Johnson's Hairstreak Surveys in Oregon and Washington (2010)

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Abstract

In 2010 the Interagency Special Status/Sensitive Species Program (ISSSSP) funded widespread field surveys in Oregon and Washington to document presence of Johnson's Hairstreak butterfly (*Callophrys johnsoni*) at locations where species presence was currently unknown, but likely based on habitat modeling and specific survey site selection criteria. The larval survey method developed and tested in 2009 was the preferred method for surveying as it has the highest probability for detecting the species at sites that are occupied. However, in some parts of its range, the Johnson's Hairstreak overlaps with the range of Thicket Hairstreak (*C. spinetorum*). While visually distinguishing between the two species is easily done in the adult stage, it is problematic in the larval stages. Adult specimens from both species collected during 2009 surveys were sent for DNA analysis. The results of that analysis successfully developed genetic markers that now allow for identification of the species in their larval stages. The results of the 2010 survey and phylogenetic analysis indicate that larval surveys that are informed through the use of habitat models are useful tools for the surveying and conservation of this species.

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INTRODUCTION

The Johnson's Hairstreak (*Callophrys johnsoni*) is a rare butterfly that inhabits the Pacific Northwest region of the United States of America. Prior to this survey, there were a total of 103 documented sites of adults or larvae, scattered throughout Oregon and Washington, dating back to 1891. All but 44 of these sightings predated the implementation of the Northwest Forest Plan in 1994 (USDA/USDI 1994), which provided increased protection of late-successional forests over the main portion of this species' range. This survey added 13 additional sites, for a total of 116 sites known from Oregon and Washington (fig. 1). Older coniferous forests, especially those with a heavy component of western hemlock (*Tsuga heterophyla*) that are infected by dwarf mistletoe (*Arceuthobium tsugense*) appear to be its key habitat. However, it is also found in other forest types, most notable, in ponderosa pine (*Pinus ponderosa*) forests infected with *A. campylopodum* in a disjunct population located in northeastern Oregon, near the Idaho border. It also has been documented to use *A. abietinum* in true firs such as white fir (*Abies concolor*).

A draft habitat model (v1.0) and survey protocol (v1.0, Davis et al. 2009) were developed in 2008 and tested in 2009. The primary objective of the 2009 survey was to field-test different survey methods for detecting presence of this butterfly at locations where species presence was likely based on habitat modeling and site-specific survey area selection criteria. The results of these surveys were used to estimate site occupancy (site = transect) and detection probabilities for two survey methods. The results of the occupancy analysis (Davis 2010) found that the probability of detecting *Callophrys* spp.[1] using the adult survey protocol was on average 33 percent, whereas the larval survey technique resulted in a much higher (near 100%) probability of detection at occupied sites. While visual identification of *C. johnsoni* is relatively easy in its adult stage, it is not in the larval stages. Adult vouchers of both *C. johnsoni* and the Thicket Hairstreak (*C. spinetorum*), a closely related look-alike species, for which there is currently no known way to visually distinguish between the two species in the larval stages, were sent out for DNA sequencing and genetic analysis. The genetic analysis was successful at identifying genetic markers to distinguish between the two species, and that new information was put to the test in the 2010 surveys.

This report documents the results of surveys conducted throughout Oregon and Washington in 2010 using a revised habitat model (v1.1) and survey protocol (v1.1, Davis et al. 2010). The primary objective of these surveys was to document presence of *C. johnsoni*, by means of larval surveys and genetic analysis, at locations where species presence was currently unknown, but likely based on habitat modeling. The presence of the larval host plant, dwarf mistletoe, was essential to the larval surveys. While dwarf mistletoe abundance is highest in the upper tree canopy, it can be readily found near ground level, where our survey focused for safety and logistical reasons. The larval survey was the preferred method, as it produced the highest detection rate at sites occupied by *Callophrys* spp. during 2009 surveys. However, the survey method was left to the discretion of the surveyor on whether to use the adult, larval, focused [checklist] surveys, or any combination of survey methods, depending on their site specific circumstances. The results of the genetic analysis conducted on adult specimens of *C. johnsoni* and *C. spinetorum* collected in 2009, and larvae collected during the 2010 survey are also presented in this report.

[1] Johnson's Hairstreak (*C. johnsoni*) and Thicket Hairstreak (*C. spinetorum*), which lay eggs on dwarf mistletoes.

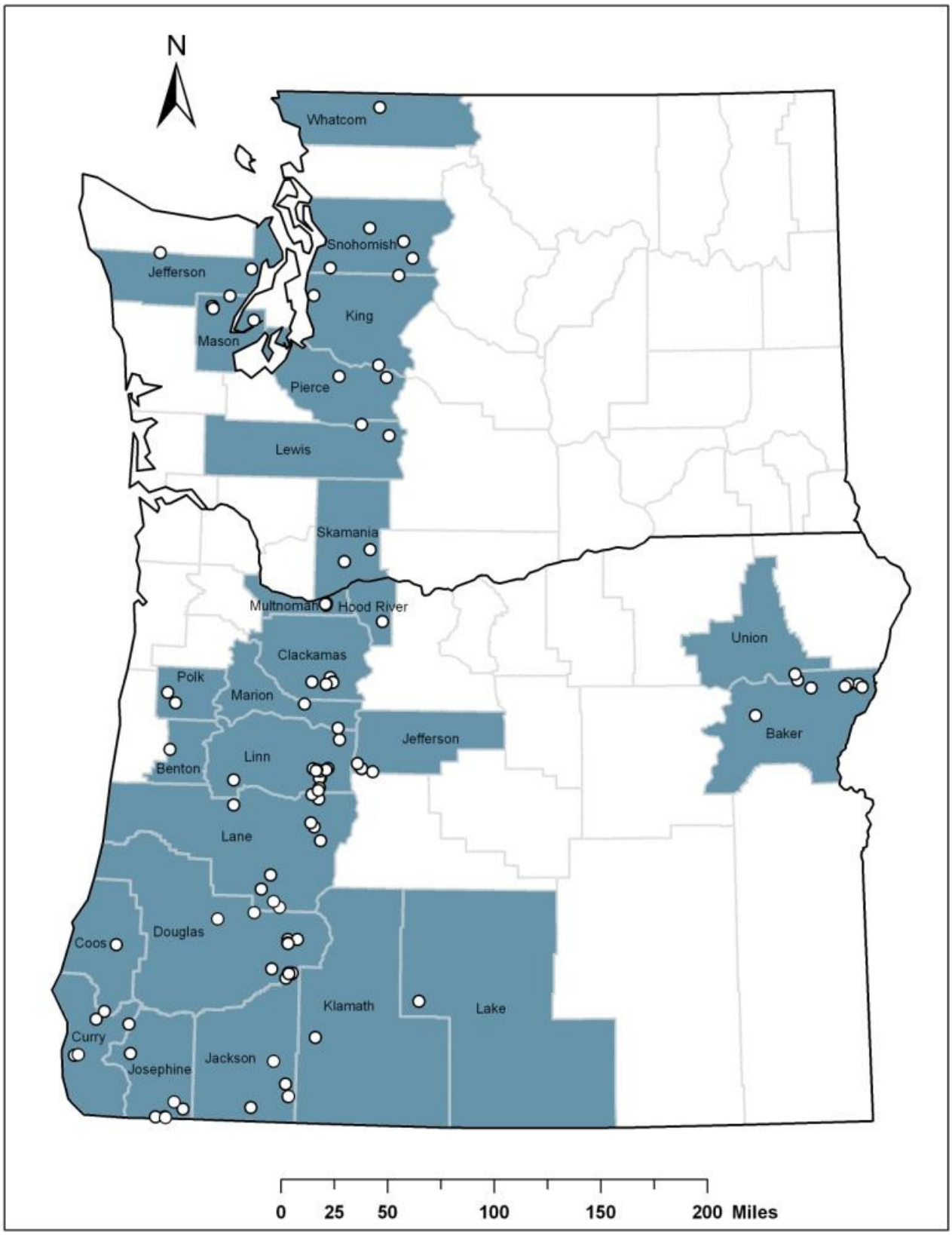

Figure 1. Current known geographic distribution of Johnson's Hairstreak butterfly (*Callophrys johnsoni*) records in Oregon and Washington (all records dating back to 1891).

METHODS

Field surveys

Field surveys were dispersed through Oregon and Washington, using the v1.1 habitat suitability map (appx. 3 in Davis et al. 2010) to focus on areas with high suitability and no known or recent presence of *C. johnsoni*. The map was to be used in conjunction with the seasonal activity maps and graphs provided in appendix 4 in Davis et al. (2010) to optimize the timing of the surveys. In general, surveyors were to focus on areas with high likelihood of occurrence and for times that correspond with peak larval seasons. Once a potential survey area was identified using these maps, ground visits prior to the larval season were to be conducted to confirm the presence of active dwarf mistletoe. Areas with low concentrations of dwarf mistletoe (<35% of transect) had a very low probability of occupancy in the surveys of 2009 (fig.4 in Davis 2010) and survey sites were to focus in areas with both high habitat suitability and high amounts of live dwarf mistletoe, however, this was not clearly stated in the v1.1 protocol.

Vouchering

Vouchering of larval specimens was conducted in the office or lab from dwarf mistletoe clippings that were collected in the field. Clippings of dwarf mistletoe with eggs or larvae were stored according to protocol in Tupperware containers until larvae hatched, and were allowed to grow for a while. Larvae and eggs were collected from these clippings and individually placed in separate sample vials containing 75% ethanol. Each vial was given a unique identifier number and the location, date, and other information were entered into a database.

DNA analysis

At the end of the field season, vouchers were consolidated and sent out for phylogenetic analysis to the University of La Verne, La Verne, California, under the supervision of Dr. Kathleen Weaver, Associate Professor of Biology. DNA extraction was performed following the manufacturer's protocol for the DNeasy Kit (Qiagen Inc., Valencia, CA) from alcohol-preserved tissue. For the adults, a small piece of tissue from the thorax was utilized. For larvae, up to half the animal was used in the extraction. For larvae under 2 mm and eggs (~1 mm diameter), the whole specimen was used, leaving no voucher. Using the genomic DNA extracted from the 137 larvae and 20 adult samples[2], an examination was conducted on the variation in mitochondrial and nuclear loci, including 12S ribosomal DNA, cytochrome oxidase I (COI), and ribosomal protein S5 (RpS5). Forward and reverse sequences were assembled using Sequencher® version 4.9 (Gene Codes Corp., Ann Arbor, MI) and alignments were conducted using Geneious Pro™ version 5.3.4 (Biomatters, New Zealand). The phylogenetic analysis of *C. johnsoni* and *C. spinetorum* specimens was conducted using maximum-likelihood in PhyML (Guindon and Gascuel 2003). Tree testing was performed using likelihood bootstrap methods (1,000 replicates) in PhyML (Guindon and Gascuel 2003). Tree visualization was performed in Geneious Pro™ version 5.3.4.

[2] Adult samples were from the 2009 survey season.

RESULTS

Field survey

Ten broad geographic areas (fig. 2, table 2 in appendix) were surveyed in 2010 between July 3rd and August 13th throughout Oregon and Washington. Surveys were conducted by 19 people with various levels of survey experience, including wildlife biologists and seasonal wildlife technicians from the Forest Service and BLM, one Forest Service entomologist, and three contract lepidopterists. A total of 137 larvae and eggs were collected from 54 of 64 survey sites[3] within seven of the geographic areas and sent for DNA analysis (fig. 2). Three of the geographic survey areas failed to locate any eggs or larvae. Those areas consistently had low to no presence of live dwarf mistletoe. Across all survey areas, only one person conducting field surveys (a contracted lepidopterist) encountered adult *C. johnsoni*, from one of five survey days; while the same person found larvae of *C. johnsoni* on three of five survey days and *Callophrys* spp. on four of five survey days.

DNA analysis

Phylogenetic analyses for the combined mitochondrial and nuclear datasets indicated moderate to strong bootstrap support for the monophyly of *C. spinetorum* and *C. johnsoni* (see appendix) of 59% and 74%, respectively. Bootstrap percentages are conservative measures of accuracy and values ≥70% usually correspond with strong support for "real" clades (Soltis and Soltis 2003). Using corrected distances (GTR+G) for all three genes, the maximum within group sequence divergence for both the *C. spinetorum* and *C. johnsoni* clade was 0.2%. The maximum sequence divergence between clades was 2.5%, which based on a recent genetic analysis of other butterfly species by Jeong et al. (2009), is considered high compared with other insect species. While sequence divergences for lepidopterans are usually greater than 3% (Hebert et al. 2003), recent studies have distinguished between butterfly species using divergences of <3% (Herbert et al. 2004, Whinnett et al. 2005). In fact, the use of the 3% threshold as a protocol to distinguish between butterfly species was disputed by Whinnett et al. (2005).

Of the 137 larval vouchers, nine percent were identified as *C. johnsoni*, while 63.5 percent were identified as *C. spinetorum*. Approximately 27.5 percent of the larval vouchers could not be successfully sequenced. Genetic sequencing was more successful for eggs and smaller larvae than for the larger specimens. The poorer results from larger specimens may be related to the amount of chitin that had developed in the larger specimens, with only 36% of larvae >12mm being successfully sequenced. Of the 54 sites where larvae were collected, *C. johnsoni* were successfully identified from ten sites and were vouchered from July 14th to July 29th. The results of the genetic analysis on adults collected in 2009 hint at the potential of hybridization between *C. spinetorum* and *C. johnsoni* with four specimens falling within the *C. johnsoni* clade based on the combined nuclear and mtDNA analysis (fig. 1 in appendix), and falling between clades when using only nuclear DNA (fig. 2 in appendix). However, given that the amount of divergence is small, these results are not enough to clearly support hybridization. There was no indication of hybridization occurring from the larval samples collected in 2010.

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[3] Here, a survey site represents a transect or focused survey site. There can be one to several survey sites in each geographic survey area.

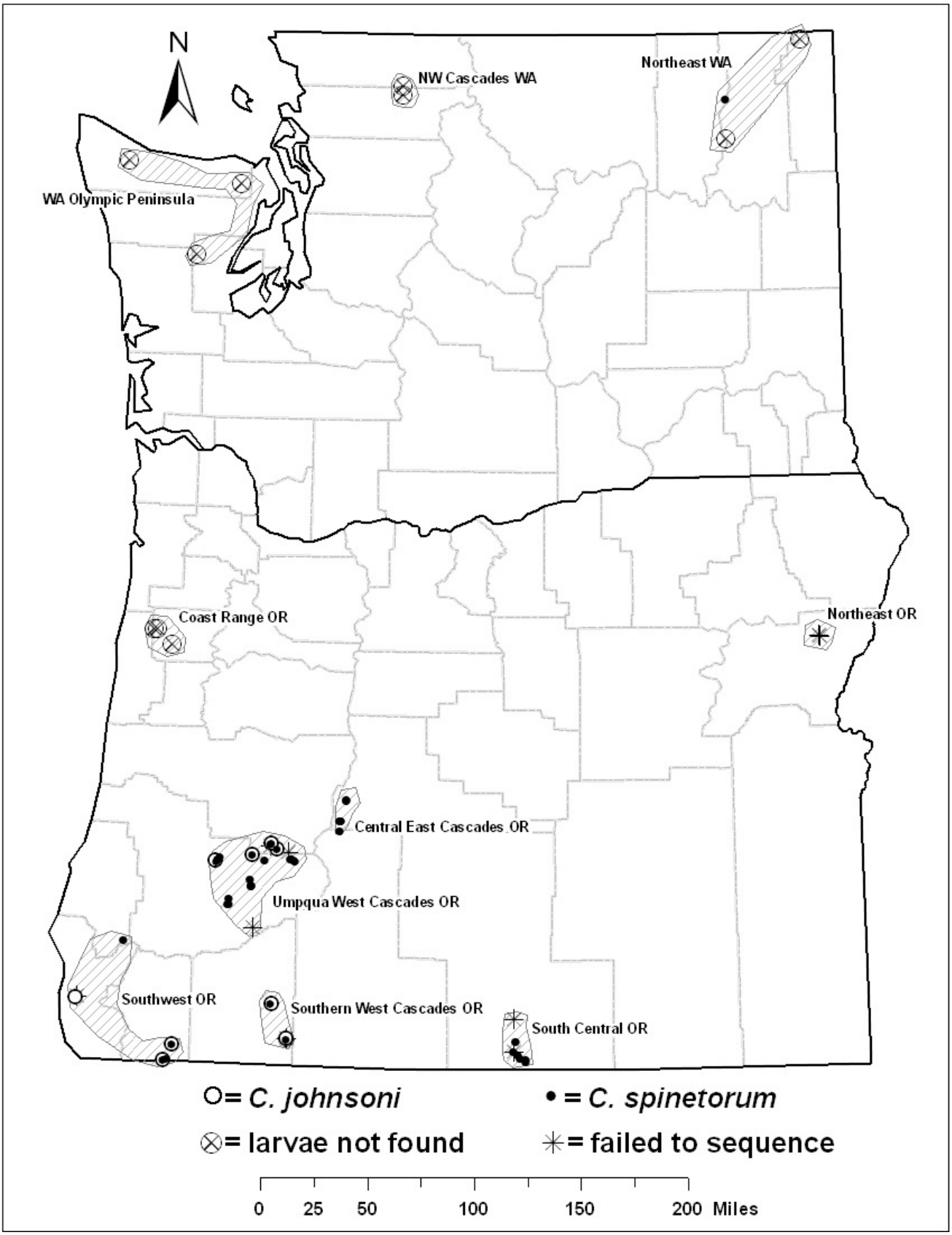

Figure 2. Geographic survey areas, survey sites and results of the 2010 larval surveys and DNA analysis.

DISCUSSION

In spite of an unseasonable wet and cool start to the survey season, the survey was a successful one. The results of this survey validated the results of the 2009 survey and analysis, which indicated that the larval survey protocol is the most effective survey technique for detecting presence of *C. johnsoni* and/or *C. spinetorum* at ground level where dwarf mistletoe is present. For example, one surveyor in 2010 conducted adult surveys to protocol (4 visits) on one transect without detecting adult *C. johnsoni*, while the larval survey of the same transect resulted in collection of two larval *C. johnsoni*. The results also suggest that focused [checklist] surveys for larvae are more effective than adult surveys for detecting this species, with one surveyor (an expert lepidopterist) detecting adult *C. johnsoni* on only one of three focused survey sites that contained *C. johnsoni* larvae. In summary, where infestations of dwarf mistletoe were abundant and accessible, *Callophrys* spp. larvae were readily collected, even when adults were not seen.

The implication of these results is important. Compared to adult surveys, larval surveys are more cost-effective, logistically flexible, and produce better results. For instance, they require only one day to conduct per site and are not dependent upon sunny weather and calm winds, as are the adult surveys. In addition, they impact a very small fraction of the larval host plant at each site, as the clipping is confined to the lower tree branches where the host plant is least abundant. They also result in the loss of fewer eggs and/or larvae from each site, whereas the vouchering of one gravid adult female could potentially result in the loss of as many as 160 fertile ova (McCorkle 1973). These are important conservation factors to consider when deciding on which survey method to use.

The ability to distinguish between species of *C. johnsoni* and *C. spinetorum* in their larval stages was crucial for making the larval survey method a viable option. Without that capability, the rearing of larvae to eclosure is required to obtain accurate species identification. The rearing of larvae to the adult stage requires a significant time commitment that most land management units are not willing or able to provide, and sometimes parasitoids destroy some of the vouchers. The cost of DNA analysis is inexpensive enough to outweigh the cost of rearing larvae to the adult stage. While some effort is still required in storing and handling clippings of dwarf mistletoe with eggs and larvae under the larval survey methods, the results of the DNA analysis indicated that the rearing of eggs and small larvae until they obtain a significantly larger size is not needed as both eggs and first instar stage larvae were successfully sequenced.

The known distribution of both hairstreak butterfly species was expanded as a result of this survey. The biggest expansion for *C. johnsoni* was a 30 mile westward expansion in the Umpqua west Cascades of Oregon, to isolated stands of old growth forest located within the Rock Creek drainage on the Roseburg BLM District. The known range of *C. spinetorum* was expanded to include a new county record in Coos County, Oregon. An area of hybridization likely occurs within the western Cascades of Oregon, where the geographic distributions of both species are sympatric (fig. 3).

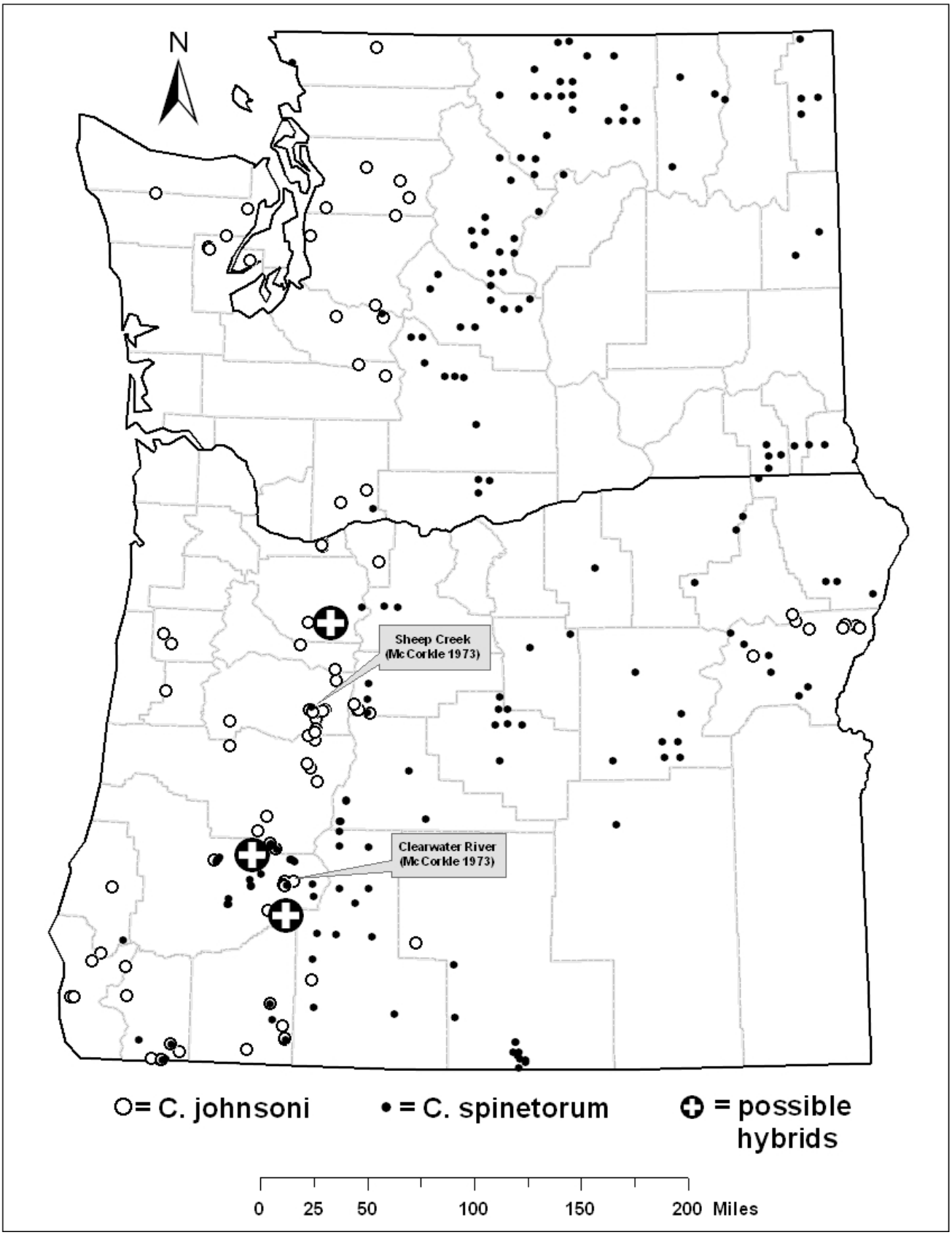

Figure 3. Current known geographic distributions of Johnson's Hairstreak (*C. johnsoni*), Thicket Hairstreak (*C. spinetorum*), and area of potential hybridization documented through DNA analysis (includes McCorkle 1973 sites).

Most of the known overlap from our surveys occurs in the western Cascades of Oregon, but to a higher degree in the southern portions of the western Cascades. This observation is supported by data collected by McCorkle (1973), who documented both species and hybridization occurring at Clearwater River, Douglas County, Oregon. The majority of specimens collected at this site were *C. spinetorum*, with a smaller portion being *C. johnsoni*. McCorkle (1973) also found sympatric populations of both species, but with a higher proportion of *C. johnsoni* than *C. spinetorum*, approximately 80 miles to the north of this site, at Sheep Creek, Linn County, Oregon (fig. 3). McCorkle (1973) concluded that both species were in competition for the same resources in these areas. Later he speculated that the *C. spinetorum* range might be expanding and could potentially displace *C. johnsoni* in certain portions of its range (McCorkle pers. comm. 2010). A potential mechanism for this change might be related to climate change, as *C. spinetorum* appears to prefer warmer/drier environments, whereas *C. johnsoni* prefers cool/moist conditions based on habitat modeling (Davis et al. 2009).

And finally, a new conifer species, Jeffrey pine (*Pinus jeffreyi*), near Signal Butte, Oregon (southwest Oregon survey area) was added to the list of conifers that harbor the larval host plant for *C. johnsoni*.

RECOMMENDATIONS

Some of the feedback received from surveyors included comments on the difficulty of finding accessible dwarf mistletoe in the field. When located, it was sometimes too high to reach with hand pruners and long-handled pruners had to be utilized. In some cases, dwarf mistletoe clippings were stored in tightly sealed plastic bags, with no air circulation, resulting in the quick formation of fungus, rendering the samples useless. On a few occasions, survey transects were established in areas that lacked adequate amounts of dwarf mistletoe. Based on figure 4 in the final report for the 2009 field surveys (Davis 2010), the suggestion that "survey areas should be selected in areas with both high habitat suitability and high amounts of dwarf mistletoe" was made. This was not included in the v1.1 survey protocol. Not surprisingly, survey sites with little to no active dwarf mistletoe failed to locate *Callophrys* spp. larvae.

We recommend revising the survey protocol to emphasize the importance of locating dwarf mistletoe prior to conducting a survey. We also recommend providing surveyors training in larval host plant identification and other aspects of the protocol, especially the clipping, storage, and inspection of dwarf mistletoe clumps. The training should also be open to forest silviculturists and botanists, which we believe would result in improved integration, awareness, and management of this species. The v1.1 survey protocol did not include specific procedures for vouchering specimens. This had to be sent out separately. We will include these procedures in the revised protocol. Based on the results of the genetic analysis, it appears that the optimal voucher size is between 2-4mm. Once larvae reach this size range they should be vouchered according to the protocol.

Future surveys should focus on areas in Figure 4 that show high probability of occurrence and are >25 miles from know or recent occurrence. In particular, areas within Washington Cascades and Olympic Peninsula, the Oregon Coast Range, the eastern Cascades of Oregon, and the area along the state border between SE corner of Washington and NE corner of Oregon, are of particular interest.

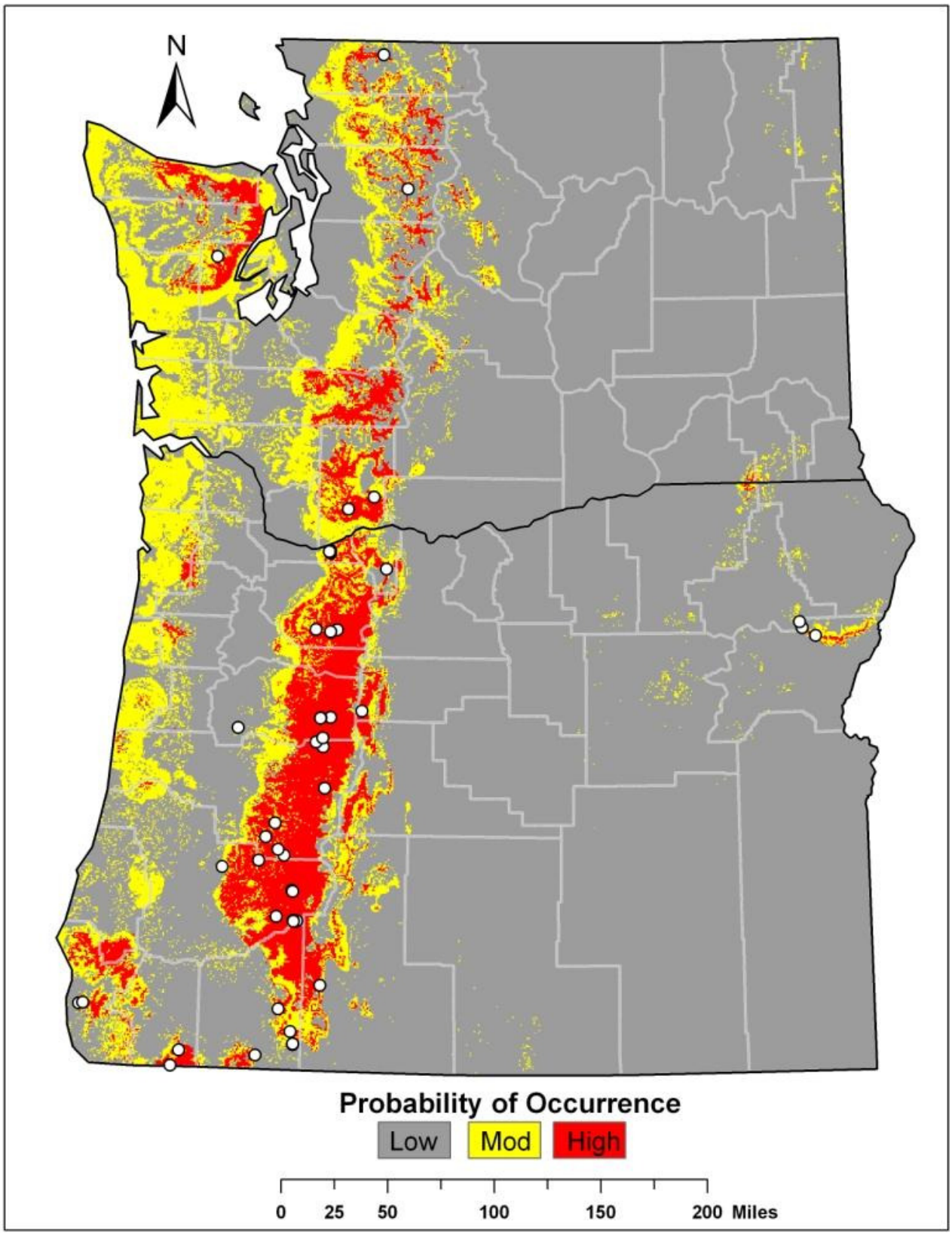

Figure 4. Field surveys for 2011 should focus first on areas with the highest probability of occurrence (red areas) with no recent documentation of *C. johnsoni* (since 1994), represented by the white circles in this figure.

REFERENCES

Davis, R., D. McCorkle, and D. Ross. 2009. Draft Survey Protocol (v1.0) for Johnson's Hairstreak butterfly (*Callophrys johnsoni*) in Washington and Oregon. Unpublished report. On file with: Interagency Special Status Species Program, USDA Forest Service and USDI Bureau of Land Management, Portland, OR. http://www.fs.fed.us/r6/sfpnw/issssp/

Davis, R.J. 2010. Johnson's hairstreak surveys in Oregon and Washington (2009). Unpublished report. On file with: Interagency Special Status Species Program, USDA Forest Service and USDI Bureau of Land Management, Portland, OR. http://www.fs.fed.us/r6/sfpnw/issssp/

Davis, R., D. McCorkle, and D. Ross. 2010. Survey protocol (v1.1) for Johnson's Hairstreak butterfly (*Callophrys johnsoni*) in Washington and Oregon. Unpublished report. On file with: Interagency Special Status Species Program, USDA Forest Service and USDI Bureau of Land Management, Portland, OR. http://www.fs.fed.us/r6/sfpnw/issssp/

Guindon S. and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology, 52(5): 696-704.

Hebert, P.D.N., A. Cywinska, S.L. Ball, and J.R. deWaard. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society B, 270: 313–321.

Hebert, P.D.N., E.H. Penton, J.M. Burns, D.H. Janzen, and W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Science USA, 101: 14812–14817.

Jeong, H.C., J.A. Kim, H.H. Im, H.U. Jeong, M.Y. Hong, J.E. Lee, Y.S. Han, and I. Kim. 2009. Mitochondrial DNA sequence variation of the swallowtail butterfly, *Papilio xuthus*, and the cabbage butterfly, *Pieris rapae*. Biochemical Genetics, 47: 165–178

McCorkle, D.V. 1973. An autecological study of *Callophrys johnsoni* with emphasis upon its relationship to dwarf mistletoe of western hemlock. Final report on contract no. 19-157, Western Oregon University, Monmouth, OR. 21 p.

McCorkle, D.V. 2010. Personal communication.

Soltis, P.S. and D.E. Soltis. 2003. Applying the bootstrap in phylogeny reconstruction. Statistical Science, 18(2): 256–267.

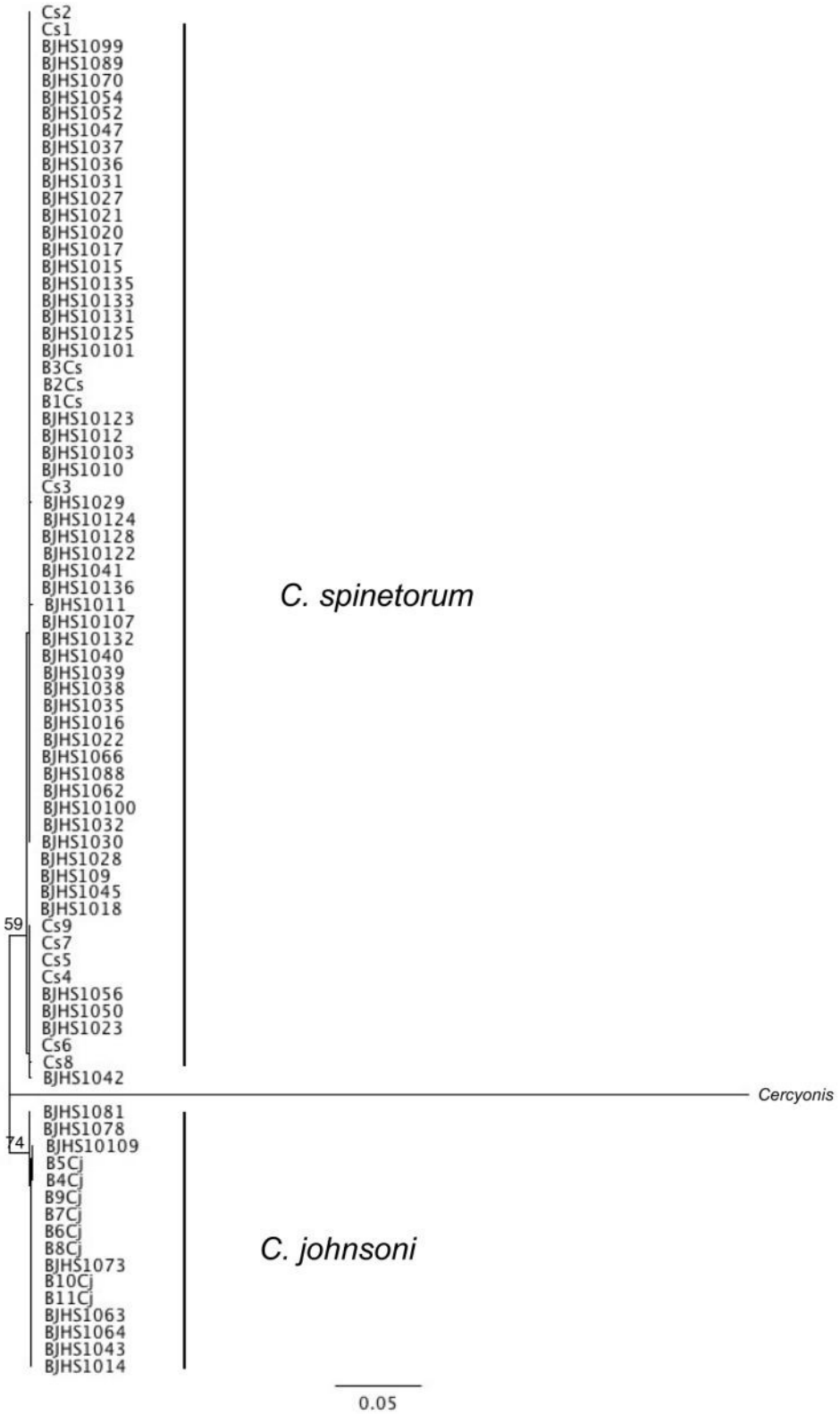

Figure 1 – Phylogenetic tree based on combined nuclear and mitochondrial DNA for adult (2009) and larval (2010) specimens.

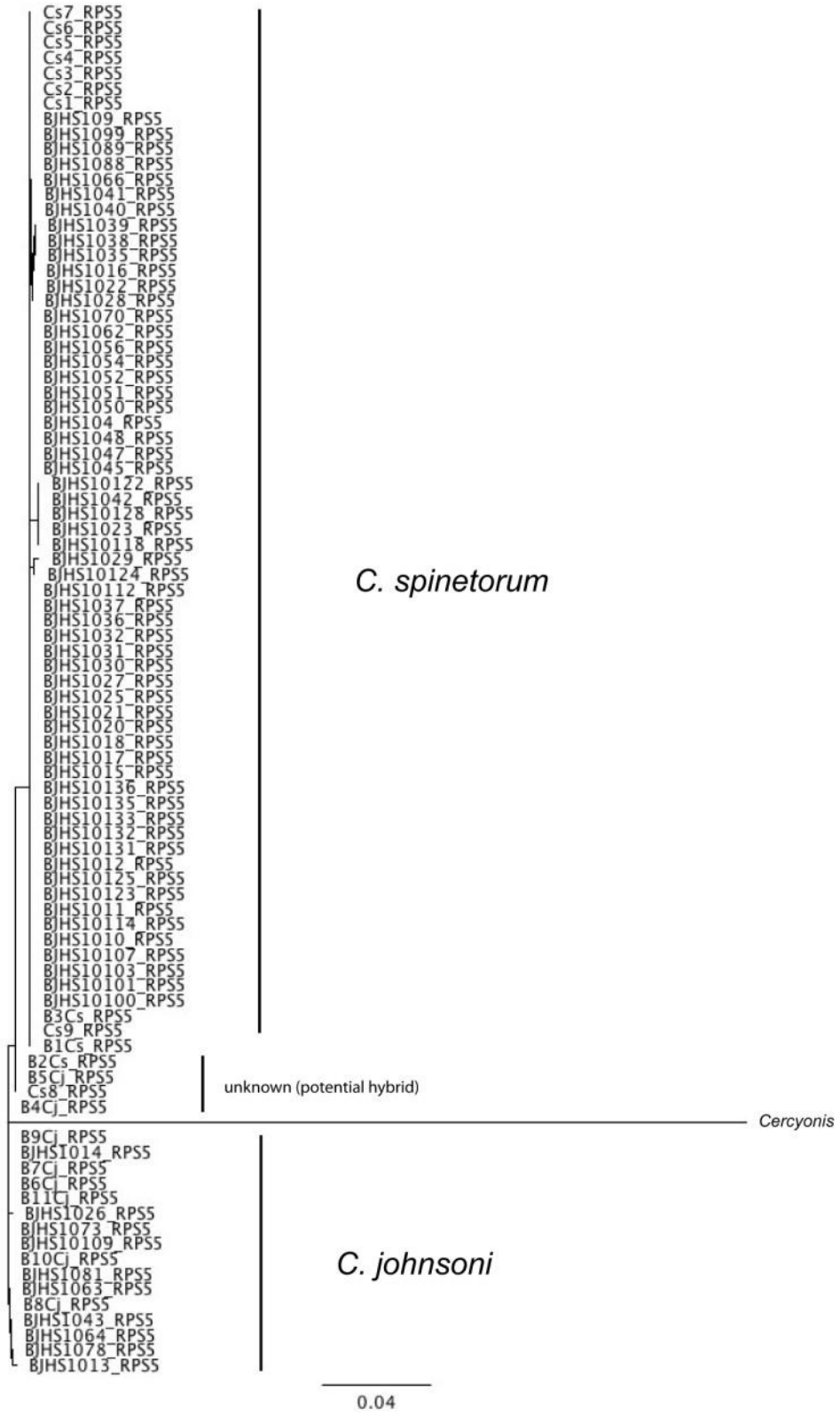

Figure 2 – Phylogenetic tree based on only nuclear DNA for adult (2009) and larval (2010) specimens.

Table 1 – Vouchers collected during the 2010 survey season.

Voucher ID	Species	Site Name	Collected By	Voucher Date	Length (mm)	Stage
BJHS101	*C. spinetorum*	Rickey Point, Columbia River, WA	C. Logger	7/3/2010	8.5	*almost pupa*
BJHS102	*C. spinetorum*	Rickey Point, Columbia River, WA	C. Logger	7/3/2010	11	*pupa*
BJHS103	---	Rickey Point, Columbia River, WA	C. Logger	7/3/2010	12.5	*larva*
BJHS104	---	Rickey Point, Columbia River, WA	C. Logger	7/3/2010	7	*larva*
BJHS105	*C. spinetorum*	Chilcoot Mtn, Umpqua NF, OR	R. Davis	7/14/2010	3	*larva*
BJHS106	*C. spinetorum*	Chilcoot Mtn, Umpqua NF, OR	R. Davis	7/14/2010	3.5	*larva*
BJHS107	*C. spinetorum*	Chilcoot Mtn, Umpqua NF, OR	R. Davis	7/14/2010	3	*larva*
BJHS108	*C. spinetorum*	Chilcoot Mtn, Umpqua NF, OR	R. Davis	7/14/2010	3.5	*larva*
BJHS109	*C. spinetorum*	Reynolds Creek, Umpqua NF, OR	R. Davis	7/15/2010	3.5	*larva*
BJHS1010	*C. spinetorum*	Reynolds Creek, Umpqua NF, OR	R. Davis	7/15/2010	2	*larva*
BJHS1011	*C. spinetorum*	Reynolds Creek, Umpqua NF, OR	R. Davis	7/15/2010	3.5	*larva*
BJHS1012	*C. spinetorum*	Reynolds Creek, Umpqua NF, OR	R. Davis	7/15/2010	3	*larva*
BJHS1013	*C. johnsoni*	Chilcoot Mtn, Umpqua NF, OR	R. Davis	7/14/2010	2	*larva*
BJHS1014	*C. johnsoni*	Calapooya Mtn, Umpqua NF, OR	R. Davis	7/15/2010	1.5	*larva*
BJHS1015	*C. spinetorum*	Chilcoot Mtn, Umpqua NF, OR	R. Davis	7/14/2010	4.5	*larva*
BJHS1016	*C. spinetorum*	Road 27- Segment 6, Umpqua NF, OR	K. Sands	7/14/2010	4	*larva*
BJHS1017	*C. spinetorum*	Road 2810- Segment 2, Umpqua NF, OR	K. Sands	7/20/2010	4	*larva*
BJHS1018	*C. spinetorum*	Road 27- Segment 4, Umpqua NF, OR	K. Sands	7/14/2010	3	*larva*
BJHS1019	---	Road 2950- Segment 7, Umpqua NF, OR	K. Sands	7/13/2010	7	*larva*
BJHS1020	*C. spinetorum*	Road 2810- Segment 2, Umpqua NF, OR	K. Sands	7/20/2010	2.5	*larva*
BJHS1021	*C. spinetorum*	Road 2810- Segment 2, Umpqua NF, OR	K. Sands	7/20/2010	4	*larva*
BJHS1022	*C. spinetorum*	Road 2810- Segment 1, Umpqua NF, OR	K. Sands	7/20/2010	3.5	*larva*
BJHS1023	*C. spinetorum*	Reynolds Creek, Umpqua NF, OR	R. Davis	7/15/2010	1	*larva*
BJHS1024	*C. spinetorum*	Reynolds Creek, Umpqua NF, OR	R. Davis	7/15/2010	1	*larva*
BJHS1025	*C. spinetorum*	Calapooya Mtn, Umpqua NF, OR	R. Davis	7/15/2010	1	*egg*
BJHS1026	*C. johnsoni*	Calapooya Mtn, Umpqua NF, OR	R. Davis	7/15/2010	1	*egg*
BJHS1027	*C. spinetorum*	Toketee 2316-B-0.6	G. Pearson	7/20/2010	1	*larva*
BJHS1028	*C. spinetorum*	Road 2792- Segment 7, Umpqua NF, OR	K. Sands	7/21/2010	3	*larva*
BJHS1029	*C. spinetorum*	Road 2810- Segment 2, Umpqua NF, OR	K. Sands	7/20/2010	3.5	*larva*
BJHS1030	*C. spinetorum*	Road 2810- Segment 2, Umpqua NF, OR	K. Sands	7/20/2010	2.5	*larva*
BJHS1031	*C. spinetorum*	Road 2810- Segment 2, Umpqua NF, OR	K. Sands	7/20/2010	6	*larva*
BJHS1032	*C. spinetorum*	Road 27- Segment 5, Umpqua NF, OR	K. Sands	7/14/2010	3	*larva*
BJHS1033	---	Road 27- Segment 5, Umpqua NF, OR	K. Sands	7/14/2010	4.5	*larva*
BJHS1034	*C. spinetorum*	Rock Creek Site 1, Roseburg BLM, OR	R. Davis	7/28/2010	4	*larva*
BJHS1035	*C. spinetorum*	Rock Creek Site 1, Roseburg BLM, OR	R. Davis	7/28/2010	3.5	*larva*
BJHS1036	*C. spinetorum*	Rock Creek Site 1, Roseburg BLM, OR	R. Davis	7/28/2010	1.5	*larva*

Table 1 – (cont).

Voucher ID	Species	Site Name	Collected By	Voucher Date	Length (mm)	Stage
BJHS1037	*C. spinetorum*	Rock Creek Site 1, Roseburg BLM, OR	R. Davis	7/28/2010	2	*larva*
BJHS1038	*C. spinetorum*	Rock Creek Site 1, Roseburg BLM, OR	R. Davis	7/28/2010	4	*larva*
BJHS1039	*C. spinetorum*	Rock Creek Site 1, Roseburg BLM, OR	R. Davis	7/28/2010	3.5	*larva*
BJHS1040	*C. spinetorum*	Rock Creek Site 1, Roseburg BLM, OR	R. Davis	7/28/2010	4	*larva*
BJHS1041	*C. spinetorum*	Rock Creek Site 1, Roseburg BLM, OR	R. Davis	7/28/2010	5	*larva*
BJHS1042	*C. spinetorum*	Station 6, Sample 5, Medford BLM, OR	L. Hale	7/23/2010	2.5	*larva*
BJHS1043	*C. johnsoni*	Station 7, Sample 6, Medford BLM, OR	L. Hale	7/23/2010	8.5	*larva*
BJHS1044	*C. spinetorum*	Road 2792- Segment 7, Umpqua NF, OR	K. Sands	7/21/2010	2.5	*larva*
BJHS1045	*C. spinetorum*	Road 2810- Segment 2, Umpqua NF, OR	K. Sands	7/20/2010	6	*larva*
BJHS1046	---	Road 2810- Segment 2, Umpqua NF, OR	K. Sands	7/20/2010	9	*larva*
BJHS1047	*C. spinetorum*	Road 2810- Segment 2, Umpqua NF, OR	K. Sands	7/20/2010	4.5	*larva*
BJHS1048	*C. spinetorum*	Toketee 2316-B-0.5	G. Pearson	7/20/2010	10	*larva*
BJHS1049	*C. spinetorum*	Toketee 2316-B-0.3	G. Pearson	7/20/2010	1	*egg/larva*
BJHS1050	*C. spinetorum*	Toketee 2316-C-0.2-0.5	G. Pearson	7/20/2010	7	*larva*
BJHS1051	*C. spinetorum*	Toketee 2316-B-0.6	G. Pearson	7/20/2010	2	*larva*
BJHS1052	*C. spinetorum*	Toketee 2316-D-0.3-0.4	G. Pearson	7/20/2010	1.5	*larva*
BJHS1053	---	Toketee 2316-C-0.2-0.5	G. Pearson	7/20/2010	13	*larva*
BJHS1054	*C. spinetorum*	Toketee 2316-B-0.6	G. Pearson	7/20/2010	2	*larva*
BJHS1055	---	Toketee 2314-B-1.6	G. Pearson	7/20/2010	3.5	*larva*
BJHS1056	*C. spinetorum*	Steamboat 3818-C-0.7-1	G. Pearson	7/23/2010	9	*larva*
BJHS1057	*C. spinetorum*	Steamboat 300-B-0.5	G. Pearson	7/23/2010	3.5	*larva*
BJHS1058	---	Steamboat 300-B-0.5	G. Pearson	7/23/2010	8.5	*larva*
BJHS1059	*C. johnsoni*	Steamboat 300-B-0.5	G. Pearson	7/23/2010	3	*larva*
BJHS1060	---	Rock Creek Site 3C, Roseburg BLM, OR	G. Pearson	7/28/2010	9.5	*larva*
BJHS1061	---	Rock Creek Site 3C, Roseburg BLM, OR	G. Pearson	7/28/2010	10	*larva*
BJHS1062	*C. spinetorum*	Rock Creek Site 3C, Roseburg BLM, OR	G. Pearson	7/28/2010	8.5	*larva*
BJHS1063	*C. johnsoni*	Rock Creek Site 3A, Roseburg BLM, OR	G. Pearson	7/28/2010	4	*larva*
BJHS1064	*C. johnsoni*	Rock Creek Site 3A, Roseburg BLM, OR	G. Pearson	7/28/2010	4.5	*larva*
BJHS1065	*C. spinetorum*	Rock Creek Site 4, Roseburg BLM, OR	G. Pearson	7/28/2010	11.5	*larva*
BJHS1066	*C. spinetorum*	Rock Creek Site 4, Roseburg BLM, OR	G. Pearson	7/28/2010	11.5	*larva*
BJHS1067	---	Rock Creek Site 4, Roseburg BLM, OR	G. Pearson	7/28/2010	10.5	*larva*
BJHS1068	---	Rock Creek Site 3C, Roseburg BLM, OR	G. Pearson	7/28/2010	9	*larva*
BJHS1069	*C. spinetorum*	Panther Creek, Rd 4714, Umpqua NF, OR	G. Pearson	7/27/2010	2	*larva*
BJHS1070	*C. spinetorum*	Rock Creek Site 2, Roseburg BLM, OR	G. Pearson	7/28/2010	7.5	*larva*
BJHS1071	*C. spinetorum*	Ross Site 7	D. Ross	7/25/2010	6.5	*larva*
BJHS1072	---	Ross Site 7	D. Ross	7/25/2010	6.5	*larva*

Table 1 – (cont).

Voucher ID	Species	Site Name	Collected By	Voucher Date	Length (mm)	Stage
BJHS1073	*C. johnsoni*	Ross Site 2	D. Ross	7/21/2010	5	*larva*
BJHS1074	---	Ross Site 2	D. Ross	7/21/2010	8	*larva*
BJHS1075	*C. spinetorum*	Ross Site 2	D. Ross	7/21/2010	8	*larva*
BJHS1076	*C. spinetorum*	Ross Site 1	D. Ross	7/21/2010	8.5	*larva*
BJHS1077	*C. spinetorum*	Ross Site 4	D. Ross	7/22/2010	12	*larva*
BJHS1078	*C. johnsoni*	Ross Site 4	D. Ross	7/22/2010	9	*larva*
BJHS1079	---	Ross Site 5	D. Ross	7/23/2010	13	*larva*
BJHS1080	---	Ross Site 6	D. Ross	7/23/2010	17.5	*larva*
BJHS1081	*C. johnsoni*	Ross Site 6	D. Ross	7/23/2010	10.5	*larva*
BJHS1082	---	Ross Site 6	D. Ross	7/23/2010	16	*larva*
BJHS1083	---	Ross Site 6	D. Ross	7/23/2010	12.5	*larva*
BJHS1084	---	Forshey 2, Wallowa Whitman NF, OR	L. Spiegel	8/2/2010	15	*larva*
BJHS1085	---	Panther Creek, Rd 4714, Umpqua NF, OR	G. Pearson	7/27/2010	3.5	*larva*
BJHS1086	---	Steamboat 350-B-0.2, Umpqua NF, OR	G. Pearson	7/23/2010	4	*larva*
BJHS1087	*C. spinetorum*	Rock Creek Site 3C, Roseburg BLM, OR	G. Pearson	7/28/2010	1.5	*larva*
BJHS1088	*C. spinetorum*	Rock Creek Site 3C, Roseburg BLM, OR	G. Pearson	7/28/2010	2	*larva*
BJHS1089	*C. spinetorum*	Rock Creek Site 3C, Roseburg BLM, OR	G. Pearson	7/28/2010	1	*larva*
BJHS1090	*C. spinetorum*	Rock Creek Site 3C, Roseburg BLM, OR	G. Pearson	7/28/2010	4	*larva*
BJHS1091	---	Rock Creek Site 3A, Roseburg BLM, OR	G. Pearson	7/28/2010	5	*larva*
BJHS1092	*C. spinetorum*	Rock Creek Site 3C, Roseburg BLM, OR	G. Pearson	7/28/2010	1	*larva*
BJHS1093	*C. spinetorum*	Rock Creek Site 3C, Roseburg BLM, OR	G. Pearson	7/28/2010	1.5	*larva*
BJHS1094	*C. spinetorum*	Rock Creek Site 3C, Roseburg BLM, OR	G. Pearson	7/28/2010	1	*larva*
BJHS1095	*C. spinetorum*	Rock Creek Site 3C, Roseburg BLM, OR	G. Pearson	7/28/2010	1	*larva*
BJHS1096	---	Forshey 6, Wallowa Whitman NF, OR	L. Spiegel	8/2/2010	12	*larva*
BJHS1097	---	Forshey 6, Wallowa Whitman NF, OR	L. Spiegel	8/2/2010	10	*larva*
BJHS1098	---	Transect 1, Segment 1, Deschutes NF, OR	W. Branum	7/20/2010	16	*larva*
BJHS1099	*C. spinetorum*	Transect 1, Segment 1, Deschutes NF, OR	W. Branum	7/20/2010	5	*larva*
BJHS10100	*C. spinetorum*	Transect 1, Segment 2, Deschutes NF, OR	W. Branum	7/20/2010	7.5	*larva*
BJHS10101	*C. spinetorum*	Transect 1, Segment 5, Deschutes NF, OR	W. Branum	7/20/2010	7.5	*larva*
BJHS10102	---	Transect 2, Segment 8, Deschutes NF, OR	W. Branum	7/12/2010	13	*larva*
BJHS10103	*C. spinetorum*	Transect 2, Segment 8, Deschutes NF, OR	W. Branum	7/12/2010	12.5	*larva*
BJHS10104	*C. spinetorum*	Transect 3, Segment 3, Deschutes NF, OR	W. Branum	7/15/2010	10	*larva*
BJHS10105	*C. spinetorum*	Transect 3, Segment 7, Deschutes NF, OR	W. Branum	7/15/2010	16	*larva*
BJHS10106	*C. spinetorum*	Transect 3, Segment 7, Deschutes NF, OR	W. Branum	7/15/2010	9	*larva*
BJHS10107	*C. spinetorum*	Transect 3, Segment 7, Deschutes NF, OR	W. Branum	7/15/2010	13	*almost pupa*
BJHS10108	---	Transect 3, Segment 7, Deschutes NF, OR	W. Branum	7/15/2010	8	*larva*

Table 1 – (cont).

Voucher ID	Species	Site Name	Collected By	Voucher Date	Length (mm)	Stage
BJHS10109	*C. johnsoni*	Site N, Medford BLM, OR	T. Manger	7/29/2010	11.5	*almost pupa*
BJHS10110	*C. johnsoni*	Site G, Medford BLM, OR	T. Manger	7/28/2010	12.0	*larva*
BJHS10111	---	Yew Springs, Site A, Medford BLM, OR	S. Godwin	7/17/2010	16.0	*larva*
BJHS10112	*C. spinetorum*	Site P, Medford BLM, OR	T. Manger	7/29/2010	10.0	*larva*
BJHS10113	*C. spinetorum*	Site P, Medford BLM, OR	T. Manger	7/29/2010	16.0	*larva*
BJHS10114	*C. spinetorum*	South Warners Mtn, Crane Cr, 012 Rd #1	C. Cavanaugh	8/13/2010	9.0	*larva*
BJHS10115	---	South Warners Mtn, Crane Cr, 012 Rd #2	C. Cavanaugh	8/13/2010	11.5	*larva*
BJHS10116	---	South Warners Mtn - 3915-013 Rd	C. Cavanaugh	8/13/2010	16.0	*larva*
BJHS10117	---	South Warners Mtn - 3915-013 Rd	C. Cavanaugh	8/13/2010	16.5	*larva*
BJHS10118	*C. spinetorum*	South Warners Mtn - 3915-013 Rd	C. Cavanaugh	8/13/2010	16.0	*larva*
BJHS10119	---	South Warners Mtn - 3915-013 Rd	C. Cavanaugh	8/13/2010	18.0	*larva*
BJHS10120	---	South Warners Mtn - 3915-024 Rd #1	C. Cavanaugh	8/13/2010	17.5	*larva*
BJHS10121	*C. spinetorum*	South Warners Mtn - 3910-023 Rd	C. Cavanaugh	8/13/2010	8.0	*larva*
BJHS10122	*C. spinetorum*	South Warners Mtn - 3910-023 Rd	C. Cavanaugh	8/13/2010	6.0	*larva*
BJHS10123	*C. spinetorum*	South Warners Mtn - 3910-023 Rd	C. Cavanaugh	8/13/2010	13.5	*larva*
BJHS10124	*C. spinetorum*	South Warners Mtn - 3910-023 Rd	C. Cavanaugh	8/13/2010	16.0	*larva*
BJHS10125	*C. spinetorum*	South Warners Mtn - 3910-023 Rd	C. Cavanaugh	8/13/2010	17.0	*larva*
BJHS10126	---	South Warners Mtn - 3910-023 Rd	C. Cavanaugh	8/13/2010	16.0	*larva*
BJHS10127	*C. spinetorum*	South Warners Mtn - 3915-024 Rd #1	C. Cavanaugh	8/13/2010	11.0	*almost pupa*
BJHS10128	*C. spinetorum*	South Warners Mtn - 3915-024 Rd #2	C. Cavanaugh	8/13/2010	19.0	*larva*
BJHS10129	---	South Warners Mtn - 4011 Rd	C. Cavanaugh	8/13/2010	16.0	*larva*
BJHS10130	---	South Warners Mtn - 4011 Rd	C. Cavanaugh	8/13/2010	17.5	*larva*
BJHS10131	*C. spinetorum*	South Warners Mtn - 4011 Rd	C. Cavanaugh	8/13/2010	14.0	*almost pupa*
BJHS10132	*C. spinetorum*	South Warners Mtn - 4011 Rd	C. Cavanaugh	8/13/2010	12.0	*almost pupa*
BJHS10133	*C. spinetorum*	South Warners Mtn - 4011 Rd	C. Cavanaugh	8/13/2010	10.0	*pupa*
BJHS10134	---	South Warners Mtn - 4011 Rd	C. Cavanaugh	8/13/2010	16.0	*larva*
BJHS10135	*C. spinetorum*	South Warners Mtn - 4011 Rd	C. Cavanaugh	8/13/2010	10.0	*almost pupa*
BJHS10136	*C. spinetorum*	South Warners Mtn - 4011 Rd	C. Cavanaugh	8/13/2010	12.0	*almost pupa*
BJHS10137	---	South Warners Mtn, Crooked Cr, 012 Rd	C. Cavanaugh	8/13/2010	9.5	*larva*

Table 2. Survey area information and summary results from 2010 surveys (UTMs in NAD27 UTM zone 10[1] and zone 11[2]).

Survey Area	Site Name	UTM X	UTM Y	Total Coll.	*CAJO*	*CASP*	Total ID
Washington Olympic Peninsula[1]	Skok Road 2362-700 Transect, Olympic NF	465942	5257415	0	0	0	0
	Quil Road 2840 Transect, Olympic NF	497366	5311547	0	0	0	0
	Solduc-Bear Cr Transect, Olympic NF	412554	5325964	0	0	0	0
Northern West Cascades of Washington[1]	Diobsub Transect, MBS NF	616489	5382610	0	0	0	0
	Bacon Transect, MBS NF	616175	5389143	0	0	0	0
Northeast Washington[2]	Gifford CG, Columbia River, WA	415435	5348532	0	0	0	0
	Colville NF, Rd 330/350	473643	5421639	0	0	0	0
	Rickey Point, Columbia River, WA	416110	5377859	4	0	2	2
Northeast Oregon[2]	Forshey 6, Wallowa Whitman NF	471900	4972156	2	0	0	0
	Forshey 2, Wallowa Whitman NF	471292	4972535	1	0	0	0
Northern Coast Range of Oregon[1]	Valley of the Giants Transect, Salem BLM	444116	4974887	0	0	0	0
	Cold Springs A.01, Salem BLM	457920	4963165	0	0	0	0
	S Line 28.01, Salem BLM	446976	4974970	0	0	0	0
Central East Cascades of Oregon[1]	Transect 1, Segment 1, Deschutes NF	589732	4834770	2	0	1	1
	Transect 1, Segment 2, Deschutes NF	589438	4834860	1	0	1	1
	Transect 1, Segment 5, Deschutes NF	588890	4835138	1	0	1	1
	Transect 2, Segment 8, Deschutes NF	589120	4827519	2	0	1	1
	Transect 3, Segment 3, Deschutes NF	593003	4851046	1	0	1	1
	Transect 3, Segment 7, Deschutes NF	593097	4850348	4	0	3	3
Umpqua Western Cascades of Oregon[1]	Chilcoot Mtn, Umpqua NF	523438	4807217	6	1	5	6
	Reynolds Cr, Umpqua NF	533576	4803578	6	0	4	4
	Calapooya Mtn, Umpqua NF	542433	4811856	3	2	1	3
	Toketee, Rd 2316-B-0.6	553416	4804597	3	0	3	3
	Toketee, Rd 2316-B-0.3	553065	4804819	1	0	1	1
	Toketee, Rd 2314-B-1.6	551809	4809990	1	0	0	0
	Toketee, Rd 2316-C-0.2-0.5	555224	4804018	2	0	1	1
	Toketee, Rd 2316-D-0.3-0.4	555832	4802970	1	0	1	1
	Toketee, Rd 2316-B-0.5	553212	4804627	1	0	1	1
	Rock Creek Site 1	499723	4804353	8	0	8	8
	Rock Creek Site 2	497985	4803156	1	0	1	1
	Rock Creek Site 4	498505	4802457	3	0	2	2
	Rock Creek Site 3A	496127	4801575	3	2	0	2
	Rock Creek Site 3C	496918	4801101	12	0	6	6
	Panther Creek, Rd 4714	522861	4788419	2	0	1	1
	Steamboat 300-B-0.5	537707	4816170	3	1	1	2
	Steamboat 350-B-0.2	537964	4814584	1	0	0	0
	Steamboat 3818-C-0.7-1.0	536662	4815174	1	0	1	1
	Road 27- Segment 4, Umpqua NF	524125	4783518	1	0	1	1
	Road 27- Segment 5, Umpqua NF	523899	4783674	2	0	1	1
	Road 27- Segment 6, Umpqua NF	523995	4784030	1	0	1	1
	Road 2792 - Segment 7, Umpqua NF	507105	4773322	2	0	2	2
	Road 2810 - Segment 1, Umpqua NF	507132	4769660	1	0	1	1
	Road 2810 - Segment 2, Umpqua NF	506876	4769566	9	0	8	8
	Road 2950 - Segment 7, Umpqua NF	526422	4751966	1	0	0	0

Table 2 – (cont).

Survey Area	Site Name	UTM X	UTM Y	Total Coll.	*CAJO*	*CASP*	Total ID
Southern West Cascades of Oregon[1]	Site N, Medford BLM	554242	4669293	1	1	0	1
	Site P, Medford BLM	554186	4669032	2	0	2	2
	Yew Springs, Site A, Medford BLM	554945	4669826	1	0	0	0
	Site G, Medford BLM, OR	554274	4669711	1	1	0	1
	Wasson Canyon, Segment 6, Medford BLM	541834	4695649	1	0	1	1
	Wasson Canyon, Segment 7, Medford BLM	542188	4695761	1	1	0	1
Southwest Oregon[1]	Ross Site 1 - Rd. 041, 0.2 mi E Kings Saddle	464604	4651109	1	0	1	1
	Ross Site 2 - Rd. 041, 0.5 mi S Althouse Mtn.	462373	4650106	3	1	1	2
	Ross Site 4 - INTSXN to Mt Elijah, Rd. 070	468357	4662096	2	1	1	2
	Ross Site 5 - Rd. 220, 1mi SE Signal Buttes	396174	4696079	1	0	0	0
	Ross Site 6 - Rd. 190, 1 mi. S Signal Buttes	394714	4695184	4	1	0	1
	Ross Site 7 - Rd. 3348 (S Fk Coquille River)	429065	4739778	2	0	1	1
South Central Oregon[1]	South Warners Mtn - 3915-013 Rd	728928	4663767	4	0	1	1
	South Warners Mtn - 4011 Rd	731021	4661280	8	0	5	5
	South Warners Mtn - 3915-024 Rd (site 1)	735511	4660160	2	0	1	1
	South Warners Mtn - 3915-024 Rd (site 2)	735288	4658949	1	0	1	1
	South Warners Mtn, Crane Cr, 012 Rd (site 2)	726330	4666046	1	0	0	0
	South Warners Mtn, Crane Cr, 012 Rd (site 1)	725973	4666215	1	0	1	1
	South Warners Mtn - 3910-023 Rd	727182	4673372	6	0	5	5
	South Warners Mtn, Crooked Cr, 012 Rd	725300	4690573	1	0	0	0