Phaeocollybia Persistence in Project Areas

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Introduction

The detection of fungi based on visual surveys is difficult because of the unpredictability of mushroom production and the ephemeral nature of these fruiting bodies. However the true test of fungus presence at a site is whether it exists in substrate such as soil, since as long as the fungal mycelium is present in the substrate, the potential for fruiting exists. The fungal mycelium (and mycorrhizae if they are present) contain DNA which can be used to identify species present in soil at a given location. In the last several years, the Oregon/Washington Bureau of Land Management (BLM) and USDA Region 6 Forest Service (USFS) have funded the development of species-specific PCR primers for 15 species of *Phaeocollybia* fungi in the Pacific Northwest, that can be used to detect these species in DNA extracted from soil samples. Each of the 15 species is either on the BLM or USFS Interagency Special Status/Sensitive Species Program (ISSSSP) lists or their Survey and Manage list or both lists. The goal of the "case study" reported here is to determine if *Phaeocollybia attenuata* and other *Phaeocollybia* species known prior to harvest through mushroom presence persisted in the soil in 1) a "buffered" species location within a thinned area, 2) a commercially thinned project area, and 3) a regeneration harvest area. In all cases we found species present before site management activities persisted in the soil after treatment. Some species that had not previously been observed at the sites were also found to be present in the soil.

Methods

Study Sites

The buffered site was on Moose Mountain in the western Cascades, off the Santiam River, about 24 km east of Sweet Home, OR (10T 545398 4917093) at an elevation of 1375' (419 m). Administratively it is in the Sweet Home Ranger District of the Willamette National Forest. On Oct. 14, 2011 two approximately perpendicular transects were sampled at this site. The transects intersected at a flagged spot where *P. attenuata* had been collected in 1999. A 15.2 m radius buffer had been set up around this spot before a thinning project was completed just downslope from the site, in 2004 or 2005. Transect A was 36 m long and 80 equally spaced samples were taken from this transect. Transect B was 30.5 m long and 20 equally spaced samples were taken from this transect. No mushrooms were seen on the day of survey.

Figure 1. Moose Mtn. buffered site, looking from within buffer to adjacent thinned area.

The thinned site was on Green Peak in the Oregon Coast Range, about 29 km SW of Corvallis, OR (10T 464001 4912801) at an elevation of 1828' (558 m). Administratively, the land is in the Salem District BLM, Mary's Peak Resource Area. The site had been thinned in 1999 and again in 2011 to a final density of 60 trees per acre. Soil samples were collected on Nov. 9, 2011 from two parallel transects. The transects were 20 m long and spaced 2 m apart. Fifty equally spaced samples were taken from each transect. *P. attenuata* was fruiting at the time of sampling, and sporocarps were present on the study site.

Figure 2. Green Peak thinned site. Yellow lines (meter tape) mark the transects.

The regeneration harvest site was also on Green Peak, about 600 m WSW of the thinned site (10T 463420 4912592) at an elevation of 2115' (605 m). This site had been clear cut in 1999 and re-planted with Douglas-fir and western hemlock in 2001. These and other young trees and shrubs were growing throughout the site. On Oct. 24, 2011 four 10 m transects were sampled at this site, each 1 m apart. Twenty five equally spaced samples were taken from each transect. No mushrooms were seen on the day of sampling. The plot was centered on the center of an old visual survey transect, on the north end where *P. attenuata* sporocarps had been seen before timber cutting at the site. *P. attenuata* has not been seen at this site since the timber harvest, although *P. Phaeogaleroides* (not a listed species) was seen.

Figure 3. Green Peak regeneration harvest site.

Field Protocol

An anchored tape measure was used to mark each transect. A 2.5 cm diameter stainless steel soil probe was used to obtain soil samples. The location of each sample on the transect was written down along with any notes about unusual soil characteristics, obstructions, proximity to mushrooms, etc. At each sampling location, two adjacent probe samples were taken to increase the soil volume tested. The maximum depth of soil that could be taken by the probe was about 35 cm. However, the probe frequently hit obstructions in the soil and the full depth could not be reached. Previous work showed that *Phaeocollybia* species were detected in both shallow and deep soil samples, so extraordinary efforts were not made to obtain full depth samples.

For each sampling point, the soil from both samples was combined in a numbered heavy-duty recloseable plastic bag. After each pair of samples, the probe was cleaned in a detergent solution, rinsed in water, and dried with a rag. Samples were stored on ice during transport to the lab.

Lab Protocol

Samples were kept chilled (1- 5^{o} C) until they were processed. After hand mixing the soil in a sample bag, 0.7 to 0.9 g of soil was transferred to a 2.0 ml centrifuge tube, and DNA was extracted using a Chelex extraction buffer. To clean the DNA, tubes containing soil and buffer were centrifuged for 2 min. Then, 150 µl of supernatant, containing the DNA and other soluble soil compounds, was removed from each sample. The DNA was isolated from the supernatant by binding to glass fiber filters in a 96-well filter plate, washing the filters, and eluting the DNA with an aqueous buffer, in a procedure similar to that described in Ivanova et al, 2006. Every extraction batch included at least one positive control and every 16th sample extracted was a blank.

DNA analysis using multiplex PCR has been previously described (Gordon, 2011). Although *P. attenuata* was specifically targeted in this study, the multiplex PCR method allows the detection of 15 different *Phaeocollybia* species in two to three PCRs. Using this method each sample was tested for the presence of all 15 listed *Phaeocollybia* species. Positive controls and blanks were run with every PCR batch to insure the integrity of the method.

Because so many positive results were found in the PCR test, and because some species were detected that had not been observed fruiting at the sites, some of the PCR amplicons were sent in to a commercial lab for sequencing. Forward and reverse sequences were aligned using Geneious Pro (version 5.5.7) and the consensus alignment was compared to known *Phaeocollybia* sequences using Geneious Pro.

Results

Results are presented in Tables III, IV, and V in Appendix A and are summarized in Table I below. The results are displayed pictorially in figures 4, 5, and 6 below. Sequencing results are shown in Table II.

Table I. Site results. All sites had 100 samples collected and analyzed.

Site	positive samples	*P. attenuata* positives	Individual *Phaeocollybia* detections	*Phaeocollybia* species detected
Buffered	45	45	58	3
Thinned	53	33	83	4
Regen	65	60	69	2

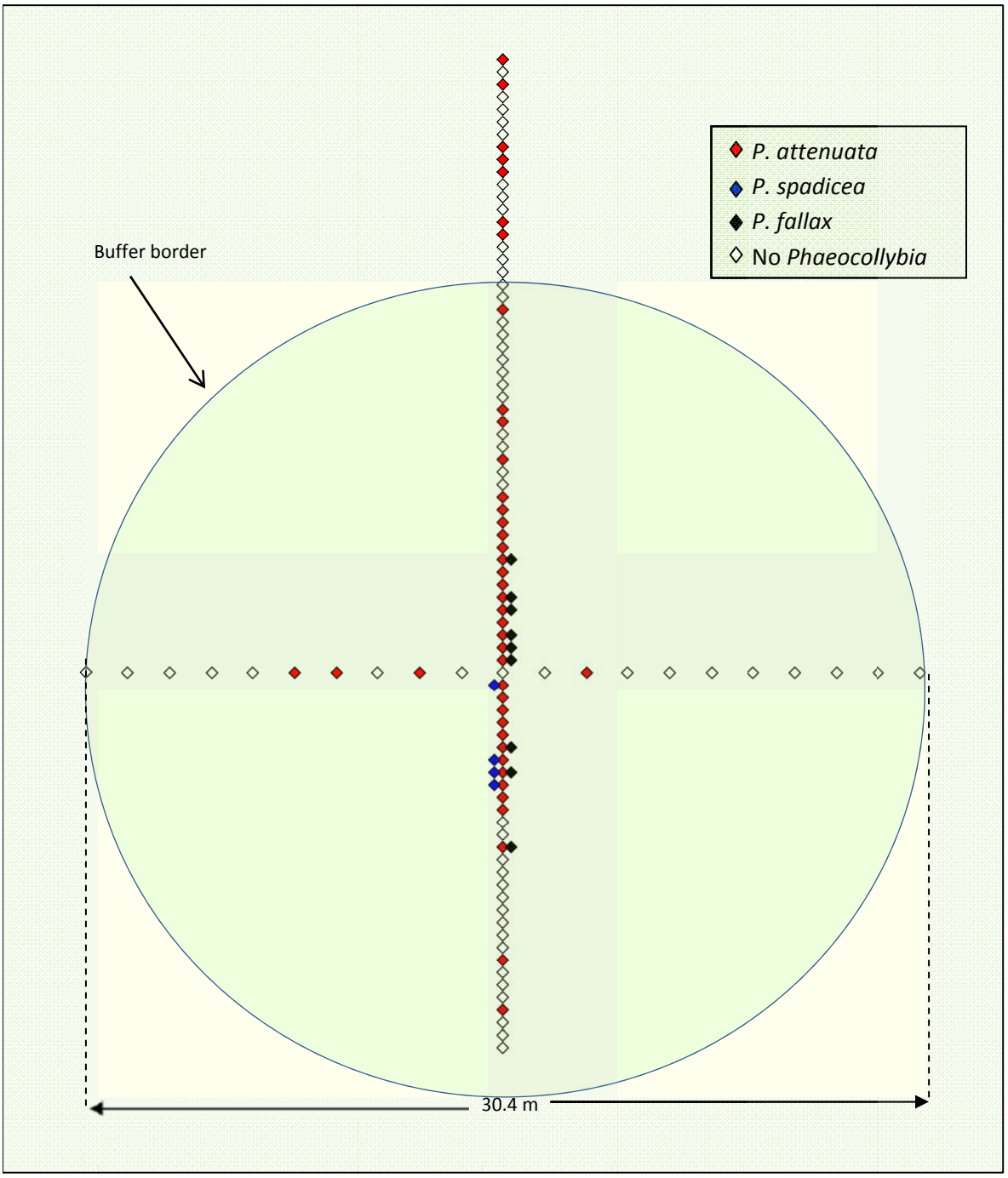

Figure 4. Sample test results, buffered site, Moose Mtn, Sweet Home Ranger District on the Willamette National Forest. The *P. attenuata* sporocarp collected prior to management activities was located at the center of the buffered site circle.

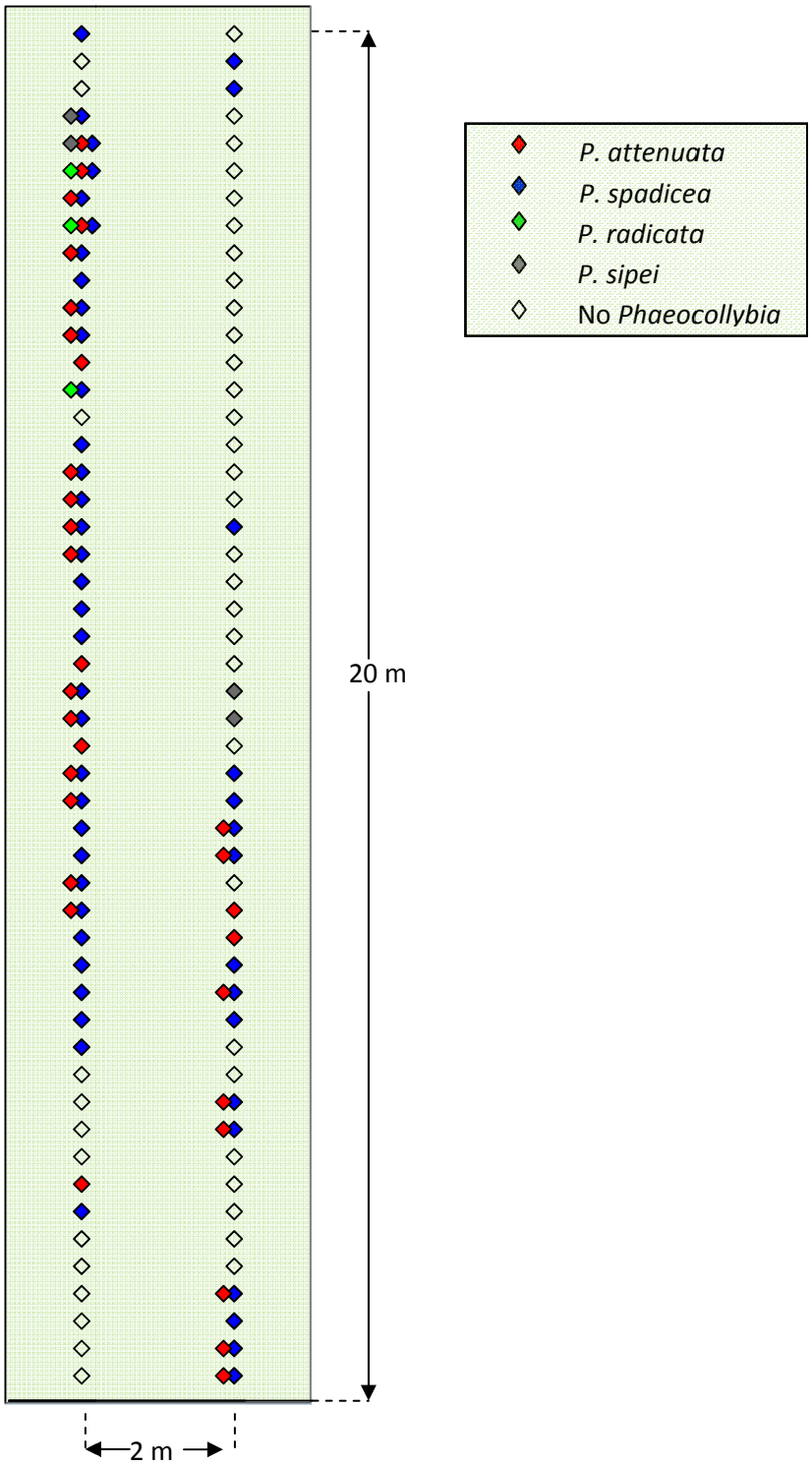

Figure 5. Sample test results, thinned site, Green Peak, Mary's Peak Resource Area on Salem District BLM. *P. attenuata* sporocarps were seen during sample collection in the upper left quadrant of the diagram.

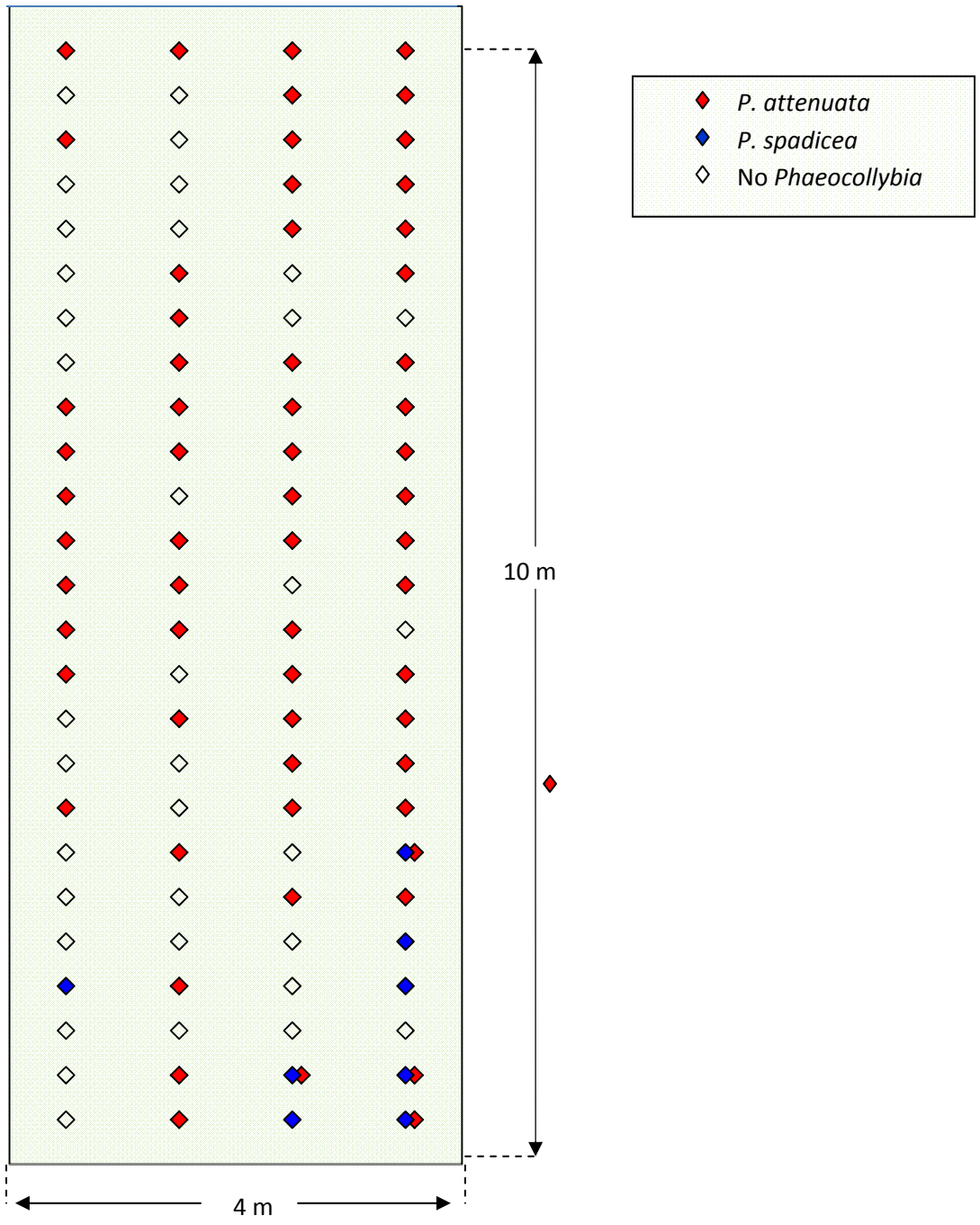

Figure 6. Sample test results, regeneration harvest site, Green Peak, Mary's Peak Resource Area on Salem District BLM. Locations of past *P. attenuata* sporocarps were not flagged, but were on or near this plot.

Table II. Sequencing results. Sequences obtained from PCR products derived from sample DNA were compared to a database of *Phaeocollybia* sequences and all were found to match.

Site	Sample #	PCR Result	Sequence closest match
Moose Mtn. buffer	03	*fallax*	*fallax*
Moose Mtn. buffer	06	*fallax*	*fallax*
Moose Mtn. buffer	07	*fallax*	*fallax*
Moose Mtn. buffer	56	*fallax*	*fallax*
Moose Mtn. buffer	57	*spadicea*	*spadicea*
Moose Mtn. buffer	64	*attenuata*	*attenuata*
Moose Mtn. buffer	64	*fallax*	*fallax*
Moose Mtn. buffer	98	*attenuata*	*attenuata*
Green Peak thin	22	*spadicea*	*spadicea*
Green Peak thin	37	*radicata*	*radicata*
Green Peak thin	38	*attenuata*	*attenuata*
Green Peak thin	40	*spadicea*	*spadicea*
Green Peak thin	43	*attenuata*	*attenuata*
Green Peak thin	43	*spadicea*	*spadicea*
Green Peak thin	70	*attenuata*	*attenuata*
Green Peak regen	08	*attenuata*	*attenuata*
Green Peak regen	25	*attenuata*	*attenuata*
Green Peak regen	38	*attenuata*	*attenuata*
Green Peak regen	41	*attenuata*	*attenuata*
Green Peak regen	45	*attenuata*	*attenuata*
Green Peak regen	61	*attenuata*	*attenuata*
Green Peak regen	63	*attenuata*	*attenuata*
Green Peak regen	80	*attenuata*	*attenuata*
Green Peak regen	94	*attenuata*	*attenuata*

Discussion

The sites examined in this case study were not genetically surveyed before management activities were undertaken, so we cannot say what the effects of these activities were, or compare the effects of different management regimes. However this study does show that some *Phaeocollybia* species that were known to be present prior to management activities, including *P. attenuata* and *P. spadicea* can persist under a variety of management regimes, including regeneration harvest.

Norvell and Exeter(2008) present strong evidence of the mycorrhizal nature of *Phaeocollybia.* It would seem to make sense that the elimination of their mycorrhizal partners, presumably the mature conifers, would take a toll on the *Phaeocollybia* present in a harvest area. But it may be that the loss of this nutrient source just makes mushroom production physiologically impossible, rather than causing the complete loss of the fungus from the soil. Some species of mycorrhizal fungi can possibly rely on shrub layer plants as mycorrhizal partners until trees mature, or they may have saprobic modes of nutrition available. At least

one study has shown that living mycorrhizae can be found on the roots of cut trees up to two years after harvest.

At the Green Peak thinned site *P. radicata*, a species that had not been seen fruiting, was found in the soil. At the Moose Mtn. buffered site *P. fallax*, which had not been observed there, was found in the soil. Both of these findings were confirmed with sequencing. These results demonstrate the value of a genetic soil survey: in one site visit it supplies a picture of the full fungal diversity at a site, provided that specific primers have been developed for all species of interest.

The regeneration harvest site and the thinned site were close (600m) to each other, yet the thinned site had 2 more species than the regeneration site. Some *Phaeocollybia* species may be sensitive to harvest intensity and may not be able to survive a regeneration harvest. This is a hypothesis that has not been tested—we do not know how many species were present in the soil at the regeneration site before harvest.

At all three sites it appears that *Phaeocollybia* species cluster together rather than occupying separate patches of ground. A previous study at two different sites (Gordon, 2011) also found a tendency for *Phaeocollybia* species to cluster. The reason for this remains unclear. It may be that one *Phaeocollybia* species establishes a favorable soil environment, for example by eliminating competitors, that other *Phaeocollybia* species, too similar to be recognized as competitors, can take advantage of.

Acknowledgements

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References

Gordon, M. 2011. Pilot project to detect *Phaeocollybia* in soil: a field study. Final report submitted to USDI Bureau of Land Management, Oregon State Office. Contract number L09PX01364. On file with: Interagency Special Status/Sensitive Species Program, USDI Bureau of Land Management, Oregon State Office. http://www.fs.fed.us/r6/sfpnw/issssp/documents/inventories

Ivanova, N.V., J.R. DeWaard, and P.D.N. Hebert. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes, 6(4): 998-1002.

Norvell, L.L., Exeter, R.L. Phaeocollybia of Pacific Northwest North America. 2008. US Dept. of Interior, Bureau of Land Management, Salem District. Salem, OR.

Appendix A

Table III. Data from Moose Mtn. buffered site

Sample #	Transect	Distance along tsect (ft)	Collection Note	Primer Set 1 Final Result	Species	Primer Set 2 Final Result	Species
g1 01	A	0.0	rocky	-	0		0
g1 02	A	1.5	rocky	+	falA	+	attA
g1 03	A	3.0	rocky	+	falA	+	attA
g1 04	A	4.5	rocky	+	falA	+	attA
g1 05	A	6.0	rocky	-	0	+	attA
g1 06	A	7.5	rocky	+	falA	+	attA
g1 07	A	9.0	rocky	+	falA	+	attA
g1 08	A	10.5	rocky	-	0	+	attA
g1 09	A	12.0	rocky	-	0	+	attA
g1 10	A	13.5	rocky	+	falA	+	attA
g1 11	A	15.0	rocky, deer trail	-	0	+	attA
g1 12	A	16.5	rocky, deer trail	-	0	+	attA
g1 13	A	18.0	rocky, deer trail	-	0	+	attA
g1 14	A	19.5	rocky, deer trail	-	0	+	attA
g1 15	A	21.0	5" soil, deer trail	-	0	+	attA
g1 16	A	22.5	shallow, deer trail	-	0	0	0
g1 17	A	24.0	rocky, deer trail	-	0	0	0
g1 18	A	25.5	rocky, deer trail	-	0	+	attA
g1 19	A	27.0	rocky, deer trail	-	0	0	0
g1 20	A	28.5	rocky	-	0	0	0
g1 21	A	30.0	rocky	-	0	+	attA
g1 22	A	31.5	rocky	-	0	+	attA
g1 23	A	33.0	rocky	-	0	0	0
g1 24	A	34.5	rocky	-	0	0	0
g1 25	A	36.0	rocky	-	0	0	0
g1 26	A	37.5		-	0	0	0
g1 27	A	39.0		-	0	0	0
g1 28	A	40.5		-	0	0	0
g1 29	A	42.0		-	0	0	0
g1 30	A	43.5	deeper soil, clay	-	0	+	attA
g1 31	A	45.0		-	0	0	0
g1 32	A	46.5	under log, drier soil	-	0	0	0
g1 33	A	48.0		-	0	0	0
g1 34	A	49.5		-	0	0	0
g1 35	A	51.0		-	0	0	0
g1 36	A	52.5		-	0	+	attA
g1 37	A	54.0		-	0	+	attA
g1 38	A	55.5	deep soil, outside buffer	-	0	0	0
g1 39	A	57.0	rocky	-	0	0	0
g1 40	A	58.5	moderate depth, clay	-	0	0	0
g1 41	A	60.0	clay	-	0	+	attA
g1 42	A	61.5		-	0	+	attA
g1 43	A	63.0	deep soil	-	0	+	attA
g1 44	A	64.5	deep and drier	-	0	0	0
g1 45	A	66.0		-	0	0	0
g1 46	A	67.5	deep	-	0	0	0
g1 47	A	69.0	deep	-	0	0	0
g1 48	A	70.5	deep	-	0	+	attA

g1 49	A	72.0	deep	-	0	0	0
g1 50	A	73.5	deep	-	0	+	attA
g1 51	B	1.5	rocky	+	spa	+	attA
g1 52	B	3.0	shallow soil	0	0	+	attA
g1 53	B	4.5	shallow soil	0	0	+	attA
g1 54	B	6.0	shallow soil	0	0	+	attA
g1 55	B	7.5	rocky	0	0	+	attA
g1 56	B	9.0	rocky	+	falA	+	attA
g1 57	B	10.5	shallow soil	+	spa	+	attA
g1 58	B	12.0	shallow soil	+ +	spa/ falA	+	attA
g1 59	B	13.5		+	spa	+	attA
g1 60	B	15.0		0	0	+	attA
g1 61	B	16.5		0	0	+	attA
g1 62	B	18.0	rocky	0	0		
g1 63	B	19.5		0	0		
g1 64	B	21.0		+	falA	+	attA
g1 65	B	22.5	rocky	0	0	0	0
g1 66	B	24.0	rocky	0	0	0	0
g1 67	B	25.5		0	0	0	0
g1 68	B	27.0		0	0	0	0
g1 69	B	28.5		0	0	0	0
g1 70	B	30.0		0	0	0	0
g1 71	B	31.5		0	0	0	0
g1 72	B	33.0		0	0	0	0
g1 73	B	34.5		0	0	+	attA
g1 74	B	36.0		0	0	0	0
g1 75	B	37.5		0	0	0	0
g1 76	B	39.0	deep soil	0	0	0	0
g1 77	B	40.5	deep soil	0	0	+	attA
g1 78	B	42.0		0	0	0	0
g1 79	B	43.5	deep soil	0	0	0	0
g1 80	B	45.0	deep soil, buffer boundary	0	0	0	0
g1 81	C	5.0		0	0	0	0
g1 82	C	10.0		0	0	+	attA
g1 83	C	15.0		0	0	0	0
g1 84	C	20.0		0	0	+	attA
g1 85	C	25.0		0	0	+	attA
g1 86	C	30.0		0	0	0	0
g1 87	C	35.0		0	0	0	0
g1 88	C	40.0		0	0	0	0
g1 89	C	45.0	deep soil	0	0	0	0
g1 90	C	50.0	~2' outside buffer	0	0	0	0
g1 91	D	5.0		0	0	0	0
g1 92	D	10.0		0	0	+	attA
g1 93	D	15.0		0	0	0	0
g1 94	D	20.0		0	0	0	0
g1 95	D	25.0		0	0	0	0
g1 96	D	30.0		0	0	0	0
g1 97	D	35.0		0	0	0	0
g1 98	D	40.0		0	0	0	0
g1 99	D	45.0		0	0	0	0
g1 100	D	50.0		0	0	0	0

Table IV. Data from Green Peak thinned site

Sample #	Transect	Distance along tsect (ft)	Collection Note	Primer Set 1 Final Result	Species	Primer Set 2 Final Result	Species
g3 01	A	63.7		-	0	-	0
g3 02	A	62.4		-	0	-	0
g3 03	A	61.1		-	0	-	0
g3 04	A	59.8		-	0	-	0
g3 05	A	58.5		-	0	-	0
g3 06	A	57.2		-	0	-	0
g3 07	A	55.9		+	spa	-	0
g3 08	A	54.6		-	0	+	attA
g3 09	A	53.3		-	0	-	0
g3 10	A	52		-	0	-	0
g3 11	A	50.7		-	0	-	0
g3 12	A	49.4	shallow	-	0	-	0
g3 13	A	48.1		+	spa	-	0
g3 14	A	46.8		-	0	-	0
g3 15	A	45.5		-	0	-	0
g3 16	A	44.2		-	0	-	0
g3 17	A	42.9		-	0	-	0
g3 18	A	41.6		+	spa	+	attA
g3 19	A	40.3		+	spa	+	attA
g3 20	A	39		+	spa	-	0
g3 21	A	37.7		+	spa	-	0
g3 22	A	36.4		+	spa	+	attA
g3 23	A	*35.1*	P. *attenuata* 2' west	+	spa	+	attA
g3 24	A	33.8		-	0	+	attA
g3 25	A	32.5	6" deep	+	spa	+	attA
g3 26	A	31.2	5" deep	+	spa	+	attA
g3 27	A	29.9		-	0	+	attA
g3 28	A	28.6		+	spa	-	0
g3 29	A	27.3		+	spa	-	0
g3 30	A	26		+	spa	-	0
g3 31	A	24.7		+	spa	+	attA
g3 32	A	23.4		+	spa	+	attA
g3 33	A	22.1	big air pocket	+	spa	+	attA
g3 34	A	20.8		+	spa	+	attA
g3 35	A	19.5		+	spa	-	0
g3 36	A	*18.2*	P. *attenuata* 2' west	-	0	-	0
g3 37	A	16.9		+	rad	+	attA
g3 38	A	*15.6*	P. *attenuata* 1' north	-	0	+	attA
g3 39	A	14.3		+	spa	+	attA
g3 40	A	13		+	spa	+	attA
g3 41	A	11.7		+	spa	-	0
g3 42	A	10.4		+	spa	+	attA
g3 43	A	*9.1*	P. attenuata 5" west	+/+	spa/rad	+	attA
g3 44	A	7.8		+	spa	+	attA
g3 45	A	6.5		+/+	spa/rad	+	attA
g3 46	A	5.2	4" deep	+/+	spa/ sip	+	attA
g3 47	A	3.9	4" deep	+	sip	+	attA
g3 48	A	2.6		-	0	-	0
g3 49	A	1.3		-	0	-	0
g3 50	A	0		+	spa	-	0
g3 51	B	63.7		+	spa	+	attA

g3 52	B	62.4		+	spa	+	attA
g3 53	B	61.1		+	spa	-	0
g3 54	B	59.8		+	spa	+	attA
g3 55	B	58.5		-	0	-	0
g3 56	B	57.2		-	0	-	0
g3 57	B	55.9		-	0	-	0
g3 58	B	54.6		-	0	-	0
g3 59	B	53.3	shallow	-	0	-	0
g3 60	B	52		+	spa	+	attA
g3 61	B	50.7		+	spa	+	attA
g3 62	B	49.4		-	0	-	0
g3 63	B	48.1		-	0	-	0
g3 64	B	46.8		+	spa	-	0
g3 65	B	45.5		+	spa	+	attA
g3 66	B	44.2		+	spa	-	0
g3 67	B	42.9		-	0	+	attA
g3 68	B	41.6		-	0	+	attA
g3 69	B	40.3		-	0	-	0
g3 70	B	39	near chantarelle	+	spa	+	attA
g3 71	B	37.7	6" deep	+	spa	+	attA
g3 72	B	36.4		+	spa	-	0
g3 73	B	35.1		+	spa	-	0
g3 74	B	33.8		-	0	-	0
g3 75	B	32.5		+	sip	-	0
g3 76	B	31.2		+	sip	-	0
g3 77	B	29.9		-	0	-	0
g3 78	B	28.6		-	0	-	0
g3 79	B	27.3		-	0	-	0
g3 80	B	26		-	0	-	0
g3 81	B	24.7		-	0	-	0
g3 82	B	23.4		+	spa	-	0
g3 83	B	22.1		-	0	-	0
g3 84	B	20.8		-	0	-	0
g3 85	B	19.5		-	0	-	0
g3 86	B	18.2		-	0	-	0
g3 87	B	16.9		-	0	-	0
g3 88	B	15.6		-	0	-	0
g3 89	B	14.3		-	0	-	0
g3 90	B	13		-	0	-	0
g3 91	B	11.7		-	0	-	0
g3 92	B	10.4		-	0	-	0
g3 93	B	9.1		-	0	-	0
g3 94	B	7.8		-	0	-	0
g3 95	B	6.5		-	0	-	0
g3 96	B	5.2		-	0	-	0
g3 97	B	3.9		-	0	-	0
g3 98	B	2.6		+	spa	-	0
g3 99	B	1.3		+	spa	-	0
g3 100	B	0		-	0	-	0

Table V. Data from Green Peak regeneration harvest site

Sample #	Transect	Distance along tsect (ft)	Collection Note	Primer Set 1 Final Result	Species	Primer Set 2 Final Result	Species
g2 01	A	1.3		-	0	-	0
g2 02	A	2.6		-	0	-	0
g2 03	A	3.9		-	0	-	0
g2 04	A	5.2		+	spa	-	0
g2 05	A	6.5	next to stump	-	0	-	0
g2 06	A	7.8	next to stump	-	0	-	0
g2 07	A	9.1	next to stump	-	0	-	0
g2 08	A	10.4		-	0	+	attA
g2 09	A	11.7		-	0	-	0
g2 10	A	13.0		-	0	-	0
g2 11	A	14.3		-	0	+	attA
g2 12	A	15.6		-	0	+	attA
g2 13	A	16.9	rocky	-	0	+	attA
g2 14	A	18.2	rocky	-	0	+	attA
g2 15	A	19.5		-	0	+	attA
g2 16	A	20.8		-	0	+	attA
g2 17	A	22.1	salal	-	0	+	attA
g2 18	A	23.4	salal	-	0	-	0
g2 19	A	24.7	salal, rocky, stump	-	0	-	0
g2 20	A	26.0		-	0	-	0
g2 21	A	27.3		-	0	-	0
g2 22	A	28.6		-	0	-	0
g2 23	A	29.9		-	0	+	attA
g2 24	A	31.2		-	0	-	0
g2 25	A	32.5	rocky	-	0	+	attA
g2 26	B	1.3		-	0	+	attA
g2 27	B	2.6		-	0	+	attA
g2 28	B	3.9	rocky	-	0	-	0
g2 29	B	5.2		-	0	+	attA
g2 30	B	6.5		-	0	-	0
g2 31	B	7.8	air pocket	-	0	-	0
g2 32	B	9.1		-	0	+	attA
g2 33	B	10.4		-	0	-	0
g2 34	B	11.7	rocky	-	0	-	0
g2 35	B	13.0	air pockets	-	0	+	attA
g2 36	B	14.3		-	0	-	0
g2 37	B	15.6		-	0	+	attA
g2 38	B	16.9		-	0	+	attA
g2 39	B	18.2		-	0	+	attA
g2 40	B	19.5		-	0	-	0
g2 41	B	20.8		-	0	+	attA
g2 42	B	22.1		-	0	+	attA
g2 43	B	23.4		-	0	+	attA
g2 44	B	24.7	next to stump	-	0	+	attA
g2 45	B	26.0		-	0	+	attA
g2 46	B	27.3	next to big stump	-	0	-	0
g2 47	B	28.6	next to big stump	-	0	-	0
g2 48	B	29.9		-	0	-	0
g2 49	B	31.2		-	0	-	0
g2 50	B	32.5		-	0	+	attA
g2 51	C	1.3		+	spa	-	0

g2 52	C	2.6	air pocket	+	spa	+	attA
g2 53	C	3.9		-	0	+	attA
g2 54	C	5.2		-	0	-	0
g2 55	C	6.5		-	0	-	0
g2 56	C	7.8		-	0	-	0
g2 57	C	9.1	next to 7 ft Doug fir	-	0	+	attA
g2 58	C	10.4		-	0	-	0
g2 59	C	11.7	rocky soil	-	0	+	attA
g2 60	C	13.0		-	0	+	attA
g2 61	C	14.3		-	0	+	attA
g2 62	C	15.6	air pocket	-	0	+	attA
g2 63	C	16.9		-	0	+	attA
g2 64	C	18.2	rocky	-	0	-	0
g2 65	C	19.5	rocky	-	0	+	attA
g2 66	C	20.8	deep soil sample	-	0	+	attA
g2 67	C	22.1		-	0	+	attA
g2 68	C	23.4	deep soil sample	-	0	+	attA
g2 69	C	24.7	deep soil sample	-	0	+	attA
g2 70	C	26.0	next to big stump	-	0	-	0
g2 71	C	27.3	touching stump	-	0	-	0
g2 72	C	28.6	17 in W of transect--large stump	-	0	+	attA
g2 73	C	29.9	12" W of stump/ transect	-	0	+	attA
g2 74	C	31.2	deep	-	0	+	attA
g2 75	C	32.5		-	0	+	attA
g2 76	D	1.3		+	spa	+	attA
g2 77	D	2.6		+	spa	+	attA
g2 78	D	3.9		+	spa	-	0
g2 79	D	5.2		+	spa	-	0
g2 80	D	6.5	rocky	-	0	+	attA
g2 81	D	7.8	rocky next to 9' Doug fir	-	0	-	0
g2 82	D	9.1		+	spa	+	attA
g2 83	D	10.4		+	spa	-	0
g2 84	D	11.7	rocky soil	-	0	+	attA
g2 85	D	13.0		-	0	+	attA
g2 86	D	14.3		-	0	+	attA
g2 87	D	15.6		-	0	+	attA
g2 88	D	16.9	next to stump	-	0	+	attA
g2 89	D	18.2	next to stump	-	0	+	attA
g2 90	D	19.5	next to stump	-	0	+	attA
g2 91	D	20.8		-	0	-	0
g2 92	D	22.1		-	0	+	attA
g2 93	D	23.4		-	0	+	attA
g2 94	D	24.7		-	0	+	attA
g2 95	D	26.0		-	0	+	attA
g2 96	D	27.3	deep	-	0	+	attA
g2 97	D	28.6	deep	-	0	+	attA
g2 98	D	29.9	deep	-	0	-	0
g2 99	D	31.2	deep, next to stump	-	0	+	attA
g2 100	D	32.5		-	0	+	attA