



Genome sequences of six *Phytophthora* species threatening forest ecosystems



Nicolas Feu^{a,*}, Greg Taylor^b, Angela L. Dale^{a,c}, Braham Dhillon^a, Guillaume J. Bilodeau^d, Inanç Birol^{b,e}, Steven J.M. Jones^{b,e,f}, Richard C. Hamelin^{a,g,**}

^a Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, British Columbia, Canada

^b Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, British Columbia, Canada

^c FPInnovations, Vancouver, British Columbia, Canada

^d Canadian Food Inspection Agency, Ottawa, Ontario, Canada

^e Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada

^f Department of Molecular Biology and Biochemistry, Simon Fraser University, Vancouver, BC, Canada

^g Institut de Biologie Intégrative des Systèmes, Université Laval, Québec, Canada

ARTICLE INFO

Article history:

Received 14 September 2016

Received in revised form 26 September 2016

Accepted 29 September 2016

Available online 03 October 2016

Keywords:

Invasive species

Oomycetes

Forest health

ABSTRACT

The *Phytophthora* genus comprises of some of the most destructive plant pathogens and attack a wide range of hosts including economically valuable tree species, both angiosperm and gymnosperm. Many known species of *Phytophthora* are invasive and have been introduced through nursery and agricultural trade. As part of a larger project aimed at utilizing genomic data for forest disease diagnostics, pathogen detection and monitoring (The TAIGA project: Tree Aggressors Identification using Genomic Approaches; <http://taigaforesthealth.com/>), we sequenced the genomes of six important *Phytophthora* species that are important invasive pathogens of trees and a serious threat to the international trade of forest products. This genomic data was used to develop highly sensitive and specific detection assays and for genome comparisons and to make evolutionary inferences and will be useful to the broader plant and tree health community. These WGS data have been deposited in the International Nucleotide Sequence Database Collaboration (DDBJ/ENA/GenBank) under the accession numbers AUPNO1000000, AUJH01000000, AUWJ02000000, AUUF02000000, AWWV02000000 and AWWV02000000.

Crown Copyright © 2016 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Specifications

Organism/cell line/tissue	Six <i>Phytophthora</i> species, see Table 1.
Sex	Not applicable
Sequencer type or array	Illumina Hi-Seq
Data format	Analyzed; i.e. raw data filtered and assembled
Experimental factors	Genomic sequence of pure microbial cultures
Experimental features	Genomic sequence of pure microbial cultures
Consent	Not applicable. Data are available without restriction
Sample source location	Various; see Table 1

<http://www.ncbi.nlm.nih.gov/bioproject/PRJNA190826>

<http://www.ncbi.nlm.nih.gov/bioproject/PRJNA190827>

<http://www.ncbi.nlm.nih.gov/bioproject/PRJNA190828>

1. Direct link to deposited data

<http://www.ncbi.nlm.nih.gov/bioproject/PRJNA190823>

<http://www.ncbi.nlm.nih.gov/bioproject/PRJNA190824>

<http://www.ncbi.nlm.nih.gov/bioproject/PRJNA190825>

* Corresponding author.

** Corresponding author at: Institut de Biologie Intégrative des Systèmes, Université Laval, Québec, Canada.

E-mail addresses: feunico@mail.ubc.ca (N. Feu), rhamelin@gmail.com (R.C. Hamelin).

2. Experimental design, materials and methods

Woodlands and trees are under serious threat from an increasing number of *Phytophthora* species [1]. Several species of these fungus-like microorganisms may attack over 100 different host species and possess the ability to infect woody tissues, making them potentially destructive in plantations and native forest ecosystems worldwide [2,3]. Early detection, monitoring and surveillance are important aspects in preventing such outbreaks but are hindered by a lack of genomic resources. Genome sequencing and comparisons should help to develop biosurveillance tools and to predict pathogenic outcome of the interaction of these microorganisms and their host.

Here, we present the draft genome sequence of six *Phytophthora* species threatening trees, which were selected on the basis of their potential to cause significant economic losses and large-scale damage to forest ecosystems. *Phytophthora lateralis* is an invasive pathogen

Table 1
Phytophthora species and isolates sequenced.

Species	Isolate	Host	Location
<i>Phytophthora alni</i> sp. <i>alni</i>	CBS_117376	<i>Alnus</i> sp., roots	Hungary
<i>P. cambivora</i>	CBS_114087	<i>Abies procera</i>	Oregon, USA
<i>P. cryptogea</i>	CBS_418.71	<i>Gerbera</i> sp.	The Netherlands
<i>P. kernoviae</i>	CBS_122049	<i>Rhododendron</i> sp.	United Kingdom
<i>P. lateralis</i>	CBS_168.42	<i>Chamaecyparis lawsoniana</i>	Oregon, USA
<i>P. pinifolia</i>	CBS_122922	<i>Pinus radiata</i> , needles	Arauco, Chile

that infects Port Orford Cedar (*Chamaecyparis lawsoniana*) and has spread throughout the natural range of the tree [2,4]. *P. lateralis* is a sister species of *P. ramorum*, a species that has been responsible for the deaths of millions of trees in North America and Europe as a result of Sudden Oak Death and Sudden Larch Death [1,5,6]. The *P. lateralis* epidemic has affected both the wood export market as Port Orford Cedar is a valued species in foreign markets as well as the nursery trade as it is also a valued horticultural species [2]. *Phytophthora alni* sp. *alni*, the cause of Alder decline has been a serious threat to riparian ecosystems in Europe over the last 20 years [7]. The emergence of this disease is linked to an interspecific hybridization event, as *P. alni* subsp. *alni*, *P. alni* subsp. *uniformis* and *P. alni* subsp. *multiformis*, initially identified as genetic variants of *P. alni* sp. *alni* [8], were shown to be the parental species of the more aggressive hybrid *P. alni* sp. *alni* [9]. *Phytophthora kernoviae* first found in 2003 in the UK, primarily causes bleeding stem lesions on *Fagus sylvatica* and foliar and stem necrosis on *Rhododendron ponticum*, but has also been found on other hosts [10]. *Phytophthora cambivora* originally associated with *Castanea* species is a widespread root and canker pathogen of many woody hosts, but is most problematic on hardwoods in Europe, in particular on European beech and European chestnut [11–13]. *Phytophthora pinifolia* was first described in Chile and caused widespread disease on the needles and shoots of *Pinus radiata* [14]. *Phytophthora cryptogea* is a widespread pathogen of numerous ornamental hosts infecting roots, stems and leaves, and is an important pathogen in the nursery industry often isolated during surveys of infected plant material [15].

Genome assemblies were obtained by generating paired-end Illumina reads using the HiSeq 2000 platform at Canada's Michael Smith Genome Sciences Centre or GSC (Vancouver, Canada). For each species, DNA was extracted from pure culture using the DNA extraction procedure of Moller et al. [16]. Two genomic DNA libraries with fragment size of approximately 250 bp and 800 bp were constructed according to British Columbia Cancer Agency Genome Sciences Centre's tube-based paired-end library protocols. One μ g of high molecular weight genomic DNA was sonicated (Covaris E210) in 60 μ L volume to 200–300 bp. The DNA fragments were end-repaired, phosphorylated and bead purified in preparation for A-tailing. Illumina sequencing adapters were ligated overnight at 16 °C. Adapter ligated products were bead purified and enriched with 10 cycles of PCR using primers containing a hexamer index that enables library pooling. Paired-end 100 base reads were sequenced per pool in a single lane of an Illumina HiSeq2000 instrument.

Illumina chastity failed reads were removed, and the remaining reads were filtered by looking for exact read matches against *Penicillium chrysogenum* (GCA_000710275.1, GCA_000523475.1, GCA_000816005.1

and GCA_000801355.1) and *P. marneffeii* (GCA_000001985.1, GCA_000227055.2 and GCA_000750115.1) genome sequences to eliminate commensal fungi contaminants. Each library was assembled into contigs using ABySS and a range of k-values from 32 to 96. ABySS was also used to scaffold the contigs, taking care to minimize the duplication of highly repeated sequences that can proliferate on the ends of scaffolds. The best assembly was then selected based on genome size and contiguity (best N50). Completeness of the genome assemblies was assessed using BUSCO (Benchmarking Universal Single-Copy Orthologs).

Raw sequence data and the sets of gene and protein models are available using the GenBank and Sequence Read Archive (SRA) accession numbers listed in Table 2. Genome assembly statistics obtained for *P. kernoviae* and *P. lateralis* were in the range of those obtained for Illumina *de novo* assemblies of two same species (*P. kernoviae* [$n = 5$], assembly size: 40.3 Mbp \pm 3.0; N₅₀: 61,035 \pm 2195; length of longest scaffold: 796,176 bp \pm 284,587 bp. *P. lateralis* [$n = 4$], assembly size: 50.6 Mbp \pm 4.8; N₅₀: 22,373 bp \pm 6314; length of longest scaffold: 300,607 bp \pm 247,251) (Table 2) [17]. Sequencing completeness was estimated using BUSCO based on a set of 429 single-copy ortholog genes common to Eukaryotes [18]. For the *P. alni* sp. *alni* assembly, quality control values were largely under those obtained for the other species with an assembly size twice the expected value of 114 Mbp [19] and a N50 under 3Kb (Table 2). Only 299 (69.0%) out of the 429 eukaryotic BUSCOs were found in this genome; a majority of these were fragmented and duplicated BUSCOs (121 fragmented and 61 duplicated; 60.9%), illustrating the difficulty to obtain an accurate *de novo* assembly for this homoploid hybrid species [19] (Table 2; Fig. 1A). With 344 (80.2%) to 355 (82.8%) BUSCO genes found all the other genomes (Table 2; Fig. 1), assemblies looked complete relative to the published *Phytophthora* and Oomycete *de novo* assemblies (Table 2; Fig. 1) [17,20–22].

These genomic data were used to develop highly sensitive and specific detection assays that will have applications in biosurveillance of potentially invasive threats [23]. These genomes complete an initial collection of forest-related *Phytophthora* species [17,21,24] and will be used in comparative studies in conjunction with transcriptomic data, to identify factors related to epidemic traits such as the capacity to attack woody tissues and multiple host species.

Acknowledgments

We would like to acknowledge members of the TAIGA team at UBC Vancouver Hesther Yueh, Stéphanie Beauseigle, Padmini Herath and from CFIA team Debbie Shearlaw, Miranda Newton for their help with

Table 2
Assembly statistics and gene content for the genome sequences reported in this study.

Species	Isolate	Genome assembly accession #	Total size (Mbp)	Genome coverage	# of scaffolds	N50 (bp)	Length of longest scaffold (bp)	BUSCO coverage
<i>P. alni</i> sp. <i>alni</i>	CBS_117376	GCA_000439335.1	236.0	113.0×	118,474	2791	47,541	299 (69.0%)
<i>P. cambivora</i>	CBS_114087	GCA_000443045.1	230.6	163.0×	72,332	4693	76,007	354 (82.5%)
<i>P. cryptogea</i>	CBS_418.71	GCA_000468175.2	63.8	345.0×	19,533	12,607	234,588	347 (80.9%)
<i>P. kernoviae</i>	CBS_122049	GCA_000448265.2	39.4	474.0×	5026	64,601	435,012	344 (80.2%)
<i>P. lateralis</i>	CBS_168.42	GCA_000500205.2	52.4	470.0×	9039	23,425	178,165	353 (82.3%)
<i>P. pinifolia</i>	CBS_122922	GCA_000500225.2	94.6	470.0×	36,928	8087	93,857	355 (82.8%)

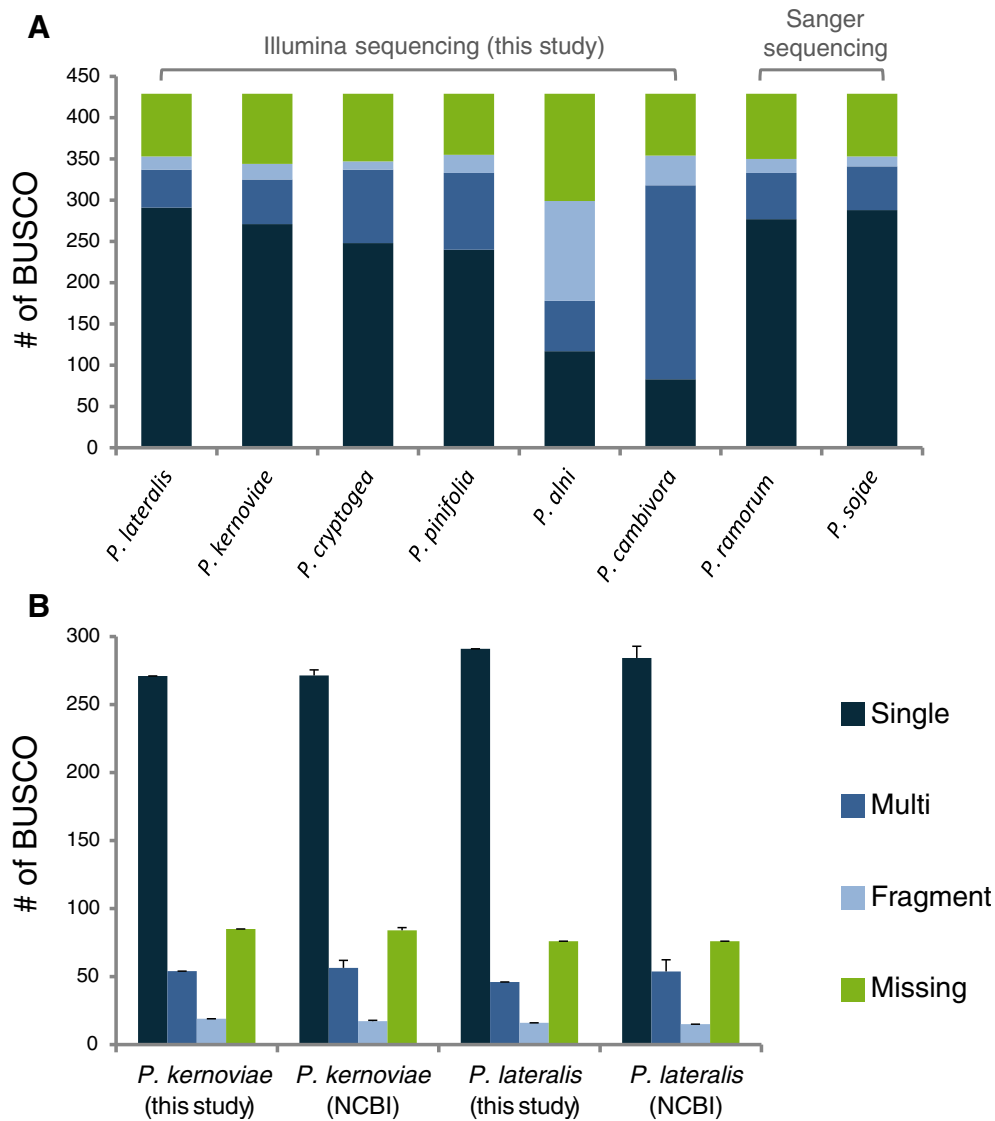


Fig. 1. Genome completeness (BUSCO results) for six *Phytophthora* genomes. Searches for single-copy Eukaryote orthologs ($n = 429$) were conducted following Augustus gene predictions. A) Comparison of the six Illumina genomes with published genome assemblies of *P. ramorum* (GCA_000149735.1) and *P. sojae* (GCA_000149755.2). B) Comparison of the *P. kernoviae* and *P. lateralis* assemblies obtained in this study with five *P. kernoviae* (GCA_000333075.2, GCA_000333095.2, GCA_000333115.2, GCA_000785725.2 and GCA_000785735.2) and four *P. lateralis* (GCA_000318465.2, GCA_000333055.2, GCA_000338795.2 and GCA_000338815.2) Illumina assemblies downloaded from NCBI.

this project. This work was funded by Genome Canada, Genome British Columbia, the Canadian Forest Service (Genomics Research and Development Initiative, GRDI), FP Innovations and the Canadian Food Inspection Agency, through a Large Scale Applied Research Program (LSARP 2112; Genome Canada grant).

References

- [1] N.J. Grünwald, M. Garbelotto, E.M. Goss, K. Heungens, S. Prospero, Emergence of the sudden oak death pathogen *Phytophthora ramorum*. *Trends Microbiol.* 20 (2012) 131–138.
- [2] E.M. Hansen, Alien forest pathogens: *Phytophthora* species are changing world forests. *Boreal Environ. Res.* 13 (2008) 33–41.
- [3] E.M. Hansen, *Phytophthora* species emerging as pathogens of forest trees. *Curr. For. Reports.* 1 (2015) 16–24.
- [4] E.M. Hansen, D.J. Goheen, E.S. Jules, B. Ullian, Managing Port-Orford-cedar and the introduced pathogen *Phytophthora lateralis*. *Plant Dis.* 84 (2000) 4–14.
- [5] C. Brasier, J. Webber, Sudden larch death. *Science* 329 (2010) 824–825.
- [6] C. Robin, D. Piou, N. Feau, G. Douzon, N. Schenck, E.M. Hansen, Root and aerial infections of *Chamaecyparis lawsoniana* by *Phytophthora lateralis*: a new threat for European countries. *For. Pathol.* 41 (2011) 417–424.
- [7] J. Aguayo, G.C. Adams, F. Halkett, M. Catal, C. Husson, Z.Á. Nagy, et al., Strong genetic differentiation between North American and European populations of *Phytophthora alni* subsp. *uniformis*. *Phytopathology* 103 (2012) 190–199.
- [8] C.M. Brasier, S.a. Kirk, J. Delcan, D.E.L. Cooke, T. Jung, W.A. Man in't Veld, *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on Alnus trees. *Mycol. Res.* 108 (2004) 1172–1184.
- [9] R. Ioos, A. Andrieux, B. Marçais, P. Frey, Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. *Fungal Genet. Biol.* 43 (2006) 511–529.
- [10] C.M. Brasier, P.a. Beales, S.a. Kirk, S. Denman, J. Rose, *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. *Mycol. Res.* 109 (2005) 853–859.
- [11] B.S. Grandall, The distribution and significance of the chestnut root rot phytophthoras, *P. cinnamomi* and *P. cambivora*. *Plant Dis. Rep.* 34 (1950) 194–196.
- [12] C.M. Brasier, S.A. Kirk, Comparative aggressiveness of standard and variant hybrid alder phytophthoras, *Phytophthora cambivora* and other *Phytophthora* species on bark of *Alnus*, *Quercus* and other woody hosts. *Plant Pathol.* 50 (2001) 218–229.
- [13] T. Jung, G.W. Hudler, S.L. Jensen-Tracy, H.M. Griffiths, F. Fleischmann, W. Osswald, Involvement of *Phytophthora* species in the decline of European beech in Europe and the USA. *Mycologist* 19 (2005) 159.
- [14] A. Durán, M. Gryzenhout, B. Slippers, R. Ahumada, A. Rotella, F. Flores, et al., *Phytophthora pinifolia* sp. nov. associated with a serious needle disease of *Pinus radiata* in Chile. *Plant Pathol.* 57 (2008) 715–727.
- [15] A.J. Leonberger, C. Speers, G. Ruhl, T. Creswell, J.L. Beckerman, A survey of *Phytophthora* spp. in midwest nurseries, greenhouses, and landscapes. *Plant Dis.* 97 (2013) 635–640.
- [16] E.M. Möller, G. Bahnweg, H. Sandermann, H.H. Geiger, A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Res.* 20 (1992) 6115–6116.

- [17] D.J. Studholme, R.L. McDougal, C. Sambles, E. Hansen, G. Hardy, M. Grant, et al., Genome sequences of six *Phytophthora* species associated with forests in New Zealand. *Genomics Data*. 7 (2015) 54–56.
- [18] F.A. Simão, R.M. Waterhouse, P. Ioannidis, E.V. Kriventseva, E.M. Zdobnov, BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31 (2015) 3210–3212.
- [19] C. Husson, J. Aguayo, C. Revellin, P. Frey, R. Ios, B. Marçais, Evidence for homoploid speciation in *Phytophthora alni* supports taxonomic reclassification in this species complex. *Fungal Genet. Biol.* 77 (2015) 12–21.
- [20] H. Berger, A. Yacoub, J. Gerbore, D. Grizard, P. Rey, A. Sessitsch, et al., Draft genome sequence of biocontrol agent *Pythium oligandrum* strain Po37, an Oomycota. *Genome Announc.* 4 (2016) e00215–e00216.
- [21] L. Quinn, P.a. O'Neill, J. Harrison, K.H. Paskiewicz, A.R. McCracken, L.R. Cooke, et al., Genome-wide sequencing of *Phytophthora lateralis* reveals genetic variation among isolates from Lawson cypress (*Chamaecyparis lawsoniana*) in Northern Ireland. *FEMS Microbiol. Lett.* 2008 (2013) 179–185.
- [22] B.M. Tyler, S. Tripathy, X. Zhang, P. Dehal, R.H.Y. Jiang, A. Aerts, et al., *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Science* 313 (2006) 1261–1266.
- [23] J. Lamarche, A. Potvin, G. Pelletier, D. Stewart, N. Feau, D.I.O. Alayon, et al., Molecular detection of 10 of the most unwanted alien forest pathogens in Canada using real-time PCR. *PLoS One* 10 (2015), e0134265.
- [24] L. de la Mata Saez, A.R. McCracken, L.R. Cooke, P. O'Neill, M. Grant, D.J. Studholme, Draft genome sequences of seven isolates of *Phytophthora ramorum* EU2 from Northern Ireland. *Genomics Data*. 6 (2015) 191–192.