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## Molecular phylogenomics and population structure of

### *Phytophthora pluvialis*

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## ABSTRACT

*Phytophthora pluvialis* is an oomycete that was first isolated from soil, water, and tree foliage in mixed Douglas-fir - tanoak forests of the US Pacific Northwest (PNW). It was then identified as the causal agent of red needle cast (RNC) of radiata pine (*Pinus radiata*) in New Zealand (NZ). Genotyping-by-sequencing (GBS) was used to obtain 1,543 single nucleotide polymorphisms (SNPs) across 145 *P. pluvialis* isolates to characterize the population structure in the PNW and NZ. We tested the hypothesis that *P. pluvialis* was introduced to NZ from the PNW using genetic distance measurements and population structure analyzes among locations between countries, . The low genetic distance, population heterozygosity, and lack of geographic structure in NZ, suggest a single colonization event from the US followed by clonal expansion in NZ. The PNW Coast Range was proposed as a presumptive center of origin of the currently known distribution of *P. pluvialis* based on its geographic range and position as the central cluster in a minimum spanning network. The Coastal cluster of isolates were located at the root of every US cluster, and emerged earlier than all NZ clusters. The Coastal cluster had the highest degree of heterozygosity ( $H_s = 0.254$ ) and median pairwise genetic distance (0.093) relative to any other cluster. Finally, the rapid host diversification between closely related isolates of *P. pluvialis* in New Zealand, indicate that this pathogen has the potential to infect a broader range of hosts than is currently recognized.

## INTRODUCTION

The introduction of exotic plant pathogens poses risks to agroecosystems, natural environments, and human health (Fisher et al. 2012, Wingfield et al. 2015, Corredor-Moreno and Saunders 2019, Scott et al. 2019). For example, the introduction of *Phytophthora infestans* (Montagne) de Bary to Europe from Mexico in the nineteenth century, caused the Irish Potato Famine. This famine was a result of the near-total failure of Ireland's primary food staple, the potato, following an outbreak of *P. infestans*. The loss of potatoes led to widespread starvation and the Irish diaspora (Goss et al. 2012). Recently, introduced plant pathogens such *Phytophthora ramorum* Werres, De Cock & Man in 't Veld, the cause of sudden oak death (Cobb et al. 2012), and *Cronartium ribicola* J.C. Fisch, the cause of white pine blister rust (Kinloch 2003), have had devastating impacts to natural ecosystems. These and other reported introductions of plant pathogens have been linked to human-mediated movement of infected plants and infested soil (Kamvar et al. 2015; Wingfield et al. 2015; Tabima et al. 2020). Understanding the source-sink dynamics of these introductions informs policy, prevents future introductions, and can be used to mitigate disease impacts.

Microorganisms coevolve with sympatric plant species, and can cause little to no disease in their hosts within their native range (Stukenbrock and McDonald 2008). When their geographic distribution shifts, they come into contact with naïve plant species, potentially leading to new disease outbreaks (Grünwald and Flier 2005, Stukenbrock and McDonald 2008). The source of exotic pathogens can provide important information about the genetic diversity of the microbe, its native hosts, and potential sources of resistance. For example, the establishment of the center of origin of *P. infestans* in Mexico led to the identification of wild *Solanaceae* spp. with co-evolved resistance to the pathogen. These species have been used to identify resistance genes that have been integrated into commercial potato varieties (Grünwald and Flier 2005). In

the case of forest ecosystems, *Bretziella fagacearum* (T. W. Bretz) J. Hunt, the causal agent of oak wilt, is another example. The current range of *B. fagacearum* is restricted to the US, but population genetic analyses indicate that the genetic diversity of the species is very limited in the US and likely a result of a single clonal introduction. It has therefore been hypothesized to have been introduced from either Mexico, Central America, or northern South America despite the lack of reports from these regions (Juzwik et al. 2008).

A similar example of long-distance movement of a plant-associated microbe is *Phytophthora pluvialis* Reeser, Sutton, & Hansen. This oomycete was first isolated from baited streams, soil, and canopy drip in Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirbel] Franco)/tanoak (*Notholithocarpus densiflorus* [Hook. & Arn.] Manos, Cannon, & Oh) forests in southwestern Oregon (OR) (Reeser et al. 2013, Hansen et al. 2017). In 2014, *P. pluvialis* was confirmed as the cause of red needle cast (RNC), a disease of radiata pine (*Pinus radiata* [D. Don]) that was first identified on the North Island of New Zealand (NZ) in 2008 (Dick et al. 2014). Radiata pine is a tree species native to coastal California (CA) and was introduced to NZ in the nineteenth century for use in plantation forestry (Burdon et al. 2017). Symptoms of RNC start as discrete olive-colored lesions and black resinous bands on needles. As the disease progresses the lesions change color from yellow to red brown. Needles on affected trees eventually turn a reddish color and senesce. Premature defoliation of the lower crown and occasionally the entire tree is observed. In addition, *Phytophthora pluvialis* has been isolated from olive-colored Douglas-fir needles growing near radiata pine plantations in NZ (Gómez-Gallego et al. 2019).

On the west coast of the United States (US) *P. pluvialis* has been isolated from scattered regions in Washington (WA), Oregon (OR), and CA (Hansen et al. 2015; Gómez-Gallego et al.

2019; Brar et al. 2018). A variety of approaches have been used to collect isolates including canopy drip, symptomatic needle tissue, soil baiting, and stream baiting (Gómez-Gallego et al. 2019; Reeser et al. 2013, Hansen et al. 2017). In NZ, collections have been made from Douglas-fir plantations across both islands (Gómez-Gallego et al. 2019). The symptoms on Douglas-fir are more cryptic than those found on radiata pine. Isolations can frequently be made from olive-colored and asymptomatic needles. Since its initial description, *P. pluvialis* has been isolated from symptomatic Douglas-fir and tanoak in the US and radiata pine, Douglas-fir, patula pine (*Pinus patula* Schiede ex Schltdl. & Cham.), and Eastern white pine (*Pinus strobus* L.) in NZ.

The discovery of *P. pluvialis* in both OR and NZ suggests human-mediated migration across the Pacific Ocean. Like other Clade 3 *Phytophthora* spp., *P. pluvialis* disperses aerially over relatively short distances, via rain splash and wind-driven rain (Dick et al. 2014, Hansen et al. 2017). Recent efforts to identify the pathway of introduction of *P. pluvialis* used 28 single nucleotide polymorphisms (SNPs). The analysis suggested two introductions of *P. pluvialis* from OR to NZ (Brar et al. 2018). The authors hypothesized that the introduction was likely due to movement of soil, water, or asymptomatic plant material (Brar et al. 2018). Similar mechanisms of anthropogenic-mediated spread have been suggested for *P. ramorum* and other plant pathogens (Davidson et al. 2008, Grünwald et al. 2012). In order to test the hypothesis of multiple introductions the authors suggested that increasing the number of SNP and the geographic range of the sampling would be necessary.

Building on Brar et al. (2018), the sampling in the forests of the US PNW was expanded and additional samples from NZ were added. These samples were collected from various hosts across the current distribution of the pathogen. Our overarching goal was to develop a better understanding of the population dynamics of *P. pluvialis* in NZ and the US. The specific

objectives were to: 1) characterize the population structure of *P. pluvialis* in the US and NZ; 2) study the genetic diversity of *P. pluvialis* at the state/location level within these geographic regions; and 3) examine the interaction between genetic structure and host range. To meet these objectives 157 isolates of *P. pluvialis* were genotyped using genotyping-by-sequencing (GBS) and a population genomics framework was used to address the objectives.

## **MATERIALS AND METHODS**

**Isolate collection.** A total of 157 curated isolates of *P. pluvialis* from NZ (n=91) and the US (n=66) were selected for genotyping. Selections were made in order to maximize the temporal (2008-2018) and geographic range of sampling (Supp. Table 1). In NZ samples were collected from across the North and South Island from 14 regions as defined by Crosby (1976): Northland (ND; n = 5), Gisborne (GB; n = 36), Bay of Plenty (BP; n = 11), Wairarapa (WA-NZ; n = 5), Rangitikei (RI; n = 3), Taupo (TO; n = 7), Taranaki (TK; n = 1), Auckland (AK; n = 1), Coromandel (CL; n = 2), Hawkes Bay (HB; n = 4), Nelson (NN; n = 11), Wellington (WN; n = 3), Waikato (WO; n = 1), and Wanganui (WI; n = 1). The US isolates were collected from 14 regions in Brookings (n = 6), Cascades (n = 4), Central OR (n = 10), Del Norte Co. (n = 3), Florence (n = 1), Humboldt (n = 5), Little Lobster (n = 2), Medocino (n = 9), North CA (n = 4), Port-Orford (n = 14), Redwood national trail (n = 1), South OR (n = 1), South WA (n = 1), Washington (n = 3), Wheeler (n = 1). These regions were spread across three states. WA (n = 5), OR (n = 39) and CA (n = 22). All isolates of *P. pluvialis* were grown in clarified carrot broth for 4-5 days in the dark at 17°C. Mycelia were harvested, washed twice, transferred to 96-well collection tubes (Qiagen, USA) and sent to the Center for Genome Research and Biocomputing (CGRB) at Oregon State University (Corvallis, USA) for genomic DNA extraction and GBS.

**Genotyping-by-sequencing, read mapping, and quality filtering.** To reduce the complexity of the genome, a double restriction enzyme digestion was performed with the endonucleases PstI and MstI (New England Biolabs, Ipswich, MA). All samples were sequenced on the Illumina HiSeq 3000 platform using 150 base pair (bp) single end reads following the Illumina protocol (San Diego, CA). Sequenced libraries were demultiplexed using the program *sabre* (<https://github.com/najoshi/sabre>). All reads are available at the NCBI SRA repository (accession number: PRJNA638875).

The reads from the sequenced isolates were aligned to the reference genome of *P. pluvialis* type strain LC-9 (Studholme et al. 2015). Alignments were made using bowtie 2, with the “-very careful” option as the sole parameter added to the program (Langmead and Salzberg 2012). In order to identify which of the genetic variants represent the ancestral state, reads from the genome of *Phytophthora agathidicida* B.S. Weir, Beever, Pennycook & Bellgard strain NZFS 3770 were also mapped to the *P. pluvialis* reference (Studholme et al. 2015). The resulting SAM file was converted into a BAM file, sorted, and indexed using SAMTools V. 2.1.2 (Li et al. 2009). *P. agathidicida* is a sister species found in Phytophthora Clade 5 and is the most closely related *Phytophthora* species with a reference genome. This polarization approach will allow more accurate prediction of the earliest emerging populations of *P. pluvialis* (US or NZ).

Genomic variants were predicted using GATK V. 4.0 (McKenna et al. 2010). Initially, each sample was independently genotyped with GATK’s HaplotypeCaller and the GVCF option. All samples were pooled, and common variants identified in the CombineGVCFs program. Finally, the likelihood of each variant was estimated using the GenotypeGVCFs program for all samples. The final variant files were filtered for quality using the *vcfR* package (Knaus and Grünwald 2016) in R (R Core Team 2019). Two variant files were created for subsequent



analysis: (i) *P. pluvialis* and *P. agathidicida* reads (combined-species dataset); and (ii) *P. pluvialis* reads only (single-species dataset).

Variants were filtered in a three-stage process. In stage one, for the combined-species dataset variant positions missing in *P. agathidicida* were removed. In stage two, for both the combined-species and single-species datasets, filtering was performed to remove individual variants with a minimum read depth (DP) of  $< 4x$  and greater than the 95th percentile of each sample DP distribution. In stage three, variants were filtered by mapping quality (MQ = 44), minimum allele frequency (MAF  $< 0.05$ ), and missing data. All variant positions with any missing data were removed and finally samples with more than 70% missing data were eliminated. The final single-species and combined-species files were used in all subsequent analysis.

**Population differentiation of *P. pluvialis* between countries.** To characterize the population dynamics between the US and NZ and identify a presumptive center of origin, we measured the degree of genetic differentiation across *P. pluvialis* samples in a hierarchical manner. Initially, comparisons between countries were made among geographic regions within countries. To identify the earliest emerging population of *P. pluvialis* from our sampled populations, a phylogenetic tree using maximum likelihood was reconstructed in RAxML (Stamatakis et al. 2007) on both the combined-species dataset and single-species dataset. In both cases, *P. agathidicida* was the outgroup used to polarize the tree. The MULTIGAMMAI model of substitution with 1,000 bootstraps was used. The degree of genetic connectivity among populations was evaluated using a minimum spanning network (MSN) constructed in the R package poppr 2.1.5 (Kamvar et al. 2014; 2015b). Finally, population differentiation was evaluated at the different hierarchies with Nei's  $G_{st}$ , an  $F_{st}$  analog estimated in the mmod 1.13

package (Winter, 2012). Population heterozygosity was estimated and statistically contrasted for each population using the `Hs.test` function with 499 replicates using the same package (Winter, 2012). Population differentiation and population heterozygosity were measured in the single-species dataset

**Population dynamics in the US.** For the single-species dataset the number of genetic clusters (K) of *P. phuvialis* was estimated using the `find.clusters` function of the `adegenet` package in R (Jombart 2008). The optimal number of clusters was automatically determined by goodness of fit with Bayesian Inference Criterion (BIC) across all values of  $K = 2 - 10$ . A discriminant analysis of principal components (DAPC; Jombart et al. 2010) was performed for each sample using the predicted genetic clusters as a population index.

For each genetic cluster, heterozygosity was calculated using the `Hs` function in the `mmod` package (Winter 2012). The distribution and median estimates of pairwise genetic distance within each population were calculated using the `bitwise.distance` function in `poppr` (Kamvar et al. 2014). The index of association ( $I_A$ ) was calculated for each genetic cluster to identify the degree of linkage within populations.  $I_A$  was calculated with the `RbarD` index implemented in `poppr` (Kamvar et al. 2014). A total of 999 `RbarD` permutations were performed.

To identify the degree of genetic differentiation across the predicted genetic clusters, Nei's  $G_{st}$  was estimated for each pairwise combination of genetic clusters as described above (Winter 2012). To evaluate the relatedness of the predicted genetic clusters, the maximum likelihood phylogenetic reconstruction from the previous step was colored by genetic cluster in `ggtree` (Yu et al. 2017). Similarly, the MSN was colored by genetic cluster and geographic regions in order to observe how different geographic regions were grouped within each predicted genetic cluster. To identify if the predicted genetic clusters were grouped geographically, a map

of the samples colored by genetic cluster was constructed using the ggmap package in R (Khale and Wickham 2013).

**Population structure and host specificity of *P. pluvialis*.** To test the relatedness of *P. pluvialis* samples from different hosts in NZ, the host information was superimposed on the MSN and phylogenetic reconstruction of the single species dataset. The overlay was performed by creating a stratified data frame of hosts in the genind object of the single species SNP dataset in poppr (Kamvar et al. 2014) nodes were colored by host. The ML tree was colored by host using the strata host table in ggtree (Yu et al. 2017).

## RESULTS

**Summary statistics from genotyping-by-sequencing.** A total of 1,543 SNPs were obtained from 145 isolates of *P. pluvialis* after variant filtering by DP, MQ, MAF and missing data. Eight samples were removed from the single species dataset. The combined species dataset had considerably fewer variants and isolates: 156 SNPs across 78 samples after variant filtering.

**Genetic differentiation between US and NZ populations of *P. pluvialis*.** Differences between the US and the NZ populations were observed across all analyses performed. The phylogenetic reconstruction with *P. agathidicida* as a root reveals that all samples from the US are clustered in two early emerging clades, while the NZ samples were grouped in a single terminal clade (Supp. Fig. 1A), with the exception of pluv-3635. The US clades have a longer average branch length than the NZ clade, indicating a higher average number of substitutions per site. The MSN also separated the US samples from the NZ samples (Supp. Fig. 1B). Pairwise genetic distances from the MSN showed that the US samples had an overall greater genetic distance among samples than those collected in NZ. Population heterozygosity metrics indicated significantly higher heterozygosity in the US (0.285) compared to NZ (0.239) (Hs.test,  $p = 0.002$ ). The NZ sample

pluv-3635 is clustered in a different clade than the other NZ samples. It is more closely related to MG-AND1 and MG-YUK-4, two samples from the Cascades cluster in OR. This placement is consistent in both the phylogenetic reconstruction and the MSN.

**Genetic differentiation among regions in the US and NZ.** Stratification of isolates by region within countries revealed low pairwise differences in genetic diversity between regions (Supp. Figs. 3 and 4). In most cases, the closer the regions the less genetic differentiation. However, there were several exceptions (Supp. Figs. 2 and 3). Across the US, the Central OR, South WA, Florence OR and WA-state regions were all similar ( $G_{st} < 0.05$ ), compared to the other geographic regions in the US. Additionally, samples from the Port-Orford region had the highest genetic differentiation ( $G_{st} > 0.12$ ) when compared to all US regions, with the exception of South OR and Florence OR. In the case of NZ, the only geographic area with strong evidence for genetic differentiation was ND. Pairwise comparisons of genetic differentiation between ND and GB, BP, WA-NZ, RI, and TO regions had an  $G_{st} > 0.12$  (Crosby, 1976). The pairwise genetic differentiation indicates low differentiation between regions within countries. Two regions from NZ, TK and WI, were weakly differentiated ( $G_{st} < 0.1$ ) when compared with all the US regions with the exception of Central OR and Port-Orford.

**Population dynamics of *P. pluvialis* between its US center of origin and NZ.** Five genetic clusters were identified across the US ( $n=3$ ) and NZ ( $n=2$ ) (Figure 1A; Fig 2A and 2B). The Coastal, Central, and Port-Orford genetic clusters grouped most US samples. The remaining samples were from the Cascades region, and clustered within NZ1. The Coastal cluster grouped all southern OR and northern CA coastal samples. The Central cluster grouped all northern OR and WA samples. Port-Orford samples grouped in their own cluster with a single sample from the Cascades. NZ1 grouped all samples from the regions of ND, AK, TK, three from NN, two

from HB, one from WN in NZ, and three samples from the OR Cascades region in the US. NZ2 was exclusive to NZ and grouped all samples from GB, BP, WA, RI, four samples from TO, eight from NN, two from HB, two from WN, and one from CL.

The Coastal genetic cluster is the most diverse of the genetic clusters, both in terms of heterozygosity and pairwise genetic distance, while the Port-Orford and NZ2 clusters are the least diverse (Table 1, Supp. Fig. 4). The Coastal genetic cluster had the lowest genetic differentiation when compared to any other genetic cluster ( $G_{st} < 0.15$ , Table 2). All other pairwise comparisons had similar levels of genetic differentiation ( $G_{st} > 0.18$ ). The  $I_A$  simulations indicate that the NZ2 and Port-Orford clusters have the largest simulated values of  $R_{barD}$  compared to the other clusters. This indicates a higher degree of genetic linkage within these populations and a higher degree of clonal reproduction compared to the other populations (Supp. Fig. 5).

The Coastal genetic cluster had more connections to other genetic clusters in the MSN. Phylogenetic reconstruction revealed that the Coastal cluster was always positioned with the earliest emerging clade, suggesting the Coastal cluster is the center of origin of *P. pluvialis*. All population clustering metrics indicated that MG-YUK-4, MG-CASC-5, and MG-AND-1, isolated from Douglas-fir in the Cascades region of OR, are the most closely related to the NZ samples clustered within NZ1.

**Genetic structure and host associations in New Zealand *P. pluvialis* samples.** Host overlay on the MSN indicates no correlation between the genetic cluster, phylogenetic clade, or geographic region of the isolate with host specificity (Figure 3). All samples from the US were exclusively isolated from Douglas-fir and as a result not included in this analysis. The phylogenetic tree indicates no host specificity or directional selection to date.

## DISCUSSION

The hypothesis of a single colonization of *P. pluvialis* giving rise to the NZ RNC epidemic is supported by the analysis and results described above. A demographic analysis of the 147 isolates indicates that unidirectional migration from the US to NZ is the most parsimonious explanation for the observed genetic patterns (Supp. Fig. 2). The source of this colonization event was likely the Cascades region in OR. Weak genetic differentiation among isolates from the Cascades region in the US and the Northland and Nelson regions in NZ resulted in their grouping into a single genetic cluster. In addition, all measures of genetic diversity from US samples were greater than the samples from NZ, regardless of the metric used for comparison (Supp. Figure 3).

The increased number of molecular markers, as a result of the GBS, improved the characterization of the US and NZ source-sink dynamics. This can best be seen when comparing the single-species versus combined-species datasets. The analysis with the combined-species dataset groups one NZ isolate within the US samples (Supp. Fig. 1A). In this reduced dataset the sample labelled pluv-3635 is more closely related to the US samples than to NZ samples. When the same analysis is conducted with the larger single-species dataset (1,543 SNP) this isolate is repositioned within the NZ1 genetic cluster. The likely explanation for this change is the small number of molecular markers retained ( $n = 156$ ) when using the distantly related *P. agathidicida* to polarize the ML tree (Supp. Fig. 1A). Furthermore, a similar pattern was noted by Brar et al. (2018) who suggested that the NZ outbreak was a result of two separate introductions. Their analysis used 27 SNP rather than the 1,543 SNPs identified in the single-species dataset described herein. The combination of the number of markers in this analysis and the population

genetics metrics (Figs. 1B, 2A and 2B) strongly support the hypothesis of a single introduction of *P. pluvialis* into NZ.

The pattern of introduction followed by clonal expansion observed between the US and NZ can be seen at a smaller spatial scale within the US. The samples from the US have high levels of genetic association over small geographic distances. This pattern resembles small, local epidemic events following initial migration of a small number of individuals into a new region. A good example of this is the regional outbreak of *P. pluvialis* reported for the Coast Range of Oregon in 2015 (Hansen et al. 2017). The Central genetic cluster appears to have originated from the Coastal genetic cluster followed by clonal expansion within the central region. This hypothesis is supported by the low genetic diversity, high population differentiation, and strong population structure found in the Central genetic cluster (Table 1). The Port-Orford genetic cluster exhibits similar characteristics. This cluster appears to have emerged in a manner similar to what was described above, movement from the Coastal cluster followed by local clonal expansion (Figs. 1 and 2). Both of these examples, occurring at different times (Port-Orford in 2013-2014 and Central in 2017) suggest that local environmental conditions may be driving clonal expansion and outbreak development. Similar scenarios have been proposed for local dispersal of the invasive oomycete *P. ramorum* in the western US (Peterson et al. 2014). Continued sampling both within and beyond the regions sampled in this study, and implementation of a demographic analysis would be required to detect the putative origin of *P. pluvialis*.

The question remains as to how and when *P. pluvialis* was introduced to NZ. The ubiquitous presence of *P. pluvialis* in the Coast Range of Oregon suggests many possible modes of introduction from Douglas-fir needles to infested soil. Increased biosecurity restrictions in NZ

since the late 1990's and early 2000's have significantly curtailed the risk of pathogen movement from overseas, suggesting an introduction which occurred prior to these restrictions. However, once established in NZ, few measures are in place to minimize the spread of pathogens among forested areas (Froud 2018; Hood 2019).

Reports of *P. pluvialis* in the US are limited to the forests of the PNW (Hansen et al. 2015; Gómez-Gallego et al. 2020; Brar et al. 2018). All of the US samples were collected from a single conifer species, Douglas-fir; whereas the isolates collected in NZ came from four different conifer species. In NZ, isolates were collected from Douglas-fir (n=9), radiata pine (n=79), Eastern white pine (n=1), and patula pine (n=1) (Supp. Table 1). However, there was no correlation between genetic or geographic cluster and host specificity. Although the isolates predominantly came from the most abundant host, *P. radiata*, multiple shifts between Douglas-fir and radiata pine were observed within the same genetic cluster and phylogenetic clade (Fig. 3) suggesting a broader host range than originally described (Hansen et al. 2017). The historic range of radiata pine spans from the northern San Francisco Bay Area in CA to northern Baja in Mexico (Millar 1999). This overlaps with the southern range of Douglas-fir. However, *P. pluvialis* has not been recovered from radiata pine in CA as either a pathogen or endophyte. Although RNC has never been reported in radiata pine's native range. Further sampling efforts to determine if *P. pluvialis* can be found in alternate coniferous hosts throughout North America is warranted. Finally, it is also important to note that *P. pluvialis* is less noticeable when infecting Douglas-fir compared to radiata pine where it causes RNC. However, infected Douglas-fir needles are cast prematurely and severe defoliation in the lower canopy has been noted on occasion in both the US and NZ (Hansen et al. 2015). This may suggest a coevolutionary history between the two species whereby *P. pluvialis* plays a role in the suppression of understory



species, a pattern that is characteristic of Douglas-fir stands (Gómez-Gallego et al. 2020). This potential coevolution and mutualistic ecological interactions between both species requires further exploration.

The population structure approach presented here suggests that *P. pluvialis* could have originated in the coastal Douglas-fir forests of the PNW where the population exhibits some characteristics of a center of origin. From this region, *P. pluvialis* was introduced at least once to NZ. Following the introduction, the pathogen was able to infect a wider range of host species than described in the PNW. These rapid host shifts may have been a contributing factor in its establishment and impact on the exotic radiata pine plantations grown throughout temperate regions of NZ. However, more evidence should be presented to support this hypothesis. The recent discovery of *P. pluvialis* has not permitted extensive sampling in areas of North America and NZ. As a result, the potential exists for undiscovered populations of *P. pluvialis* outside of these locations. In order to establish where *P. pluvialis* may have originated, more extensive sampling over a wide geographic area is required. This may reveal both the center of origin of this pathogen and other possible routes of migration.

Future work should focus on increased sample size, in terms of both geographic area and hosts sampled, in North America and NZ. A focus on whole genome resequencing with a higher depth of coverage would further increase the number of alleles that can be detected and provide a clearer view of the genetic diversity of this pathogen. Finally, demographic modeling of migration scenarios and history to test different routes of introduction and migration would be valuable. This work highlights the importance of phylogenomics to identify sources of forest pathogens that can potentially endanger naïve hosts.

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## Figures and Tables.

**Figure 1.** Population structure of *P. pluvialis* in the US and NZ using the genetic variants extracted from the reads mapped to the *P. pluvialis* reference genome A). Bar plot result of the discriminant analysis of principal components. Each color represents the posterior probability membership value per sample to one of the five predicted genetic clusters B). Maximum likelihood phylogenetic reconstruction. Bootstrap support values over 70 are shown in the nodes. Tip color represent genetic cluster assignment per sample. Region abbreviations for NZ: Northland (ND), Gisborne (GB), Bay of Plenty (BP), Wairarapa (WA-NZ), Rangitikei (RI), Taupo (TO), Taranaki (TK), Auckland (AK), Coromandel (CL), Hawkes Bay (HB), Nelson (NN), Wellington (WN), Waikato (WO), and Wanganui (WI).

**Figure 2.** Population dynamics of *P. pluvialis*. A). Minimum spanning network reconstructed using pairwise distances between samples. Node color represents geographic regions of each sample. Edge thickness represents genetic distance between samples. Overlaying polygons represent each predicted genetic cluster. B). Geographic distribution of samples used in this study. Colors represent sample membership to each predicted genetic cluster. Region abbreviations for NZ: Northland (ND), Gisborne (GB), Bay of Plenty (BP), Wairarapa (WA-NZ), Rangitikei (RI), Taupo (TO), Taranaki (TK), Auckland (AK), Coromandel (CL), Hawkes Bay (HB), Nelson (NN), Wellington (WN), Waikato (WO), and Wanganui (WI).

**Figure 3.** Host shifts in NZ samples of *P. pluvialis*. Each color represents the host where each sample was isolated from. A). Subset of the minimum spanning network reconstructed in figure

2 representing samples on NZ. B). Subset of the phylogenetic reconstruction in figure 1 representing samples of NZ.

**Table 1.** Summary statistic for genetic diversity and linkage measurements for *P. pluvialis* genetic clusters.

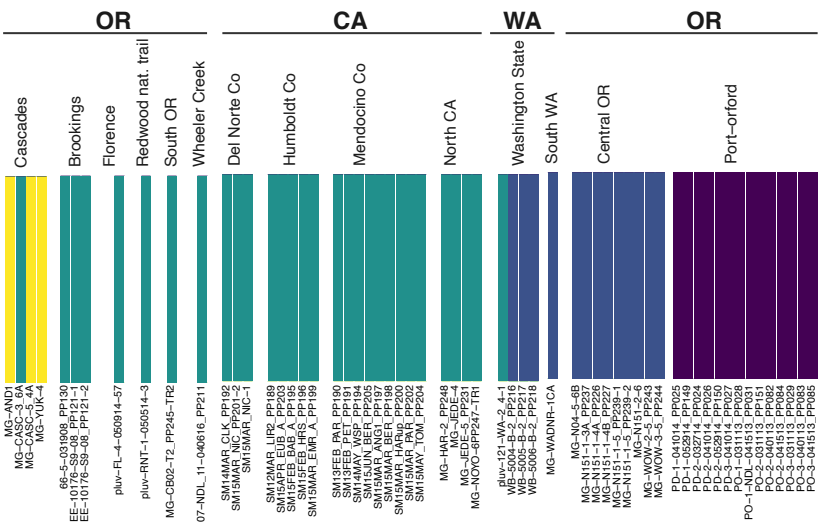
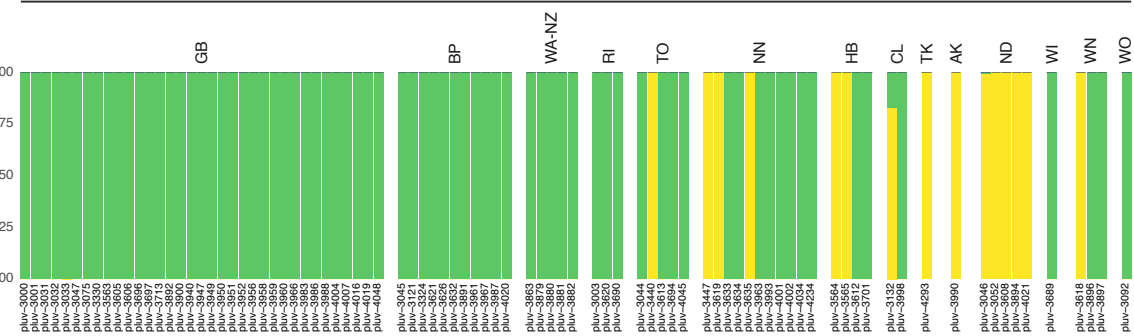
<b>Genetic cluster</b>	<b>Heterozygosity</b>	<b>Median pairwise genetic distance</b>	<b>Index of association (RbarD)</b>
<b>Coastal</b>	0.254	0.093	0.037
<b>Central</b>	0.228	0.054	0.034
<b>Port-Orford</b>	0.201	0.032	0.047
<b>NZ1</b>	0.213	0.044	0.032
<b>NZ2</b>	0.207	0.040	0.135

**Table 2.** Population differentiation via Nei's  $G_{ST}$  for all pairwise comparisons of genetic clusters predicted for *P. pluvialis*

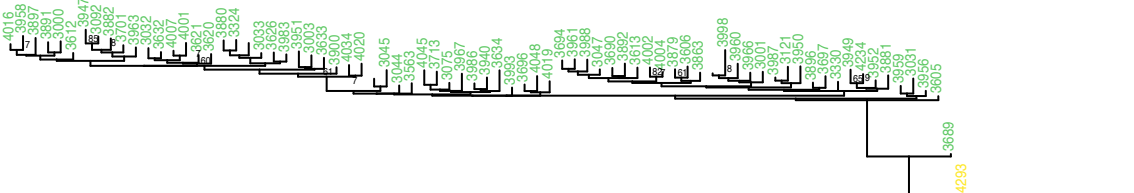
	<b>Coastal</b>	<b>NZ2</b>	<b>Central</b>	<b>Port-Orford</b>
<b>NZ2</b>	0.144	-	-	-
<b>Central</b>	0.155	0.258	-	-
<b>Port-Orford</b>	0.086	0.223	0.235	-
<b>NZ1</b>	0.153	0.184	0.231	0.230

Posterior membership probability

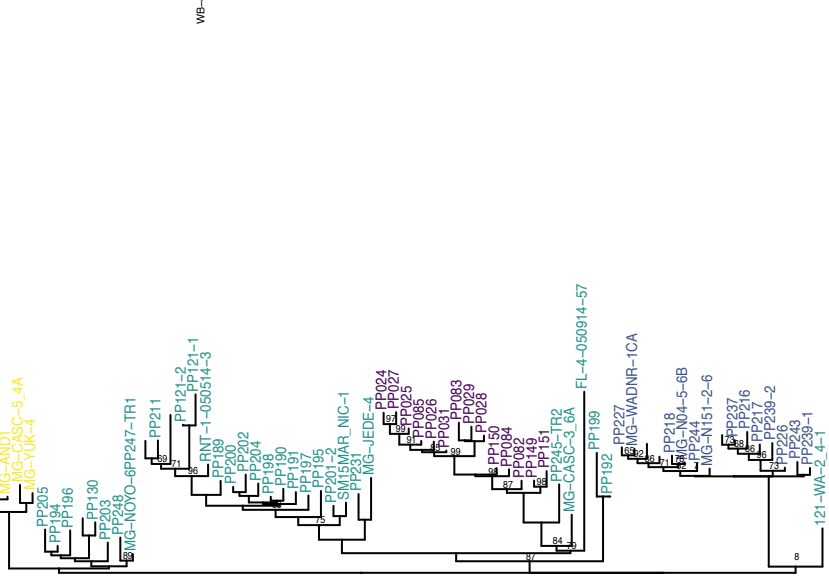
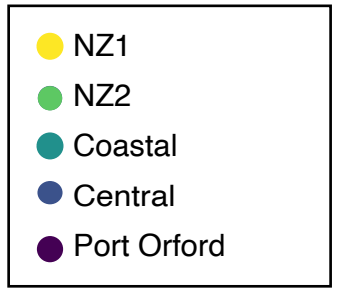
New Zealand



B.



Genetic cluster



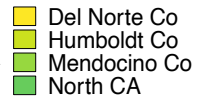
A.

## Site of collection (Circles)

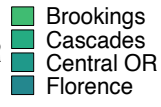
## New Zealand



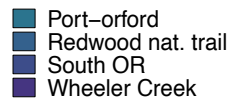
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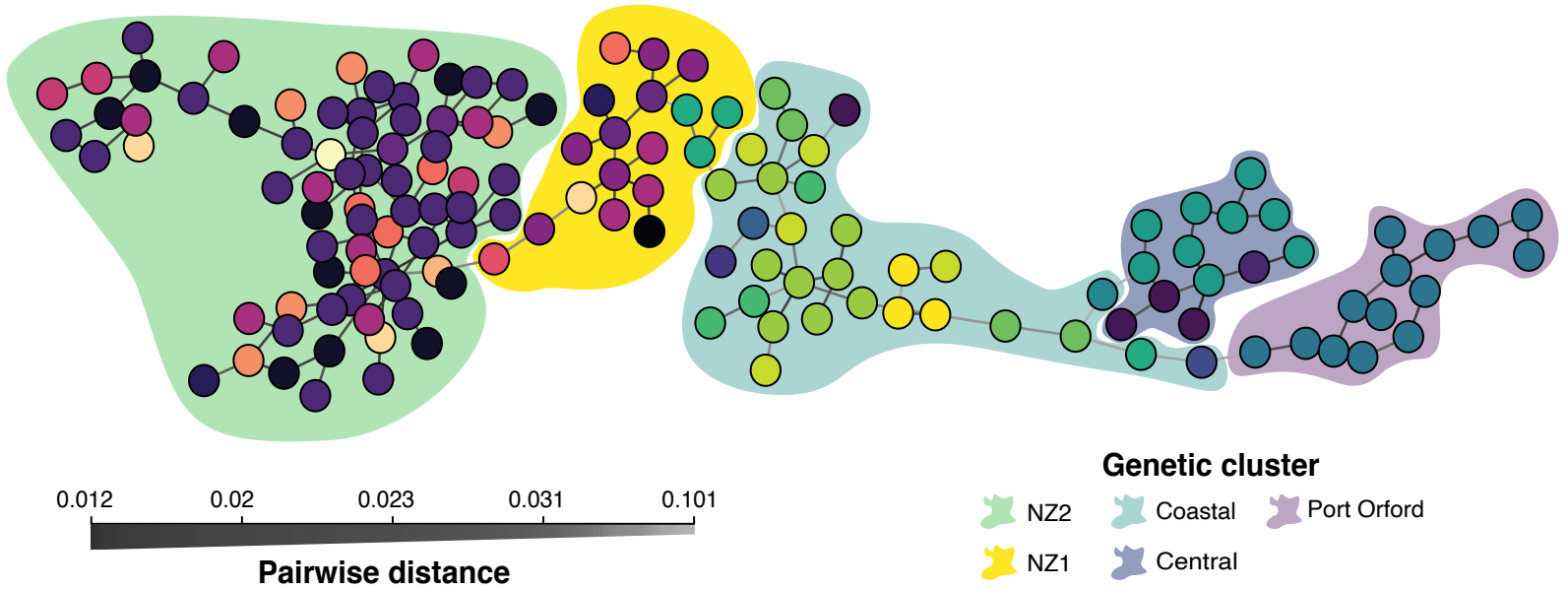
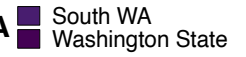
## OR



## USA

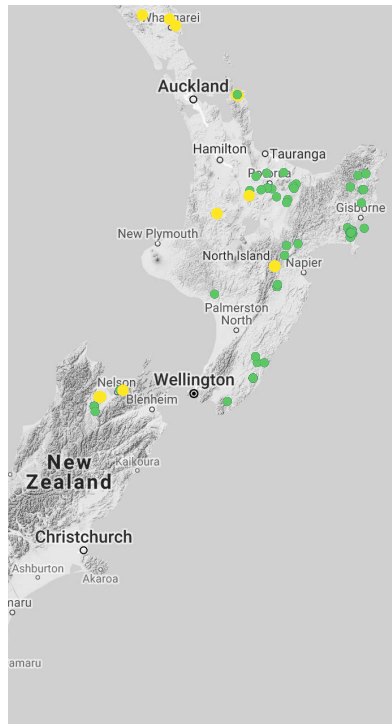


## WA

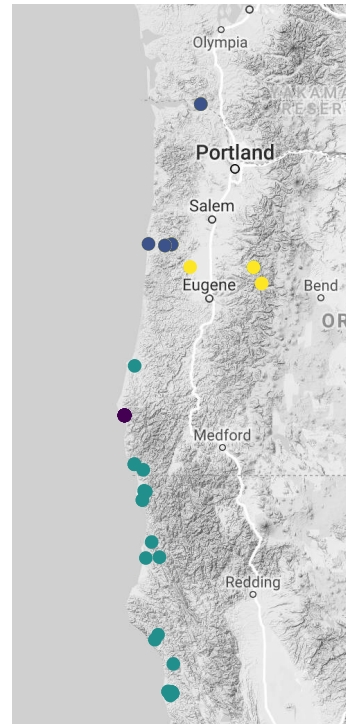


B.

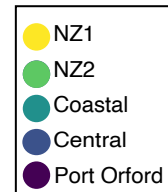
## New Zealand

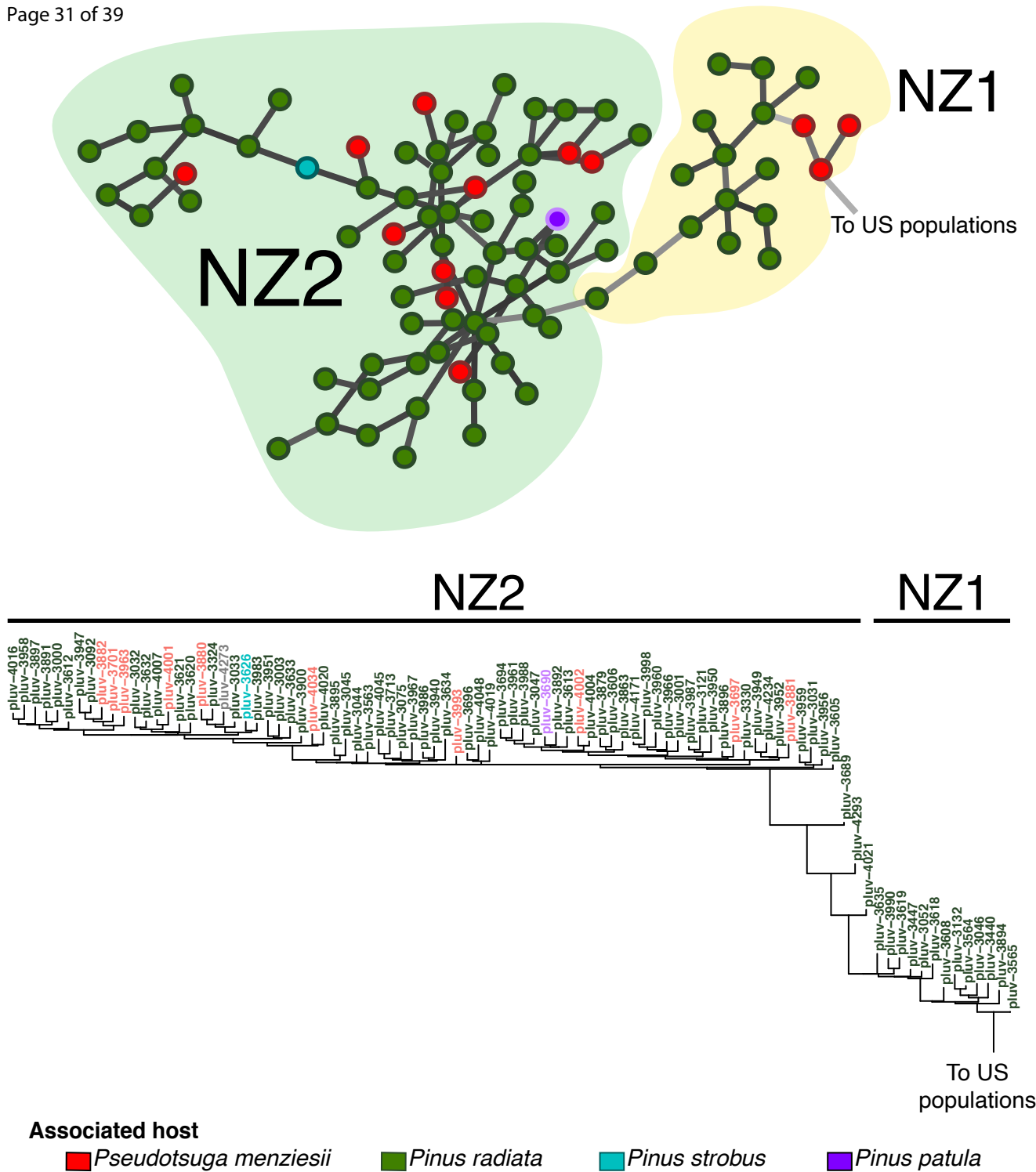


## U.S.A P.N.W



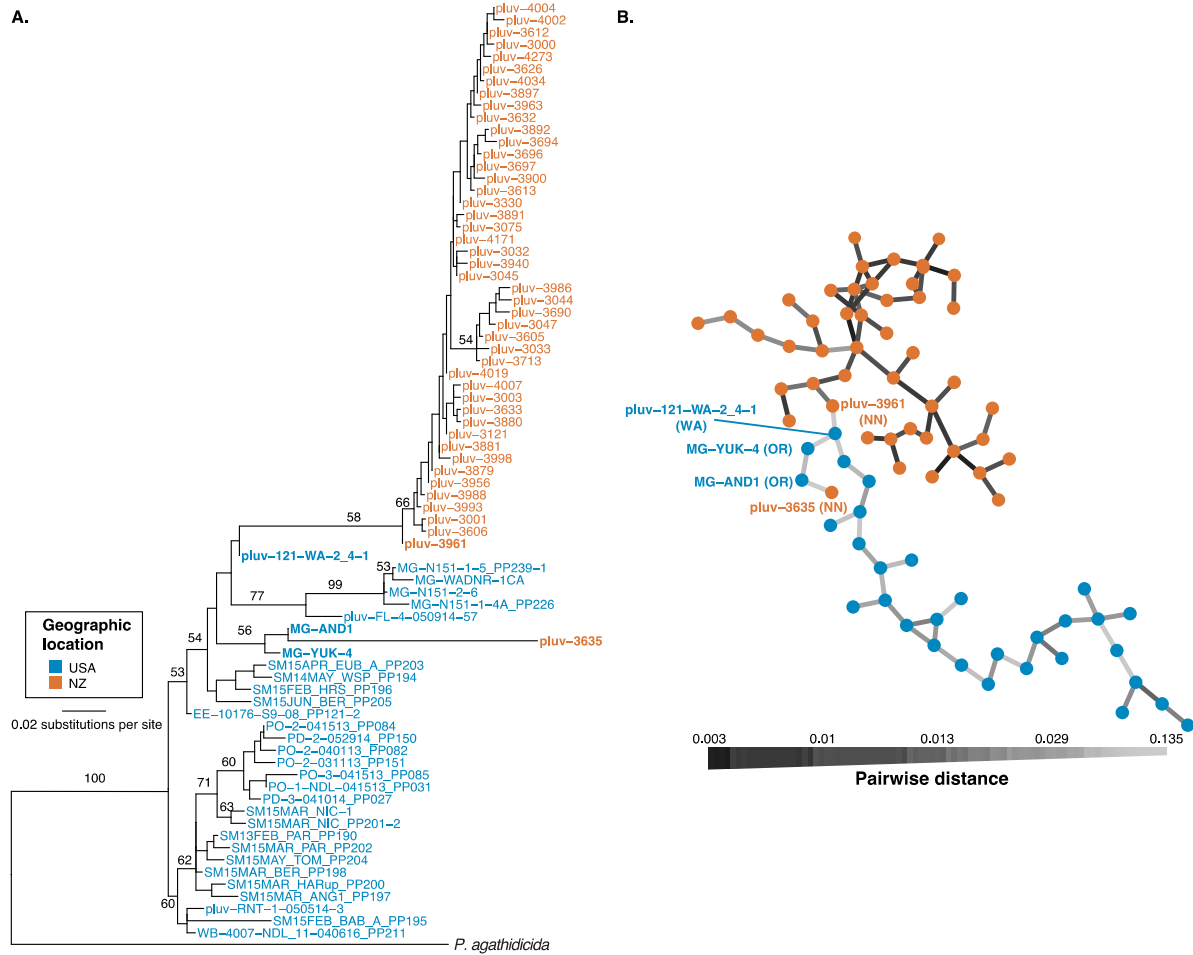
## Genetic cluster



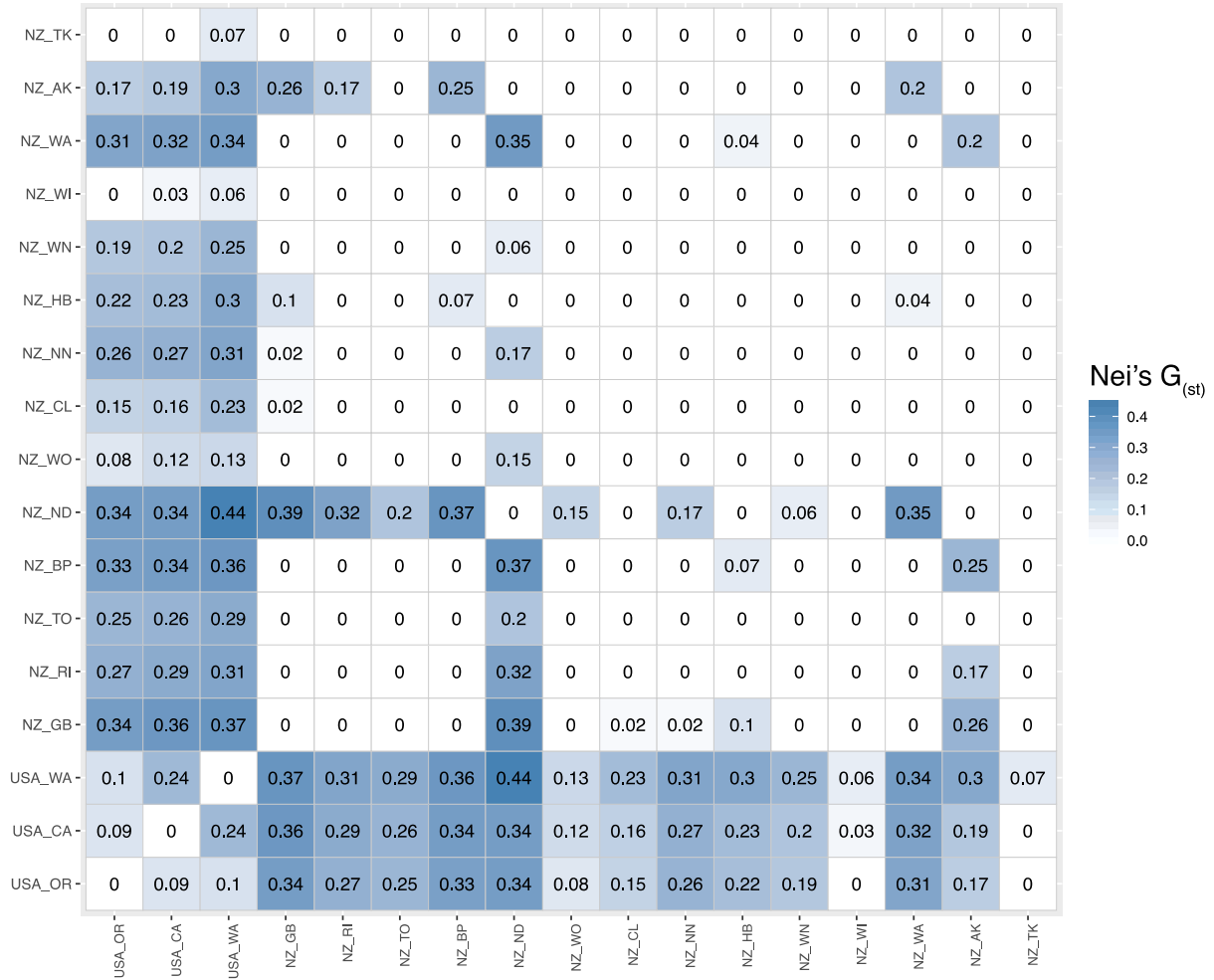




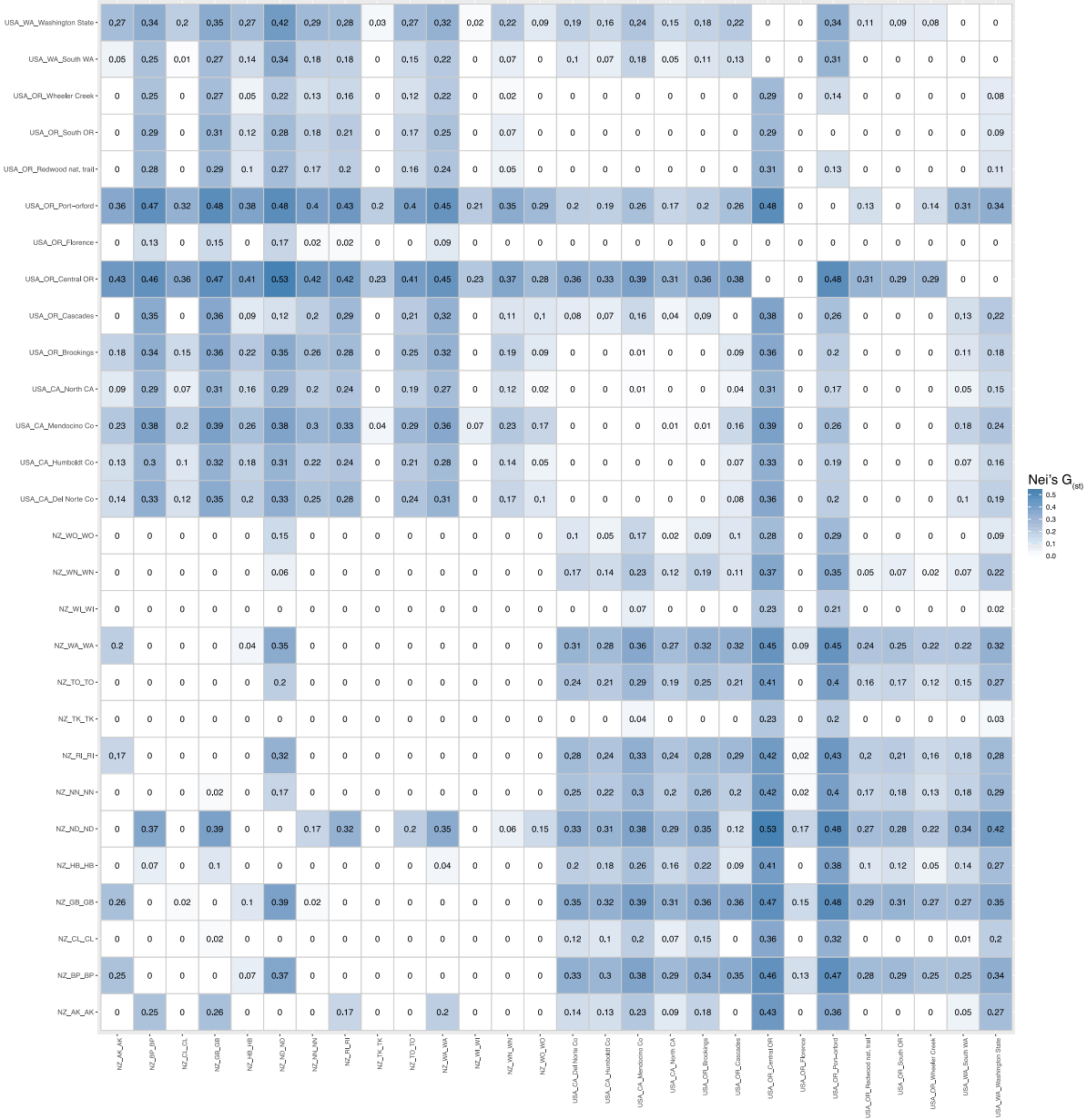
## Supplementary Figures and Tables. (Tabima et al. 2020).



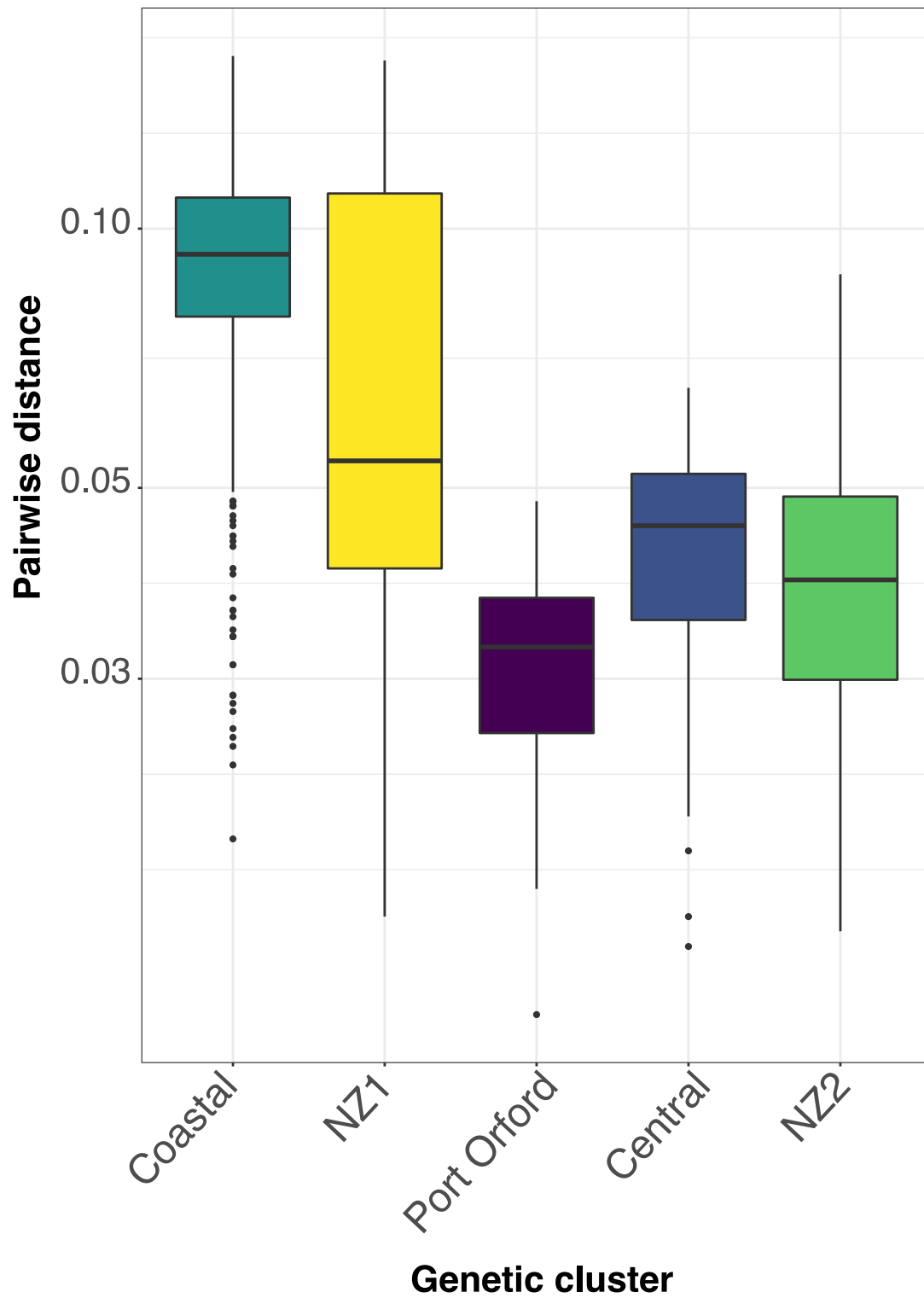
**Supplementary Figure 1.** Population structure of *P. pluvialis* in the US and NZ using the genetic variants extracted from the reads mapped to the *P. agathidicida* reference genome A). Maximum likelihood phylogenetic reconstruction. Bootstrap support values over 70 are shown in the nodes. Tip color represent country of origin. B). Minimum spanning network reconstructed using pairwise distances between samples (*P. agathidicida* was removed from the analysis). Node color represents country of origin of each sample. Edge thickness represents genetic distance between samples.



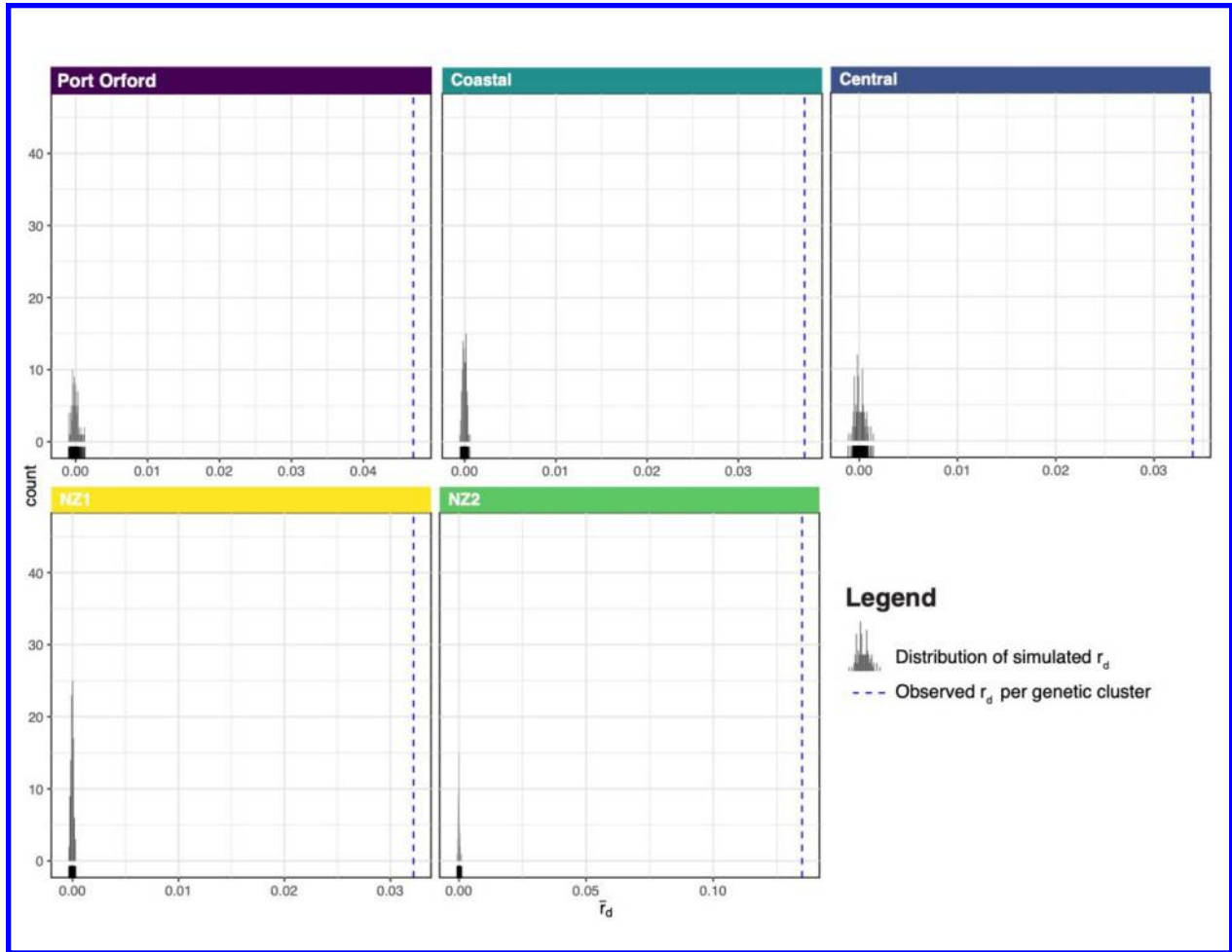
**Supplementary Figure 2.** Genetic differentiation based on pairwise  $G_{st}$  estimation at the hierarchical level of US states/NZ geographic locations. Colors represent  $G_{st}$  values, where darker colors represent higher genetic differentiation between locations.



**Supplementary Figure 3.** Genetic differentiation based on pairwise  $G_{ST}$  estimation at the hierarchical level of location within each state and country. Colors represent  $G_{ST}$  values, where darker colors represent higher genetic differentiation between states.



**Supplementary Figure 4.** Distribution of pairwise genetic distance measured by bitwise.distance function of the poppr package across the predicted genetic clusters of *P. phuvialis*.



**Supplementary Figure 5.** Index of Association (RbarD) estimation the predicted genetic clusters of *P. pluvialis*. Distributions represent 999 permutations of RbarD simulations, and vertical line represents the estimated values of RbarD per genetic cluster.

Supp Table 1. Samples used in this study

Sample <sup>a</sup>	Country	State	Region	Latitude	Longitude	Isolation source
pluv-4171	NZ	NZ	Taupo (TO)	-38.67539	176.44520	<i>Pinus radiata</i>
pluv-4273	NZ	NZ	Taupo (TO)	-38.259982	175.8606	<i>Pinus radiata</i>
pluv-3990	NZ	NZ	Auckland (AK)	-36.804268	175.651518	<i>Pinus radiata</i>
pluv-3045	NZ	NZ	Bay of Plenty (BP)	-38.168896	176.779757	<i>Pinus radiata</i>
pluv-3121	NZ	NZ	Bay of Plenty (BP)	-37.993311	176.53988	<i>Pinus radiata</i>
pluv-3324	NZ	NZ	Bay of Plenty (BP)	-38.001729	176.209895	<i>Pinus radiata</i>
pluv-3621	NZ	NZ	Bay of Plenty (BP)	-38.404855	176.618723	<i>Pinus radiata</i>
pluv-3626	NZ	NZ	Bay of Plenty (BP)	-38.242728	176.306942	<i>Pinus strobus</i>
pluv-3632	NZ	NZ	Bay of Plenty (BP)	-38.205504	176.718861	<i>Pinus radiata</i>
pluv-3891	NZ	NZ	Bay of Plenty (BP)	-38.050239	176.001762	<i>Pinus radiata</i>
pluv-3961	NZ	NZ	Bay of Plenty (BP)	-38.228751	176.221057	<i>Pinus radiata</i>
pluv-3967	NZ	NZ	Bay of Plenty (BP)	-38.228751	176.221057	<i>Pinus radiata</i>
pluv-3987	NZ	NZ	Bay of Plenty (BP)	-38.256896	176.09933	<i>Pinus radiata</i>
pluv-4020	NZ	NZ	Bay of Plenty (BP)	-38.235523	176.739133	<i>Pinus radiata</i>
pluv-3132	NZ	NZ	Coromandel (CL)	-36.796902	175.623673	<i>Pinus radiata</i>
pluv-3998	NZ	NZ	Coromandel (CL)	-36.790311	175.637221	<i>Pinus radiata</i>
pluv-3000	NZ	NZ	Gisborne (GB)	-38.869783	177.819637	<i>Pinus radiata</i>
pluv-3001	NZ	NZ	Gisborne (GB)	-38.869783	177.819637	<i>Pinus radiata</i>
pluv-3031	NZ	NZ	Gisborne (GB)	-38.842413	178.104533	<i>Pinus radiata</i>
pluv-3032	NZ	NZ	Gisborne (GB)	-38.881695	177.855329	<i>Pinus radiata</i>
pluv-3033	NZ	NZ	Gisborne (GB)	-38.880972	177.872417	<i>Pinus radiata</i>
pluv-3047	NZ	NZ	Gisborne (GB)	-38.255539	178.070845	<i>Pinus radiata</i>
pluv-3075	NZ	NZ	Gisborne (GB)	-38.21425	177.83473	<i>Pinus radiata</i>
pluv-3330	NZ	NZ	Gisborne (GB)	-38.458015	178.051855	<i>Pinus radiata</i>
pluv-3563	NZ	NZ	Gisborne (GB)	-38.254348	178.088993	<i>Pinus radiata</i>
pluv-3605	NZ	NZ	Gisborne (GB)	-38.037182	177.982285	<i>Pinus radiata</i>
pluv-3606	NZ	NZ	Gisborne (GB)	-38.833167	177.779304	<i>Pinus radiata</i>
pluv-3696	NZ	NZ	Gisborne (GB)	-38.977135	177.835688	<i>Pinus radiata</i>
pluv-3697	NZ	NZ	Gisborne (GB)	-38.012511	178.138193	<i>Pseudotsuga menziesii</i>
pluv-3713	NZ	NZ	Gisborne (GB)	-38.922781	177.883422	<i>Pinus radiata</i>
pluv-3892	NZ	NZ	Gisborne (GB)	-38.896755	177.875965	<i>Pinus radiata</i>
pluv-3895	NZ	NZ	Gisborne (GB)	-38.912777	177.833036	<i>Pinus radiata</i>
pluv-3900	NZ	NZ	Gisborne (GB)	-38.911281	177.831563	<i>Pinus radiata</i>
pluv-3940	NZ	NZ	Gisborne (GB)	-38.90359	177.835519	<i>Pinus radiata</i>
pluv-3947	NZ	NZ	Gisborne (GB)	-38.912777	177.833036	<i>Pinus radiata</i>
pluv-3949	NZ	NZ	Gisborne (GB)	-38.889205	177.874822	<i>Pinus radiata</i>
pluv-3950	NZ	NZ	Gisborne (GB)	-38.889205	177.874822	<i>Pinus radiata</i>
pluv-3951	NZ	NZ	Gisborne (GB)	-38.904708	177.839344	<i>Pinus radiata</i>
pluv-3952	NZ	NZ	Gisborne (GB)	-38.889205	177.874822	<i>Pinus radiata</i>
pluv-3956	NZ	NZ	Gisborne (GB)	-38.905794	177.83792	<i>Pinus radiata</i>
pluv-3958	NZ	NZ	Gisborne (GB)	-38.912777	177.833036	<i>Pinus radiata</i>
pluv-3959	NZ	NZ	Gisborne (GB)	-38.904708	177.839344	<i>Pinus radiata</i>
pluv-3960	NZ	NZ	Gisborne (GB)	-38.909245	177.835481	<i>Pinus radiata</i>
pluv-3966	NZ	NZ	Gisborne (GB)	-38.909907	177.834281	<i>Pinus radiata</i>
pluv-3983	NZ	NZ	Gisborne (GB)	-38.907074	177.837684	<i>Pinus radiata</i>
pluv-3986	NZ	NZ	Gisborne (GB)	-38.871645	177.857277	<i>Pinus radiata</i>
pluv-3988	NZ	NZ	Gisborne (GB)	-38.889205	177.874822	<i>Pinus radiata</i>
pluv-4004	NZ	NZ	Gisborne (GB)	-38.889205	177.874822	<i>Pinus radiata</i>
pluv-4007	NZ	NZ	Gisborne (GB)	-38.889205	177.874822	<i>Pinus radiata</i>
pluv-4016	NZ	NZ	Gisborne (GB)	-38.909287	177.835417	<i>Pinus radiata</i>
pluv-4019	NZ	NZ	Gisborne (GB)	-38.907063	177.837677	<i>Pinus radiata</i>
pluv-4048	NZ	NZ	Gisborne (GB)	-38.909062	177.846542	<i>Pinus radiata</i>
pluv-3564	NZ	NZ	Hawkes Bay (HB)	-39.413303	176.364163	<i>Pinus radiata</i>
pluv-3565	NZ	NZ	Hawkes Bay (HB)	-39.413302	176.364163	<i>Pinus radiata</i>
pluv-3612	NZ	NZ	Hawkes Bay (HB)	-39.076077	176.817507	<i>Pinus radiata</i>
pluv-3701	NZ	NZ	Hawkes Bay (HB)	-39.252954	176.556203	<i>Pseudotsuga menziesii</i>
pluv-3046	NZ	NZ	Northland (ND)	-35.2812	173.830536	<i>Pinus radiata</i>
pluv-3052	NZ	NZ	Northland (ND)	-35.219206	173.798511	<i>Pinus radiata</i>
pluv-3608	NZ	NZ	Northland (ND)	-35.604258	174.31654	<i>Pinus radiata</i>
pluv-3894	NZ	NZ	Northland (ND)	-35.709001	174.437952	<i>Pinus radiata</i>
pluv-4021	NZ	NZ	Northland (ND)	-35.542232	173.789672	<i>Pinus radiata</i>

pluv-3447	NZ	NZ	Nelson (NN)	-41.348279	172.947959	<i>Pinus radiata</i>
pluv-3619	NZ	NZ	Nelson (NN)	-41.350218	172.978392	<i>Pinus radiata</i>
pluv-3633	NZ	NZ	Nelson (NN)	-41.247197	173.352377	<i>Pinus radiata</i>
pluv-3634	NZ	NZ	Nelson (NN)	-41.253842	173.364333	<i>Pinus radiata</i>
pluv-3635	NZ	NZ	Nelson (NN)	-41.252332	173.414333	<i>Pinus radiata</i>
pluv-3963	NZ	NZ	Nelson (NN)	-41.486022	172.855989	<i>Pseudotsuga menziesii</i>
pluv-3993	NZ	NZ	Nelson (NN)	-41.486022	172.855989	<i>Pseudotsuga menziesii</i>
pluv-4001	NZ	NZ	Nelson (NN)	-41.486022	172.855989	<i>Pseudotsuga menziesii</i>
pluv-4002	NZ	NZ	Nelson (NN)	-41.486022	172.855989	<i>Pseudotsuga menziesii</i>
pluv-4034	NZ	NZ	Nelson (NN)	Unknown	Unknown	<i>Pinus radiata</i>
pluv-4234	NZ	NZ	Nelson (NN)	-41.561909	172.8738	<i>Pinus radiata</i>
pluv-3003	NZ	NZ	Rangitikei (RI)	-39.719075	176.40844	<i>Pinus radiata</i>
pluv-3620	NZ	NZ	Rangitikei (RI)	-39.718903	176.41004	<i>Pinus radiata</i>
pluv-3690	NZ	NZ	Rangitikei (RI)	-39.687564	176.41991	<i>Pinus patula</i>
pluv-4293	NZ	NZ	Taranaki (TK)	-38.61826	175.24105	<i>Pinus radiata</i>
pluv-3044	NZ	NZ	Taupo (TO)	-38.880972	177.872417	<i>Pinus radiata</i>
pluv-3440	NZ	NZ	Taupo (TO)	-38.346272	175.867125	<i>Pinus radiata</i>
pluv-3613	NZ	NZ	Taupo (TO)	-39.102989	176.595443	<i>Pinus radiata</i>
pluv-3694	NZ	NZ	Taupo (TO)	-38.365217	176.400563	<i>Pinus radiata</i>
pluv-4045	NZ	NZ	Taupo (TO)	-38.452007	176.595888	<i>Pinus radiata</i>
pluv-3863	NZ	NZ	Wairarapa (WA)	-40.845812	176.023596	<i>Pinus radiata</i>
pluv-3879	NZ	NZ	Wairarapa (WA)	-40.848207	176.163234	<i>Pinus radiata</i>
pluv-3880	NZ	NZ	Wairarapa (WA)	-41.073545	175.943922	<i>Pseudotsuga menziesii</i>
pluv-3881	NZ	NZ	Wairarapa (WA)	-41.073545	175.943922	<i>Pseudotsuga menziesii</i>
pluv-3882	NZ	NZ	Wairarapa (WA)	-41.073545	175.943922	<i>Pseudotsuga menziesii</i>
pluv-3689	NZ	NZ	Wanganui (WI)	-39.831458	175.193992	<i>Pinus radiata</i>
pluv-3618	NZ	NZ	Wellington (WN)	-41.350218	172.978392	<i>Pinus radiata</i>
pluv-3896	NZ	NZ	Wellington (WN)	-41.414553	175.442424	<i>Pinus radiata</i>
pluv-3897	NZ	NZ	Wellington (WN)	-40.757033	175.990807	<i>Pinus radiata</i>
pluv-3092	NZ	NZ	Waikato (WO)	-38.267747	175.8793	<i>Pinus radiata</i>
PP192	US	CA	Del Norte Co	41.810119	-124.11201	Stream-bait
PP201-2	US	CA	Del Norte Co	41.700806	-124.13839	Stream-bait
SM15MAR_NIC-1	US	CA	Del Norte Co	41.700806	-124.13839	Stream-bait
PP189	US	CA	Humboldt	41.009763	-124.08422	Stream-bait
PP195	US	CA	Humboldt	41.019489	-123.86756	Stream-bait
PP196	US	CA	Humboldt	40.023522	-123.94099	Stream-bait
PP199	US	CA	Humboldt	41.200914	-123.99177	Stream-bait
PP203	US	CA	Humboldt	40.08443	-123.88914	Stream-bait
PP190	US	CA	Mendocino	39.369521	-123.65879	Stream-bait
PP191	US	CA	Mendocino	39.387762	-123.6816	Stream-bait
PP194	US	CA	Mendocino	39.368769	-123.65947	Stream-bait
PP197	US	CA	Mendocino	39.728773	-123.6477	Stream-bait
PP198	US	CA	Mendocino	39.384445	-123.67399	Stream-bait
PP200	US	CA	Mendocino	39.387333	-123.73155	Stream-bait
PP202	US	CA	Mendocino	39.369521	-123.65879	Stream-bait
PP204	US	CA	Mendocino	39.342091	-123.69312	Stream-bait
PP205	US	CA	Mendocino	39.384445	-123.67399	Stream-bait
MG-JEDE-4	US	CA	North CA	41.80034	-124.08604	<i>Pseudotsuga menziesii</i>
MG-NOYO-6PP247-TR1	US	CA	North CA	39.38177	-123.6703	<i>Pseudotsuga menziesii</i>
PP231	US	CA	North CA	41.80045	-124.08614	<i>Pseudotsuga menziesii</i>
PP248	US	CA	North CA	39.39314	-123.72926	<i>Pseudotsuga menziesii</i>
pluv-RNT-1-050514-3	US	CA	Brookings	Unknown	Unknown	Unknown
pluv-66-2-022068-129	US	OR	Brookings	42.12326	-124.26535	Soil-bait
pluv-EE-10253-58-09-126	US	OR	Brookings	42.12326	-124.26535	Soil-bait
pluv-EE-2559-513-09-122	US	OR	Brookings	42.12326	-124.26535	Soil-bait
PP121-1	US	OR	Brookings	42.12326	-124.26535	Soil-bait
PP121-2	US	OR	Brookings	42.12326	-124.26535	Soil-bait
PP130	US	OR	Brookings	42.12346	-124.2654	Raintrap-bait
MG-AND1	US	OR	Cascades	44.216388	-122.24417	<i>Pseudotsuga menziesii</i>
MG-CASC-3_6A	US	OR	Cascades	44.39989	-122.3734	<i>Pseudotsuga menziesii</i>
MG-CASC-5_4A	US	OR	Cascades	44.40004	-122.37352	<i>Pseudotsuga menziesii</i>
MG-YUK-4	US	OR	Cascades	44.403889	-123.38	<i>Pseudotsuga menziesii</i>
MG-N04-5-6B	US	OR	Central OR	44.66537	-124.04219	<i>Pseudotsuga menziesii</i>
MG-N04-506A	US	OR	Central OR	44.66537	124.04219	<i>Pseudotsuga menziesii</i>
MG-N151-2-6	US	OR	Central OR	44.65978	-123.67484	<i>Pseudotsuga menziesii</i>
PP226	US	OR	Central OR	44.65983	-123.67525	<i>Pseudotsuga menziesii</i>

PP227	US	OR	Central OR	44.65983	-123.67525	<i>Pseudotsuga menziesii</i>
PP237	US	OR	Central OR	44.65983	-123.67525	<i>Pseudotsuga menziesii</i>
PP239-1	US	OR	Central OR	44.65983	-123.67525	<i>Pseudotsuga menziesii</i>
PP239-2	US	OR	Central OR	44.65983	-123.67525	<i>Pseudotsuga menziesii</i>
PP243	US	OR	Central OR	44.646944	-123.78038	<i>Pseudotsuga menziesii</i>
PP244	US	OR	Central OR	44.646944	-123.78038	<i>Pseudotsuga menziesii</i>
pluv-FL-4-050914-57	US	OR	Florence	Unknown	Unknown	Unknown
pluv-LL-5-051213-58	US	OR	Little Lobster	Unknown	Unknown	<i>Pseudotsuga menziesii</i>
pluv-LL-5-NDL-042713-2	US	OR	Little Lobster	Unknown	Unknown	<i>Pseudotsuga menziesii</i>
PP024	US	OR	Port-Orford	42.69623	-124.42282	Raintrap-bait
PP025	US	OR	Port-Orford	42.69623	-124.42282	Raintrap-bait
PP026	US	OR	Port-Orford	42.69623	-124.42282	Raintrap-bait
PP027	US	OR	Port-Orford	42.69623	-124.42282	Raintrap-bait
PP028	US	OR	Port-Orford	42.69614	-124.42296	Raintrap-bait
PP029	US	OR	Port-Orford	42.69614	-124.42296	Raintrap-bait
PP031	US	OR	Port-Orford	42.69614	-124.42296	<i>Pseudotsuga menziesii</i>
PP082	US	OR	Port-Orford	42.69614	-124.42296	Raintrap-bait
PP083	US	OR	Port-Orford	42.69614	-124.42296	Raintrap-bait
PP084	US	OR	Port-Orford	42.69614	-124.42296	Raintrap-bait
PP085	US	OR	Port-Orford	42.69614	-124.42296	Raintrap-bait
PP149	US	OR	Port-Orford	42.69623	-124.42282	Raintrap-bait
PP150	US	OR	Port-Orford	42.69623	-124.42282	Raintrap-bait
PP151	US	OR	Port-Orford	42.69614	-124.42296	Raintrap-bait
PP245-TR2	US	OR	Brookings	43.27168	-124.26174	<i>Pseudotsuga menziesii</i>
PP211	US	OR	Brookings	42.05785	-124.12458	Raintrap-bait
pluv-121-WA-2_4-1	US	WA	Brookings	Unknown	Unknown	Unknown
MG-WADNR-1CA	US	WA	Washington	46.22242	-123.21182	<i>Pseudotsuga menziesii</i>
PP216	US	WA	Washington	46.22248	-123.21191	Unknown
PP217	US	WA	Washington	46.22242	-123.21182	Bait screen
PP218	US	WA	Washington	46.22248	-123.21197	Bait screen

<sup>a</sup> All New Zealand *P. pluvialis* isolate numbers refer to those from the New Zealand Forest Health culture collection (NZFS) held at Scion (NZFRI, Ltd.), Rotorua, New Zealand.