

WORK PLAN

Plant Protection and Quarantine, Science and Technology

Title: DNA barcoding of downy mildew specimens on flowering plants present in the U.S. National Fungus Collection.

Prior Year FAIN: N/A

Period of Performance: August 1, 2026 – July 31, 2027.

I. Introduction

This Work Plan reflects a cooperative relationship between USDA Agriculture Research Service (ARS) and the Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ). It outlines the project specific goals, objectives, and anticipated accomplishments as well as the approach to follow for the suggestion “DNA barcoding of downy mildew specimens on flowering plants present in the U.S. National Fungus Collection” and the related roles and responsibilities of the parties as negotiated.

II. Background

The primary purpose of this agreement is to support goal 3 “Increase Identification Capacity and Strengthen Pest Detection Technology”, Strategy 3 by generating high-quality sequence data for specimens of downy mildew species in the genera *Hyaloperonospora*, *Peronospora*, and *Plasmopara*, including type specimens, present in the U.S. National Fungus Collections, associated with nursery and floral crops plants and their wild relatives.

The activities proposed in this project address long-standing taxonomic challenges caused by outdated morphological classifications, taxonomic synonymization errors, and lack of molecular data for many downy mildew species. Downy mildews cause significant economic losses in the floriculture industry, and accurate identification is critical for quarantine and diagnostic efforts. By identifying key specimens from the U.S. National Fungus Collections and optimizing both DNA extraction and PCR amplification methods for herbarium-preserved material, the project will produce reliable Sanger sequences for major genera including *Hyaloperonospora*, *Peronospora*, and *Plasmopara*. These sequences will support updated phylogenetic analyses and be shared with APHIS PPQ and public repositories, ultimately improving the speed and accuracy of pathogen identification and strengthening diagnostic capabilities for plant-health programs.

Potential barriers or obstacles to progress that could be anticipated may include those originating from the USDA Restructuring Plan.

Dr. Catalina Salgado-Salazar is a Research Mycologist at the Mycology & Nematology Genetic Diversity and Biology Laboratory, USDA-ARS, in Beltsville, Maryland. Dr. Salgado-Salazar has 20 years of experience performing and leading laboratory and field-based research projects on systematics and taxonomy, biogeography, ecology, evolution, and genomics of higher fungi and oomycete downy mildew species important for plants and ecosystem health.

She has more than ten years of experience at USDA-ARS both leading and collaborating on interdisciplinary projects related to the classification of fungal and oomycete plant pathogens. Dr. Salgado has a strong background in systematic studies of higher fungi and oomycete downy mildews using morphological and molecular and whole genome data, particularly in how their phylogenetic relationships relate to current taxonomic classifications and how this knowledge can be applied for safeguarding plant resources. Dr. Salgado-Salazar also has experience in whole genome sequencing, genome analyses, and comparative genomics. In this project, Dr. Salgado-Salazar will focus on the generation of the DNA barcoding resources for downy mildew specimens affecting nursery and floral crops present in the U.S. National Fungus Collection.

Dr. Priscila Chaverri is an Associate Professor at the Department of Natural Sciences, Bowie State University in Bowie, MD. Dr. Chaverri is a mycologist/plant pathologist with 20 years of experience leading research in fungal systematics and plant pathology. Her research portfolio, comprising over 110 publications, provides strong evidence of her relevant expertise for this project. Her portfolio demonstrates hands-on experience in systematics, taxonomy, phylogenetics, genomics, bioinformatics, and plant pathology. She has collaborated with USDA-APHIS on multiple projects focused on identifying quarantine and foreign fungal pathogens intercepted at U.S. ports of entry. In this project, Chaverri will contribute with postdoc supervision and mentoring, study design, and bioinformatic analyses.

Key studies on oomycete barcoding and phylogenetics provide the framework for this project by demonstrating the effectiveness of mitochondrial markers such as *cox1* and *cox2* for species-level resolution in downy mildews (e.g., Choi et al. 2015; Robideau et al. 2011). Foundational phylogenetic work on Peronosporaceae (Göker et al. 2003, 2007; Riethmüller et al. 2002; Thines 2014) highlights persistent taxonomic uncertainty, underscoring the need for high-quality sequence data from expertly identified historical BPI specimens—a core objective of the funded barcoding project 1. Research on herbarium DNA recovery (Marinček et al. 2022; Raxworthy & Smith 2021) informs our optimization of extraction and PCR protocols for degraded archival material. Finally, recent discoveries of new and emergent downy mildew species in the U.S. (Salgado-Salazar et al. 2020–2025; Davis and Salgado-Salazar 2026; Havan et al. 2025) demonstrate the dynamic nature of these pathogens and reinforce the importance of building a robust, voucher-linked barcode library to enhance APHIS diagnostic capacity—central to this PPA 7721 project’s mission.

Brief literature review:

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Roggenkamp EM, Check JC, Biswal AK, *et al.* 2024. Development of a qPCR assay for species-specific detection of the tar spot pathogen *Phyllachora maydis*. *PhytoFrontiers* 4:61-71

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Salgado-Salazar C, Rodriguez-Salamanca LM, Romberg MK, Davis WJ, McConnell M, Havan AE, Peduto-Hand F. 2025. Where flowers blow, so do downy mildews: New species and new records of *Hyaloperonospora*, *Peronospora* and *Plasmopara* species on ornamental and wild plants in the U.S. *Plant Health Progr.* 26:260-283.

Salgado-Salazar C, Hudelson B. 2025. First observation of *Peronospora digitalis* causing downy mildew on Culver's root (*Veronicastrum virginicum*) in the USA. *J. Plant Dis. Prot.* 132:54.

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Salgado-Salazar C, Thines M. 2022. Two new species of *Plasmopara* affecting wild grapes in the USA. Mycol. Progr. 21:63.

Salgado-Salazar C, LeBlanc N, Wallace EC, *et al.* 2020. *Peronospora monardae*, *Hyaloperonospora daughtreyae* and *H. iberidis*: new species associated with downy mildew diseases affecting ornamental plants in the United States. Eur. J. Plant Pathol. 157:311-326.

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III. Goals and Objectives –

Due to the lack of comprehensive DNA sampling of historical downy mildew species affecting the floral and nursery plant industry, the main goal of this project is to sequence traditional molecular DNA barcodes for downy mildews using sanger sequencing techniques. The barcode datasets generated in this way can be used to improve the speed and accuracy of identification of these plant pests and support future studies aimed at developing diagnostic tools that will increase the capacity for detecting these plant pests. Specifically, we aim to:

1. Identify specimens of downy mildew species in the genera *Hyaloperonospora*, *Peronospora*, *Plasmopara*, including type specimens, present in the U.S. National Fungus Collections, associated with nursery and floral crops plants and their wild relatives.
2. Optimization of a molecular method of DNA extraction of downy mildew specimens, allowing to maximize the chances of obtaining high quality DNA for PCR amplification
3. Identify the most appropriate PCR reagents and methods suitable for the amplification and production of quality DNA sequences of herbarium-stored downy mildew specimens
4. DNA sequence data processing, reconstruction of phylogenetic relationships, and data release to APHIS PPQ personnel and public data repositories.

IV. Milestones –

Objective 1: Identify specimens of downy mildew species in the genera *Hyaloperonospora*, *Peronospora*, *Plasmopara*, including type specimens, present in the U.S. National Fungus Collections, associated with nursery and floral crops plants and their wild relatives.

<i>Milestone</i>	<i>Quarter</i>				<i>Performance Measure</i>
	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	
<i>1.1 Gather updated list of downy mildew specimens' collections available at the U.S. National Fungus Collection using Mycoportal database.</i>	<i>x</i>				<i>Database of Hyaloperonospora, Peronospora and Plasmopara</i>
<i>1.2 Visual inspection and selection of downy mildew specimens according to the criteria established.</i>	<i>x</i>				<i>Collection of downy mildew specimens to be processed further.</i>
<i>Final Report</i>		<i>x</i>			<i>Report on results for objective</i>

Objective 2: Optimization of a molecular method of DNA extraction of downy mildew specimens, allowing to maximize the chances of obtaining high quality DNA for PCR amplification.

<i>Milestone</i>	<i>Quarter</i>				<i>Performance Measure</i>
	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	
<i>2.1. Test trials of traditional and kit-based DNA extraction methods</i>	<i>x</i>	<i>x</i>			<i>Selection of most appropriate methods for DNA extraction of downy mildew specimens.</i>
<i>Final Report</i>			<i>x</i>		<i>Report on results for objective</i>

Objective 3. Identify the most appropriate PCR reagents and methods suitable for the amplification and production of quality DNA sequences of herbarium-stored downy mildew specimens.

<i>Milestone</i>	<i>Quarter</i>				<i>Performance Measure</i>
	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	
<i>3.1. Test trials of different PCR reagents</i>		<i>x</i>	<i>x</i>		<i>PCR reagent suitable for the PCR amplification of DNA extracted from downy mildew specimens</i>
<i>3.2 Test trials of different PCR amplification protocols</i>		<i>x</i>	<i>x</i>		<i>PCR amplification protocol suitable for the DNA extracted from downy mildew specimens</i>

<i>3.3 Application of selected PCR reagents and amplification protocols on selected downy mildew specimens.</i>		x	x		<i>DNA extraction and PCR amplification performed for downy mildew specimens</i>
<i>3.5 Sanger sequencing of positive PCR amplification products.</i>		x	x		<i>DNA sequences of barcodes for downy mildew specimens</i>
<i>Final Report</i>			x		<i>Report on results for objective</i>

Objective 4. DNA sequence data processing, reconstruction of phylogenetic relationships, and data release to APHIS PPQ personnel and public data repositories.

<i>Milestone</i>	<i>Quarter</i>				<i>Performance Measure</i>
	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	
<i>4.1 DNA sequences postprocessing</i>		x	x		<i>DNA consensus sequences for downy mildew specimens</i>
<i>4.2 Phylogenetic analysis</i>			x	x	<i>Phylogenetic tree data and species identification based on genetic relationships.</i>
<i>4.3 Data release to APHIS-PPQ and public data repositories.</i>				x	<i>DNA datasets for use for further identification processes.</i>
<i>4.4 Specimen annotation.</i>			x		<i>Downy mildew specimens in U.S. National Fungus Collection with updated classifications/identity.</i>
<i>Final Report</i>			x		<i>Final report of project.</i>

V. Methods –

1. Specimen selection from U.S. National Fungus Collection (BPI fungaria).

Specimen search is carried out using the Mycology Collections Data Portal (<https://www.mycportal.org/portal/>), searching the collection corresponding to the USDA United States National Fungus Collection. After the lists of specimen records have been obtained for each of the three genera, *Hyaloperonospora*, *Peronospora* and *Plasmopara*, priority will be given to:

- Type specimens
- Species with no DNA sequence data available in public data repositories

- c. Specimens whose geographic location or host species has no representation in public data repositories.

Before sampling, specimens will be visually inspected to determine if there is enough material so as not to deplete the specimen. Specimens with little material will not be sampled. For those species of downy mildews with a high representation of specimens in the collection, for example *Plasmopara halstedii* and *Plasmopara viticola*, only those specimens on hosts other than Sunflower (*Helianthus annuus*) and Blackeye Susan (*Rudbeckia* sp.) for *Plasmopara halstedii* and on hosts other than winegrapes (*Vitis vinifera*) for *Plasmopara viticola*, will be chosen.

Genus *Hyaloperonospora*

Selection of specimens will focus on those labeled as *Hyaloperonospora* sp., *Hyaloperonospora parasitica* reported on hosts other than type host (*Capsella bursa-pastoris*), and specimens still labeled with the older name “*Peronospora parasitica*”. There are currently 12 specimens’ records corresponding to *Hyaloperonospora* sp. and *Hyaloperonospora parasitica*, and 944 specimen records still associated with the name “*Peronospora parasitica*” (Supplementary Table 1).

Genus *Peronospora*

Selection of specimens will focus on those labeled as *Peronospora* sp. and other species, except those labeled as “*Peronospora parasitica*”. There are currently 6,420 specimens’ records corresponding to *Peronospora* (Supplementary Table 2).

Genus *Plasmopara*

Selection of specimens will focus on those labeled as *Plasmopara* sp. and those labeled as other species. There are currently 1,959 specimen records corresponding to *Plasmopara*. Two species in *Plasmopara*, namely *P. halstedii* and *P. viticola* constitute around 35% of the total number of *Plasmopara* specimens. See above how the specimens corresponding to these two species will be processed (Supplementary Table 3).

2. Sample collection and DNA extraction.

After a specimen has been deemed appropriate for sampling, 5 – 10 mm² pieces of plant sample colonized by downy mildew and bearing characteristic structures (mycelia, sporangia) will be excised using a sterile razor blade. The plant samples will be put in a 1.5 mL Eppendorf tube and stored at -80 °C until processing. DNA extraction is one of the most challenging steps when working on DNA barcoding of historical archived collections. To increase the chances of obtaining high quality DNA for PCR amplification, several traditional methods of DNA extraction, and commercial DNA extraction kits designed to work with ancient/forensic DNA will be used. Traditional and kit-based DNA extraction methods will be pre-tested on preserved downy mildew specimens not part of the U.S. National Fungus Collection. These specimens

represent a wide array of species, hosts and collection years. The methods producing the best results will be used with the fungarium specimens.

a. Traditional methods:

i. Phenol-chloroform by Parada-Rojas and Quesada-Ocampo (2018):

1. Homogenize sample using liquid nitrogen maceration
2. Add 600 μ l of extraction buffer (0.5 M Tris pH 8.0, 5 M NaCl, 0.5 M EDTA, 10% SDS) + 4 μ l RNase A
3. Incubate 10 min at room T°, with occasional mixing
4. Add 500 μ l of phenol, mix and centrifuge at 14,000 rpm for 10 min.
5. Transfer supernatant to new 1.5 ml Eppendorf tube. Add equal volume of phenol:chloroform. Mix by gentle immersion. Centrifuge at 14,000 rpm for 10 min.
6. Transfer supernatant to new 1.5 ml Eppendorf tube. Add 0.8 volumes of isopropanol. Incubate tubes overnight at -20 °C.
7. Centrifuge tubes 15 min at 14,000 rpm. Discard supernatant and wash pellet with 80% ethanol. Centrifuge tubes 3 min at 14,000 rpm. Discard supernatant.
8. Resuspend pellet in 100 μ l of PCR-grade water. Store at -20 °C until processing.

ii. CTAB protocol by Godinez-Vidal and Groen

([dx.doi.org/10.17504/protocols.io.14egn9y8pl5d/v1](https://doi.org/10.17504/protocols.io.14egn9y8pl5d/v1))

1. Prepare fresh CTAB buffer in advance and keep it warm (~ 62 °C).
 - a. CTAB reagent (final concentration): Tris-HCL pH8 (100 mM), NaCl (1.4 M), EDTA (20 mM), β -mercaptoethanol (0.2%), CTAB (2%).
2. Homogenize sample using liquid nitrogen maceration and resuspend sample on 500 μ L of CTAB buffer
3. Incubate samples with light occasionally shaking for 60 min at 60-65 °C.
4. Open the tubes carefully and add an equal volume of isoamyl alcohol:chloroform (24:1).
5. Make sure the tubes are well closed. Mix the suspension well with vortexing until it turns white.
6. Centrifuge at 14,000 rpm for 10 min at room temperature.
7. Recover supernatant without touching the interphase (white layer) and add it to a new 1.5 ml Eppendorf tube.
8. Add an equal volume of isoamyl alcohol:chloroform (24:1), mix the suspension by vortexing. Centrifuge at 14,000 rpm for 10 min at room temperature. Repeat this step a total of 3-4 times until the interface is completely transparent or clear. Be careful not to touch the interface to avoid contaminating the DNA with proteins and/or carbohydrates.
9. Recover supernatant without touching the interphase (white layer) and add it to a new 1.5 ml Eppendorf tube.

10. Add an equal volume of absolute isopropanol and incubate from 30 to 40 min at -20 °C. (This step can be overnight).
 11. Centrifuge at 14,000 rpm for 15 min at room temperature. Discard supernatant.
 12. Wash the pellet with 500 µL of absolute ethanol and vortex until it comes off the bottom of the tube.
 13. Centrifuge at 14,000 rpm for 7 min at room temperature. Discard supernatant
 14. Wash the pellet with 70% ethanol and centrifuge at 14,000 rpm for 7 min at room temperature.
 15. Decant the ethanol and leave the tubes inverted on a paper towel to dry the pellet for no more than 5 min.
 16. Dissolve the pellet by adding 50-100 µl nuclease-free water and incubating the tubes at 65 °C for 10 min.
- b. DNA extraction kits
- i. E.Z.N.A. ® Tissue DNA kit. Omega Biotek. Previously known as the E.Z.N.A. ® Forensic DNA kit. DNA extraction kit designed. (<https://www.omegabiotek.com>) .
 - ii. GENE CLEAN® for Ancient DNA Kit. Optimized for preserved tissues. (<https://www.mpbio.com/us>)
 - iii. DNeasy Plant Mini Kit. Optimized to reduce inhibitors present in DNA (<https://www.qiagen.com/us>).
 - iv. Monarch Spin gDNA Extraction Kit. Focuses on cell lysis, RNA removal and purification of DNA (<https://www.neb.com/en-us/>)
 - v. Genome DNA Purification Kit. Designed for a wide array of samples including plants (<https://www.thermofisher.com>).
 - vi. Plant and Fungal DNA Kit. Universal kit for the isolation of total DNA from plants, fungi and lichens. (<http://roboklon.com>).
 - vii. Soil DNA kit. Optimized to reduce inhibitors present in DNA (<http://roboklon.com>)

3. PCR amplification and barcoding primers.

PCR products will be generated using the following primer pairs: **cox2-F/Cox-RC4** (*cox2*; Choi et al. 2015; Hudspeth et al. 2000), **OomCox1-levup/OomCox1-levlo** (*cox1*; Robideau et al. 2011), **LR0R** (Vilgalys and Hester 1990) and **LR6-O** (ncLSU rDNA D1–5 region; Riethmüller et al. 2002), **ITS1-O** (Rouxel et al. 2013) and **LR-O** (ncITS rDNA; Moncalvo et al. 1995), **LR6-OR2C** (Thines et al. 2019) and **LR9** (ncLSU rDNA D6–8 region, Hopple and Vilgalys 1999), **FM79-C1** (Choi et al. 2015) and **FMPH-10b** (*cox1-cox2* spacer, Martin et al. 2005), and **Prv9-F** and **Prv9-R** (*rps10* mtDNA, Martin and Coffey 2012).

To improve the chances of getting positive amplifications from the DNA obtained from the downy mildew specimens, different PCR master mix using different types of Taq polymerases will be tried, especially those designed to optimize amplification of low target DNA, and the possible presence of inhibitors. Below is a list of mixes to try:

- a. Platinum™II Hot-Start PCR Master Mix (2X) (<https://www.thermofisher.com>).

- b. Platinum™ SuperFi II PCR Master Mix (<https://www.thermofisher.com>)
- c. KAPA2G Robust HS RM+dye (6.25ml) (<https://www.lifescience.roche.com/en-us>)
- d. Q5 Hot Start High-Fidelity 2X Master Mix (<https://www.neb.com>)
- e. One Taq Hot Start Quick-Load 2x Master Mix with standard buffer (<https://www.neb.com>)
- f. HotStarTaq Master Mix Kit (250 U) (<https://www.qiagen.com/us>)
- g. sparQ HiFi PCR Master Mix (<https://www.quantabio.com/>)
- h. PrimeSTAR® Max DNA Polymerase Ver.2 (<https://www.takarabio.com/>)

Once the adequate PCR reaction mix has been identified, PCR reactions will be performed in 25-50 µl volumes containing 12.5-25 µl of selected PCR Master Mix, 0.4-0.8 µl of each primer (10 µM), 5–10 ng of template DNA (1–2 µl), and 7–8 µl of PCR-grade water. Amplification was performed in a C1000 Touch PCR Thermal Cycler (Bio-Rad, Hercules, CA) using touchdown PCR cycle conditions, or as follows: initial denaturation at 94 °C for 2 min, 35 cycles of 94 °C for 15 s, 60 °C for 15 s, and 68 °C for 30 s, final extension at 72 °C for 10 min. Amplicons were bi-directionally sequenced using BigDye™3.1 Terminator Cycle sequencing kit on an Applied Biosystems SeqStudio Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

4. Barcode DNA sequences postprocessing and analysis.

Sequences were quality trimmed and assembled using CLC Main Workbench v.24 (QIAGEN, Inc, Germantown, MD, USA) using default settings. The barcode DNA sequences obtained for *Hyaloperonospora*, *Peronospora* and *Plasmopara*, as well as sequence data from related species downloaded from GenBank will be analyzed independently. Individual alignments will be obtained using MAFFT program online version 7 (<http://mafft.cbrc.jp/alignment/server/>) (Katoh et al. 2019) using the algorithm G-INS-1. The amino acid substitution model best fitting each dataset will be estimated using ModelTest-NG v0.1.7 as implemented by RAxML GUI v.2.0.10 (Darriba et al. 2020; Edler et al. 2020; Kozlov et al. 2019; Silvestro and Michalak 2012; Stamatakis 2014) based on the Akaike Information Criterion AIC. Phylogenetic analyses will be run individually for each gene and as concatenated partitions using two different methods. Maximum likelihood (ML) phylogenetic analyses will be performed using RAxML GUI v.2.0.10 (Darriba et al. 2020; Edler et al. 2020; Kozlov et al. 2019; Silvestro and Michalak 2012; Stamatakis 2014) with 1,000 bootstrap replicates. Bayesian inference (BI) phylogenetic trees will be obtained using MrBayes version 3.2.7a (Ronquist and Huelsenbeck 2003). Bayesian inferences will be initiated from random starting trees, run for 10 million generations with four chains and sampled every 1000th generation for a total of 10,000 tree samples per run. Default priors will be used on all analyses, and two independent Bayesian inference (BI) analyses will be run. To evaluate stationarity and convergence between runs, log-likelihood scores will be plotted using TRACER version 1.7.2 (Rambaut et al. 2018). After stationarity evaluation, 25% of the trees will be removed from the analysis. The remaining trees will be used to calculate posterior probabilities (PP) and summarized in a 50% majority rule consensus tree. Phylogenetic trees will be visualized using FigTree v1.4.4 (Rambaut 2014). Species in the genera *Perofascia*, *Pseudoperonospora* and *Plasmoverna* will be used as outgroup in the phylogenetic analyses of *Hyaloperonospora*, *Peronospora* and *Plasmopara*, respectively.

5. Data management and specimen annotation

All the DNA sequences generated during the performance of this project will be made available to personnel at USDA-APHIS Plant Protection and Quarantine and also deposited at GenBank (<https://www.ncbi.nlm.nih.gov/>). Whenever necessary, fungaria specimens of downy mildew will be annotated to reflect the changes in species identifications resulting from the analysis of the data generated.

VI. Deliverables –

- A curated list of identified downy mildew specimens (*Hyaloperonospora*, *Peronospora*, *Plasmopara*), including verification of type and historic specimens from the U.S. National Fungus Collections.
- An optimized DNA extraction protocol tailored for degraded herbarium material, documented for future use by APHIS and diagnostic partners.
- Standardized PCR amplification workflow and reagent recommendations for generating high-quality Sanger sequences from historical downy mildew specimens.
- A set of validated DNA barcode sequences (e.g., *cox1*, *cox2*, ITS when possible) deposited in public databases and provided directly to APHIS PPQ personnel.
- Phylogenetic analyses reconstructing relationships among the sampled species to assist in accurate identification and taxonomic clarification. Data release package containing sequences, metadata, specimen information, and methodological documentation for APHIS PPQ.
- A concise final report summarizing accomplishments, methods, challenges, and recommendations for developing future diagnostic tools that leverage the generated barcode dataset.

VII. Budget – [Complete S&T Cooperative Agreement Budget Worksheet](#)

VIII. Data – Note: All data will be made available to USDA APHIS upon request.

- **Specimen metadata:** For each *Hyaloperonospora*, *Peronospora*, and *Plasmopara* specimen examined, information such as host plant, collection date, locality, collector, type status and final species identification will be recorded on excel spreadsheets, and will be transferred to external hard drive or using Microsoft OneDrive
- **Laboratory workflow data:** Records from DNA extraction trials—including extraction methods, reagents, and success metrics—support the optimization objective listed in the work plan. This data will be transferred to an external hard drive or using Microsoft OneDrive
- **PCR and sequencing data:** Primer sets, reagent conditions, amplification success, and final Sanger sequences will be captured as part of generating high-quality barcode data. DNA sequences. This data will be provided transferred to external hard drive or using Microsoft OneDrive.
- **Sequence-based analyses:** Alignments, curated sequence files, phylogenetic trees, and interpretation notes, reflecting the project's goal of reconstructing phylogenetic relationships. These data will be provided transferred in external hard drive or using Microsoft OneDrive

- **Administrative and reporting data:** Project progress information and documentation required for compliance with PPA 7721 reporting timelines described. Final report will be provided as electronic file.

IX. Geographical Considerations – none.

X. Statement of Federal Involvement – none.

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