Molecular Marker Development for *Phaeocollybia* Species

Final Report to the Interagency Special Status/Sensitive Species Program

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Introduction

Species-specific molecular markers can be used to detect fungal species in soil when no sporocarp is present. In this paper the term molecular marker will be used to describe segments of DNA of a specific size, that are amplified in a PCR (polymerase chain reaction), and visualized on an agarose gel that separates DNA fragments by size. Species-specific primers are used in the PCR to generate the species-specific markers. Primers are short segments of DNA (oligonucleotides) that can bind to DNA in a sample, for example from DNA extracted from soil, when a segment of the DNA in the sample exactly matches the sequence of the primer. By binding to the DNA, the primer creates a starting point for replication to occur in PCR. A second primer downstream from the first primer creates a starting point for replication in the opposite direction. Together the two primers define a segment of DNA of a specific length. If one or both of the primers is targeted to a DNA sequence that is unique to one particular species, it is a species-specific set of primers. If the DNA of the target species is present in a PCR with the species-specific primer, a marker of the specific length will be generated in the PCR. This species-specific marker will be seen when the products of the reaction are separated by size in an agarose gel.

In order to facilitate the detection of *Phaeocollybia* species in areas of interest, the Interagency Special Status/Sensitive Species Program (ISSSSP) of the OR/WA BLM and Region 6 Forest Service contracted for the development of four species-specific molecular markers for four species of *Phaeocollybia* that are on the sensitive species list for Oregon. In order to target unique DNA segments with species-specific primers, it is essential to have knowledge of the genetic sequence of the target species and of all closely related species. The internally transcribed spacer (ITS) region of the gene that codes for ribosomal RNA in fungi has long been targeted for the development of species-specific markers in fungi, because this region is variable, but typically remains consistent within species. A preliminary review of the ITS sequences of *Phaeocollybia* species available in GenBank indicated that there was good variation of this region between *Phaeocollybia* species, so this region was targeted for sequencing, with the idea of finding variable regions that could be used to design species-specific primers.

This project had three phases:

1. Sequence the ITS region for up to ten collections of each of the 25 *Phaeocollybia* species found in the Pacific Northwest and align these sequences, and sequences in GenBank, to generate consensus sequences for each species.

2. Using these consensus sequences, design species-specific primers that generate markers of different sizes for four *Phaeocollybia* species.

3. Test these primer sets on representatives of all 25 *Phaeocollybia* species to confirm their effectiveness and specificity.

Materials and Methods

DNA was extracted from *Phaeocollybia* collections (Table I). Both recently collected material, in the possession of ISSSSP, and material in the collection of Dr. Lorelei Norvell was used (see Table III in the appendix). All ISSSSP *Phaeocollybia* collections had been identified to species by Dr. Norvell. DNA was extracted with a standard Chelex method. DNA was then run in a PCR with the primers ITS1 and ITS4b to amplify the ITS region of these fungi. Successfully amplified samples were submitted for sequencing to an outside lab (Functional Biosciences, Madison, WI). Both forward and reverse sequences were obtained for each sample. Forward and reverse sequences were aligned using the program Gap 4.10, and a single consensus sequence was determined for each sample.

In general, most samples were sequenced and aligned without difficulty. However, some species (*californica* and *rufotubulina*) had a region of repeating A's, and some species (*kauffmanii* and *redheadii*) had a region of repetitive A's and T's. These repetitive A/T regions are difficult to sequence. Some success was obtained by re-doing the PCR step for failed samples with a lower extension temperature, and submitting these amplicons for sequencing.

The 148 *Phaeocollybia* sequences obtained in this project were pooled with 74 *Phaeocollybia* ITS sequences available in GenBank to form a collection of ITS sequences from 222 Pacific Northwest *Phaeocollybia* collections. All of the individual sequences for each species were aligned and a consensus sequence was generated for each species using the program Clustal X. Groupings of taxa based on ITS sequence similarity differed in some cases from the sample names. This issue is discussed in the Results section. All species' consensus sequences were then aligned and a phylogenetic tree was generated for the entire genus using Geneious Pro 4.6.4. Geneious Tree Builder, a neighbor joining tree building method was used with a Tamura-Nei Genetic Distance Model. Bootstrap resampling was used with 250 samples. The support threshold for branches was set at 50%.

Consensus sequences for each species were compared using Geneious Pro 4.6.4. The species *P. californica, P. scatesiae, P. sipei*, and *P. spadicea* were chosen as the target species for this study. Genetic regions within the ITS region of these species were identified, and primers were designed to target these variable regions, so that each primer would bind only to one target species.

Table I. List of the 25 *Phaeocollybia* fungal species that occur in the Pacific Northwest, species abbreviations, and the 2008 ISSSSP species status.

Species Name	Species Abbreviation	ISSSSP species status*
Phaeocollybia ammiratii	amm	None
Phaeocollybia attenuata	att	SEN (FS ONLY)
Phaeocollybia benzokauffmanii	ben	None
Phaeocollybia californica	cal	OR-SEN
Phaeocollybia dissiliens	dis	OR-SEN
Phaeocollybia fallax	fal	WA-SEN (FS ONLY)
Phaeocollybia gregaria	gre	OR-SEN
Phaeocollybia kauffmanii	kau	None
Phaeocollybia lilacifolia	lil	OR-STR
Phaeocollybia luteosquamulosa	lut	None
Phaeocollybia ochraceocana	och	None
Phaeocollybia olivacea	oli	OR-SEN
Phaeocollybia oregonensis	ore	SEN
Phaeocollybia phaeogaleroides	pha	None
Phaeocollybia piceae	pic	SEN (FS ONLY)
Phaeocollybia pleurocystidiata	ple	None
Phaeocollybia pseudofestiva	pse	SEN
Phaeocollybia radicata	rad	OR-STR
Phaeocollybia redheadii	red	None
Phaeocollybia rifflipes	rif	None
Phaeocollybia rufotubulina	ruf	OR-STR
Phaeocollybia scatesiae	sca	SEN
Phaeocollybia sipei	sip	OR-SEN
Phaeocollybia spadicea	spa	SEN
Phaeocollybia tibiikauffmanii	tib	None

* = SEN is sensitive, STR is strategic, OR is Oregon, WA is Washington, FS is Forest Service

Primers were selected manually and tested *in silico* using the free programs BioMath and IDT OligoAnalyzer 3.1. Two or three potential forward primers for each species were then tested in PCRs with DNA from representatives of the four target species and species closely related to the target species. A set of primers (4 forward primers and 2 reverse primers) that was specific

and yielded products of different sizes for each of the four target species was developed through this testing.

These primers were then tested on DNA from all available collections of the target species, and with two representatives of each non-target *Phaeocollybia* species present in the Pacific Northwest to confirm their effectiveness as species-specific primers for *P. californica, P. scatesiae, P. sipei*, and *P. spadicea.*

Results

Sequencing and Alignment

The source material identification information or the GenBank accession number of all sequences used in this project are shown in Table III (Appendix). A total of 222 individual samples of the 25 *Phaeocollybia* species present in the Pacific Northwest were used.

After sequence alignment by species, it was clear that some species contained two subgroups with significant sequence differences between them. For example, Figure 1 shows an alignment of a portion of the available sequences for *tibiikauffmanii*. In this depiction, loci where all sequences agree with each other are shown with a white background, while areas of disagreement are shown with a colored background.

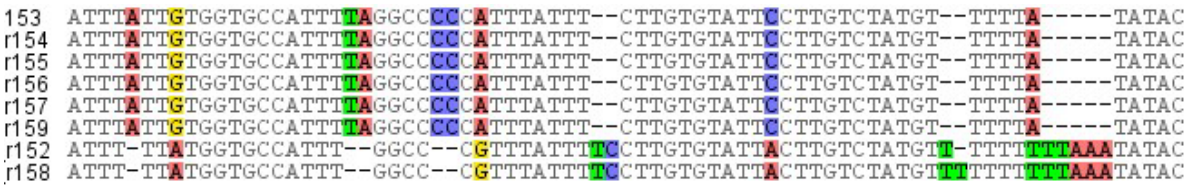

Figure 1. Alignment of a portion of the ITS1 region of 8 tibiikauffmanii collections

There are quite a few base discrepancies in the region shown, but the top 6 collections are identical to each other, and the bottom 2 collections are nearly identical, but different than the upper group. In cases like this, the species group was split. The larger subgroup was labeled with the suffix " _A", and the smaller subgroup with the suffix "_B", and a unique consensus was generated for each subgroup. Each subgroup consensus was run in the subsequent analysis as if it were an independent species.

Figure 2 is the phylogenetic tree generated using consensus sequences from each of the 25 Pacific Northwest *Phaeocollybia* species and species subgroups. Preliminary tree-building trials consistently showed *P. radicata* to be basal to the other species, so it was set as the outgroup in the tree shown in Figure 2. In this figure, three letter codes (the first three letters of

the species name) are used to identify species. These codes are listed in Table I, and may be used to refer to species elsewhere in this document.

In all cases, the two subgroups of a split species came out on distinctly different branches of the genus tree, indicating that the differences between the subgroups of these split species was as great as the differences between accepted species. The tree also shows some species and subgroups clustering at the tips of some branches, indicating that some of these taxa may be the same species. This issue will be discussed further below with regard to the cluster of species containing *P. spadicea* and cluster containing *P. californica*.

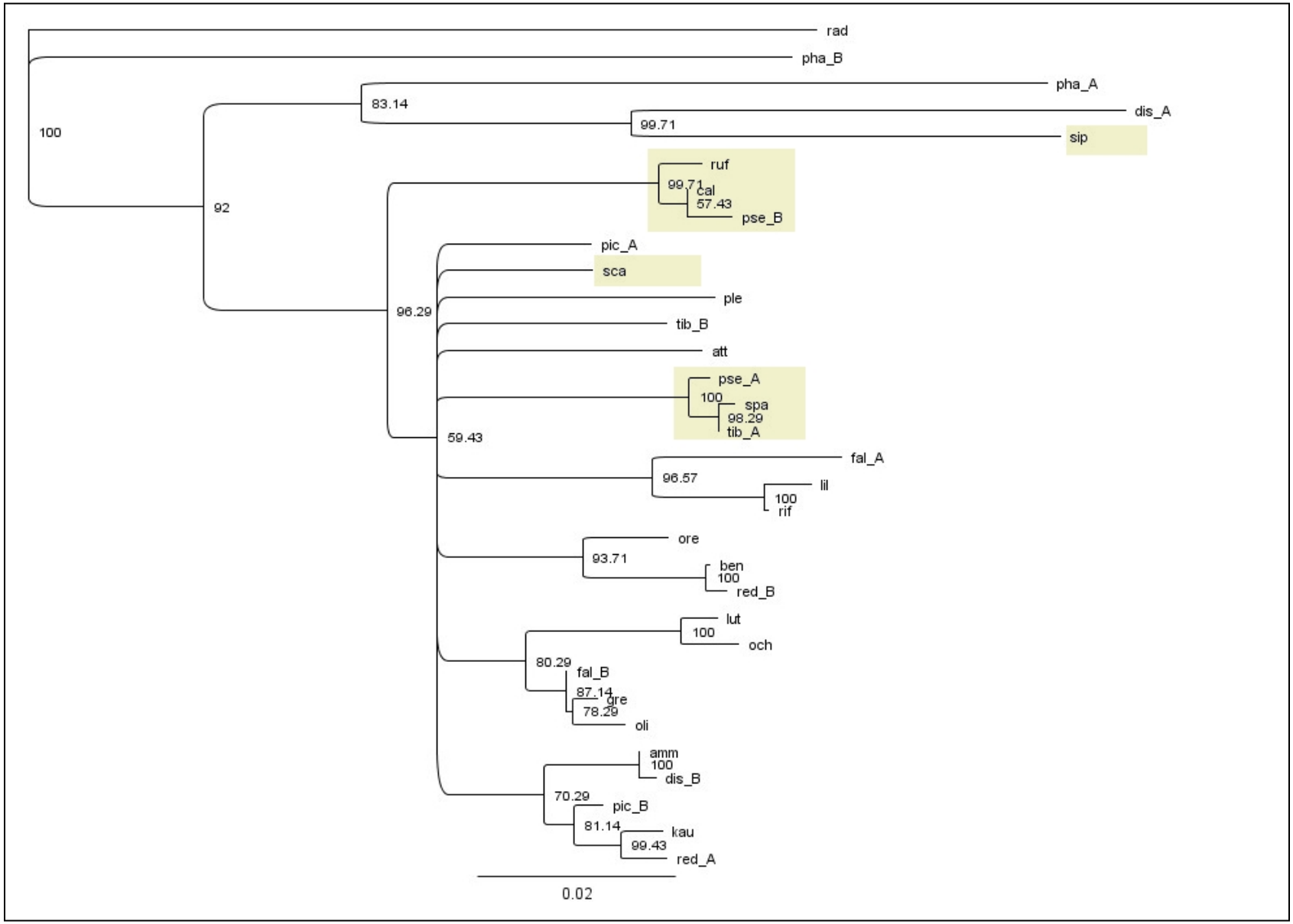

Figure 2. Phylogenetic tree for the genus *Phaeocollybia* based on ITS sequences. Branch consensus support (%) is shown. *P. radicata* is the outgroup. Taxa targeted in this study are shown in shaded boxes.

Molecular Marker Development

In this project, molecular marker development was focused on the rare species: *P. californica, P. olivacea, P. pseudofestiva, P. scatesiae, P. sipei, and P. spadicea*, with the goal of developing specific primers capable of detecting at least four of these species. The genus tree shows that *californica* sequences cluster with *rufotubulina* and pse_B. An alignment of all 17 sequences from samples in these three groups showed that there was little difference between them (pairwise identity = 99.3%), and they were collapsed together into "*californica* group" or cal_group. As a comparison, the pairwise identity between the pic_A and sca consensus sequences is 90.1%, and the pairwise identity between the closely related lut and och sequences is 95.7%. Figure 3 shows a phylogenetic tree for the *californica* group based on the ITS sequences, with *pleurocystidiata* as an outgroup. The short branch lengths for the individuals in this group show that they are all closely related. The sample pse_B_EU669240 appears to be somewhat out of the main grouping, but this is because the sequence quality for this sample was not as good as the others, and there were several ambiguous bases reported in the sequence. The ambiguous bases are all consistent with the other sequences in this group.

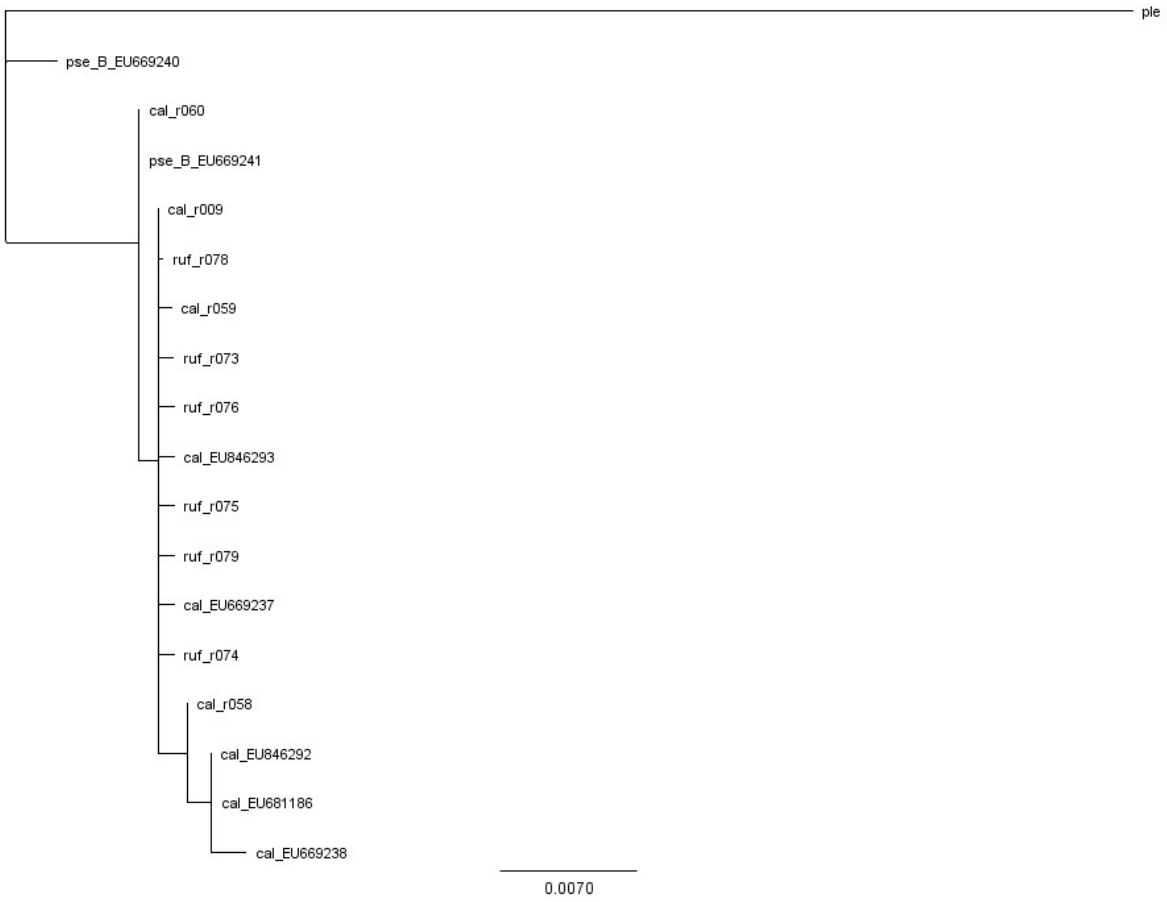

Figure 3. Phylogenetic tree for cal_group samples with *pleurocystidiata* as the outgroup.

A similar situation was found for *spadicea*, tib_A, and pse_A, which formed "*spadicea* group" or spa_group. The pairwise identity for this group of 18 sequences is 99.9%. The consensus tree, with *gregaria* as the outgroup, is shown in Figure 4.

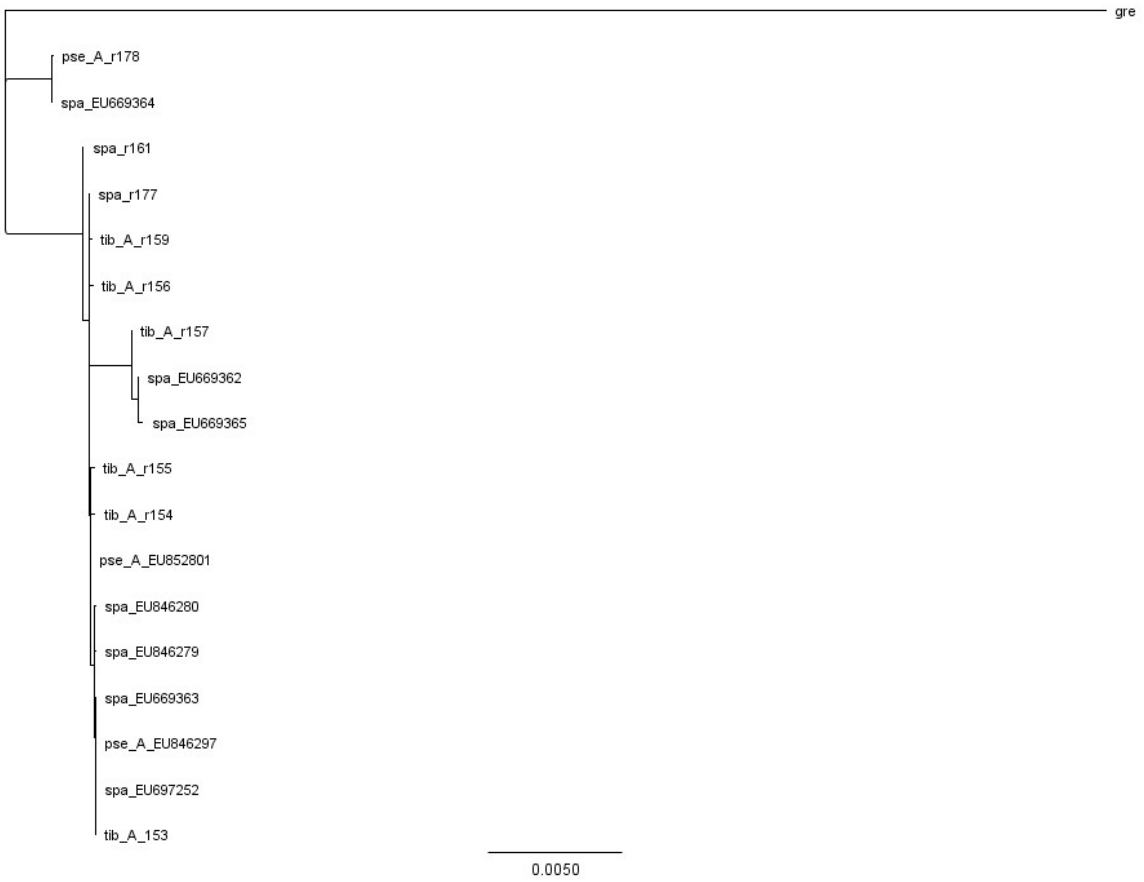

Figure 4. Phylogenetic tree for spa_group samples with *gregaria* as the outgroup.

Although cal_group and spa_group each incorporated all or part of three species, there were distinct differences between these groups and all other taxa on the tree. So along with *sipei* and *scatesiae*, cal_group and spa_group were chosen as target species for marker development.

The four consensus sequences for these target species were aligned, and the sequences inspected for variable areas. As discussed in the Introduction, two primers, an upstream or "forward" primer and a downstream or "reverse" primer are needed to produce a species-specific marker in PCR. Much inter-species variability was seen in the 5' or upstream part of the ITS sequences, where the forward primer bonds. It was decided to design a common reverse primer, and use the 5' variability to design 4 unique forward primers. A non-specific primer such as ITS4 or ITS4b could be used as a reverse primer, but a unique reverse primer was designed to enhance the specificity of the primer set. The 3' or downstream part of these sequences and other sequences in the genus were compared and a reverse primer was designed based on a region that was unique to the four target species and a few other *Phaeocollybias*. The region was not present in two other genera in the Cortinariaceae family.

The *P. sipei* sequence had minor non-homologies with the group reverse primer (gr_1r), so a second reverse primer (gr_2r), corresponding to the same region but specific to *P. sipei*, was designed and tested. In initial testing, the gr_1r primer worked fine on *P. sipei* samples, indicating that the sequence differences are not consequential. However, final testing was done with both primers gr_1r and gr_2r in all PCR reaction mixes. Further testing may indicate that primer gr_2r is not needed.

Candidate forward primers for the four target species were tested in PCRs with the two reverse primers, and a group of four compatible forward primers was developed (Table II).

Table II. Species-specific primers developed in this study. Primer names ending in f are forward primers, and names ending in r are reverse primers.

Primer Name	Target	Sequence	Product Size
cal_1f	*californica* group	GAACTCATTTATTCCTCGAGTATTTCTC	382
sca_1f	*scatesiae*	AGACAAGAAAGAAAAAGGGATTTATTCCTT	423
sip_3f	*sipei*	GTGGTTGTAGCTGGCAGTTTA	503
spa_8f	*spadicea* group	GAGTGTCATTAAATTATCAAAAAAAGATCTTC	131
gr_1r	all four spp.	GATAATTATCACACCAATAGACAAAGTC	
gr_2r	*sipei*	GATAATTATCACACCAAATAGACCAAGTC	

Running a PCR with more than one primer set and targeting more than one sequence in the same reaction is referred to as multiplex PCR. Figure 5 shows the results of multiplex PCR using all of the primers in Table II with *Phaeocollybia* DNA. In this gel, lanes 5 and 22 contained *P. scatesiae* DNA, lanes 8 and 9 contained pse_A (spa group) DNA, lanes 16 and 17 contained tib_A (spa group) DNA, lane 10 contained tib_B (cal group) DNA, lane 18 contains *rufotubulina* (cal group) DNA, lanes 19, 20, 21, and 23 contain *californica* DNA, and lanes 24 and 26 contain *sipei* DNA. All of these were strongly positive with a product of the expected size for the species present in the reaction, except for lanes 18 and 19. The DNA in each of these lanes came from collections that had been made in 1956, and were not expected to show significant amplification. Other species represented on this gel are *phaeogaleroides, piceae, pleurocystidiata, radicata, redheadii, rifflipes,* and *fallax*. All of these non-target DNA samples were negative in reactions that contained all of the primers in Table II.

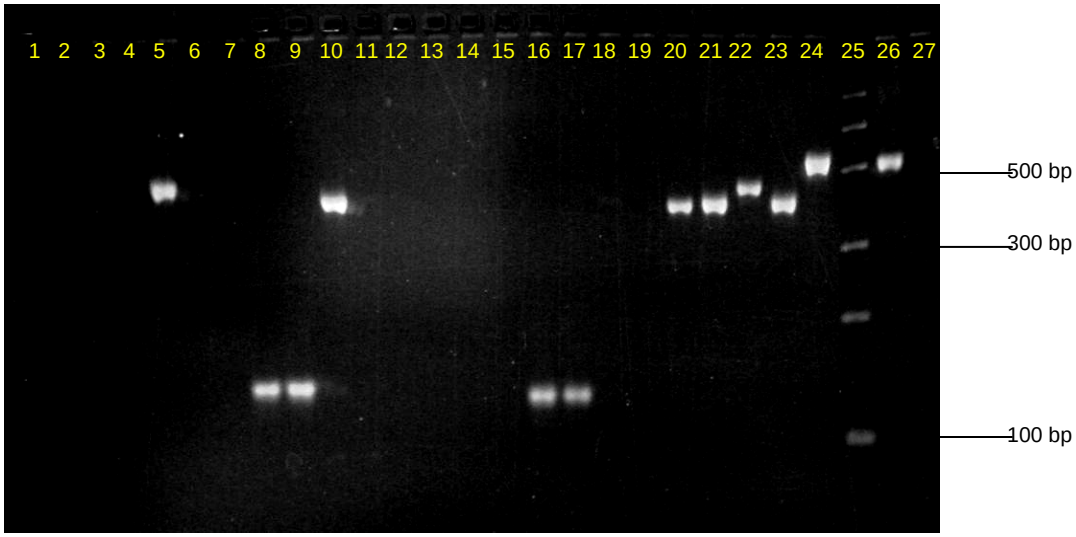

Figure 5. Gel of PCR products from reactions *of Phaeocollybia* DNA with reactions containing all primers in Table I. See text for identities of species tested.

Final Testing

The final testing plan was to test all available DNA samples in the four target species, and to test two samples from each of the non-target species. Some *Phaeocollybia* DNA samples were available from a previous project, so some extra target species samples were drawn from that collection for testing in this project. The results of all testing are shown in Table IV.

Table IV. Results of testing *Phaeocollybia* DNA with specific primers.

Test Id.	Species	Sample #	Result
90414_13	amm	r001	-
90414_14	amm	r017	-
90416_13	att	r030	-
90408_7	att	r031	-
90418_29	att	r033	-
90418_30	att	r034	-
90414_15	ben	r025	-
90414_16	ben	r048	-
90410_1	cal	r009	+
90418_19	cal	r041	-
90418_20	cal	r058	+
90418_21	cal	r059	+
90418_23	cal	r060	+
90407_8	dis	r013	-
90407_9	dis	r092	-
90416_14	dis	r093	-
90418_27	fal_A	p329	-
90418_28	fal_B	p327	-
90414_17	gre	r090	-
90414_18	gre	r121	-
90416_20	gre	r123	-
90414_25	kau	r169	-
90414_26	kau	r179	-
90414_23	lil	r012	-
90414_24	lil	r113	-
90410_9	lut	r136	-
90410_10	lut	r144	-
90414_27	och	r004	-
90414_28	och	r146	-
90410_6	oli	r125	-
90416_21	oli	r126	-
90410_7	oli	r150	-
90416_19	ore	r089	-
90416_22	ore	r132	-
90418_1	pha_A	r105	-
90418_2	pha_B	r112	-
90418_3	pic_A	r035	-
90418_4	pic_A	r039	-
90418_6	ple	r064	-
90418_7	ple	r087	-
90418_9	pse_A	p371	+
90418_8	pse_A	p372	+
90416_10	pse_A	r178	+
90418_10	pse_B	p369	+
90418_11	rad	r114	-
90418_12	rad	r115	-
90418_14	red	r003	-

Test Id.	Species	Sample #	Result
90418_13	red	r085	-
90418_15	rif	r010	-
90408_2	ruf	r073	+
90416_1	ruf	r074	+
90416_2	ruf	r075	+
90416_3	ruf	r076	+
90418_18	ruf	r077	-
90416_4	ruf	r078	+ wk
90416_5	ruf	r079	+
90414_7	ruf	r080	+
90414_1	sca	p275	+
90414_2	sca	p276	+
90414_3	sca	p280	+
90414_22	sca	r081	+
90418_22	sca	r096	+
90416_15	sca	r098	+
90416_16	sca	r099	-
90416_17	sca	r100	+
90416_18	sca	r101	+
90414_20	sip	p355	+
90418_24	sip	p359	+
90418_25	sip	p360	+
90414_21	sip	p362	+
90418_26	sip	p363	+
90414_12	sip	r134	+
90410_15	sip	r135	+
90408_1	spa	r161	+
90416_9	spa	r177	+
90407_11	tib_A	r153	+
90418_16	tib_A	r154	+
90414_8	tib_A	r155	+
90414_9	tib_A	r156	+
90414_10	tib_A	r157	+
90418_17	tib_A	r159	+
90414_11	tib_B	r158	-
90416_6	tib_B	r152	-

A total of 81 non-target samples were tested, and all of them came out negative. A total of 41 target species samples were tested, 38 of them were positive and 3,were negative (shown in red in Table IV). Two of them, a *californica* (r041) and a *rufotubulina* (r077) collection, were discussed above. They were DNA from 50+ year old collections that were only weakly positive when tested with ITS primers. The third negative, a *scatesiae* (r099) was from a 16 year old collection, and again had only given a weak positive response with ITS primers. I believe that

these three failures were due to poor DNA condition in the sample and did not indicate a failure of the primers.

Conclusion

Four species-specific primer sets were developed, for *P. californica, P. scatesiae, P. sipei*, and *P. spadicea*. Each of these primer sets amplifies a marker of a different size when its target species is present. These four primer sets can be used in one multiplex PCR to test for all four species simultaneously. The primers were tested against representatives of all known *Phaeocollybia* species present in the Pacific Northwest and were found to be specific to their target species.

As a practical test of these primers, it would be useful to test soil samples known to contain the target species. One way to accomplish this is to spike soil samples with a known amount of target species tissue from a sporocarp. Soil samples with different levels of sporocarp tissue could be tested to quantify the sensitivity of the method.

Appendix

Table III. *Phaeocollybia* collections whose ITS sequences were used in this study. Samples with a project number were extracted, amplified and sequenced in this study. Others were taken from GenBank. GenBank reference numbers (all beginning "EU…") are given in the 2nd column, otherwise the collection reference number is given.

Project Number	Reference	Species
r001	TJR F13F	ammiratii
r007	TJR F13D	ammiratii
r014	RLE 2007 105	ammiratii
r015	RLE 2007 117	ammiratii
r023	RLE 2007 042	ammiratii
r024	RLE 2007 036	ammiratii
r016	RLE 2007 161	ammiratii
r017	RLE 2007 032	ammiratii
r018	RLE 2007 103	ammiratii
r019	LLN 2071029-25	ammiratii
r020	LLN 51018-1	ammiratii
r030	RLE 2007 176	attenuata
r031	LN 207 1018-11	attenuata
r032	RLE 2002 42	attenuata
r033	RLE 2007 144	attenuata
r034	RLE 2007 162	attenuata
	EU697254	attenuata
	EU846287	attenuata
	EU846288	attenuata
	EU846289	attenuata
	EU846290	attenuata
	EU846291	attenuata
r022	LLN 92.11.20-1	benzokauffmanii
r025	a2061114o1I	benzokauffmanii
r026	RLE 2007 018	benzokauffmanii
r027	A2001101O1-01	benzokauffmanii
r028	LLN2071018-06	benzokauffmanii
r029	RLE 2007 147	benzokauffmanii
r047	RLE 2007 150	benzokauffmanii
r048	RLE 2007 035	benzokauffmanii
r049	RLE 2007 075	benzokauffmanii
r021	LLN 92.10.23-1 ben2)	benzokauffmanii
r009	F7 JS 39	californica
r058	RLE 2007 135	californica
r059	RLE 2007 138	californica
r060	RLE 2007 177	californica
	EU846293	californica
	EU846292	californica
	EU669238	californica

Project Number	Reference	Species
	EU669237	californica
	EU681187	californica
r013	F7 JS 19	dissiliens
r092	RLE 2007 169	dissiliens
r093	RLE 2008 138	dissiliens
	EU846273	dissiliens
	EU846272	dissiliens
	EU846271	dissiliens
	EU669357	dissiliens
	EU669356	dissiliens
	EU669355	dissiliens
	EU669354	dissiliens
	EU846286	fallax
	EU846285	fallax
	EU846284	fallax
	EU846283	fallax
	EU846282	fallax
	EU846281	fallax
	EU669366	fallax
	EU669338	fallax
	EU669312	fallax
	EU697253	fallax
r116	RLE 2007 59	gregaria
r117	RLE 2007 104	gregaria
r118	RLE 2007 049	gregaria
r119	RLE 2006 06	gregaria
r120	RLE 2007 019	gregaria
r090	CH2 JP8	gregaria
r121	RLE 2007 061	gregaria
r122	LLN 2071029-27	gregaria
r123	RLE 2008 26	gregaria
r124	RLE 2007 121	gregaria
r002	TJR F10	kauffmanii
r088	W29 11/7/7	kauffmanii
r164	RLE 2008-27	kauffmanii
r165	RLE 2006-10	kauffmanii
r167	LLN 92.11.24-12	kauffmanii
r169	LLN 2001105-02	kauffmanii
r012	F7 JS 18	lilacifolia
r113	RLE 2008 47 b	lilacifolia
r040	LLN 207-1018-12	lilacifolia
r042	RLE 2007 020	lilacifolia
r043	RLE 2007 021	lilacifolia
r044	LN 207 1018-13	lilacifolia
r045	RLE 2007 022	lilacifolia

Project Number	Reference	Species
r136	RLE 2007-016	luteosquamulosa
r137	RLE 2007-125	luteosquamulosa
r138	RLE 2007-051	luteosquamulosa
r139	RLE 2007-050	luteosquamulosa
r140	LLN2071029-43	luteosquamulosa
r141	LLN2071029-33	luteosquamulosa
r142	LLN2071029-24	luteosquamulosa
r143	LLN2071029-46	luteosquamulosa
r004	CH JP7	ochraceocana
r005	30 V	ochraceocana
r146	RLE 2007-009	ochraceocana
r147	RLE 2007-033	ochraceocana
r148	RLE 2007-057	ochraceocana
r125	GAL-JP2 (BLMOR110)	olivacea
r126	MCM2CN6 (BLMOR080)	olivacea
r150	RLE 2008-129	olivacea
r151	LLN 92.11.22-4	olivacea
	EU846266	olivacea
	EU846265	olivacea
	EU846264	olivacea
	EU846263	olivacea
	EU669235	olivacea
	EU669234	olivacea
r089	CH2 JP3	oregonensis
r128	UEC 153	oregonensis
r130	Ex198-101	oregonensis
r131	RLE 2007-130	oregonensis
r132	RLE 2006-16	oregonensis
r127	LLN 2001105-01	oregonensis
	EU846274	oregonensis
	EU697251	oregonensis
	EU697250	oregonensis
	EU846273	oregonensis
r103	RLE 2008 003	phaeogaleroides
r105	LN 2071029-53	phaeogaleroides
r106	LN 2071029-14	phaeogaleroides
r107	RLE 1999-64	phaeogaleroides
r108	RLE 2007 152	phaeogaleroides
r109	LLN 2000426-01	phaeogaleroides
r110	g2041122c2-1	phaeogaleroides
r111	RLE 2007 93	phaeogaleroides
r112	g2051026c1-6	phaeogaleroides
r039	RLE 2007 027	piceae
r035	RLE 2007 178	piceae

Project Number	Reference	Species
r037	RLE 2007 143	piceae
r175	LLN 92.10.09-7	piceae
	EU846267	piceae
	EU697249	piceae
	EU846268	piceae
	EU669236	piceae
r086	MCM3 CN6	pleurocystidia
r087	LIM3 JPS	pleurocystidia
r061	LLN 93.05.16-1	pleurocystidia
r062	RLE 2008 002	pleurocystidia
r063	LLN 94.03.30-2	pleurocystidia
r064	RLE 2007-001	pleurocystidia
r065	RLE 2005-002	pleurocystidia
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r069	RLE 2008 050	pseudofestiva
r070	RLE 2007 164	pseudofestiva
r178	F7-JS-38E-11/27/07	pseudofestiva
	EU846297	pseudofestiva
	EU852801	pseudofestiva
	EU669241	pseudofestiva
	EU669240	pseudofestiva
	EU669239	pseudofestiva
	EU697255	pseudofestiva
r114	RLE 2008 131	radicata
r115	RLE 2006 19	radicata
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r010	F7 JS 32	rifflipes
r091	4592 SL	rifflipes
r054	RLE 2006 043	rifflipes

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r055	Ex 199- M	rifflipes
r057	A202 1113 O1-02	rifflipes
r050	LLN 92.11.11-6	rifflipes
r051	A 202 1204 O1-07	rifflipes
r052	A 201 1031 Y1	rifflipes
r053	A206 1114 O1e	rifflipes
r095	RLE 2008 44	rifflipes
r078	LLN 97.10.31-1	rufotubulina
r079	LLN 92.11.24-2	rufotubulina
r073	LLN 92.11.16-1	rufotubulina
r074	SAR 7500	rufotubulina
r075	LLN 92.11.21-6	rufotubulina
r076	LLN 92.11.17-8	rufotubulina
r077	AHSmith 55611	rufotubulina
r081	LLN 95.11.09-27b	scatesiae
r096	LLN 95.11.09-27b	scatesiae
r098	RLE 2007 151	scatesiae
r099	LLN 92.10.15-19	scatesiae
r100	LLN 95.10.14-4	scatesiae
r101	LLN 93.11.04-9	scatesiae
	EU846270	scatesiae
	EU846269	scatesiae
	EU669353	scatesiae
	EU669352	scatesiae
r134	RLE 2007-123	sipei
	EU846294	sipei
	EU846296	sipei
	EU846295	sipei
	EU644708	sipei
	EU644707	sipei
	EU644706	sipei
	EU644705	sipei
r161	RLE 2007-141	spadicea
r177	07-TJR-F02-10/25/07	spadicea
	EU846280	spadicea
	EU846279	spadicea
	EU669365	spadicea
	EU669364	spadicea
	EU669363	spadicea
	EU669362	spadicea
	EU697252	spadicea
	EU846278	spadicea
r152	RLE2007-129	tibiikauffmanii
r153	RLE 2008-55	tibiikauffmanii

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r154	RLE 2007-060	tibiikauffmanii
r155	RLE 2007-165	tibiikauffmanii
r156	Dcalver 200-13	tibiikauffmanii
r157	a2061114o2-O	tibiikauffmanii
r158	RLE 2007-074	tibiikauffmanii
r159	a2011031ox-01	tibiikauffmanii