sci. Rep.* 10:11043. doi: 10.1038/s41598-020-66761-9

Kiss, L. (2012). Limits of nuclear ribosomal DNA internal transcribed spacer (ITS) sequences as species barcodes for Fungi. *Proc. Natl. Acad. Sci. U.S.A.* 109:E1811. doi: 10.1073/pnas.1207143109

Kiss, L., Vaghefi, N., Bransgrove, K., Dearnaley, J. D. W., Takamatsu, S., Tan, Y. P., et al. (2020). Australia: A Continent Without Native Powdery Mildews? The First Comprehensive Catalog Indicates Recent Introductions and Multiple Host Range Expansion Events, and Leads to the Re-discovery of *Salmonomyces* as a New Lineage of the Erysiphales. *Front. Microbiol.* 11:1571. doi: 10.3389/fmicb.2020.01571

Kovács, G. M., Jankovics, T., and Kiss, L. (2011). Variation in the nrDNA ITS sequences of some powdery mildew species: do routine molecular identification procedures hide valuable information? *Eur. J. Plant Pathol.* 131, 135–141.

Liu, M., Braun, U., Takamatsu, S., Hambleton, S., Shoukouhi, P., Bisson, K. R., et al. (2021). Taxonomic revision of *Blumeria* based on multi-gene DNA sequences, host preferences and morphology. *Mycoscience* 62, 143–165. doi: 10.47371/mycosci.2020.12.003

Liu, S. Y., Jin, D., Götz, M., Bradshaw, M., Liu, M., Takamatsu, S., et al. (2021). Phylogeny and taxonomy of *Podosphaera filipendulae* (Erysiphaceae) revisited. *Mycoscience* 62, 390–394. doi: 10.47371/mycosci.2021.07.001

Marmolejo, J., Siahaan, S. A. S., Takamatsu, S., and Braun, U. (2018). Three new records of powdery mildews found in Mexico with one genus and one new species proposed. *Mycoscience* 59, 1–7. doi: 10.1016/j.myc.2017.06.010

Matsuda, Y., Takeuchi, K., Obase, K., and Ito, S. (2015). Spatial distribution and genetic structure of *Cenococcum geophilum* in coastal pine forests in Japan. *FEMS Microbiol. Ecol.* 91:fiv108. doi: 10.1093/femsec/fiv108

Meyer, W., Irinyi, L., Hoang, M. T. V., Robert, V., Garcia-Hermoso, D., Desnos-Ollivier, M., et al. (2019). Database establishment for the secondary fungal DNA barcode translational elongation factor 1α (TEF1α)1. *Genome* 62, 160–169. doi: 10.1139/gen-2018-0083

Mori, Y., Sato, Y., and Takamatsu, S. (2000). Evolutionary Analysis of the Powdery Mildew Fungi Using Nucleotide Sequences of the Nuclear Ribosomal DNA. *Mycologia* 92:74. doi: 10.2307/3761452

Ouhibi, S., Santos, C., Ghali, R., Soares, C., Hedhili, A., Paterson, R., et al. (2018). (2018) *Penicillium tunisiense* sp. nov., a novel species of *Penicillium* section Ramosa discovered from Tunisian orchard apples. *Int. J. Syst. Evol. Microbiol.* 68, 3217–3225. doi: 10.1099/ijsem.0.002962

Qiu, P.-L., Liu, S.-Y., Bradshaw, M., Rooney-Latham, S., Takamatsu, S., Bulgakov, T. S., et al. (2020). Multi-locus phylogeny and taxonomy of an unresolved, heterogeneous species complex within the genus *Golovinomyces* (Ascomycota, Erysiphales), including *G. ambrosiae, G. circumfusus* and *G. spadiceus*. *BMC Microbiol.* 20:51. doi: 10.1186/s12866-020-01731-9

Saenz, G. S., and Taylor, J. W. (1999). Phylogeny of the Erysiphales (powdery mildews) inferred from internal transcribed spacer ribosomal DNA sequences. *Can. J. Bot.* 77, 150–168. doi: 10.1139/b98-235

Saito, S., Margosan, D., Michailides, T. J., and Xiao, C. L. (2016). *Botrytis californica*, a new cryptic species in the *B.cinerea* species complex causing gray mold in blueberries and table grapes. *Mycologia* 108, 330–343. doi: 10.3852/15-165

Samson, R. A., Visagie, C., Houbraken, J., Hong, S.-B., Hubka, V., Klaassen, C. H., et al. (2014). Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Stud. Mycol.* 78, 141–173. doi: 10.1016/j.simyco.2014.07.004

Seifert, K. A., Wingfield, B. D., and Wingfield, M. J. (1995). A critique of DNA sequence analysis in the taxonomy of filamentous Ascomycetes and ascomycetous anamorphs. *Can. J. Bot.* 73, 760–767.

Shin, H. D., Meeboon, J., Takamatsu, S., Adhikari, M. K., and Braun, U. (2019). Phylogeny and taxonomy of *Pseudoidium pedaliacearum*. *Mycol. Prog.* 18, 237–246. doi: 10.1007/s11557-018-1429-y

Shirouzu, T., Takamatsu, S., Hashimoto, A., Meeboon, J., and Ohkuma, M. (2020). Phylogenetic overview of Erysiphaceae based on nrDNA and MCM7 sequences. *Mycoscience* 61, 249–258. doi: 10.1016/j.myc.2020.03.006

Silvestro, D., and Michalak, I. (2012). RaxmlGUI: A graphical front-end for RAxML. *Organ. Divers. Evol.* 12, 335–337. doi: 10.1007/s13127-011-0056-0

Simon, U. K., and Weiss, M. (2008). Intragenomic variation of fungal ribosomal genes is higher than previously thought. *Mol. Biol. Evol.* 25, 2251–2254. doi: 10.1093/molbev/msn188

Stielow, J. B., Lévesque, C. A., Seifert, K. A., Meyer, W., Iriny, L., Smits, D., et al. (2015). One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia* 35, 242–263. doi: 10.3767/003158515X689135

Suchard, M. A., Lemey, P., Baele, G., Ayres, D. L., Drummond, A. J., and Rambaut, A. (2018). Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evol.* 4:Vey016. doi: 10.1093/ve/vey016

Tamura, K., Stecher, G., and Kumar, S. (2021). MEGA11: molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* 38, 3022–3027. doi: 10.1093/molbev/msab120

Tanabe, Y., Watanabe, M. M., and Sugiyama, J. (2005). Evolutionary relationships among basal fungi (Chytridiomycota and Zygomycota): insights from molecular phylogenetics. *J. Gen. Appl. Microbiol.* 51, 267–276. doi: 10.2323/jgam.51.267

Tymon, L., Bradshaw, M., Gotz, M., Braun, U., Peever, T. L., and Edmonds, R. L. (2022). Phylogeny and Taxonomy of *Erysiphe* spp. on *Rhododendron* with a special emphasis on North American species. *Mycologia*

Vélez, J. M., Morris, R. M., Vilgalys, R., Labbé, J., and Schadt, C. W. (2021). Phylogenetic diversity of 200+ isolates of the ectomycorrhizal fungus *Cenococcum geophilum* associated with *Populus trichocarpa* soils in the Pacific Northwest, USA and comparison to globally distributed representatives. *PLoS One* 16:e0231367. doi: 10.1371/journal.pone.0231367

Vu, D., Groenewald, M., de Vries, M., Gehrmann, T., Stielow, B., Eberhardt, U., et al. (2019). Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. *Stud. Mycol.* 92, 135–154. doi: 10.1016/j.simyco.2018.05.001

Walsh, P., Metzger, D., and Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* 10, 506–513.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.