

Article

# Phylogenetic Relationships among Species of *Phellinus sensu stricto*, Cause of White Trunk Rot of Hardwoods, from Northern North America

## Nicholas J. Brazee

Center for Agriculture, Food and the Environment, University of Massachusetts, Amherst, MA 01002, USA; E-Mail: nbrazee@umass.edu; Tel.: +1-413-545-2826

Academic Editor: Jan Stenlid

Received: 30 September 2015 / Accepted: 4 November 2015 / Published: 18 November 2015

Abstract: Species in *Phellinus* s.s. are some of the most important wood-decaying fungal pathogens in northern temperate forests, yet data on species incidence in North America remains limited. Therefore, phylogenetic analyses were performed using four loci (ITS, nLSU, *tef1* and *rpb2*) with isolates representing 13 species. Results of phylogenetic analyses using maximum likelihood and Bayesian inference revealed that eight species of Phellinus s.s. occur in North America, and include: P. alni, P. arctostaphyli, P. betulinus, P. lundellii, P. nigricans, P. tremulae and two undescribed species, P. NA1 and P. NA2. Meanwhile, P. tuberculosus, P. igniarius s.s., P. populicola, P. laevigatus s.s. and P. orienticus were not detected and appear restricted to Europe and/or Asia. The tefl dataset outperformed all other loci used and was able to discriminate among all 13 of the currently known Phellinus s.s. species with significant statistical support. The internal transcribed spacer (ITS) region performed well but a high level of intraspecific variation could lead to inflated taxa recognition. Phellinus alni exhibited the broadest host range, as demonstrated previously, and appears to be the most common species in northern hardwood (Acer-Betula-Fagus), northern floodplain (Fraxinus-Populus-Ulmus) and coastal alder (Alnus) forests of North America.

Keywords: northern hardwood; aspen; wood decay; heart rot; Basidiomycetes

## 1. Introduction

Based on several independent studies that utilized the morphological, biological and phylogenetic species concepts, it is clear that *Phellinus sensu stricto* (s.s.) is composed of a limited number of species that cause a delignifying trunk rot of deciduous hardwoods, with minor exceptions [1–12]. Numerous other species previously classified as *Phellinus* [13] are now members of various segregate genera (e.g., *Fomitiporia, Fuscoporia, Inonotus, Onnia, Phellinidium* and *Porodaedalea*, among others) [7–9].

White trunk rot of hardwoods, caused by *Phellinus* s.s., has been described as the single most important rot of hardwoods in North America [14]. Boyce ([15] p. 413) noted: "The false tinder fungus causes more loss than any other wood destroyer of hardwoods". In North America, *Phellinus* s.s. is known to be most destructive in northern hardwood (*Acer-Betula-Fagus*) and aspen (*Populus*) forest types [14–16]. The presence of a single basidiocarp on the trunk of an infected tree can indicate a column of decay approximately 2 m in length for American beech (*Fagus grandifolia*) and sugar maple (*Acer saccharum*), and nearly 3 m in length for yellow birch (*Betula alleghaniensis*) [17]. *Phellinus tremulae*, one of the first species to be distinguished from the *P. igniarius sensu lato* (s.l.) complex [18], acts as an aggressive pathogen of aspen (*Populus*; especially *P. tremuloides*) and has been shown to cause significant losses of merchantable timber in the U.S. Lake States and Intermountain West [19,20].

For decades, white trunk rot of hardwoods was attributed to the actions of a single species, *P. igniarius*, even though several varieties or types were described [21,22]. While still referred to as the "*P. igniarius* group", there are up to nine species of *Phellinus* s.s. known or suspected to occur in North America, and include: *P. alni*, *P. acrtostaphyili*, *P. igniarius* s.s., *P. laevigatus*, *P. lundellii*, *P. nigricans*, *P. populicola*, *P. tremulae*, *P. tuberculosus* [8,9,16,23,24]. There are additional species still described as "*Phellinus*" in North America (*P. bicuspidatus* and *P. spiculosus*, for example) that prior studies have shown are not members of *Phellinus* s.s. [8,10]. Ironically, it has been hypothesized that *P. igniarius* s.s. is a European species restricted to *Salix* [6,9]. However, a broader survey of species incidence by Tomšovský *et al.* [12] demonstrated that while *P. igniarius* s.s. is most common on *Salix*, it also occurs on *Populus* and *Malus* in Europe and Asia, signifying this species could potentially occur on all members of the Salicaceae and Rosaceae growing in northern temperate regions. An additional *Phellinus* species found only on *Populus*, *P. populicola*, is genetically similar to *P. igniarius* s.s. and is present throughout northern Europe and Asia [6,12]. To date, it remains unclear if either *P. igniarius* s.s. or *P. populicola* occur in North America.

Phylogenetic analyses have vastly improved the ability to discriminate genera and species in *Phellinus* s.l., yet most studies that include *Phellinus* s.s. have primarily utilized isolates collected from Europe [6–9,12], leaving North American researchers to speculate about species distribution and host specificity. The goals of this study were to determine the incidence and host distribution of *Phellinus* s.s. species using isolates that originated from northern temperate forests in North America. It was hypothesized that eight species of *Phellinus* s.s. occur in North America: *P. alni, P. arctostaphyli, P. nigricans, P. laevigatus, P. lundellii, P. populicola, P. tremulae* and *P. tuberculosus*.

#### 2. Experimental Section

## 2.1. Isolates Used, DNA Extraction, PCR, and DNA Sequencing

A total of 57 isolates, representing eight presumed species from northern North America, Europe and Asia were chosen for this study and include: *P. alni*, *P. arctostaphyli*, *P. nigricans*, *P. laevigatus*, *P. lundellii*, *P. populicola*, *P. tremulae* and *P. tuberculosus*. In addition, isolates representing segregate genera in *Phellinus* s.l. and known to infect deciduous hardwoods were also used and include: *Fuscoporia* spp., *Inocutis dryophila*, *Inonotus vaninii* and *Phellinopsis conchata*. *Fomes fomentarius* was chosen to represent the outgroup. Most isolates were either field-collected by the author or obtained from the USDA Forest Service, Center for Forest Mycology Research (CFMR) herbarium. Isolates from the CFMR herbarium where chosen based on presumed species, geographic disparity and host diversity from northern North American forests that encompass the northern hardwood, aspen and alder forest types, among others (Table 1).

DNA extraction and PCR protocols have been described previously [25]. To generate PCR amplicons, the following primer pairs were used: ITS1-F and ITS4 [26] for the internal transcribed spacer (ITS); LR0R [27] and LR5 [28] the nuclear large subunit (nLSU) domains one to three; EF595F and EF1160R for partial elongation factor 1-alpha (*tef1*) [29]; and bRPB2-6F and bRPB2-7.1R [30] for partial RNA polymerase II, second largest subunit (*rpb2*) sequences. PCR products were visualized on 1% agarose gels stained with SYBR Safe (Life Technologies, Carlsbad, CA, USA) to ensure the presence of single amplicons. After visualizing the *tef1* amplicons, six of the ten *P. alni* isolates chosen for study failed to amplify using the EF595F/EF1160R primer pair. Additional *tef1* primers frequently used in phylogenetic studies of Basidiomycetes (983F/2218R) [31] were then used in the following combination to generate amplicons: (i) 983F/1160R; and (ii) EF595F/2218R. The first primer pair again failed, while the second primer pair produced single amplicons, approximately 1120 bp in size. The sequence analysis (described below) showed that isolates of *P. alni* can have two C/T mismatches in the EF1160R priming region, preventing successful annealing.

Prior to sequencing, PCR products were purified using ExoSAP-IT (USB, Cleveland, OH, USA). Isolates were sequenced using the Big Dye Sequencing Kit v. 3.1 on an ABI 3130xl capillary sequencer (Applied Biosystems, Foster City, CA, USA) at the Genomics Resource Laboratory, University of Massachusetts, Amherst. GenBank accession numbers are listed in Table 1.

#### 2.2. Sequence Alignment and Phylogenetic Analysis

Raw sequences were edited using CodonCode v. 4.0.4 (CodonCode Corporation, Dedham, MA) and aligned with MAFFT v. 7 using the L-INS-i option for ITS sequences and the FFT-INS-i option for the remaining datasets [32]. Phylogenetic reconstructions were performed using maximum likelihood (ML) and Bayesian inference (BI). For ML and BI, the best-fit nucleotide substitution model was chosen using log-likelihood (*-lnL*) scores generated using jModelTest v. 2 [33,34]. For all data sets, the model that produced the highest *-lnL* score was the general time reversible (GTR) substitution model [35]. Sequences generated in this study were combined with data from previous studies [7,9,12,36,57]. Information for these isolates is provided in Table 1.

	Hest			D		GenBank Accession Nos.			
Species & Code	Host	State/Province	Country	Reference	ITS	nLSU	rpb2	tef1	
			Phellinus alni						
DLL2009-140	Acer rubrum	MN	USA	This study	KU139159	KU139211	KU139282	KU139331	
NJB2011-SM1	Acer rubrum	MA	USA	This study	KU139161	KU139217	KU139279	KU139324	
NJB2011-SM4	Acer rubrum	MA	USA	This study	KU139163	KU139219	KU139284	KU139326	
NJB2011-SM3	Acer saccharum	MA	USA	This study	KU139162	KU139218	KU139286	KU139325	
FP-134638-Sp	Alnus sp.	ID	USA	This study	KU139167	KU139213	KU139280	KU139330	
HHB-15085-Sp	Alnus sp.	AK	USA	This study	KU139160	KU139216	KU139285	KU139328	
NJB2011-WEN2	Betula lenta	MA	USA	This study	KU139164	KU139220	KU139283	KU139327	
FP-125027-T	Betula papyrifera	NH	USA	This study	KU139166	KU139212	KU139281	KU139329	
FP-70831-T	Fagus grandifolia	MI	USA	This study	KU139165	KU139214	KU139278	KU139322	
NJB2011-GR3	Fagus grandifolia	MA	USA	This study	KU139168	KU139215	KU139287	KU139323	
BRNM 714864	Alnus incana	Mikkeli, Kakriala	Finland	[12]	GQ383775			GQ383821	
BRNM 714865	Alnus incana	Červený Kláštor	Slovakia	[12]	GQ383730			GQ383840	
BRNM 714891	Betula sp.	Vyborg	Russia	[12]	GQ383770			GQ383853	
MJ 51/96	Fagus sylvatica	Havlíčkův Brod	Czech Republic	[12]	GQ383756			GQ383831	
BRNM 714881	Sorbus cf. intermedia	Uppsala	Sweden	[12]	GQ383732			GQ383848	
TN3301	Betula sp.	n/a	Finland	[9]	AY340040				
TW-162	Laburnum anagyroides	n/a	Germany	[7]		AF311025			
		Ph	ellinus arctostaphyli						
FP-94140-R	Arctostaphylos manzanita	OR	USA	This study	KU139143	KU139250	KU139264	KU139348	
FP-94186-R	Arctostaphylos patula	CA	USA	This study	KU139145	KU139252	KU139266	KU139350	
M-92-2	Arctostaphylos patula	OR	USA	This study	KU139144	KU139251	KU139265	KU139349	
		Ph	ellinus laevigatus s.l.						
			P. betulinus						
FP-105325-Sp	Betula alleghaniensis	WV	USA	This study	KU139154	KU139239	KU139312	KU139369	
DLL2009-143	Betula alleghaniensis	MN	USA	This study	KU139146	KU139236	KU139308	KU139371	
DVB-Betula	Betula nigra	VA	USA	This study	KU139151	KU139246	KU139314	KU139365	

Table 1.	Information and	GenBank acce	ession numbers	for isolates	of Phellinus s s
	mormation and	OuriDank acco	ssion numbers	101 15014105	01 1 пенниз 5.5

	Hast		Country			GenBank Accession Nos.		
Species & Code	Host	State/Province	Country	Reference	ITS	nLSU	rpb2	tef1
NJB2009-FpG	Betula papyrifera	MA	USA	This study	KU139153	KU139248	KU139311	KU139368
NJB2009-FpE	Betula papyrifera	MA	USA	This study	KU139152	KU139247	KU139307	KU139370
DLL2009-175	Betula papyrifera	MN	USA	This study	KU139155	KU139237	KU139309	KU139367
RLG-5835-T	Betula papyrifera	MT	USA	This study	KU139150	KU139240	KU139306	KU139364
RLG-645-T	Betula papyrifera	MT	USA	This study	KU139147	KU139238	KU139313	KU139366
			P. laevigatus s.s.					
NJB2011-PLa1-F	Betula pubescens	Hame	Finland	This study	KU139148	KU139241	KU139305	KU139372
NJB2011-PLa2-F	Betula pubescens	Hame	Finland	This study	KU139149	KU139242	KU139310	KU139373
BRNM 714875	Betula sp.	<b>Bohemian Forest</b>	Czech Republic	[12]	GQ383779			GQ383857
BRNM 714867	Betula sp.	<b>Bohemian Forest</b>	Czech Republic	[12]	GQ383778			GQ383856
BRNM 714877	Betula sp.	Uppsala	Sweden	[12]	GQ383777			GQ383855
TN-3260	Betula pubescens	n/a	Finland	[7]		AF311034		
			P. orienticus					
TN-6392	<i>Betula</i> sp.	Jilin	China	This study	KU139156	KU139243		KU139374
TN-6432	Betula costa	Jilin	China	This study	KU139158	KU139245		KU139376
TN-6425	<i>Betula</i> sp.	Jilin	China	This study	KU139157	KU139244		KU139375
		1	Phellinus lundellii					
NJB2011-SM2	Betula alleghaniensis	MA	USA	This study	KU139184	KU139235	KU139299	KU139335
NJB2011-WEN1	Betula alleghaniensis	MA	USA	This study	KC551835	KC551859	KU139302	KC551884
DLL2011-321	Betula alleghaniensis	MI	USA	This study	KU139181			
NJB2011-GR1	Betula lenta	MA	USA	This study	KU139182	KU139232	KU139301	KU139336
JJW-694	Betula sp.	NY	USA	This study	KU139183	KU139233	KU139298	KU139334
NJB2011-PLu-F	Betula pubescens	Hame	Finland	This study	KU139185	KU139234	KU139300	KU139337
BRNU 604719	Betula carpatica	Jeseníky Mountains	Czech Republic	[12]	GQ383704			
		P	Phellinus nigricans					
ННВ-15513-Т	Betula nana	AK	USA	This study	KU139171	KU139226	KU139295	KU139347
FP-62186-T	Betula occidentalis	WA	USA	This study	KU139176	KU139227	KU139290	KU139343

Service & Code	Uest	State / <b>D</b>	Constant	D.f		GenBank A	Accession Nos.		
Species & Code	Host	State/Province	Country	Kelefence	ITS	nLSU	rpb2	tef1	
FP-140068-T	Betula papyrifera	MN	USA	This study	KU139175	KU139225	KU139292	KU139345	
DMR-94-13	Betula papyrifera	MN	USA	This study	KU139173	KU139221	KU139291	KU139344	
FP-135209-R	Betula papyrifera	MI	USA	This study	KU139174	KU139224	KU139293	KU139346	
OKM-3356	Betula papyrifera	ID	USA	This study	KU139178	KU139228	KU139288	KU139342	
ННВ-12617-Т	Betula papyrifera	AK	USA	This study	KU139172	KU139230	KU139294	KU139338	
RLG-5844-Sp	Betula sp.	MT	USA	This study	KU139177	KU139229	KU139289	KU139341	
NJB2011-PA1-F	Betula pubescens	Hame	Finland	This study	KU139169	KU139222	KU139297	KU139340	
NJB2011-PA2-F	Betula pubescens	Hame	Finland	This study	KU139170	KU139223	KU139296	KU139339	
MJ 557/94	Betula sp.	Rondane	Norway	[12]	GQ383724			GQ383801	
BRNM 714879	Betula sp.	Uppsala	Sweden	[12]	GQ383721			GQ383803	
BRNM 714883	Betula sp.	Vyborg	Russia	[12]	GQ383719			GQ383851	
85-917	Betula pubescens	n/a	Germany	[7]		AF311027			
		Р	hellinus populicola						
TN-526 <sup>(1)</sup> (ATCC 36122; CBS 638 75)	Populus tremula	Uusimaa	Finland	This study	KU139179	KU139231	KU139303	KU139333	
BRNM 714885	Populus tremula	Uppsala	Sweden	[12]	GQ383706			GQ383785	
BRNM 714890	Populus tremula	Uppsala	Sweden	[12]	GQ383707			GQ383786	
MJ 92/96	Populus alba	Lanžhot	Czech Republic	[12]	GQ383705			GQ383787	
84-61	Populus alba	n/a	Germany	[7]		AF311038			
		Ph	ellinus igniarius s.s.						
MJ 19/94	Populus nigra	Jihlava	Czech Republic	[12]	GQ383718			GQ383793	
BRNM 714866	Populus nigra	Zábřeh	Czech Republic	[12]	GQ383710			GQ383792	
BRNM 714889	Salix alba	Brno	Czech Republic	[12]	GQ383709			GQ383791	
MJ 40/07	Salix caprea	Jihlava	Czech Republic	[12]	GQ383712			GQ383790	
BRNM 714884	Salix caprea	Uppsala	Sweden	[12]	GQ383715			GQ383795	
83-1110a	Salix fragilis	n/a	Germany	[7]		AF311033			

	II (			D.C		GenBank A	ccession Nos.	
Species & Code	Host	State/Province	Country	Reference	ITS	nLSU	tef1	
			Phellinus tremulae					
FP-135820-T	Populus grandidentata	WI	USA	This study	KU139136	KU139206	KU139274	KU139363
FP-59023-T	Populus tremuloides	NH	USA	This study	KU139135	KU139205	KU139270	KU139358
FP-135202-T	Populus tremuloides	MI	USA	This study	KU139134	KU139207	KU139271	KU139359
FP-140050-T	Populus tremuloides	MN	USA	This study	KU139131	KU139204	KU139272	KU139362
A-17	Populus tremuloides	СО	USA	This study	KU139137	KU139200	KU139273	KU139361
FP-105919-R	Populus tremuloides	SD	USA	This study	KU139130	KU139203	KU139275	KU139360
NJB2011-PT1-F	Populus tremula	Hame	Finland	This study	KU139132	KU139201	KU139276	KU139357
NJB2011-PT2-F	Populus tremula	Hame	Finland	This study	KU139133	KU139202	KU139277	KU139356
MJ 45/07	Populus tremula	Havlíčkův Brod	Czech Republic	[12]	GQ383782			GQ383860
BRNM 714886	Populus tremula	Třeboň	Czech Republic	[12]	GQ383780			GQ383862
MJ 32/07	Populus tremula	Jihlava	Czech Republic	[12]	GQ383781			GQ383861
Dai2352	Populus sp.	n/a	China	[9]	AY340063			
89-826c	Populus tremula	n/a	Estonia	[7]		AF311042		
Dai-Pt	n/a	n/a	China	Unpublished		HQ328536		
		I	Phellinus tuberculosi	IS				
TN-449 (ATCC 38666)	Prunus domestica	Nauvo	Finland	This study	KU139142	KU139254	KU139263	KU139352
TN-236 (ATCC 38665)	Prunus insititia	Tammisaari	Finland	This study	KU139141	KU139253	KU139262	KU139351
MJ 47/07	Prunus domestica	Havlíčkův Brod	Czech Republic	[12]	GQ383784			GQ383859
MJ 44/07	Prunus spinosa	Havlíčkův Brod	Czech Republic	[12]	GQ383783			GQ383858
			Phellinus NA1					
OKM-4173	Populus tremuloides	ID	USA	This study	KU139180	KU139249	KU139304	KU139332
			Phellinus NA2					
SRM-158-Sp	Prunus americana	NE	USA	This study	KU139140	KU139210	KU139267	KU139353
FP-103293-R	Prunus angustifolia	SC	USA	This study	KU139138	KU139208	KU139268	KU139355
FP-105670-R	Prunus sp.	GA	USA	This study	KU139139	KU139209	KU139269	KU139354

	II		C	D.C.		GenBank A	ccession Nos.	
Species & Code	Host	State/Province	Country	Reference	ITS	nLSU	rpb2	tef1
			Inonotus vaninii					
DMR-95-1-T	Populus tremuloides	MN	USA	This study	KU139198	KU139258	KU139318	KU139380
DLL2010-102	Populus tremuloides	MN	USA	[57]	KU139197			
			Inocutis dryophila					
DLL2012-001	Quercus alba	WI	USA	This study	KU139186	KU139255	KU139317	
		Pi	hellinopsis conchata					
DLL2009-149	Acer sp.	MN	USA	[57]	KU139187	KU139256	KU139316	KU139378
L-7601	Fraxinus nigra	NY	USA	This study	KU139188	KU139257	KU139315	KU139377
89-1014	Salix sp.	—	Germany	[7]		AF311028		
			Fuscoporia ferrea					
FP-133592-Sp	Alnus sp.	OR	USA	This study	KU139189	KU139259	KU139319	KU139379
DLL2009-035	Populus tremuloides	MN	USA	[57]	KU139190			
			Fuscoporia gilva					
DLL2011-109	Acer saccharum	WI	USA	[36]	KU139195			
DLL2011-147	Acer saccharum	WI	USA	[36]	KU139196			
			Fuscoporia sp. 1					
DLL2009-025	Populus tremuloides	MN	USA	[57]	KU139193			
DLL2011-211	Prunus serotina	WI	USA	[36]	KU139194			
			Fuscoporia sp. 2					
DLL2011-140	Acer saccharum	WI	USA	[36]	KU139192			
DLL2011-256	Ostrya virginiana	WI	USA	[36]	KU139191			
		Ì	Fomes fomentarius					
NJB2011-KD3	Fagus grandifolia	MA	USA	This study	KU139199	KU139260	KU139320	KU139382
NJB2011-GR2	Populus grandidentata	MA	USA	This study		KU139261	KU139321	KU139381

<sup>(1)</sup> Type specimen.

ML analyses were carried out in MEGA v. 6.06 [37] using the GTR+I+G substitution model with the subtree-pruning-regrafting (SPR level 5) heuristic search method and the branch swap filter set to "moderate". The gamma shape parameter value was estimated directly from the data within MEGA. Significance was assessed over 1000 bootstrap replicates and clades with BS values >70% were considered significant. BI of phylogenies was determined using MrBayes 3.2.4 [38]. The analyses were performed using the GTR substitution model with gamma distributed rate variation and a proportion of invariable sites (INVGAMMA). Posterior probability distributions were obtained with two independent runs of Markov Chain Monte Carlo (MCMC), each with four chains (three heated and one cold) for 10,000,000 generations and a sampling frequency every 1000th generation. Posterior probability (PP) values were calculated after excluding 25% (burn-in) of the trees produced during the MCMC analysis. Estimated Sample Size (ESS) values for the parameters were then assessed in Tracer v. 1.6.0 (http://tree.bio.ed.ac.uk/software/tracer/) to assure convergence. Sufficient sampling was accomplished if ESS values >200. Trees sampled after convergence of the two runs (when average standard deviation of split frequencies <0.01 and average potential scale reduction factor for parameter values >1) were used to reconstruct a 50% majority rule consensus tree and obtain PP values. Trees were viewed in FigTree v. 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/) and clades supported by PP values ≥0.95 were considered significant.

The disparity index (*I*<sub>D</sub>) test of pattern heterogeneity [39] was performed to determine if significant differences in substitution rates existed among the chosen loci. Only the isolates for which all datasets were available (ITS, nLSU, *tef1* and *rpb2*) were used. The test was performed using 1000 Monte-Carlo replications with significance assessed at p = 0.05. Pairwise analysis of all sequences in each dataset determined that two sets of loci evolved with similar substitution rates: (i) ITS and *tef1*; and (ii) nLSU and *rpb2*. However, phylogenetic reconstructions carried out separately for each dataset highlighted significant differences in tree topology (BS values  $\geq$ 70% and PP values  $\geq$ 0.95 at the nodes; see Results). Therefore, none of the individual datasets were concatenated for further phylogenetic analysis.

## 3. Results

## 3.1. Phylogenetic Analysis and Species Identification

Successful amplification and sequencing was achieved for nearly all isolates used in this study. For certain isolates representing segregate genera in *Phellinus* s.l., only ITS sequences were generated (Table 1). Based on the results of the analyses performed here, 13 phylogenetic species in *Phellinus* s.s. were differentiated from isolates that originated from northern North America, Europe and Asia (Table 1; Figures 1–4). Of these 13 species, eight are present in North America, and include: *P. alni*, *P. arctostaphyli*, *P. betulinus*, *P. lundellii*, *P. nigricans*, *P. tremulae* and two undescribed species, hereafter referred to as *P.* NA1 and *P.* NA2 (Figures 1–4).



**Figure 1.** Maximum likelihood tree based on rDNA ITS sequences of *Phellinus* s.s. species. Bootstrap support values  $\geq$ 70% and PP values  $\geq$ 0.95 are shown next to the nodes. Thickened branches represent BS/PP values  $\geq$ 99%/1. Intraspecific variation by geographic origin is defined by the following codes: NA, North America; EU, Europe; AS, Asia; NA-NC, north-central North America; NA-AK, Alaska; NA-W, western North America.



**Figure 2.** Maximum likelihood tree based on nLSU sequences of *Phellinus* s.s. species. Bootstrap support values  $\geq$ 70% and PP values  $\geq$ 0.95 are shown next to the nodes. Thickened branches represent BS/PP values  $\geq$ 99%/1.



**Figure 3.** Maximum likelihood tree based on *rpb2* sequences of *Phellinus* s.s. species. Bootstrap support values  $\geq$ 70% and PP values  $\geq$ 0.95 are shown next to the nodes. Thickened branches represent BS/PP values  $\geq$ 99%/1.



**Figure 4.** Maximum likelihood tree based on *tef1* sequences of *Phellinus* s.s. species. Bootstrap support values  $\geq$ 70% and PP values  $\geq$ 0.95 are shown next to the nodes. Thickened branches represent BS/PP values  $\geq$ 99%/1.

The results demonstrate that three species suspected of having a Holarctic distribution are, based on this dataset, restricted to Europe and Asia with a sister species present in North America. Specifically, *P. laevigatus* s.s., *P. populicola* and *P. tuberculosus* were not detected from North America (Table 1; Figures 1–4). In their place, *P. betulinus*, *P.* NA1 and *P.* NA2 occur with the same host preference: *Betula, Populus* and *Prunus* (Table 1; Figures 1–4). Based on the *tef1* dataset, *P. laevigatus* s.l. is composed of three unique phylogenetic species (*P. betulinus*, *P. orienticus* and *P. laevigatus* s.s.) (Figure 4). The remaining three loci were unable to fully discriminate the *P. laevigatus* s.l. complex, although the ITS dataset did distinguish *P. orienticus* as distinct from *P. betulinus* and *P. laevigatus* s.s. (Figures 1–3). Finally, *P. igniarius* s.s. was not detected from the North American dataset, although one of its primary hosts in Europe (*Salix*) was not analyzed here. However, a closely-related species, *P.* NA1, was discovered but is represented by only a single isolate (Figures 1–4).

Overall, the four loci used produced similar tree topologies with one significant difference. Trees reconstructed using the ITS, nLSU and *rpb2* datasets all grouped isolates representing the *P. laevigatus* s.l. complex distal to all other *Phellinus* s.s. species (Figures 1–3). Only the *tef1* dataset was not in agreement (Figure 4), grouping *P. laevigatus* s.l. in a superclade with *P. tremulae*, *P. NA2*, *P. tuberculosus* and *P. arctostaphyli*. Additionally, all four loci demonstrated a close relationship between two sets of species: (i) *P. tremulae* and *P. NA2* and; (ii) *P. tuberculosus* and *P. arctostaphyli*. These four species consistently grouped together regardless of locus or analysis method (Figures 1–4).

#### 3.2. Phylogenetic Relationships by Locus

The four loci used in this study (ITS, nLSU, *rpb2* and *tef1*) had varying levels of utility in discriminating *Phellinus* s.s. species. The ITS dataset was the most extensive in the number of sequences available from different geographic regions and hosts (Table 1; Figure 1). While ITS sequences demonstrated an ability to differentiate most *Phellinus* s.s. species, this dataset also exhibited high intraspecific variation. For example, isolates representing *P. alni*, *P. nigricans* and *P. igniarius* s.s. were divided into subclades with significant statistical support based on their geographic origin (Figure 1). In other cases, ITS provided only limited utility in differentiating several closely-related species groups, such as *P. igniarius* s.s.-*P. populicola-P.* NA1 and *P. tremulae-P.* NA2 (Figure 1). The ITS dataset was able to distinguish *P. orienticus* as a unique phylogenetic species from the *P. laevigatus* s.l. group but could not differentiate between *P. betulinus* and *P. laevigatus* s.s. (Figure 1).

Much like the ITS dataset, the 5' region of the nLSU is able to differentiate among most *Phellinus* species, but support for some clades was weak with both ML and BI (Figure 2). As expected, there was limited intraspecific variation in the nLSU compared to the ITS region, but closely-related species are once again undifferentiated (Figure 2). Sequences from domains six to seven in the *rpb2* gene comprised the lowest number of total sequences due to the lack of additional data in public databases (Figure 3). This dataset provided higher BS and PP values compared to the ITS and nLSU datasets but again, lacked resolution among closely-related species (Figure 3).

The *tef1* dataset was superior to all other loci used in this study (Figure 4). Nearly all of the species analyzed were supported with significant BS and PP values with most supported with very high confidence (BS  $\geq$  99% and PP = 1). In conjunction with sequences generated by Tomšovský *et al.* [12], *tef1* grouped *Phellinus* s.s. species into two main superclades (Figure 4). The *tef1* dataset was also able to

discriminate members among the closely-related *P. laevigatus* s.l. complex (*P. betulinus*, *P. laevigatus* s.s. and *P. orienticus*) with strong statistical support (Figure 4). Despite the high overall resolution, this dataset, like all others, failed to differentiate *P. igniarius* s.s., *P. populicola* and *P.* NA1 with consistent, significant support (Figure 4).

#### 3.3. Phylogenetic Relationships by Host

The results show that most *Phellinus* s.s. species primarily infect a single host genus (Table 2). Five species (*P. betulinus*, *P. laevigatus* s.s., *P. orienticus*, *P. lundellii* and *P. nigricans*) are specific to *Betula* and three (*P. betulinus*, *P. lundellii* and *P. nigricans*) have overlapping host ranges in North America. *Prunus* has two associated species (*P. tuberculosus* and *P.* NA2) while *Populus* has three (*P. populicola*, *P. tremulae* and *P.* NA1). Lastly, *P. arctostaphyli* is known only from the woody shrub genus *Arctostaphylos*. Two remaining *Phellinus* species, meanwhile, are notable exceptions to this trend: (i) *P. alni*, which has a broad host range that includes *Acer*, *Alnus*, *Betula* and *Fagus* and in this particular study; and (ii) *P. igniarius* s.s., which is still unconfirmed from North America.

Phellinus Species	Known Range	Known Host Genera	Known Host Families		
<b>^</b>		Acer, Alnus, Betula, Carpinus,	Betulaceae, Fagaceae,		
		Corylus, Fagus, Fraxinus,	Juglandaceae, Oleaceae,		
P. alni	Holarctic	Juglans, Laburnum, Malus,	Rosaceae, Sapindaceae		
		Padus, Prunus and Sorbus	& Ulmaceae		
P. lundellii	Holarctic	Betula	Betulaceae		
P. nigricans	Holarctic	Betula	Betulaceae		
P. tremulae	Holarctic	Populus	Salicaceae		
P. arctostaphyli	North America	Arctostaphylos	Ericaceae		
P. betulinus	North America	Betula	Betulaceae		
<i>P</i> . NA1	North America	Populus	Salicaceae		
<i>P</i> . NA2	North America	Prunus	Rosaceae		
P. igniarius s.s.	Europe	Populus, Salix and Malus	Salicaceae and Rosaceae		
P. populicola	Europe & Asia	Populus	Salicaceae		
P. tuberculosus	Europe & Asia	Prunus	Rosaceae		
P. laevigatus s.s.	Europe & West Asia	Betula	Betulaceae		
P. orienticus	East Asia	Betula	Betulaceae		

Table 2. Known range, host genera and host families of *Phellinus* s.s. species.

#### 4. Discussion

The primary objective of this study was to better understand the assemblage of *Phellinus* s.s. species present in North America using a multilocus dataset composed of isolates representing a broad geographic area. As hypothesized, at least eight species of *Phellinus* s.s. occur in North America, yet the specific species assemblage was not accurately predicted. Up to this point, nearly all of the work aimed at elucidating the species in *Phellinus* s.s. has been performed in Europe, albeit with some isolates originating from North America [4,6–9,12,40]. While some *Phellinus* s.s. species have Holarctic distributions, the results of this study demonstrate clear differences in the North American assemblage that were not previously known.

In North America, P. alni has the widest host range of any Phellinus s.s. species, occurring on Acer, Alnus, Betula and Fagus. Including its known host range in Europe, P. alni inhabits at least 14 host genera, including Fraxinus, Juglans, Prunus and Ulmus [6,12]. This broad host range is unique within the genus, as most members of *Phellinus* s.s. exhibit a very narrow host range (see Table 2). Considering this, P. alni is likely the most common species found in northern hardwood (Acer-Betula-Fagus), northern floodplain (Fraxinus-Populus-Ulmus) and coastal Alnus forests in North America. It is most likely widespread in *Quercus*-dominated forests as well, since many of the minor tree species in the Ouercus forest types are hosts for P. alni [41]. Phylogenetically, all four loci grouped P. alni with P. lundellii, P. igniarius s.s., P. NA1, P. nigricans and P. populicola. Of this group, P. igniarius s.s., P. NA1 and *P. populicola* are known only from woody plants in the Salicaceae and Rosaceae. Interestingly, both Fischer [6] and Tomšovský et al. [12] found that P. alni does not infect members of the Salicaceae in Europe and this trend was observed in North America as well. Yet, Populus was the only member of Salicaceae examined in this study and more targeted sampling of *Salix* and *Populus* species is necessary to conclude if *P. alni* can attack these hosts. Additionally, this targeted sampling will help to conclude if *P. igniarius* s.s. and *P. populicola* are indeed present in North America. At present, these two species are known only from Europe and Asia. Attempts to utilize "Phellinus" specimens from the CFMR herbarium that were collected from *Salix* proved these isolates belong to segregate genera such as Fuscoporia (results not shown).

Based on variations in morphological characters, Parmasto [40] proposed that *P. laevigatus* s.l. is composed of two species: *P. laevigatus* s.s. from Europe and *P. betulinus* from North America and Asia. Furthermore, Parmasto believed that *P. betulinus* can be further divided into two subspecies: ssp. *betulinus* from North America and ssp. *orienticus* from Asia. The results presented here show that *P. laevigatus* s.s., *P. betulinus* and *P. orienticus* each represent a unique phylogenetic species. While this assertion is supported by only one dataset (*tef*1), prior studies have shown *tef*1 to be the only marker capable of discriminating between very closely-related species in basidiomycete genera like *Armillaria* and *Trametes* [42–44]. Additionally, the ITS dataset was able to discriminate *P. orienticus* from *P. betulinus*-P. *laevigatus* s.s. with a high level of support (BS/PP = 99/1). Thus, the results presented here support the elevation of *P. orienticus* and *P. betulinus* to species status. *Phellinus orienticus* has been previously treated as a distinct species based solely on morphological characters [45]. Additionally, Wagner and Fisher [8] treated *P. orienticus* as a unique species in their study to deconstruct *Phellinus* s.l. using nLSU sequences. In this study, nLSU sequences were unable to discriminate among members of *P. laevigatus* s.l.

Isolates collected from *Prunus* in North America that were thought to represent *P. tuberculosus* were instead shown to be a separate phylogenetic species, referred to here as *P.* NA2. What is perhaps more interesting is that each locus examined in this study showed that *P.* NA2 is more closely-related to *P. tremulae* than to *P. tuberculosus*. Meanwhile, the results also reveal a close genetic relationship between *P. tuberculosus* and *P. arctostaphyli*. Regardless of its closest relative, the results suggest that *P. tuberculosus* is restricted to Europe and Asia and that *P.* NA2 requires formal description as a new North American species. Overholts [22] (pp. 64–65) did note that specimens of *P. tuberculosus* from Europe had larger basidiospores compared to specimens from North America and that: "The American plant should perhaps be designated as a distinct variety". Basidiospore variation between *P.* NA2 and *P. tuberculosus* was also confirmed by Niemelä [46] and Gilbertson and Ryvarden [5].

The first recognized description of *Phellinus* from *Prunus* was performed by Baumgarten, who described the species as *Boletus tuberculosus* [47]. This name was soon forgotten though, in favor of the epithet *B. pomaceus*, which appeared in the literature less than a decade later [48]. Both of these early descriptions utilized European strains of the pathogen. Numerous, subsequent descriptions of this species were made in North America and Europe, all using *Phellinus pomaceus* [5,22,46,49,50] until it was determined to be a synonym of *P. tuberculosus*, with the latter having precedence [51]. After this point, Riffle and Conway [52] adopted the use of *P. tuberculosus* while Ryvarden and Gilbertson [53] preferred use of the well-established *P. pomaceus*. Phylogenetic studies of *Phellinus* s.l. have consistently used *P. tuberculosus* over *P. pomaceus* [7,8,10,12], but the use of *P. pomaceus* still continues, albeit infrequently [54,55]. Adding to the confusion is the fact that *P. alni* can also be found on *Prunus* [6] along with *Fomitiporia prunicola* [56].

One of the most fascinating aspects of *Phellinus* s.s. is that five species are known to occur primarily on *Betula* (*P. betulinus*, *P. laevigatus* s.s., *P. lundellii*, *P. nigricans*, and *P. orienticus*). Three of these species (*P. betulinus*, *P. lundellii* and *P. nigricans*) are present in North American forests and have overlapping host ranges. Not only do these three species occupy the same geographic area but they appear to occupy the same ecological niche, with no apparent preference for a particular species of *Betula*. Clearly, these species represent an interesting model for studies on sympatric speciation. At the same time, *P. nigricans* and *P. lundellii* were somewhat partitioned in their distribution in North America, with *P. nigricans* present in western and north-central North America while *P. lundellii* was identified from north-central and eastern North America. Because their ranges do overlap in the Lake States region and *Betula* species occur contiguously across northern North America, it's most likely both species are present across the continent as well.

A single isolate collected from Idaho in 1966 by Orson Miller, and presumed to be *P. tremulae*, represents a sister species to *P. igniarius* s.s. and *P. populicola*. This species, *P.* NA1, remains undescribed here pending further study on its distribution and host range. While white trunk rot of aspen is primarily the result of infection by *P. tremulae*, this study shows that *P.* NA1 must be considered as an additional causal agent. Further, *Inonotus vaninii* (formerly *Phellinus vaninii*) can also be found on *Populus* [57]. Even if *P.* NA1 and *I. vaninii* are uncommon on *Populus* compared to *P. tremulae*, a complex of *Phellinus* s.l. species should now be considered to cause white trunk rot of aspen.

It is entirely reasonable to believe that *Phellinus* s.s. species described here as host-specific may, at times, be found on non-preferred hosts. Because of decades of confusion and misinterpretation in the taxonomy of *Phellinus* s.s. [13], numerous reports exist of exceptions in the host-specificity reported here and in previous studies [6,12]. Yet, these reports of incidence on non-preferred hosts should be carefully scrutinized as future studies shed further light on the incidence and host range of *Phellinus* s.s. across northern temperate forests.

#### Acknowledgments

I am grateful to Daniel Lindner and Tuomo Niemelä for their assistance in obtaining isolates used in this study. Funding was provided, in part, by the USDA Forest Service, Center for Forest Mycology Research.

# **Conflicts of Interest**

The author declares no conflict of interest.

# References

- 1. Niemelä, T. On Fennoscandian Polypores. II. *Phellinus laevigatus* (Fr.) Bourd. & Galz. and *P. lundellii* Niemelä, n. sp. *Ann. Bot. Fennici* **1972**, *9*, 41–59.
- 2. Niemelä, T. On Fennoscandian Polypores. III. *Phellinus tremulae* (Bond.) Bond. & Borisov. *Ann. Bot. Fennici* **1974**, *11*, 202–215.
- 3. Niemelä, T. On Fennoscandian Polypores. IV. *Phellinus igniarius*, *P. nigricans* and *P. populicola*, n. sp. *Ann. Bot. Fennici* **1975**, *12*, 93–122.
- 4. Fiasson, J.; Niemelä, T. The Hymenochaetales: A revision of the European poroid taxa. *Karstenia* **1984**, *24*, 14–28.
- 5. Gilbertson, R.L.; Ryvarden, L. North American Polypores; Fungiflora: Oslo, Norway, 1987.
- 6. Fischer, M. Phellinus igniarius and its closest relatives in Europe. Mycol. Res. 1995, 99, 735-744.
- 7. Wagner, T.; Fischer, M. Natural groups and a revised system for the European poroid Hymenochaetales (Basidiomycota) supported by nLSU rDNA sequence data. *Mycol. Res.* 2001, *105*, 773–782.
- 8. Wagner, T.; Fischer, M. Proceedings towards a natural classification of the worldwide taxa *Phellinus* s.l. and *Inonotus* s.l., and phylogenetic relationships of allied genera. *Mycologia* **2002**, *94*, 998–1016.
- Fischer, M.; Binder, M. Species recognition, geographic distribution and host-pathogen relationships: A case study in a group of lignicolous basidiomycetes, *Phellinus* s.l. *Mycologia* 2004, 96, 799–811.
- Larsson, K.H.; Parmasto, E.; Fischer, M.; Langer, E.; Nakasone, K.K.; Redhead, S.A. Hymenochaetales: A molecular phylogeny for the hymenochaetoid clade. *Mycologia* 2006, 98, 926–936.
- 11. Sell, I. Taxonomy of the species in the Phellinus igniarius group. Mycotaxon 2008, 104, 337-347.
- Tomšovský, M.; Vampola, P.; Sedlák, P.; Byrtusová, Z.; Jankovský, L. Delimitation of central and northern European species of the *Phellinus igniarius* group (Basidiomycota, Hymenochaetales) based on analysis of ITS and translation elongation factor 1 alpha DNA sequences. *Mycol. Prog.* 2010, 9, 431–445.
- 13. Larsen, M.J.; Cobb-Poulle, L.A. *Phellinus (Hymenochaetaceae): A Survey of the World Taxa*; Fungiflora: Oslo, Norway, 1990.
- 14. Ohman, J.H.; Kessler, K.J. *White Trunk Rot of Hardwoods*; USDA Forest Service: Fort Collins, CO, USA, 1964.
- 15. Boyce, J.S. Forest Pathology, 3rd ed.; John Wiley and Sons Inc.: New York, NY, USA, 1961.
- 16. Sinclair, W.A.; Lyon, H.H. *Diseases of Trees and Shrubs*, 2nd ed.; Cornell University Press: Ithaca, NY, USA, 2005; pp. 306–308.
- 17. Silverborg, S. *Northern Hardwoods Cull Manual*; Bulletin No. 31; State University of New York: Syracuse, NY, USA, 1954; pp. 4–5.

- 18. Bondartsev, A.S. *The Polyporaceae of the European USSR and Caucasia*; Israel Program for Scientific Translations: Jerusalem, Israel, 1953; p. 358.
- 19. Jones, A.C.; Ostry, M.E. Estimating white trunk rot in aspen stands. *North. J. Appl. For.* **1998**, *15*, 33–36.
- 20. Worrall, J.J.; Fairweather, M.L. *Decay and Discoloration of Aspen*; USDA Forest Service: Fort Collins, CO, USA, 2009.
- 21. Verrall, A.F. *Variation in Fomes igniarius (L.) Gill*; Minnesota Agricultural Experiment Station: MN, USA, 1937.
- 22. Overholts, L.O. *The Polyporaceae of the United States, Alaska, and Canada*; University of Michigan Press: Ann Arbor, MI, USA, 1953.
- 23. Worrall, J.J.; Lee, T.D.; Harrington, T.C. Forest dynamics and agents that initiate and expand canopy gaps in *Picea—Abies* forests of Crawford Notch, New Hampshire, USA. *J. Ecol.* **2005**, *93*, 178–190.
- 24. Lindner, D.L.; Burdsall, H.H., Jr.; Stanosz, G.R. Species diversity of polyporoid and corticioid fungi in northern hardwood forests with differing management histories. *Mycologia* **2006**, *98*, 195–217.
- 25. Brazee, N.J.; Lindner, D.L. Unraveling the *Phellinus pini* s.l. complex in North America: A multilocus phylogeny and differentiation analysis of *Porodaedalea*. For. Pathol. **2013**, 43, 132–143.
- 26. Gardes, M.; Bruns, T.D. ITS primers with enhanced specificity for basidiomycetes—Application to the identification of mycorrhizae and rusts. *Mol. Ecol.* **1993**, *2*, 113–118.
- 27. Rehner, S.; Samuels, G. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycol. Res.* **1994**, *98*, 625–634.
- Hopple, J.S.; Vilgalys, R. Phylogenetic relationships in the mushroom genus *Coprinus* and dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: Divergent domains, outgroups and monophyly. *Mol. Phylogenet. Evol.* 1999, 13, 1–19.
- 29. Kauserud, H.; Schumacher, T. Outcrossing or inbreeding: DNA markers provide evidence for type of reproductive mode in *Phellinus nigrolimitatus*. *Mycol. Res.* **2001**, *105*, 676–683.
- 30. Matheny, P.B. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; Agaricales). *Mol. Phylogenet. Evol.* **2005**, *35*, 1–20.
- Rehner, S.A.; Buckley, E. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 2005, 97, 84–98.
- 32. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780.
- 33. Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. jModelTest 2: More models, new heuristics and parallel computing. *Nat. Methods* **2012**, *9*, 772.
- 34. Guindon, S.; Gascuel, O. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Syst. Biol.* **2003**, *52*, 696–704.
- 35. Tavaré, S. Some probabilistic and statistical problems on the analysis of DNA sequences. *Lect. Math. Life Sci.* **1986**, *17*, 57–86.
- 36. Brazee, N.J.; Lindner, D.L.; D'Amato, A.W.; Fraver, S.; Forrester, J.A.; Mladenoff, D.J. Disturbance and diversity of wood-inhabiting fungi: Effects of canopy gaps and downed woody debris. *Biodivers. Conserv.* **2014**, *23*, 2155–2172.

- 37. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729.
- Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 2012, *61*, 539–542.
- 39. Kumar, S.; Gadagkar, S.R. Disparity Index: A simple statistic to measure and test the homogeneity of substitution patterns between molecular sequences. *Genetics* **2001**, *158*, 1321–1327.
- 40. Parmasto, E. *Phellinus laevigatus* s. l. (Hymenochaetales): A ring species. *Folia Cryptogam. Estonica* **2007**, *43*, 39–49.
- 41. Eyre, F.H. *Forest Cover Types of the United States and Canada*; Society of American Foresters: Washington, DC, USA, 1980.
- 42. Brazee, N.J.; Hulvey, J.P.; Wick, R.L. Evaluation of partial *tef1*, *rpb2*, and nLSU sequences for identification of isolates representing *Armillaria calvescens* and *Armillaria gallica* from northeastern North America. *Fungal Biol.* **2011**, *115*, 741–749.
- 43. Ross-Davis, A.L.; Hanna, J.W.; Kim, M.-S.; Klopfenstein, N.B. Advances toward DNA-based identification and phylogeny of North American *Armillaria* species using elongation factor-1 alpha gene. *Mycoscience* **2012**, *53*, 161–165.
- 44. Carlson, A.L.; Justo, A.; Hibbett, D.S. Species delimitation in *Trametes*: A comparison of ITS, RPB1, RPB2 and TEF1 gene phylogenies. *Mycologia* **2014**, *106*, 735–745.
- 45. Ming-Xiao, D.; Yu-Lian, W.; Yue, G. Three species of *Phellinus* (Basidiomycota, Hymenochaetaceae) new to China. *Mycosystema* **2012**, *31*, 940–946.
- 46. Niemelä, T. On Fennoscandian polypores 5, Phellinus pomaceus. Karstenia 1977, 17, 77-86.
- 47. Baumgarten, J.C.G. Flora Lipsiensis; Leipzig: Saxony, Germany, 1790; p. 635.
- 48. Persoon, C.H. Observationes Mycologicae; Leipzig: Saxony, Germany, 1799, 2, 5.
- 49. Nobles, M.K. Studies in forest pathology VI. Identification of cultures of wood-rotting fungi. *Can. J. Res.* **1948**, *26*, 281–431.
- 50. Gilbertson, R.L. The genus *Phellinus* (Aphyllophorales: Hymenochaetaceae) in western North America. *Mycotaxon* **1979**, *9*, 51–89.
- 51. Niemelä, T. Taxonomic notes on the polypore genera *Antrodiella*, *Daedaleopsis*, *Fibuloporia* and *Phellinus*. *Karstenia* **1982**, *22*, 11–12.
- Riffle, J.W.; Conway K.E. Phellinus stem decays of hardwoods. In *Diseases of Trees in the Great Plants*; Riffle, J.W., Peterson, G.W., Eds.; USDA Forest Service: Fort Collins, CO, USA, 1986; pp. 83–85.
- 53. Ryvarden, L.; Gilbertson, R.L. European Polypores, part 2; Fungiflora: Oslo, Norway, 1994; p. 507.
- Nam, B.-H.; Lee, J.-Y.; Kim, G.-Y.; Jung H.-H.; Park, H.-S.; Kim, C.-Y.; Jo, W.-S.; Jeong, S.-J.; Lee, T.-H.; Lee, J.-D. Phylogenetic analysis and rapid detection of genus *Phellinus* using the nucleotide sequences of 18S ribosomal RNA. *Mycobiology* 2003, *31*, 133–138.
- 55. Schmidt, O.; Gaiser, O.; Dujesiefken, D. Molecular identification of decay fungi in the wood of urban trees. *Eur. J. For. Res.* **2012**, *131*, 885–891.
- 56. Overholts, L.O. Diagnoses of American polypores—III. Some additional brown species, with a key to the common brown species of the United States and Canada. *Mycologia* **1931**, *23*, 117–129.

57. Brazee, N.J.; Lindner, D.L.; Fraver, S.; D'Amato, A.W.; Milo, A.M. Wood-inhabiting, polyporoid fungi in aspen-dominated forests managed for biomass in the U.S. Lake States. *Fungal Ecol.* **2012**, *5*, 600–609.

 $\bigcirc$  2015 by the author; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/4.0/).