Polymorphic SSR Markers for Plasmopara obducens (Peronosporaceae), the Newly Emergent Downy Mildew Pathogen of Impatiens (Balsaminaceae)

Authors: Salgado-Salazar, Catalina, Rivera, Yazmín, Veltri, Daniel, and Crouch, Jo Anne

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PRIMER NOTE

POLYMORPHIC SSR MARKERS FOR *PLASMOPARA OBDUCENS* (PERONOSPORACEAE), THE NEWLY EMERGENT DOWNY MILDEW PATHOGEN OF *IMPATIENS* (BALSAMINACEAE)[1]

CATALINA SALGADO-SALAZAR[2,3], YAZMÍN RIVERA[2,3], DANIEL VELTRI[2,4], AND JO ANNE CROUCH[2,5]

[2]USDA-ARS, Systematic Mycology and Microbiology Laboratory, Beltsville, Maryland 20705 USA; [3]Rutgers University, Department of Plant Biology and Pathology, New Brunswick, New Jersey 08901 USA; and [4]Oak Ridge Institute for Science and Education, ARS Research Participation Program, Oak Ridge, Tennessee 37831 USA

- *Premise of the study:* Simple sequence repeat (SSR) markers were developed for *Plasmopara obducens*, the causal agent of the newly emergent downy mildew disease of *Impatiens walleriana*.
- *Methods and Results:* A 202-Mb draft genome assembly was generated from *P. obducens* using Illumina technology and mined to identify 13,483 SSR motifs. Primers were synthesized for 62 marker candidates, of which 37 generated reliable PCR products. Testing of the 37 markers using 96 *P. obducens* samples showed 96% of the markers were polymorphic, with 2–6 alleles observed. Observed and expected heterozygosity ranged from 0.000–0.892 and 0.023–0.746, respectively. Just 17 markers were sufficient to identify all multilocus genotypes.
- *Conclusions:* These are the first SSR markers available for this pathogen, and one of the first molecular resources. These markers will be useful in assessing variation in pathogen populations and determining the factors contributing to the emergence of destructive impatiens downy mildew disease.

Key words: de novo assembly; high-throughput marker identification; ornamental impatiens; *Plasmopara obducens*; population genetics; simple sequence repeats.

Downy mildew is a newly emergent disease of *Impatiens walleriana* Hook. f. (impatiens; Balsaminaceae), a high-value, flowering annual plant contributing $105 million annually to the horticulture industry in the United States alone. This destructive disease threatens the cultivation of impatiens worldwide (Brasier, 2008). In 2011, widespread outbreaks of impatiens downy mildew (IDM) disease were observed for the first time in the United States, affecting plants grown in greenhouses, nurseries, and landscapes (e.g., Wegulo et al., 2004; Baysal-Gurel et al., 2012; Palmateer et al., 2013; Crouch et al., 2014). Similar disease outbreaks have been reported through the United Kingdom, continental Europe, and Australia (e.g., Lane et al., 2005; Cunnington et al., 2008; Toppe et al., 2010). The causal agent of IDM, *Plasmopara obducens* (J. Schröt.) J. Schröt., is one of many obligate biotrophic plant pathogens in the Oomycota (Chromalveolata, Heterokontophyta) afflicting numerous economically important plants around the world (Kamoun et al., 2015). Impatiens infected with *P. obducens* are quickly defoliated, and death occurs within weeks of disease onset. Infected plants cannot be cured, and the pathogen might be capable of persisting in the soil from one season to the next.

Despite the global impact of IDM on cultivated impatiens, there is currently no information about pathogen population structure or the factors that led to the epidemics, delaying the development of effective mitigation strategies (Plantegenest et al., 2007). Downy mildew pathogens engage in classic gene-for-gene interactions with their hosts during the infection process, producing fast-evolving elicitor molecules that in turn give rise to diverse physiological races varying in their ability to infect a given plant (e.g., Lebeda and Cohen, 2011). As such, knowledge of pathogen variability provides key information required to develop durable host disease resistance. In this study, we developed 37 simple sequence repeat (SSR) markers from the genome of *P. obducens* to support investigations of population diversity, and demonstrate the utility of these markers in a sample of 96 *P. obducens* collected throughout the United States.

METHODS AND RESULTS

Genomic DNA from *P. obducens* sample H12.14-11 (Appendix 1) was extracted from a sporangial mass using the OmniPrep DNA Kit (G-Biosciences, St. Louis, Missouri, USA) following manufacturer's instructions, then purified

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[5] Author for correspondence: Joanne.Crouch@ars.usda.gov

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Table 1. Characteristics of the 37 novel genomic SSR loci developed for *Plasmopara obducens*.

Locus	Primer sequences (5′–3′)	Repeat motif[a]	Observed allele size (bp)	T_a (°C)	N	A	H_o	H_e	PIC	GenBank accession no.	BLAST top hit description [organism][b]
Pob52	F: `ACAGGAATTCATCGGCTCAA` R: `TAACACGAGCTTGCTTGCAC`	$(TTA)_5$	222–234	65	59	2	0.695	0.486	0.485	KP704220	Predicted polyprotein [*Phytophthora infestans*]
Pob1601	F: `CTGCCCTGACTGACCTTCTC` R: `TGTTTGCTTGTTGCTAACGG`	$(TTC)_5$	142–148	65	87	3	0.023	0.023	0.022	KP704221	Conserved hypothetical protein [*Phytophthora parasitica*]
Pob1861	F: `CTCAGAGTTCCTCCGTCTGG` R: `GACTTTGAGGATCCACGAGC`	$(CTA)_5$	266–275	65	48	2	0.042	0.080	0.079	KP704222	TKL protein kinase [*Phytophthora parasitica*]
Pob2171	F: `AAGCTTGCTAGACGAGGCAG` F: `CACGAGCCACCAGCATAGTA`	$(GAC)_5$	250–262	65	89	3	0.640	0.508	0.508	KP704223	Predicted crinkler (CRN) family protein [*Phytophthora infestans*]
Pob2497	F: `CGAGGAGAACAAGCACAACA` R: `AACCTCGAAATGGTCGATTG`	$(GAA)_5$	260–272	65	75	3	0.013	0.114	0.114	KP704224	Hypothetical protein PHYSODRAFT_502025 [*Phytophthora sojae*]
Pob2739	F: `CTGCTTCTCCTGCTTGCTCT` R: `TCAAAGCCAAGGATACCCAC`	$(GGA)_5$	286–289	65	47	2	0.000	0.081	0.081	KP704225	Hypothetical protein F443_14337 [*Phytophthora parasitica*]
Pob2910	F: `GATCTTAGGCGTCATCCACG` R: `CATTTGTCCACGCTACCCTT`	$(GTAT)_5$	165–169	65	71	2	0.718	0.475	0.475	KP704226	Hypothetical protein F441_23092 [*Phytophthora parasitica*]
Pob2933	F: `CTTCGACAGGATCTGCAACA` R: `GGCCCATGCACTTGTAAAAT`	$(AGA)_5$	219–228	65	77	2	0.403	0.322	0.322	KP704227	Hypothetical protein L915_15226 [*Phytophthora parasitica*]
Pob3024	F: `TCGTGCCATCTCTGCATAAG` R: `AAGACGAGAGGATGGACGTG`	$(TTC)_5$	292–295	65	71	2	0.549	0.443	0.443	KP704228	Reverse transcriptase [*Phytophthora sojae*]
Pob3075	F: `CCTCATTCTTCGGTCTGAGC` R: `CTAGTGTCGGAACGCACGTA`	$(CCG)_7$	269–275	65	79	2	0.570	0.418	0.418	KP704229	Conserved hypothetical protein [*Phytophthora infestans*]
Pob3197	F: `GACGTTTTCTCCTGCTCGTC` R: `CAGCCATAAATATCCGCCAT`	$(TTC)_5$	266	58	35	1	—	—	—	KP704230	Hypothetical protein L915_01983 [*Phytophthora parasitica*]
Pob3896	F: `GGACGACAATGAAGAAATGGA` R: `CTGAAATTGACGCTGTGCAT`	$(CGA)_5$	280–295	64	72	3	0.069	0.167	0.167	KP704231	Hypothetical protein L915_17322 [*Phytophthora parasitica*]
Pob4176	F: `AAAAGCTTTGCCGCTCATTA` R: `GGCGGGCTCTTGTGATAATA`	$(AAT)_5$	210–222	65	49	2	0.041	0.040	0.040	KP704232	Hypothetical protein PPTG_05406 [*Phytophthora parasitica*]
Pob4357	F: `GCAATGGCAAGAAAGAGGAG` R: `GATTTAGCCAAACGCGTGAT`	$(TGG)_5$	266–272	63	81	3	0.741	0.483	0.483	KP704233	Predicted crinkler (CRN) family protein [*Phytophthora infestans*]
Pob4700	F: `TACCCACTGTCAATCCAGCA` R: `TGCAGATGCACTAAACGAGG`	$(TTC)_5$	257–269	65	82	4	0.073	0.503	0.503	KP704234	Hypothetical protein F443_20714 [*Phytophthora parasitica*]
Pob5097	F: `CCACCCGATTCTGGTATGTC` R: `GGACGCTTCCACACGTTAAT`	$(GCA)_5$	237–249	65	74	4	0.270	0.569	0.569	KP704235	Hypothetical protein F441_16564 [*Phytophthora parasitica*]
Pob5487	F: `TTTGGGAAATCGACTCTTGG` R: `TTGCGGGATTAATGGAAGAG`	$(CTT)_5$	272–284	59	70	5	0.100	0.376	0.376	KP704236	Hypothetical protein F442_04463 [*Phytophthora parasitica*]
Pob5494	F: `CTGCAACCAGGGTTCTTTTC` R: `GAGACGTCCCAGCTCGTTAG`	$(TAT)_5$	285–303	65	78	5	0.103	0.225	0.225	KP704237	Reverse transcriptase [*Phytophthora sojae*]
Pob5875	F: `GGTTCGGCAGTCGTAGAAAG` R: `GATGTTTGACGTGGATGTGC`	$(CTT)_5$	221–230	65	68	3	0.074	0.099	0.099	KP704238	Conserved hypothetical protein [*Phytophthora infestans*]
Pob6030	F: `CCTTCTTTCTGTGCTACGCC` R: `GTCTCGAGTTTCCAAGCGAC`	$(TTC)_5$	220–229	65	82	2	0.341	0.283	0.283	KP704239	Hypothetical protein PHYSODRAFT_519760 [*Phytophthora sojae*]
Pob7328	F: `GCTTTAGCTGTTCGCTACGG` R: `GGCTTTCTCGTGTCTTCGTC`	$(AGA)_5$	137–155	64	74	4	0.892	0.507	0.507	KP704240	Hypothetical protein F443_03181 [*Phytophthora parasitica*]
Pob7989	F: `AAGGAGATGGACGAGACCCT` R: `TTTTTCTTCTTGTCGTCGCC`	$(AAG)_6$	202	63	8	1	—	—	—	KP704241	Hypothetical protein F444_13637 [*Phytophthora parasitica*]
Pob8649	F: `TGGATCCATTCTCCTTCTGG` R: `TAATGCCAATTCGTGCACAT`	$(TCG)_5$	159–174	65	78	3	0.603	0.425	0.425	KP704242	Hypothetical protein PPTG_04971 [*Phytophthora parasitica*]
Pob10169	F: `TCAGATAGCCTTCCCCCTTT` R: `TAACACCAGCGTAGCGATTG`	$(GAC)_7$	293	65	64	1	—	—	—	KP704243	Hypothetical protein L915_12540 [*Phytophthora parasitica*]
Pob11069	F: `CAACATCCACCATTAGCGTG` R: `GGTGGTGTGTCCTCCTTAGC`	$(CTT)_5$	188–200	65	80	5	0.563	0.501	0.501	KP704244	Hypothetical protein F441_08549 [*Phytophthora parasitica*]
Pob11700	F: `CATCGACAAAGAGTGGCTCA` R: `CCAGCAAATAATCCAGCTCC`	$(AAT)_6$	272–299	65	74	5	0.541	0.746	0.746	KP704245	Predicted carbon-nitrogen hydrolase [*Phytophthora infestans*]
Pob11993	F: `CGACAGTTGGATGCAAACAC` R: `AATTTCTTGGCTTCTGCTGC`	$(TTA)_5$	208–217	65	69	2	0.072	0.070	0.070	KP704246	Hypothetical polyprotein [*Phytophthora infestans*]

Table 1. Continued.

Locus	Primer sequences (5′–3′)	Repeat motif[a]	Observed allele size (bp)	T_a (°C)	N	A	H_o	H_e	PIC	GenBank accession no.	BLAST top hit description [organism][b]
Pob12309	F: `GCCAAGTCGGCAATATCTGT` R: `TTTGACAACAGGTGACCCAA`	$(AGT)_5$	270–282	65	74	4	0.257	0.605	0.605	KP704247	Conserved hypothetical protein [*Phytophthora infestans*]
Pob14678	F: `GTCTACCACCAGACGCCAAC` R: `GCAAAGTGAAGAGAGGGTGC`	$(GTC)_5$	208–220	65	78	2	0.397	0.318	0.318	KP704248	Conserved hypothetical protein [*Phytophthora infestans*]
Pob21005	F: `GTGTACACCACCTGAACCCC` R: `GTTCAGGTCCTTGGCAATGT`	$(TCTTGTCTCCAGC)_4$	134–163	65	88	2	0.670	0.489	0.489	KP704249	Hypothetical protein PPTG_07274 [*Phytophthora parasitica*]
Pob29057	F: `CGACCAGGGAGTCCAAGATA` R: `GGAGGCAGAAGAAAGTGCTG`	$(GTT)_5$	251–260	65	83	2	0.325	0.272	0.272	KP704250	Hypothetical protein L914_08176 [*Phytophthora parasitica*]
Pob33638	F: `CGCTTCCTCCTTCTTCTCCT` R: `GACGAAACGGAAGACGAAAA`	$(CTT)_{10}$	166–196	65	50	6	0.280	0.486	0.486	KP704251	Hypothetical protein PHYSODRAFT_353608 [*Phytophthora sojae*]
Pob36128	F: `AGATTGGCCTTGCGACTCTA` R: `TGGCTGAGAGCTAAGACGCT`	$(ATTTA)_5$	198–214	65	47	3	0.021	0.102	0.102	KP704252	Hypothetical protein H257_19342 [*Aphanomyces astaci*]
Pob47245	F: `ACCCGAGATAGACGTTGTCG` R: `CTTGTGACCCCTGTTCACCT`	$(GAAA)_5$	262–274	62	58	2	0.431	0.338	0.338	KP704253	Hypothetical GK15001 protein [*Albugo laibachii*]
Pob48178	F: `CGGATAAGTACGCAACCGAT` R: `TGGCTACAGTTGTGAGTCGC`	$(CGA)_9$	214–226	65	77	3	0.831	0.553	0.553	KP704254	Di-trans,poly cis-decaprenylcistransferase [*Phytophthora parasitica*]
Pob52381	F: `ATGAGACGACGGTCGAGACT` R: `CACCGTCCTTTTCTTCTTGC`	$(AAG)_6$	173–179	65	70	2	0.714	0.459	0.459	KP704255	Hypothetical protein PPTG_06711 [*Phytophthora parasitica*]
Pob60359	F: `TGGAATCTGGAGGACTGACC` R: `TTCCTGCACATGCAATCTTC`	$(ATA)_5$	200–203	65	70	2	0.000	0.459	0.459	KP704256	Hypothetical protein F444_17394 [*Phytophthora parasitica*]

Note: A = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; N = number of isolates that positively amplified; PIC = polymorphism information content; T_a = annealing temperature.

[a] Repeat motif is given based on number of units in the reference genome of *P. obducens* H12.14-11.

[b] Putative identifications based on BLASTN and BLASTX searches of the NCBI GenBank nonredundant database (threshold E-value = 1.0E-06).

using the Zymo DNA Clean and Concentrator kit (Zymo Research, Irvine, California, USA). DNA was sheared to 600 bp using the Covaris M220 ultrasonicator (Covaris, Woburn, Massachusetts, USA), and then used to construct a library with the Illumina TruSeq DNA LT Sample Prep kit (Illumina, San Diego, California, USA). Library sequencing was conducted on an Illumina MiSeq instrument (Illumina) using 600-cycle sequencing cartridges, run as 2 × 300-bp paired-end reads. Reads were processed using CLC Genomics Workbench version 7.5.1 (CLC Bio, Boston, Massachusetts, USA), and a de novo assembly was performed after removal of adapters, indices, bases with low-quality scores (limit = 0.05), and runs of ambiguous bases >2 bp. The assembly measured 202 Mb, contained in 137,754 scaffolds (N50 = 1486), with an average depth of coverage of 120.76×.

Using PrimerPro version 1.0 (http://webdocs.cs.ualberta.ca/~yifeng/primerpro/), the *P. obducens* H12.14-11 genome assembly was mined for SSR motifs, screened for optimal PCR primer pairs, and BLASTN searched to ensure unique priming sites. Motif size search ranged from mono- to tridecanucleotides, with minimum repeat units set as follows: mononucleotides ≥10; di-, tri-, tetra-, penta-, and hexanucleotides ≥5; the remaining repeat motifs ≥5. The genome assembly contained 13,483 SSR motifs. Dinucleotide repeats were the most abundant class, followed by mononucleotides and trinucleotides. SSRs averaged 17.8 bp in length, with 78% smaller than 21 bp. Repeats averaged 7.4 ± 4.15 units/SSR. SSR relative abundance (# SSRs/genome size [Mb]) was 66.9, and SSR density (combined length of SSRs [bp]/genome size [Mb]) was 1083.5.

From the set of candidate SSR loci suitable for marker development (e.g., those found as a single copy in the genome assembly, with repeat units of trinucleotide or greater, and unique priming sites), we identified loci that were heterozygous in the genome assembly of H12.14-11 by performing probabilistic variant detection using CLC Genomics, then visually inspecting candidate regions. Because *P. obducens* is an obligate biotroph and the H12.14-11 sporangial sample was collected directly from the surface of the host plant, candidate markers were further assessed by performing BLAST searches of the National Center for Biotechnology Information (NCBI) GenBank nonredundant (nr) database to ensure the sequences were not derived from the plant host or other environmental components. This filtering yielded 189 primer sets, from which 62 primer sets were tested for amplification using DNA extracted from *P. obducens* sporangial sample PA1-1 (Appendix 1). Twenty-five primer sets were discarded due to lack of amplification, or the production of stutter and/or multiple bands. The 37 remaining markers represented a wide variety of repeat motif and length (Table 1) and were located on 37 different contigs. All but three of the markers contained trinucleotide motifs. When tested on *I. walleriana* DNA, none of the markers produced an amplicon. The 37 microsatellite loci were used to perform BLAST searches against the NCBI GenBank database to determine putative functions, as summarized in Table 1. Sequence contigs containing microsatellite loci shared homology to predicted proteins of different oomycete plant pathogens (Table 1).

A total of 96 *P. obducens* samples collected between 2012 and 2014 from *I. walleriana* (n = 73) and from four additional *Impatiens* species (n = 23) at different localities in the United States were used for marker validation (Appendix 1). DNA was extracted from leaves visibly afflicted with downy mildew using the DNeasy Plant Kit (QIAGEN, Germantown, Maryland, USA). PCR amplifications were performed as described (Schuelke, 2000) in 10-μL volumes: 6.5 μL of 2× Mango Mix (Bioline Inc., Tauton, Massachusetts, USA), 1 μL of DNA (2–10 ng/μL), 7 μM of forward primer with 5′ M13 tail, 13 μM of reverse primer, 7 μM of dye-labeled M13 (FAM, PET, VIC, NED), and 25 mM of $MgCl_2$. Fragment sizing was performed by adding 1 μL amplicon to 9 μL of Hi-Di Formamide (Applied Biosystems, Carlsbad, California, USA) containing GeneScan 500 LIZ Size Standard (Applied Biosystems), denaturing at 95°C for 2 min, then injecting onto an ABI 3730xl DNA Analyzer (Applied Biosystems). Results were analyzed using GeneMarker version 2.6.3 (SoftGenetics, State College, Pennsylvania, USA); GenAlEx version 6.5 (Peakall and Smouse, 2012) was used to generate summary statistics. Allele frequencies were used to calculate polymorphism information content (PIC; Botstein et al., 1980).

Only three of the SSR markers (Pob3197, Pob7989, and Pob10169) were monomorphic across the 96 *P. obducens* samples. Marker Pob10169 could be amplified from just 8% of the *P. obducens* samples; therefore, the monomorphic data might be an artifact of the small sample size. The 34 polymorphic markers displayed 2–6 alleles, for a total of 104 alleles (Table 1). Observed heterozygosity ranged from 0.000–0.892 (mean = 0.355), while expected heterozygosity ranged from 0.023–0.746 (mean = 0.354) from polymorphic loci. The PIC ranged from 0.022–0.746 (mean = 0.354), with 18 of the markers moderately informative (PIC > 0.40) and one marker highly informative (PIC > 0.70; Pob11700). Analysis in GenClone version 2.0

(http://www.ccmar.ualg.pt/maree/software.php?soft=genclon) showed that just 17 of the 37 SSR markers (45.9%) were sufficient to identify all multilocus genotypes.

CONCLUSIONS

The oomycete *P. obducens* is one of many obligate biotrophic plant pathogens currently impacting the health of economically important plants worldwide. The SSR markers developed here are the first molecular resource available for *P. obducens*. The high level of polymorphism present in these markers will enhance efforts to monitor pathogen population genetic structure and diversity over time, trace source populations, and understand the role of pathogen physiological races on host susceptibility.

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Appendix 1. *Plasmopara obducens* samples collected from *Impatiens* and used to screen microsatellite markers developed in this study. Voucher specimens corresponding to the samples used in this study were deposited in the U.S. National Fungus Collections (Herbarium BPI), Beltsville, Maryland, USA.

Accession no.	Origin	Host^a	Collection date	Collector
1300252A	Cattaraugus, NY	*I. walleriana*	07/23/13	G. Sphar, M. Daughtery
1300252F	Cattaraugus, NY	*I. walleriana*	07/23/13	G. Sphar, M. Daughtery
1300272G	Rockland, NY	*I. walleriana*	07/16/13	M. Formichelli, M. Daughtery
1300315F	Westchester, NY	*I. walleriana*	08/04/13	M. Formichelli, M. Daughtery
CA1-A	Santa Clara Co., CA	*I. walleriana*	08/21/13	Jane Trolinger
CA1-B	Santa Clara Co., CA	*I. walleriana*	08/21/13	Jane Trolinger
CA2-B	Santa Clara Co., CA	*I. walleriana*	08/21/13	Jane Trolinger
CA3-A	Santa Clara Co., CA	*I. walleriana*	08/21/13	Jane Trolinger
COL1	Silver Spring, MD	*I. walleriana*	2013	Jo Anne Crouch
CT1-A	New Haven, CT	*I. walleriana*	07/1/13	Yonghao Li
CT1-C	New Haven, CT	*I. walleriana*	07/1/13	Yonghao Li
CT1-F	New Haven, CT	*I. walleriana*	07/1/13	Yonghao Li
CT1-G	New Haven, CT	*I. walleriana*	07/1/13	Yonghao Li
DE1-6	Frederick, MD	*I. balsamina*	08/29/13	Nina Shiskoff
DE1-7	Frederick, MD	*I. balsamina*	08/29/13	Nina Shiskoff
DE1-I	Frederick, MD	*I. balsamina*	08/29/13	Nina Shiskoff
FL14A	Homestead, FL	*I. walleriana*	Winter 2013	Aaron Palmateer
FL14B	Homestead, FL	*I. walleriana*	Winter 2013	Aaron Palmateer
FL18	Homestead, FL	*I. walleriana*	Winter 2013	Aaron Palmateer
FL23	Homestead, FL	*I. walleriana*	Winter 2013	Aaron Palmateer
FL26	Homestead, FL	*I. walleriana*	Winter 2013	Aaron Palmateer
FL33	Homestead, FL	*I. walleriana*	Winter 2013	Aaron Palmateer
FL39C	Homestead, FL	*I. walleriana*	Winter 2013	Aaron Palmateer
FL45	Homestead, FL	*I. walleriana*	Winter 2013	Aaron Palmateer
FL49	Homestead, FL	*I. walleriana*	Winter 2013	Aaron Palmateer
FL7	Homestead, FL	*I. walleriana*	Winter 2013	Aaron Palmateer
H12.14-11	Harbor Springs, MI	*I. walleriana*	08/14/12	Mary Hausbeck
HI10-2	Keneohe, HI	*I. walleriana*	06/03/13	Becky Azama
HI10-5	Keneohe, HI	*I. walleriana*	06/03/13	Becky Azama
HI10-8	Keneohe, HI	*I. walleriana*	06/03/13	Becky Azama
HI11-11	Honolulu, HI	*I. walleriana*	06/06/13	Mann Ko
HI11-8	Honolulu, HI	*I. walleriana*	06/06/13	Mann Ko
HI12-7	Manoa, HI	*I. walleriana*	06/19/13	Christopher Lao

APPENDIX 1. Continued.

Accession no.	Origin	Host[a]	Collection date	Collector
HI13-4	Kailua, HI	*I. walleriana*	06/19/13	Christopher Lao
HI14-4	Hilo, HI	*I. walleriana*	05/29/13	Mann Ko
HI15-1	Kailua-Kona, HI	*I. walleriana*	06/01/13	R.T. Curtis III
HI15-4	Kailua-Kona, HI	*I. walleriana*	06/01/13	R.T. Curtis III
I10-D	Orange Co., NY	*I. walleriana*	09/11/13	Brian Eshenaur
I11-A	Orange Co., NY	*I. walleriana*	09/11/13	Betsy Lamb
I11-G	Orange Co., NY	*I. walleriana*	09/11/13	Betsy Lamb
I13-A	Suffolk Co., NY	*I. walleriana*	09/17/13	Marie Camenares
I13-C	Suffolk Co., NY	*I. walleriana*	09/17/13	Marie Camenares
I13-D	Suffolk Co., NY	*I. walleriana*	09/17/13	Marie Camenares
I15-F	Barnstable Co., MA	*I. walleriana*	09/19/13	Paul Lopes
I17-B	Barnstable Co., MA	*I. walleriana*	09/19/13	Paul Lopes
I18-B	Franklin Co., MA	*I. walleriana*	09/20/13	Tina Smith
I19-A	Highland Park, NJ	*I. walleriana*	09/26/13	Ira Grasgreen
I19-B	Highland Park, NJ	*I. walleriana*	09/26/13	Ira Grasgreen
I19-E	Highland Park, NJ	*I. walleriana*	09/26/13	Ira Grasgreen
I20-A	Monroe Co., NY	*I. walleriana*	10/01/13	Brian Eshenaur
I21-B	West Lafayette, IN	*I. walleriana*	10/09/13	Nora Catlin
I22-A	Newport Co., RI	*I. walleriana*	10/10/13	Heather Faubert
I22-C	Newport Co., RI	*I. walleriana*	10/10/13	Heather Faubert
I4-B	Centre Co., PA	*I. walleriana*	08/27/13	Andrea Skirpan
I5-I	Tompkins Co., NY	*I. walleriana*	08/15/13	Betsy Lamb
I5-H	Tompkins Co., NY	*I. walleriana*	08/15/13	Betsy Lamb
I7-G	Staten Island, NY	*I. walleriana*	09/04/13	Joe Parent
I8-C	Rochester, NY	*I. walleriana*	09/06/13	Brian Eshenaur
I9-A	Orange Co., NY	*I. walleriana*	09/10/13	Margery Daughtrey
I9-D	Orange Co., NY	*I. walleriana*	09/10/13	Margery Daughtrey
IB1-C	Buffalo, NY	*I. balsamina*	08/09/13	Betsy Lamb
IB2-B	Niagara Co, NY	*I. balsamina*	09/10/13	John Farfaglia
IB2-D	Niagara Co, NY	*I. balsamina*	09/10/13	John Farfaglia
IB3-H	Buffalo, NY	*I. balsamina*	09/28/13	Betsy Lamb
IB3-J	Buffalo, NY	*I. balsamina*	09/28/13	Betsy Lamb
IB3-L	Buffalo, NY	*I. balsamina*	09/28/13	Betsy Lamb
IMPARG3312B	Riverhead, NY	*I. arguta*	10/15/12	Margery Daughtrey
IMPAUR3012C	Riverhead, NY	*I. auricoma*	10/13/12	Margery Daughtrey
IMPOM3812	Riverhead, NY	*I. omeiana*	10/15/12	Margery Daughtrey
IMPBA3712	Riverhead, NY	*I. arguta* ‘blue angel’	10/15/12	Margery Daughtrey
IMPC1512C	Riverhead, NY	*I. capensis*	10/13/12	Margery Daughtrey
IMPC1612	Riverhead, NY	*I. capensis*	10/12/12	Margery Daughtrey
IMPC2012	Riverhead, NY	*I. capensis*	10/12/12	Margery Daughtrey
IMPC2112A	Riverhead, NY	*I. capensis*	10/15/12	Margery Daughtrey
IMPC2112B	Riverhead, NY	*I. capensis*	10/15/12	Margery Daughtrey
IMPC2212A	Riverhead, NY	*I. capensis*	10/15/12	Margery Daughtrey
IMPF2412A	Riverhead, NY	*I. flanaganae*	10/12/12	Margery Daughtrey
IMPF2512E	Riverhead, NY	*I. flanaganae*	10/12/12	Margery Daughtrey
IMPF2812A	Riverhead, NY	*I. flanaganae*	10/17/12	Margery Daughtrey
IMPH3412B	Riverhead, NY	*I. hochstetteri*	09/07/12	Margery Daughtrey
IMPW0112A	Westchester Co., NY	*I. walleriana*	05/31/12	Margery Daughtrey
IMPW0312A	Riverhead, NY	*I. walleriana*	06/18/12	Margery Daughtrey
IMPW0312D	Riverhead, NY	*I. walleriana*	06/18/12	Margery Daughtrey
IN3-E	Tippelanoe Co., IN	*I. balsamina*	08/27/13	Margery Daughtrey
IN5-A	Terre Haute, IN	*I. walleriana*	07/01/13	Tom Creswell
IN5-F	Terre Haute, IN	*I. walleriana*	07/01/13	Tom Creswell
IN5-I	Terre Haute, IN	*I. walleriana*	07/01/13	Tom Creswell
MA1-9	Barnestable Co., MA	*I. walleriana*	07/18/13	Geoffrey Njue
MA2-11	Tewksbury, MA	*I. walleriana*	08/01/13	Karen McNaughton
MA8-C	Barnestable Co., MA	*I. walleriana*	08/26/13	Paul Lopes
NJ1-1	Cream Ridge, NJ	*I. walleriana*	08/01/14	Cristi Palmer
NJ1-6	Cream Ridge, NJ	*I. walleriana*	08/01/14	Cristi Palmer
NY10-A	Oneida Co., NY	*I. walleriana*	07/30/13	Margery Daughtrey
NY10-B	Oneida Co., NY	*I. walleriana*	07/30/13	Margery Daughtrey
PA1-1	Highland, NY	*I. walleriana*	06/16/14	Teresa Rusinek
TN1-3	Davidson Co., TN	*I. walleriana*	07/18/13	Alan Windham
TN1-7	Davidson Co., TN	*I. walleriana*	07/18/13	Alan Windham
TN1-8	Davidson Co., TN	*I. walleriana*	07/18/13	Alan Windham

[a] The following *Impatiens* species were sampled: *I. arguta* Hook. f. & Thomson, *I. auricoma* Baill., *I. balsamina* L., *I. capensis* Meerb., *I. flanaganae* Hemsl., *I. hochstetteri* Warb., *I. omeiana* Hook. f., and *I. walleriana* Hook. f.

Appendix 2. Summary of simple sequence repeat (SSR) motifs identified from the de novo genome assembly constructed for *Plasmopara obducens* H12.14-11.

Item	No. of motifs identified
Total no. of sequences examined	137,754
Total length of examined sequences (bp)	201,342,680
Total no. of identified SSRs	13,483
Total no. of contigs containing SSRs	9860
No. of contigs containing more than one SSR	1950
No. of SSRs present in compound formation	1185
No. of SSRs with effective primer modeling	11,940
Mononucleotide	3312
Dinucleotide	7360
Trinucleotide	2317
Tetranucleotide	218
Pentanucleotide	58
Hexanucleotide	76
Heptanucleotide	75
Octanucleotide	19
Nonanucleotide	20
Decanucleotide	8
Undecanucleotide	5
Dodecanucleotide	12
Tridecanucleotide	3