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Insights into the Biogeography of the Pacific Northwest of North America: Evidence from the Phylogeography of *Salix melanopsis*

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ABSTRACT. The disjunction of the mesic coniferous forests of the Pacific Northwest (PNW) has long been of interest to biogeographers, and several hypotheses have been posed to explain the disjunct distribution pattern. Analysis of intraspecific chloroplast DNA variation (1785 bp of *mat*K and 400 bp of *rpl16*) in *Salix melanopsis* allows these hypotheses to be tested. Our study confirmed the existence of three genetic races (uncorrected sequence divergence ranged from 0.7-1.1%) within the species, which differ in distribution and ecology. The mesic race, associated with mesic coniferous forests, was the focus of this study. This race consists of two major lineages (uncorrected sequence divergence ranged up to 0.28%), one of which is associated with an apparent glacial refugium south of glaciation in the northern Rocky Mountains. The three haplotypes that comprise the first lineage are largely segregated into separate river canyons that comprise the ''Greater Clearwater Refugium''. The other major lineage includes three haplotypes that occur throughout the PNW, except in the core of the Clearwater refugium. Vicariance is implicated in the origin of this latter lineage. Dispersal both before and during the Holocene appears to have occurred: the colonization of the Cascade Range from the Rocky Mountains, and later the dispersal of the Cascade haplotype into the area formerly buried by Cordilleran ice. More limited dispersal of Rocky Mountain haplotypes has resulted in contact zones with elevated haplotype diversity. The results of this study allow us to refine previous phylogeographic hypotheses of the PNW. Phylogenetic relationships within *Salix* sect. *Longifoliae*, the group that includes *S. melanopsis*, were also inferred. The sequence data produced phylogenetic hypotheses that were congruent with those obtained from an earlier analysis of cpDNA restriction site data.

KEYWORDS: biogeography, cpDNA, Pacific Northwest mesic forests, phylogeography, Salicaceae.

The origin of the disjunct mesic coniferous forests of the Pacific Northwest (PNW) has intrigued biogeographers for over a century (Leiberg 1900; Daubenmire 1952; Mack et al. 1978a; Detling 1968), and has generated ideas and hypotheses that have now been formally proposed (Brunsfeld et al. 2001) and tested (Carstens et al. 2005; Brunsfeld and Sullivan 2005). The Pacific Northwest (Fig. 1) can be divided longitudinally into three distinct regions from west to east: the Cascade Mountains, the Columbia Plateau, and the Northern Rocky Mountains. The Cascades and northern Rockies support expanses of mesic coniferous forest that are isolated by more than 300 km of xeric shrub and desert steppe on the Columbia Plateau. Western hemlock (*Tsuga heterophylla*) and western redcedar (*Thuja plicata*) dominate mesic coniferous forests late in succession. The southern extension of the mesic coniferous forest in the Rocky Mountains is the Clearwater drainage in Idaho, an area that has long been hypothesized to be a refugium due to the presence of endemics and species with coastal forest affinities (Daubenmire 1975). The ecosystem extends south in the Cascades to southwest Oregon.

Since the orogeny of the Rocky Mountains during the Eocene (45-36 million years ago), a series of complex geological and climatic events have had a major effect on shaping the current distributions of vegetation in the PNW (Graham 1993, 1999). The recent uplift of the Cascade/Sierra chain during the Pliocene (5-2 mya) and the subsequent xerification of the intervening Columbian Plateau presumably split a formerly continuous coniferous forest (Graham 1993, 1999). More recently, Pleistocene glaciation has also been a major force affecting the population genetic structure of organisms in the Pacific Northwest (e.g. Soltis et al. 1997). Milankovitch cycles, 100,000-year cycles created by variation in the orbit of Earth, resulted in glaciation lasting 90,000 years alternating with interglacial periods lasting 10,000 years (Pielou 1991). As a result, more than half of the PNW was repeatedly covered with cordilleran and alpine ice, pushing climatic zones to lower latitudes and depressing treelines by as much as 1000 m (Pielou 1991). However, the vegetation in low elevation basins was not necessarily composed of forest species that moved down from higher elevations. Fossil evidence suggests that a cold dry steppe ecosystem was followed by pine woodland steppe in the Columbia Plateau until the late Holocene (Mack et al. 1978a, c). Pollen deposits suggest that mesic coniferous forests occurred along the coast south of the maximum glacial extent (Heusser 1985), and that western redcedar migrated northward from a southern coastal refugium during the Holocene

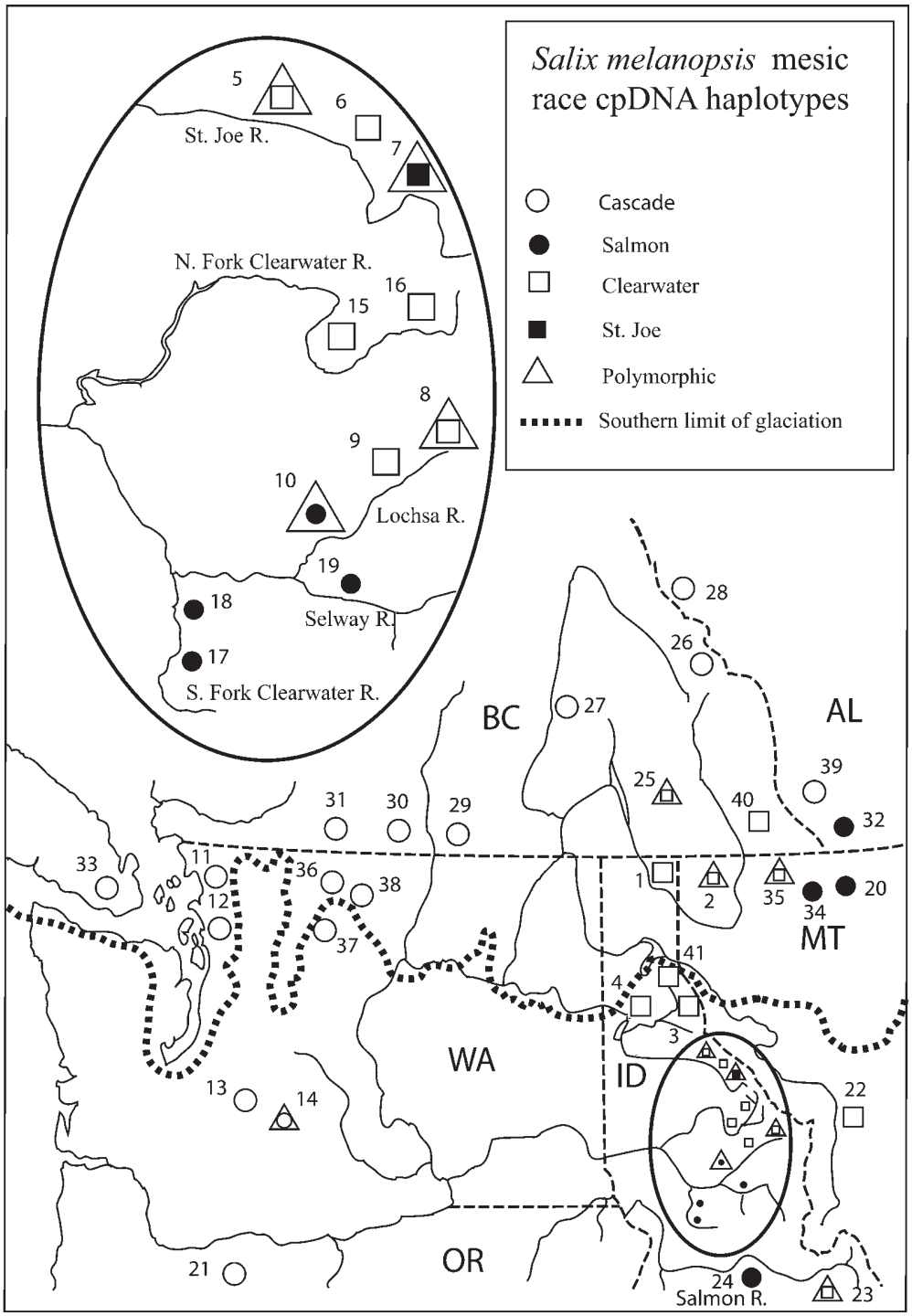

FIG. 1. Map showing distribution of the haplotypes of the mesic race of *Salix melanopsis*. For locations with polymorphism, dominant haplotype appears within the triangle. Locations of Naches and Lochsa haplotypes are in population numbers 14 and 8, respectively.

TABLE 1. Empirical investigations of species in the PNW mesic forest. Ho are the hypotheses supported by the study, where AV is ancient vicariance, RDN is recent dispersal by a northern route, and RDS is recent dispersal by a southern route.

Common name	scientific name	Data type	Ho	Reference
Douglas-fir	*Pseudotsuga menziesii*	allozymes	AV	Li and Adams 1989
Tailed frogs	*Ascaphus spp.*	mtDNA	AV	Nielson et al. 2001
Pacific Giant salamanders	*Dicamptodon spp.*	mtDNA	AV	Steele et al. 2005
Van Dyke's salamanders	*Plethodon vandykei / P. idahoensis*	mtDNA	AV	Carstens et al. 2004
Constance's Bittercress	*Cardamine constancei*	cpDNA	AV	Brunsfeld and Sullivan 2005
Coolwort	*Tiarella trifoliata*	cpDNA	RDN	Soltis et al. 1997
Sword Fern	*Polystichum munitum*	cpDNA	RDN	Soltis et al. 1997
Fringecup	*Tellima grandiflora*	cpDNA	RDN	Soltis et al. 1997
Western white pine	*Pinus monticola*	isozymes	RDN	Steinhoff et al.1983
Water vole	*Microtus richardsoni*	mtDNA	RDN	Carstens et al. 2005
Red Alder	*Alnus rubra*	cpDNA	RDS	Strenge 1994
Whitebark pine	*Pinus albicaulis*	mtDNA, cpDNA	RDN	Richardson et al. 2002

(Barnosky et al. 1987). In the northern Rocky Mountains, western hemlock appears very recently (~3000 years BP) in pollen profiles from southern glaciated areas (Mack et al. 1978a–c). Unfortunately, no pollen data exist for areas south of glaciation in the northern Rocky Mountains that currently support mesic coniferous forest (i.e. the St. Joe and Clearwater drainages).

The disjunct mesic coniferous forests in the PNW are the result of either ancient vicariance that separated populations from the coast and northern Idaho, or more recent dispersal from a refugium in the Cascades to the northern Rocky Mountains, or the opposite direction. The Ancient Vicariance hypothesis (Brunsfeld et al. 2001) is founded on the existence of a continuous mesic coniferous ecosystem sundered by cascadian orogeny 2-5 mya. Support for this hypothesis would be inferred if genetic markers were highly divergent between Pacific coast and northern Rocky Mountain populations of a species. Brunsfeld et al. (2001) also proposed two corollary hypotheses to Ancient Vicariance. First, multiple refugia may have existed in separate river canyons that drain the west slope of the range. The multiple refugia hypothesis would be supported by the presence of haplotype differentiation among separated drainages, caused by limited gene flow and small populations in shrinking mesic habitats during glaciation (Brunsfeld et al. 2001). A second corollary is that population isolation east and west of the Bitterroot crest could also have fostered genetic differentiation within the northern Rockies (Brunsfeld et al 2001). Support for the alternative hypothesis, Recent Dispersal, is based primarily on paleoecological data (Mack et al. 1978a–c), which suggests that mesic coniferous forest elements of the northern Rocky Mountains were assembled since the recession of glaciers. Basic predictions of the Recent Dispersal hypothesis are minimal genetic divergence between coastal and inland populations, declining levels of haplotype polymorphism with increasing distance from the coast, and genetic uniformity throughout the northern Rockies (Brunsfeld et al. 2001). Support for each of these hypotheses has been found in empirical systems from a variety of plants and animals (Table 1).

The dusky willow, *Salix melanopsis*, is a common member of riparian communities in western North America. Brunsfeld et al. (1992) identified three distinct intraspecific races using chloroplast DNA restriction site mapping. All three putative lineages were geographically and ecologically distinct, and apparently correspond to lowland, mesic, and subalpine habitats. The current study sought to confirm the existence of this intraspecific cpDNA variation with DNA sequencing and greatly expanded population sampling. The mesic race, if confirmed to be a robust entity, appeared to an excellent candidate for our phylogeographic focus for two reasons. First, it is seems to be closely associated with mesic coniferous forests, and has a distribution that is disjunct between the Cascades and the northern Rocky Mountains. Furthermore phylogeographic analysis of the mesic race should provide new insights into recolonization patterns into glaciated habitats to the north. Here we collect cpDNA sequence data, document the geographic distribution of haplotypes within *S. melanopsis*, and demonstrate the monophyly of the mesic race of this species.

MATERIALS AND METHODS

Data Collection. Numerous samples of *S. melanopsis* were obtained from throughout its range (Fig. 1; Table 1) during this and previous studies (Brunsfeld et al. 1992). Intensive sampling was directed at the mesic race of *S. melanopsis*, generally including several populations per drainage from both glaciated and unglaciated regions. Samples were obtained from herbarium collections to augment field collections.

Fresh growth was collected from 10 shrubs per population where densities allowed. Samples were alternatively taken

from male and female plants along a linear transect to avoid collecting multiple samples of the same genetic individual. Total genomic DNA was isolated by a modified CTAB method (Brunsfeld et al. 1992). The remainder of the material was deposited as voucher specimens in the Stillinger Herbarium (ID).

Two segments of chloroplast DNA, the *matK* gene and *rpl16* spacer region, were amplified using the polymerase chain reaction (PCR) in 25 µl reactions consisting of 20 ng of genomic DNA, 2 mM $MgCl_2$, 1 mM BSA, 1mM of each primer, 200mM of each dNTP, and 0.5 units of DNA Taq polymerase. Amplifications were carried out by heating reaction mixtures to 94°C for 1 minute (min), 94°C for 2 min, 50°C for 45 seconds, and 72°C for 3 min for a total of 35 cycles. Primer pairs used for PCR amplification of *matK* were: *matK* 710F (5′- GTA TCG CAC TAT GTW TCA TTT GA) - *mat*K 1556R (5′- GAG GCT GTC CCC CCA ATC C); *mat*K 1272F (5′- CGC TAT TGG GTG AAA GAT SCC) - *mat*K 2100R (5′- CGA GCC AAA GTT TTA ACA CA); and *mat*K 2000F (5′- TCT TTC TCA TTA TTA TAG CGG) - *trn*K-2R (5′- AAC TAG TCG GAT GGA GTA G). Primers used for amplification of *rpl16* were *rpl16*-int-f-2 (5′-AAT AAT CGC CCG CGA AGA TTT T) and *rpl16* 1516R (5′- CCC TTC ATT CTT CCT CTA TGT TG).

An ExoSAP-IT (USB) cleaning reaction was performed on PCR products, and sequencing reactions were performed using Big Dye terminator (Applied Biosystems) and the above primers. Sequence reaction products were run through an ABI 377 automated sequencer. Due to a lack of variation in the 3′end of the *mat*K gene, PCR products from the primer pair *mat*K 2000F – *trn*K-2R were not used for surveying mesic race haplotypes.

Phylogenetic Data Analysis. After a preliminary analysis of a large Salix data set, we pruned the matrix to 20 OTUs for computational efficiency, including six unique haplotypes of the mesic race, samples of the other two S. melanopsis races, exemplars of the other species of section Longifoliae, and Populus deltoides as the outgroup (Table 1). The analysis of section Longifoliae was intended to confirm the intraspecific variation in S. melanopsis revealed by an earlier restriction site analysis (Brunsfeld, et al. 1992). Sequence data for the matK gene were aligned with Sequencher (Genecodes). Clustal was used to align rpl16 sequences due to a large number of insertions/deletions in the outgroup, Populus deltoides, relative to members of Salix sect. Longifoliae. Both maximum likelihood (ML) and maximum parsimony (MP) techniques were employed for phylogenetic analysis, using the program PAUP*(v 4.0b10: Swofford 2002).

DT-ModSel (Minin et al. 2003) was used to choose a model of sequence evolution for ML analysis. This program evaluates models based on performance and penalizes for over-parameterization. Using the chosen model, we conducted a heuristic search with stepwise addition and TBR branch swapping. We also conducted MP analysis, using a heuristic search, TBR branch swapping and equally-weighted characters. To assess nodal support, a ML bootstrap analysis (Felsenstein 1985) was conducted using the same sequence evolution model and 100 replicates. In addition, nodal posterior probabilities were estimated by Bayesian analysis (MrBayes ver.3.0, Huelsenbeck and Ronquist 2001). We ran four chains of two million generations with uniform priors, sampling every 100 generations, with the first 10,000 generations discarded as the burn-in.

Monophyly of the Mesic Race. We investigated the monophyly of the mesic race of *Salix melanopsis* using two statistical approaches that complemented the bootstrap values and Bayesian posterior probabilities of the node in question. First, parametric bootstrapping (using maximum likelihood as an optimality criterion) was used to test the null hypothesis that the mesic race of *S. melanopsis* was not monophyletic. We also calculated the posterior probability of the monophyly of the mesic race by computing the proportion of trees from the posterior distribution in the Bayesian search that were compatible with a monophyletic mesic race (see Carstens et al. 2004 for detailed description of these approaches).

Nested Clade Analysis. Nested Clade Analysis (NCA) was used to attempt to place intraspecific phylogenetic relationships in a geographic context (Templeton et al. 1995; Templeton 1998, 2004). The number of substitutions with a 95% probability of being parsimonious was determined with TCS (v1.6: Clement et al. 2000; Templeton et al. 1992). A minimum spanning network was then constructed by nesting haplotypes into hierarchical clades following the method of Templeton et al. (1987) and Templeton and Sing (1993). NCA was then conducted with GEODIS (v2.0: Posada et al. 2000) using 10,000 permutations. The null hypothesis is no association between geography and clades (Templeton et al. 1995).

RESULTS

Phylogenetic Analysis. Sequence data from *mat*K and *rpl16* were obtained for a total of 201 samples. Of these, 160 individuals belonged to the mesic race, and six unique haplotypes were identified (Table 1). For the 20 taxa used in the phylogenetic analyses, the data matrix consisted of 1785 and 400 nucleotides for the *mat*K and *rpl16* regions, respectively. The 20 *mat*K and 20 *rpl16* sequences were submitted to GenBank, and the numbers are given in Appendix 1. The sequences and tree were also submitted to TreeBASE (study number S1652). For the 160 samples of the mesic race, approximately 1000 and 400 nucleotides from the *mat*K and *rpl16* regions, respectively, were surveyed for diagnostic characters. Uncorrected sequence divergence among the three races of *S. melanopsis* ranged from 0.7 – 1.1%. Divergence between the two mesic race lineages ranged up to 0.28%, whereas the divergence between the Salmon and Cascade haplotypes was 0.05%. For the entire dataset, including outgroups, 94 (4.5%) of the 2185 sites varied, 27 (1.3%) of which were parsimony informative. Four length mutations occur in the data set: three insertions and one deletion based on outgroup comparisons. They were treated as missing data in both analyses. One insertion and one deletion (I-3, D-1) support the recognition of the Clearwater Clade, whereas the other two (I-1, I-2) are autapomorphic (Fig. 2).

Parsimony analysis yielded 900 MP trees (122 steps, consistency index = 0.975, retention index = 0.939). The best model of sequence evolution, as chosen by DT-MODSEL, was the two substitution type F84 variant, with a transition/transversion ration of 0.833 and empirical base frequencies. The ML heuristic search produced a likelihood score of -1n L = -3684.95, and the tree shown in Fig. 2. The ML, MP, and MrBayes analyses all produced trees with similar topologies, except for a few cases

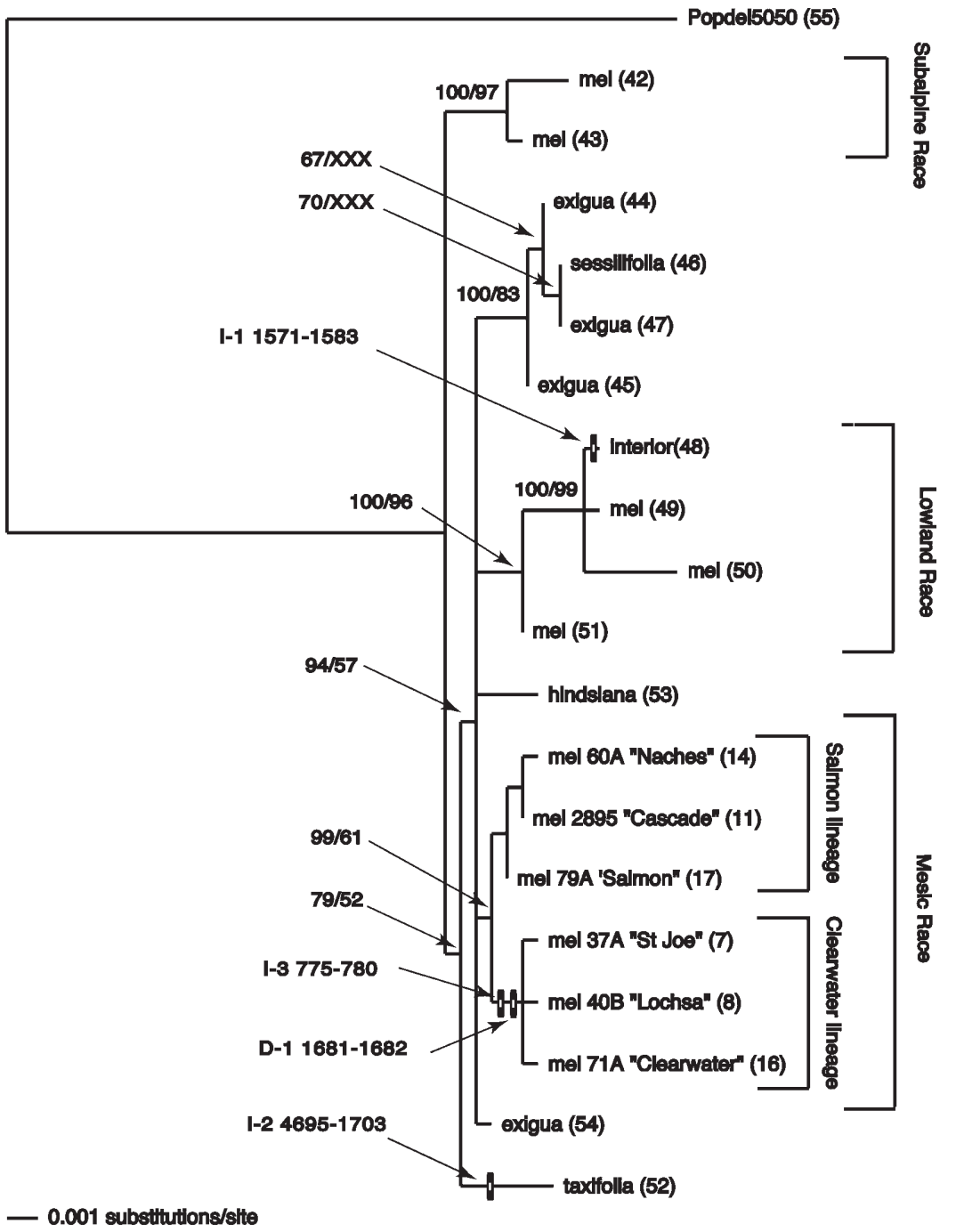

FIG. 2. Phylogenetic tree of 19 *Salix* taxa based on Maximum Likelihood (ML) analysis of *mat*K and *rpl16* DNA sequences, with *P. deltoides* as outgroup. Branch supports greater than 50% are reported as Bayesian posterior probabilities / ML bootstrap values. Insertions and deletions are plotted, but were not included in analysis.

where weakly supported nodes collapse. Bayesian posterior probabilities and ML bootstrap values greater than 50% are shown on Fig. 2.

As in an earlier analysis of cpDNA restriction site data (Brunsfeld et al. 1992), *S. melanopsis* is represented by three lineages in the current

analyses. The subalpine race is moderately supported (0.79/52%, Bayes/ML bootstrap) as sister to the rest of the group. The strongly differentiated Mexican taxon, *S. taxifolia*, is sister to the remainder of the samples, with strong to moderate support (0.94/57%). The remainder of the ingroup contains five lineages that form a polytomy. Two of these five are southwestern entities, *S. hindsiana* from west of the Sierra Nevada and a race of *S. exigua* from the deserts of Texas to southern California. Another of the lineages is well supported (1.0/83%) and includes two members of the *S. exigua* group, *S. exigua s.s.* and *S. sessilifolia* (Brunsfeld et al. 1992). Also in this clade are two samples of a narrow-leaved glabrous entity that has been considered a variety of *S. melanopsis* (var. *tenerrima*; Ball 1952). Earlier restriction site data (Brunsfeld et al. 1992) also suggested that this taxon is a variant of *S. exigua* rather than *S. melanopsis*. The fourth of the five clades is strongly supported (1.0/96%) and corresponds to the lowland race *of S. melanopsis* and *S. interior* from eastern North America. This clade was also revealed in an earlier analysis of cpDNA restriction site data (Brunsfeld et al. 1992). The last clade contains the strongly to moderately supported (0.99/61%) mesic race of *S. melanopsis*, the focus of this study. The lineage contains two major, well supported clades, the Salmon Clade and the Clearwater Clade. The Salmon Clade is strongly to moderately supported (1.0/63%) and includes three haplotypes: Salmon, Cascade, and Naches (a private haplotype within the range of the Cascade haplotype). The Clearwater Clade is strongly supported (1.0/85%), and contains three haplotypes from the northern Rockies (Clearwater, St. Joe, and Lochsa). The Lochsa haplotype, like the Naches, was found in a single plant in the drainage.

Monophyly Of The Mesic Race. Since the phylogeny with the best log-likelihood score contained a monophyletic mesic race of *S. melanopsis*, but had relatively low bootstrap support, we tested the robustness of this node using two additional approaches. Using a parametric bootstrap, we rejected a null hypothesis that the mesic race was not monophyletic ($p < 0.001$). The mesic clade was present in over 99% of all trees sampled from the posterior distribution of trees in the Bayesian analysis. While these approaches differ in their methodological assumptions, both find strong support for the monophyly of the mesic race in spite of the relatively low bootstrap support for the node uniting this clade.

Nested Clade Analysis. Haplotypes connected by up to six substitutional steps had a 95% probability of a parsimonious connection for 2185 nucleotides. The minimum spanning network for the mesic race (not shown) contained two two-step clades identical to the clades produced in phylogenetic analyses. The NCA failed to reject the null hypothesis of no geographic structure in any clade, probably due to the wide range of two of the most common haplotypes.

Discussion

Our analysis of almost 2000 bases of cpDNA revealed phylogenetic relations within *Salix* sect. *Longifoliae* congruent with those obtained in an earlier restriction site analysis (Brunsfeld et al. 1992). The restriction analysis, however, provided more clade support and some additional resolution of branching. The fact that restriction enzymes sampled approximately twice as many chloroplast bases may explain the difference.

Daubenmire (1975) inferred a continuous mesic ecosystem across the PNW, which was split into an eastern and western segment during the Pliocene by the Cascadian orogeny and the resulting rainshadow. The Rocky Mountain segment of the ecosystem therefore would have survived in isolation for at least two million years, during all of the Pleistocene glacial cycles. A contrasting history was suggested by Mack et al. (1978a, 1978b) based on the relatively recent arrival (approx. 1500 years ago) of western hemlock, a late successional dominant, in pollen deposits at glaciated sites in the northern Rocky Mountains. These data lead to an alternative hypothesis of relatively recent eastward dispersal of the ecosystem (Brunsfeld et al. 2001). Unfortunately, whether populations of disjunct taxa survived in the northern Rocky Mountains south of glaciation, or were extirpated from the region during episodes of glacial advance remains clouded by a lack of suitable fossil pollen study sites in the unglaciated region.

Vicariance. The two major clades of the mesic race of S. melanopsis exhibit a level of nucleotide divergence consistent with an ancient vicariance event. Indeed the 0.28% sequence divergence between the two clades is more than twice the intraspecific values summarized by Byrne et al. (1999) for a group of 15 plant species. Because of the Pliocene Cascadian orogeny and resulting rainshadow, mesic forests are expected to have experienced an east-west ancient vicariance, with concomitant high levels of genetic divergence (Brunsfeld et al. 2001). This is the pattern seen, for example, in three amphibian groups endemic to the mesic forest (Nielson et al. 2001; Steele et al. 2005; Carstens et al. 2005). Unexpectedly, in S. melanopsis the oldest vicariance appears to have occurred north-south within the Rocky Mountains.

The ''Clearwater Clade'' is distributed in the Clearwater, St. Joe and Coeur d'Alene River canyons within mesic forests of the northern Rocky Mountains. The other major lineage, referred to as the ''Salmon Clade'', comprises two major haplotypes, ''Salmon'' and ''Cascade''. The former occurs largely south of the Clearwater Clade, including in the Salmon River drainage, and in adjacent habitats to the north and east (Fig. 1). The Cascade haplotype occurs in the northern Cascade Mountains and north into glaciated habitats in western Canada. Because the Salmon haplotype has the ancestral sequence, we hypothesize an ancient vicariance between populations in northern drainages (the Clearwater drainage sensu lato) and those in the canyons of the Salmon River drainage to the south. Like the Clearwater drainage, the Salmon River drainage contains an abundance of endemic species (Brunsfeld and Nesom 1989), which indicates an isolated environment. Nielson et al. (2001) also found a Clearwater-Salmon vicariance in the tailed frog (Ascaphus montanus), although these clades exhibited much less genetic divergence than was found between populations in the northern Rocky Mountains and Cascades. We find little support for the alternative corollary hypothesis of vicariance caused by the Bitterroot Divide (Brunsfeld et al. 2001), due to the lack of endemic haplotypes east of the Bitterroot Mountains (Fig. 1). The lesser divergence (0.05%) between the Salmon and disjunct Cascade haplotypes may represent a more recent vicariance event within the Salmon lineage, or westward dispersal prior to post-glacial recolonization of northern latitudes (see below).

The haplotype diversity within the Clearwater clade supports the theory that the mesic race of *S. melanopsis* persisted within the northern Rocky Mountains for much of the Pleistocene. The Clearwater haplotype is the predominant lineage in the Clearwater Refugium, which we define as all the drainages from the southern limit of glaciation (see Fig. 1) to the Clearwater River. However, the St. Joe River, which lies within the Clearwater Refugium, contains a unique haplotype (St. Joe) at high frequency. This suggests that this river canyon was a separate compartment of the greater Clearwater Refugium (Brunsfeld et al. 2001). The northern Rocky Mountain endemic plant *Cardamine constancei* also provides evidence of a compartmentalization of the mesic forest ecosystem among drainages of the Clearwater Refugium (Brunsfeld and Sullivan 2005). The multitude of endemic and coastal disjunct plant and animal species associated with mesic forests south of glaciation in the northern Rocky Mountains (Daubenmire 1975, Lorain 1988) is also consistent with the existence of a Clearwater Refugium. Given the uniqueness of some of the endemics (e.g. *Dasynotus daubenmirei*, a monotypic genus), this habitat may predate Pleistocene glaciation.

Dispersal. Dispersal after the recession of Cordilleran glaciation has been shown to have played an important role in present day patterns of genetic diversity in both Europe and North America (Ferris et al. 1998, Petit et al. 1997, King and Ferris 1998, Demesure et al. 1996, Broyles 1998, Walter and Epperson 2001). The current distribution of haplotypes in regions south of glaciation does not support a recent dispersal hypothesis, at least not into the unglaciated canyons of the Clearwater Refugium because populations in the Clearwater region are not genetically similar to those in the Cascades. However, a haplotype common to the Cascades is found in northern Rocky Mountain sites that were formerly glaciated. This suggests that this haplotype dispersed recently (post-glacial) into the formerly glaciated northern Rocky Mountains from the unglaciated southern Cascades rather than from the proximal Clearwater Refugium (see Fig. 1). Due to the absence of the mesic race of *S. melanopsis* in the Oregon Cascades, dispersal along a southern route appears unlikely.

Haplotype Diversity. High haplotype diversity is often detected in regions that have either retained historical accumulations of variation (i.e. refugia), or have attained their high variation through secondary admixture via dispersal (Petit et al. 2003; Taberlet et al. 1998). Our study has identified two areas of high haplotypic diversity. One includes the drainages south of glaciation in the northern Rocky Mountains, with the three haplotypes of the Clearwater clade largely confined to this area. Two of these (St. Joe and Lochsa) are confined to single drainages. The Salmon haplotype also occupies the southern reaches of the Clearwater drainage, probably because of northward migration. Thus, the Clearwater drainage contains diversity derived from older resident and more recent immigrant haplotypes. The glaciated region north of the Clearwater drainage is also an area of high haplotypic diversity, containing the Clearwater, Salmon, and Cascade haplotypes. This recently colonized area is apparently a suture zone (Hewitt 2000, Remington 1968) where colonists from both the Cascades and the Rocky Mountains are intermingled.

Expanded Hypotheses of Pacific Northwest Phylogeography. Brunsfeld et al. (2001) proposed testable alternative hypotheses for the origin of the disjunction of mesic coniferous forests between the

mountains of the Coast and Cascade Ranges and the northern Rocky Mountains. These core hypotheses posit ancient vicariance produced by Cascadian orogeny, or recent colonization of one of the regions via dispersal from the other. Our analysis of *S. melanopsis* demonstrates that simple ''either/or'' models, although useful, can fail to explain fully the complexity that has marked the evolutionary history of species.

An important characteristic of the disjunct mesic forest ecosystem is that approximately half the current range was covered by Cordilleran ice until approximately 10-15,000 years BP, when the last glacial retreat began (Booth 1987). Thus, elements of the ecosystem survived glaciation somewhere to the south, and whether they occupied areas in the Rocky Mountains, the Cascades, or both can be inferred from genetic patterns, as outlined in the core hypotheses of Brunsfeld et al. (2001). These hypotheses do not, however, address glaciated regions, whose biogeographic history is primarily a function of the source and rate of dispersal of ecosystem members. Unglaciated areas may have served as refugia, but a lack of glaciation, per se, does not mean that conditions necessary for the existence of a mesic forest were present.

The current study found that at least one component of mesic forests, the mesic race of *S. melanopsis*, likely survived glaciation in the river canyons just south of glaciation in the Rocky Mountains. The association of the highly diverged Clearwater Clade with the region indicates an early and prolonged presence. Another major insight of this study is the apparent importance of north-south vicariance involving the two major canyon systems – the Clearwater and the Salmon - of the northern Rocky Mountains. Brunsfeld et al. (2001) hypothesized that ancient vicariance might be manifested as a series of river canyon refugia occupied by genetically differentiated populations, but these authors were focusing only on the mesic forests of the Clearwater River region. Although the Salmon River drainage is more arid (at low elevation) than the Clearwater, species that span both drainages might be hypothesized to have experienced vicariance, as *S. melanopsis* apparently has. Both of these drainages likely had a major role in the survival of biodiversity during glaciation, and each has its own suite of endemics and disjuncts (Daubenmire 1952; Brunsfeld and Nesom 1989).

Salix melanopsis data also provide unexpected evidence of two ages of vicariance and dispersal. First, a split between the two major clades apparently resulted from an ancient isolation of mesic race populations in the two major river systems of the region. The lesser divergence between the Salmon haplotype and the Cascade type suggests either more recent vicariance, or dispersal to the coast followed by divergence, perhaps during the Pleistocene. Other hypothesized dispersal is more recent, involving colonization of deglaciated landscapes.

The extension of phylogeographic hypotheses of individual species to the understanding of ecosystems offers considerable challenges. An ancient vicariance hypothesis assumes that a species was continuously distributed and genetically homogeneous before being sundered by an isolating barrier such as the Cascade Range. For example, several endemic amphibians exhibit large degrees of sequence divergence and reciprocal monophyly between coastal and Rocky Mountain populations (Nielson et al. 2001; Steele et al. 2005; Carstens et al. 2004), and are apparently examples of species that were formerly continuously distributed and underwent vicariance as a result of the Cascadian orogeny and subsequent xerification of the Columbia basin. However, these amphibans likely do not have the dispersal capabilities of many plant species. Paleobotanical evidence from eastern North America indicates that the composition of communities changed over long periods of time as species migrated following glaciation (Delcourt and Delcourt 1991). It should be expected, therefore, that some species may have entered the mesic coniferous forest via the central Rocky Mountains, whereas others entered the Cascades from the Sierra Nevada. Dispersal to the remainder of the potential range may not have occurred before the ancient sundering event or at any time until recently, or not at all. Thus ancient vicariance between mesic forest ecosystems produced by the rise of the Cascade Range is part of a continuum and not expected in every species analyzed. A prolonged occupation of the northern Rocky Mountains followed by later dispersal to the coast (as hypothesized for *S. melanopsis*) should have a similar (although reversed) genetic signature as an ancient colonization of the Cascades followed by later dispersal to the Rocky Mountains. The timing of the dispersal would determine whether ancient vicariance or a recent formation of the disjunct ecosystem was supported. In both cases, we hypothesize considerable differentiation among drainages in one mountain range, compared to the relative homogeneity among drainages in the more recently colonized mountain range. The latter populations should be nested with those from the source geographic area in an intraspecific phylogeny. Only those species that have experienced long vicariance would exhibit differentiation among

drainages in both mountain ranges and reciprocal monophyly among ranges.

Salix melanopsis appears to represent a case of early Rocky Mountain occupation and later (but not extremely recent) dispersal to the coast, whereas there are a number of cases in which the coastal mountains may have been colonized first. The north-south haplotype divergence in a number of Cascade-Sierran species (at a boundary we call the ''Soltis Line''), and existence of either northern or southern haplotypes disjunct in the Rocky Mountains (summarized by Soltis et al. 1997; Brunsfeld et al. 2001) may reflect an early coastal presence and later dispersal to the Rocky Mountains from either northern or southern sources. We are pursuing a strategy of analyzing species from multiple kingdoms, by gathering genetic data that are used to test a common set of hypotheses. This provides a mechanism for comparing the phylogeographic structure of codistributed organisms with vastly different life history characteristics. In this way we hope to discern how much of the ecosystem shared a common history of interaction, and how the community may have changed over time due to the independent arrival of species. Whether a species is part of an ancient refugium or a recent colonist has enormous implications for amount and distribution of biodiversity in a region. Refugia revealed by comparative phylogeographic analysis likely possess an abundance of cryptic genetic and organismal diversity, and such areas represent opportunities to manage ecosystem rather than single-species novelty.

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APPENDIX 1. *Salix melanopsis* samples. Population numbers (in bold) correspond to those found in Fig. 1. Voucher specimens are deposited in one of the following herbaria - ID: Stillinger Herbarium, University of Idaho, ALTA: University of Alberta Vascular Plant Herbarium, University of Alberta, WS: Marion Ownbey Herbarium, Washington State University. Numbers in parentheses following haplotypes indicate the proportion of each haplotype at the location. N= indicates the number of samples sequenced. GenBank accession numbers (*mat*K; *rpl16*) for the *Salix melanopsis* mesic race haplotypes are A: Salmon (DQ922722, DQ060273), B: Cascade (DQ875035, DQ060271), C: Naches

(DQ922773, DQ060272), D: Clearwater (DQ922769, DQ-060276), E: St. Joe (DQ922771, DQ060274), F: Lochsa (DQ922770, DQ060275). GenBank accession numbers for the other samples are given following their locality information.

1. *S. melanopsis* A(1), D(4), Moyie R., ID, *Miller 26* (ID), 48° 49′ 11″ N, 116° 08′ 46″ W, N=5. **2.** *S. melanopsis* A(2), D(3), Yaak R., MT, *Miller 28* (ID), 48° 38′ 39″ N , 115° 53′ 07″ W, N=5. **3.** *S. melanopsis* D(5), C′dA R., ID, *Miller 30* (ID), 47° 41′ 52″ N , 115° 56′ 28″ W, N=5. **4.** *S. melanopsis* D(5), N.Fork C′dA R., ID, *Miller 32* (ID), 47° 39′ 11″ N, 116° 21′ 49″ W, N=5. **5.** *S. melanopsis* D(8), E(2) , St Joe R., ID, *Miller 34* (ID), 47° 16′ 06″ N , 116° 05′ 19″ W, N=10. **6.** *S. melanopsis* D(5), Upper St Joe R., ID, *Miller 35* (ID), 47° 09′ 32″ N , 115° 25′ 16″ W, N=5. **7.** *S. melanopsis* D(1), E(4) , Upper St Joe R., ID. , *Miller 37* (ID), 47° 12′ 56″ N , 115° 33′ 27″ W, N=5. **8.** *S. melanopsis* D(4), F(1), Upper Lochsa R., ID, *Miller 40* (ID), 46° 28′ 37″ N , 114° 52′ 54″ W, N=5. **9.** *S. melanopsis* D(5), Upper Lochsa R., ID, *Miller 42* (ID), 46° 20′ 06″ N , 115° 20′ 37″ W, N=5. **10.** *S. melanopsis* A(9), D(1), Lochsa R., ID, *Miller 48* (ID), 46° 13′ 37″ N , 115° 28′ 22″ W, N=10. **11.** *S. melanopsis* B(8), Skagit R. WA , *Miller 54/Brunsfeld 2895* (ID), 48° 35′ 04″ N , 121° 24′ 01″ W, N=8. **12.** *S. melanopsis* B(10), Skykomish R., WA , *Miller 56* (ID), 47° 43′ 45″ N , 121° 24′ 19″ W, N=10. **13.** *S. melanopsis* B(4), White R., WA., *Miller 58* (ID), 47° 09′ 18″ N , 121° 40′ 22″ W, N=4. **14.** *S. melanopsis* B(10), C(1) , Naches R., WA , *Miller 60* (ID), 46° 59′ 22″ N , 121° 05′ 39″ W, N=11. **15.** *S. melanopsis* D(5), N. Fork Clearwater R, ID, *Miller 69* (ID), 46° 45′ 47″ N , 115° 29′ 48″ W, N=5. **16.** *S. melanopsis* D(5), N. Fork Clearwater R., ID, *Miller 71* (ID), 46° 44′ 56″ N , 115° 14′ 10″ W, N=5. **17.** *S. melanopsis* A(4), S. Fork Clearwater R, ID, *Miller 79* (ID), 45° 48′ 46″ N , 115° 37′ 53″ W, N=4. **18.** *S. melanopsis* A(5), S. Fork Clearwater R., ID, *Miller 81* (ID), 45° 38′ 44″ N , 115° 24′ 27″ W, N=5. **19.** *S. melanopsis* A(4), Selway R., ID, *Miller 84* (ID), 46° 02′ 53″ N, 115° 13′ 43″ W, N=4. **20.** *S. melanopsis* A(1), Glacier NP, MT, *Brunsfeld 3081* (ID), 48° 42′ 14″ N, 113° 48′ 21″ W, N=1. **21.** *S. melanopsis* B(1), Clackamas R., OR, *Brunsfeld 7084E* (ID), 45° 11′ 27″ N , 122° 12′ 32″ W, N=1. **22.** *S. melanopsis* D(2), Bitterroot R., MT, *Brunsfeld 7100* (ID), 45° 51′ 14″ N , 114° 01′ 10″ W, N=2. **23.** *S. melanopsis* A(2), D(5), Salmon R., ID, *Brunsfeld 7101* (ID), 45° 27′ 56″ N , 113° 59′ 54″ W, N=7. **24.** *S. melanopsis* A(2), D(2), Salmon R., ID , *Brunsfeld 7102* (ID), 45° 10′ 03″ N , 114° 09′ 40″ W, N=4. **25.** *S. melanopsis* A(1), D(2), St. Maries R., BC, *Brunsfeld 7113* (ID), 49° 34′ 16″ N , 115° 48′ 12″ W, N=3. **26.** *S. melanopsis* B(4), Vermillion R, BC, *Brunsfeld 7114* (ID), 51° 08′ 01″ N , 116° 08′ 03″W, N=4. **27.** *S. melanopsis* B(6), Kuskanax Cr., BC, *Brunsfeld 7120* (ID), 50° 14′ 59″ N , 117° 48′ 38″ W, N=6. **28.** *S. melanopsis* B(2), Jasper NP, AL, *Argus 14015,14018* (ID), 53° 01′ 00″ N , 118° 05′ 00″ W, N=2. **29.** *S. melanopsis* B(1), Kettle R., BC, *Argus 8470* (ALTA), 49° 03′ 00″ N, 118° 12′ 00″ W, N=1. **30.** *S. melanopsis* B(1), Cathedral PP, BC, *Hainault 7732* (ALTA), 49° 04′ 00″ N , 120° 11′ 00″ W, N=1. **31.** *S. melanopsis* B(1), Manning PP, BC, *Scoggan 15785* (ALTA), 49° 04′ 00″ N, 120° 47′ 00″ W, N=2. **32.** *S. melanopsis* A(1), Waterton Lakes NP, Al, *Ball 605* (ALTA), 49° 05′ 00″ N , 113° 52′ 00″ W, N=7. **33.** *S. melanopsis* B(1), Vancouver Island, BC, *Calder 16404* (WS), 48° 46′ 00″ N , 123° 38′ 00″ W, N=1. **34.** *S. melanopsis* A(1), Flathead NF, MT, *Hitchcock 18470* (WS), 47° 25′ 30″ N, 113° 24′ 13″ W, N=1. **35.** *S. melanopsis* A(1), B(1), D(1), Flathead R., MT, *Rogers 904,906,907* (WS), 48° 41′ 21″ N , 114° 11′ 40″ W, N=3. **36.** *S. melanopsis* B(1), Okanogan Co., WA, *Burnett 37* (WS), 48° 56′ 50″ N , 120° 16′ 14″ W, N=1. **37.** *S. melanopsis* B(1), Okanogan NF, WA, *Kovalchik 0632* (ID), 48° 49′ 37″ N, 120° 28′ 15″ W, N=1. **38.** *S. melanopsis* B(1), Okanogan NF, WA, *Kovalchik 0172* (ID), 48° 59′ 41″ N, 120° 25′ 56″ W, N=1. **39.** *S. melanopsis* B(1), Gold Cr., AL, *Argus 8392* (WS), 49° 36′ 00″ N, 114° 25′ 00″ W, N=1. **40.** *S. melanopsis* D(1), Elk R., BC, *Argus 8394* (WS), 49° 30′ 00″ N, 115° 04′ 00″ W, N=1. **41.** *S. melanopsis* A(1), Clark Fork R., ID, *Brunsfeld 7126* (ID), 48° 10′ 48″ N, 116° 09′ 44″ W, N=1. **42.** *S. melanopsis* Subalpine race, McCall, ID, *Brunsfeld2965* (ID), 44° 58′ 02″ N, 115° 57′ 04″ W, N=1. DQ875024, DQ875038. **43.** *S. melanopsis* Subalpine race, Napias Cr., ID, *Brunsfeld 7102B* (ID), 45° 10′ 03″ N, 114° 09′ 40″ W, N=1. DQ875033, DQ875047. **44.** *S. melanopsis* (tennerinum), Ada Co., ID. Boise R., , *Brunsfeld 2930* (ID), 43° 33′ 29″ N, 116° 06′ 13″ W, N=1. DQ875025, DQ875039. **45.** *S. melanopsis* (tennerinum), Sun Valley, ID, *Brunsfeld 5089B* (ID), 43° 40′ 17″ N, 114° 21′ 57″ W, N=1. DQ875034, DQ875048. **46.** *S. sessifolia*, Yamhill Co., OR. Willamette R., , *Brunsfeld 2926* (ID), 45° 05′ 25″ N, 123° 02′ 43″ W, N=1. DQ875026, DQ875040. **47.** *S. exigua*, Benton Co., WA. Columbia R., vic Richland, *Brunsfeld 2914* (ID), 46°16′ 10 ″ N, 119° 15′ 49″ W, N=1. DQ875028, DQ875042. **48.** *S. interior*, St. Croix Co., WI. St. Croix R., vic Hudson, *Brunsfeld 2956* (ID), 44° 57′ 54″ N, 92° 45′ 16″ W, N=1. DQ875027, DQ875041. **49.** *S. melanopsis*, Jasper NP, AL, *Argus 14016* (ID), 53° 01′ 00″ N , 118° 05′ 00″ W, N=1. DQ875032, DQ875046. **50.** *S. melanopsis* Lowland race, Clackamas R., OR, *Brunsfeld 7084A* (ID), 45° 11′ 27″ N , 122° 12′ 32″ W, N=1. DQ060270, DQ060277. **51.** *S. melanopsis*, Scott Cr., BC, *Argus 8774* (ALTA), 55° 45′ 00″ N, 123° 32′ 00″ W, N=1. DQ875031, DQ875045. **52.** *S. taxifolia*, Vera Cruz, Mexico. Rio Tablazos, vic Tlapacoyan, *Brunsfeld 3008* (ID), 19° 12′ 00″ N, 96° 08′ 00″ W, N=1. DQ875029, DQ875043. **53.** *S. hindsiana*, Curry Co., OR. Lwr Rogue R., vic Gold Beach, *Brunsfeld 2828* (ID), 42° 27′ 45″N, 124° 22′ 00″ W, N=1. DQ875030, DQ875044. **54.** *S. exigua*, San Miguel Co., NM, *Brunsfeld 2870* (ID), 35° 28′ 01″ N, 104° 24′ 58″ W, N=1. DQ875036, DQ875049. **55.** *Populus deltoides*, Chautauqua Co., NY. Chautauqua R., I90, *Brunsfeld 5050* (ID), 42° 19′ 58″ N, 79° 35′ 35″ W, N=1. DQ875023, DQ875037.