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Phylogenetic relationships among *Porodaedalea pini* from Poland and related *Porodaedalea* species

Research Article

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Abstract: *Porodaedalea pini* has been found to be a pathogen of *Pinus banksiana* (1 specimen) and *Pinus sylvestris* (39 specimens) in north-western Poland. This fungus was initially identified by its host preferences and morphological characters of sporophores and basidiospores. The ITS 1/2 rDNA region was sequenced and analysed using the neighbor-joining, maximum likelihood and maximum parsimony methods. All *P. pini* from Poland, *P. pini* neotype and other *P. pini* isolates from Europe grouped together forming a moderately supported monophyletic clade. The clade included two groups which did not correlate with geographic ranges. Nucleotide polymorphism of the Polish isolates of *P. pini* was small. This study provides evidence for the taxonomy of some isolates of the *Porodaedalea* Holarctic Group in North America: grouping with *P. laricis* or with *P. gilbertsonii* suggests that the isolates belong to these species. The absence of *P. pini* (in a form recognized in Europe) in North America is suggested. Sequencing of the ITS 1/2 rDNA region with the basidiomycete-specific primers (ITS1-F and ITS4-B) proved to be a suitable and sufficient method for differentiation of species within the genus *Porodaedalea.*

Keywords: *ITS 1/2 rDNA • Morphology • Pinus banksiana • Pinus sylvestris • Porodaedalea pini • Sequencing*

1. Introduction

Porodaedalea Murrill (*Phellinus pini* group) is a taxonomically difficult complex of a few morphologically similar species. In Europe, the genus is represented by *P. chrysoloma* (Fr.) Fiasson & Niemelä, *P. laricis* (Jacz. ex Pilát) Niemelä and *P. pini* (Brot.) Murrill [1-4]. *Porodaedalea chrysoloma* occurs on *Picea, P. laricis* on *Larix, Picea* and *Pinus*, and *P. pini* on *Larix* and *Pinus*, mostly in the north-eastern part of Europe. In North America, the genus is currently represented by *P. cancriformans* (M.J. Larsen, Lombard & Aho) T. Wagner & M. Fisch., *P. gilbertsonii* Larsen (= *Phellinus gilbertsonii* M.J. Larsen), *P. piceina* (Peck) Niemelä and *P. pini* s.l. [5-7]. *Porodaedalea cancriformans* occurs on *Abies, P. gilbertsonii* on *Pseudotsuga, P. piceina* on *Picea*, and *P. pini* s.l. on *Abies, Larix, Picea, Pinus, Pseudotsuga* and *Tsuga,* mostly in the northern regions of the USA. In Poland, *P. pini* and *P. chrysoloma* occur mainly in central and north-eastern regions [8,9].

Porodaedalea is one of the most important trunk rot pathogens of conifers. In managed forests, the pathogen is economically significant on pines, mostly in old-growth forests [10-12] although severe damage has also been found in 30–40-year-old stands [13]. Seventy-seven percent of 150–180-year-old *Pinus sylvestris* L. stands in Poland are infected with *Porodaedalea* [14]. The fungus is also of interest from an ecological perspective as a component in the decomposition cycle in temperate forests.

Disease development takes a long time, and external symptoms of infection are undetectable for at least a decade. The first signs of trunk decay occur when bracket-shaped sporophores develop on older trees 10–20 years after infection, often around knotholes or beneath dead branches. These areas of decay then result in tree breakage.

Although *Porodaedalea* is a well-known pathogen, its geographical distribution and genetic variation have rarely been studied in Europe [3,8]. Recognition of *Porodaedalea* species based on phenotypic characters

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is difficult because of the morphological similarity of their sporophores, sporophore morphological transition [15], possible compatibility of species [16] and lack of specific microscopic characters useful for identification. Interspecific hybridization between *P. pini* and *P. laricis* is possible [3] and may be a source of morphological variation observed among *Porodaedalea* sporophores. In addition, recent studies [3] have revealed the presence of an undescribed species within *Porodaedalea*. The complex phylogeographical structure of this group is becoming clearer with the application of molecular techniques [3,17].

The aims of the present study were to elucidate the identification of *Porodaedalea* occurring on pines in Poland using sequences of the ITS1/2 rDNA region and to examine their genetic variation. It was also expected that new information would contribute to a better understanding of the origin and source of phenotypic and genetic variation of *Porodaedalea* in Poland. Additionally, the study was expected to contribute to the identification of some isolates of the *Porodaedalea* Holarctic Group in North America [17] and to confirm the effectiveness of the ITS1/2 rDNA region in distinguishing inter- and intraspecific variation within *Porodaedalea*.

2. Experimental Procedures

2.1 Material studied

Sporophores of *Porodaedalea* from one living *Pinus banksiana* Lamb. and from 39 *P. sylvestris* trees were collected in north-western Poland in 2008-2010, from an area of approximately 100 000 km^2 (Figure 1, Table 1). The sporophores were collected at a height of 5-10 m on the infected tree trunks. Between one and three sporophores were available and collected from each tree.

2.2 Morphological studies

Morphological characters of sporophores, *i.e.* basidiospore dimensions (µm), hymenial setae dimensions (µm), pore widths and pore density (mm^{-1}),

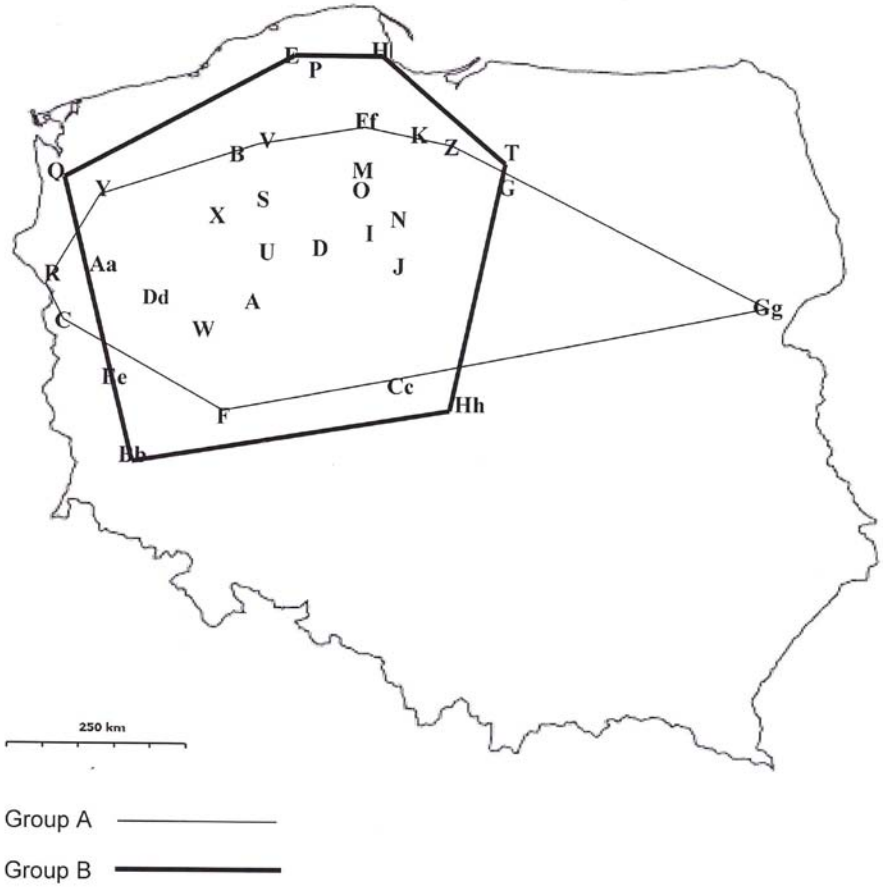

Figure 1. Map of Poland with locations where *P. pini* was collected.

Isolate	Locality	Location on Figure 1	Host tree	Age of tree	GenBank Accession number	Percentage of similarity to FJ775555
4-2010	Zielonka	A	*Pinus sylvestris*	110	HE971100	99
7-2010	Zielonka	A	*P. sylvestris*	110	HE971103	99
8-2010	Szubin	D	*P. sylvestris*	94	HE971105	99
11-2010	Zielonka	A	*P. sylvestris*	110	HE971108	99
14-2010	Czarne Człuchowskie	B	*P. sylvestris*	128	HE971111	99
16-2010	Rzepin	C	*P. sylvestris*	85	HE971114	99
17-2010	Damnica	E	*P. sylvestris*	108	HE971116	100
2-2011	Góra Śląska	F	*P. sylvestris*	96	HE971098	99
4-2011	Lubichowo	H	*P. sylvestris*	105	HE971101	100
5-2011	Żołędowo	I	*P. sylvestris*	64	HE971102	100
7-2011	Gniewkowo	J	*P. sylvestris*	33	HE971104	99
8- 2011	Starogard Gdański	K	*P. sylvestris*	70	HE971106	99
10-2011	Góra Śląska	F	*P. sylvestris*	118	HE971107	99
11-2011	Woziwoda	M	*P. sylvestris*	67	HE971109	99
13-2011	Toruń	N	*P. sylvestris*	90	HE971110	99
14-2011	Tuchola	O	*P. sylvestris*	76	HE971112	99
15-2011	Cewice	P	*P. sylvestris*	115	HE971113	100
16-2011	Góra Śląska	F	*P. sylvestris*	-	HE971115	99
21-2011	Mieszkowice	R	*P. sylvestris*	78	HE971117	99
23-2011	Rudka	Gg	*P. sylvestris*	55	HE971118	99
24-2011	Mieszkowice	R	*P. sylvestris*	128	HE971119	99
25-2011	Lipka	S	*P. sylvestris*	160	HE971120	99
26-2011	Miłomłyn	T	*P. sylvestris*	76	HE971121	99
27-2011	Podanin	U	*P. sylvestris*	110	HE971122	99
28-2011	Gniewkowo	J	*P. sylvestris*	103	HE971123	99
29-2011	Trzebież	Q	*P. sylvestris*	-	HE971124	100
30-2011	Niedźwiady	V	*P. sylvestris*	89	HE971125	99
31-2011	Grodzisk	W	*P. sylvestris*	107	HE971126	99
32-2011	Płytnica	X	*Pinus banksiana*	-	HE971127	99
34-2011	Dobrzany	Y	*P. sylvestris*	90	HE971128	99
36-2011	Poddębice	Hh	*P. sylvestris*	70	HE971129	100
37-2011	Bogdaniec	Aa	*P. sylvestris*	45	HE971130	100
39-2011	Dobrzany	Y	*P. sylvestris*	105	HE971131	100
43-2011	Węgliniec	Bb	*P. sylvestris*	67	HE971132	100
44-2011	Turek	Cc	*P. sylvestris*	92	HE971133	99
45-2011	Międzychód	Dd	*P. sylvestris*	98	HE971134	99
46-2011	Kościerzyna	Ff	*P. sylvestris*	135	HE971135	99
47-2011	Lidzbark	G	*P. sylvestris*	82	HE971136	99
51-2011	Krzystkowice	Ee	*P. sylvestris*	109	HE971137	99
52-2011	Kwidzyn	Z	*P. sylvestris*	68	HE971138	99

Table 1. *Porodaedalea pini* specimens from Poland.

were evaluated microscopically from fragments of fresh sporophores mounted in Melzer's reagent, under a Zeiss Imager A.1 light microscope with ×1000 magnification and under a Zeiss Stemi 2000 stereomicroscope. Measurements were taken with Quickphoto Micro 2.3 software (Olympus). Ten to 30 basidiospores, setae and pores from 1-3 sporophores from each tree were measured. Significance of differences in dimensions of basidiospores and setae from different isolates was determined by one-way ANOVA with pair-wise comparison of means (Tukey's HSD test) using SPSS software.

2.3 DNA extraction, PCR and sequencing

Axenic, dikaryotic (n + n) cultures were obtained from the fresh sporophore context material taken from above the hymenium with a sterile scalpel and plated on Goldfarb's selective medium (malt agar 15 g L^{-1}, prochloraz 1 mg L^{-1}, benomyl 1 mg L^{-1}, thiobendazole 1 mg L^{-1}, streptomycin 1 mg L^{-1}, rose Bengal 1 mg L^{-1}). Fungi were incubated at 24°C in darkness. After 7-14 d they were transferred to 1.5% malt medium (malt extract 15 g L^{-1}, peptone 5 g L^{-1}, agar 15 g L^{-1}, Difco) and maintained at 4°C in darkness.

For DNA extraction, axenic cultures were grown in L-broth (sodium chloride 10 g L^{-1}, tryptone 10 g L^{-1}, yeast extract 5 g L^{-1}) at 25°C for 10 d. Mycelium from each isolate was lyophilized and frozen at -80°C. The total DNA was extracted [18] and purified with Sure Clean (Bioline Ltd, London, Cat. no. BIO37042). PCR amplification of the ITS 1/2 rDNA was done with DNA diluted (10^{-2}) in deionized water. Primers used were ITS1-F (5'CTT GGT CAT TTA GAG GAA GTA A) and ITS4-B (5' CAGGAGACTTGTACACGGTCCAG). The two primers are specific to fungi and the Basidiomycota, respectively [19]. Each 25 µl PCR mixture consisted of 0.2 µmol L^{-1} of each primer, 0.25 U of Taq polymerase (MBI Fermentas, St. Leon-Rot, Germany), buffer (10 mmol L^{-1} Tris–HCl pH 8.8, 50 mmol L^{-1} KCl, 0.08% Nonidet P–40, 0.1 mg ml^{-1} BSA, 1.5 mmol L^{-1} $MgCl_2$), 0.2 mmol L^{-1} deoxyribonucleoside triphosphates (dNTPs) and 2 µl of DNA. PCR conditions included an initial denaturation step at 94°C for 10 min, followed by 30 cycles of 94°C for 30 s, 42°C for 1 min and 72°C for 2 min. This was followed by a final extension of 72°C for 10 min [19]. The amplified fragments were sequenced at the Centre of DNA Studies in Poznań, Poland. Sequences were queried against the GenBank database using BLAST.

2.4 Sequence alignment and phylogenetic analysis

Representatives (11-2010, 15-2011, 44-2011) from three groups of identical sequences of *Porodaedalea* were used for alignment. These were automatically aligned with sequences of other *Porodaedalea* species (Table 2) using ClustalW [20]. Alignment of all isolates was accomplished for the entire 517-nucleotide base-pair length of the ITS1/2 rDNA region. Gaps and missing data were excluded from the analysis. Phylogenetic analyses were performed by the neighbor-joining (NJ), maximum likelihood (ML) and maximum parsimony (MS) methods. The neighbor-joining analysis utilized an input distance matrix specifying the distance between each pair of isolates. The maximum likelihood analysis was performed with the Tamura-Nei model using the heuristic search procedure (Nearest-Neighbour-Interchange, NNI) with an initial tree generated automatically by applying NJ and BJONJ algorithms. Maximum parsimony analysis was performed using a heuristic search procedure (Subtree-Pruning-Regrafting (SPR)) with the widest search level (level 3) with 10 replications in the Random Additions method for the initial tree. The percentages of replicate trees in which associated taxa clustered as determined by bootstrap analysis (1000 replicates) were indicated at the nodes on the phylogram [21]. Bootstrap support (BS) values >60% were considered significant in this study. Similar bootstrap support values (>50 and >70%) were considered significant in other studies on recognition of *Porodaedalea* species [3,17]. The evolutionary distances were computed using the maximum composite likelihood method and were expressed as number of base substitutions per site. Phylogenetic analysis was performed using MEGA 5.0 [22]. *Onnia tomentosa* (Fr.) P. Karst. was chosen as an outgroup based on previous studies [17,23]. The sequences obtained from our studies have been deposited in GenBank.

3. Results

3.1 Morphological studies

Host range (*P. banksiania* and *P. pini*) and the following morphological characters suggest that all 40 specimens studied belong to *P. pini*: morphological characters of sporophores (perennial, effused-reflexed to pileate, solitary to imbricate, corky to woody, rust brown to dark grey on the upper surface, with the poroid surface ochre brown or rust brown to umber brown, and more or less shining), shape of pores (circular to angular, tending to split and becoming irregular to daedaleoid), abundant occurrence of setae in hymenophore, dimitic hyphal system, shape and size of basidiospores (subglobose, smooth, mean dimensions 5.25–6.25 × 4.12–5.30 µm), and shape and size of setae (straight, pointed, thick-walled, dark brown in KOH, mean dimensions

Species & isolate code	Host	State/ Province	Country	Collector	ITS	Depositor of ITS sequence
Porodaedalea cancriformans						
1-Sp	*Abies concolor*	California	USA	L.R. Carpenter	JX110042	N. J. Brazee & D.L. Lindner[5]
FP-133112-R	*Abies magnifica*	Oregon	USA	M.J. Larsen	JX110043	N. J. Brazee & D.L. Lindner
***Porodaedalea chrysoloma* s.s.**						
FP-102121-T	*Picea abies*	Sumava	Czech Republic	A. Cerny	JX110031	N. J. Brazee & D.L. Lindner
	Picea abies	Uppsala	Sweden	J. Stenlid & M. Larsen	AF123440	J. Stenlid[6]
FP-135952	*Picea abies*	Uppsala	Sweden	J. Stenlid	JX110033	N. J. Brazee & D.L. Lindner
NJB2011-Fin1	*Picea abies*	Uusimaa	Finland	N.J. Brazee & T. Niemelä	JX110034	N. J. Brazee & D.L. Lindner
Porodaedalea gilbertsonii						
H7002004	*Pseudotsuga menziesii*	West	USA	M.J. Larsen	FJ775560	M. Tomšovský, P. Sedlák & L. Jankovský[7]
Porodaedalea laricis						
H7002007	*Larix* sp.	Bashkortostan	Russia	M. Tomšovský, P. Sedlák & L. Jankovský	FJ775562	M. Tomšovský, P. Sedlák & L. Jankovský
UPS F-120580	*Larix* sp.	Komi Republic	Russia		FJ775563	M. Tomšovský, P. Sedlák & L. Jankovský
CCBAS 735	*Pinus cembra*	NP High Tatras[4]	Slovakia	M. Tomšovský, P. Sedlák & L. Jankovský	FJ775565	M. Tomšovský, P. Sedlák & L. Jankovský
PRM 911471	*Pinus mugo*	NP Bohemian Forest	Czech Republic	M. Tomšovský, P. Sedlák & L. Jankovský	FJ775566	M. Tomšovský, P. Sedlák & L. Jankovský
PRM 892094	*Larix decidua*	Pelvoux	France	M. Tomšovský, P. Sedlák & L. Jankovský	FJ775567	M. Tomšovský, P. Sedlák & L. Jankovský
***Porodaedalea pini* s.s.**						
BRNM 712793	*Pinus sylvestris*	NP Curonian Spit	Lithuania	M. Tomšovský, P. Sedlák & L. Jankovský	FJ775552	M. Tomšovský, P. Sedlák & L. Jankovský
BRNM 712787	*Larix decidua*	NP Podyjí	Czech Republic	M. Tomšovský, P. Sedlák & L. Jankovský	FJ775553	M. Tomšovský, P. Sedlák & L. Jankovský
BRNM 712792	*Pinus halepensis*	Isle of Korcula	Croatia	M. Tomšovský, P. Sedlák & L. Jankovský	FJ775554	M. Tomšovský, P. Sedlák & L. Jankovský
BRNM 712785	*Pinus sylvestris*	NP Podyjí	Czech Republic	M. Tomšovský, P. Sedlák & L. Jankovský	FJ775555	M. Tomšovský, P. Sedlák & L. Jankovský
BRNM 712791	*Pinus sylvestris*	Uppsala	Sweden		FJ775556	M. Tomšovský, P. Sedlák & L. Jankovský

Table 2. *Porodaedalea* isolate description and GenBank accession numbers for *Porodaedalea* included in phylogenetic analysis.

[1]Neotype isolate.
[2]Initially presumed to represent P. pini s.l.
[3]Initially presumed to represent P. laricis.
[4]NP – refers to National Park.
[5]N. J. Brazee, D.L. Lindner - UMass Extension, Center for Agriculture, University of Massachusetts, 101 University Drive, Suite A7, Amherst, MA 01002, USA.
[6]J. Stenlid – Department of Forest Mycology & Pathology, SLU, Box 7026, S-750 07 Uppsala, Sweden.
[7]M. Tomsovsky, P. Sedlak, L. Jankovsky - Faculty of Forestry and Wood Technology, Mendel University of Agriculture and Forestry in Brno, Zemedelska 3, Brno 61300, Czech Republic.

Species & isolate code	Host	State/ Province	Country	Collector	ITS	Depositor of ITS sequence
TFC 1971-6	*Pinus sylvestris*	Tartu county	Estonia	M. Tomšovský, P. Sedlák & L. Jankovský	FJ775557	M. Tomšovský, P. Sedlák & L. Jankovský
BRNM 712786	*Pinus rotundata*	Borkovická blata	Czech Republic	M. Tomšovský, P. Sedlák & L. Jankovský	FJ775558	M. Tomšovský, P. Sedlák & L. Jankovský
74-64/2	*Pinus sylvestris*	Svealand	Sweden	F. Roll-Hansen	JX110035	N. J. Brazee & D.L. Lindner
FP-102122-T	*Pinus pallasiana*	Crimea	Ukraine	A. Cerny	JX110036	N. J. Brazee & D.L. Lindner
No-6170-T[1]	*Pinus pinaster*	Lisbon	Portugal	I. Melo & J. Cardoso	JX110037	N. J. Brazee & D.L. Lindner
Porodaedalea yamanoi						
TFC 1971-24	*Picea* sp.		Russia		FJ775551	M. Tomšovský, P. Sedlák & L. Jankovský
***Porodaedalea* sp. 1**						
FP-103366-T[2]	*Pinus virginiana*	Georgia	USA	A.S. Rhoads	JX110038	N. J. Brazee & D.L. Lindner
FP-71757[2]	*Pinus virginiana*	Virginia	USA	N.E. Hawes	JX110039	N. J. Brazee & D.L. Lindner
***Porodaedalea* sp. 2**						
AZ-10-T[2]	*Pinus strobiformis*	Arizona	USA	D. Rizzo	JX110040	N. J. Brazee & D.L. Lindner
AZ-14-T[2]	*Pinus strobiformis*	Arizona	USA	D. Rizzo & Berhandt	JX110041	N. J. Brazee & D.L. Lindner
***Porodaedalea* sp.**						
PRM 876397	*Cedrus atlantica*		Marocco		FJ775550	M. Tomšovský, P. Sedlák & L. Jankovský
***Porodaedalea* Holarctic Group**						
1470/5[3]	*Pinus sylvestris*	Akershus	Norway	Unknown	JX110044	N. J. Brazee & D.L. Lindner
Colo-247-R[2]	*Pinus contorta*	Colorado	USA	R.W. Davidson	JX110046	N. J. Brazee & D.L. Lindner
FP-104211-R[2]	*Pinus lambertiana*	California	USA	R.W. Davidson	JX110048	N. J. Brazee & D.L. Lindner
FP-135418-T	*Pseudotsuga menziesii*	Idaho	USA	M.J. Larsen	JX110054	N. J. Brazee & D.L. Lindner
FP-135945-T	*Pseudotsuga menziesii*	California	USA	M.J. Larsen	JX110057	N. J. Brazee & D.L. Lindner
FP-59059-T[2]	*Pinus strobus*	Virginia	USA	R.W. Davidson	JX110058	N. J. Brazee & D.L. Lindner
FP-71681-T[2]	*Larix laricina*	Minnesota	USA	C.C. Christensen	JX110060	N. J. Brazee & D.L. Lindner
NJB2011-Fin2[3]	*Picea abies*	Hame	Finland	N.J. Brazee & T. Niemelä	JX110069	N. J. Brazee & D.L. Lindner
Onnia tomentosa						
Bud-551-C-1	*Tsuga canadensis*	Ontario	Canada	G.E. Englerth	JX110072	N. J. Brazee & D.L. Lindner

continued **Table 2.** *Porodaedalea* isolate description and GenBank accession numbers for *Porodaedalea* included in phylogenetic analysis.

[1]Neotype isolate.
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[5]N. J. Brazee, D.L. Lindner - UMass Extension, Center for Agriculture, University of Massachusetts, 101 University Drive, Suite A7, Amherst, MA 01002, USA.
[6]J. Stenlid – Department of Forest Mycology & Pathology, SLU, Box 7026, S-750 07 Uppsala, Sweden.
[7]M. Tomsovsky, P. Sedlak, L. Jankovsky - Faculty of Forestry and Wood Technology, Mendel University of Agriculture and Forestry in Brno, Zemedelska 3, Brno 61300, Czech Republic.

42.0–58.0 x 8.20–13.50 µm. The low standard deviation values observed indicated that the size of the basidiospores and setae were typically average (Table 3). Larger spores, albeit not significantly, were recorded from isolates 5-2011, 27-2011, 28-2011, 37-2011 and 43-2011 (Table 3). The first three of these isolates occurred in close vicinity to each other (locations I, J and U, Figure 1). The low standard deviation values observed indicated that the size of the basidiospores and setae were typically average (Table 3), and no significant differences were observed between isolates (ANOVA with Tukey's HSD, $P< 0.05$).

3.2 Phylogenetic analysis

Identification of *P. pini* from Poland was confirmed by phylogenetic analysis based upon the sequencing of the ITS 1/2 rDNA region. Nine sequences had 100% similarity and 31 sequences had 99% similarity to the sequence of *P. pini* from *P. sylvestris* in the Czech Republic (FJ775555) (Table 1). The phylogram generated with the neighbor-joining method included four main clades and two one-isolate lineages (Figure 2). Representative isolates of *P. pini* from Poland grouped together with the *P. pini* neotype from *Pinus pinaster* Aiton in Portugal (JX110037),with *P. pini* from *Larix* (FJ775553), and with different species of *Pinus* (FJ775552, FJ775554, FJ775555, FJ775556, FJ775557, FJ775558, JX110035, JX110036) (Table 2). All of these isolates formed a monophyletic clade comprised of two relatively cohesive groups (A, B). *Porodaedalea cancriformans* from *Abies* in the USA created a well-supported clade (BS 99%). Six representatives of the *Porodaedalea* Holarctic Group from *Larix* (JX110060), *Picea* (JX110069), different species of *Pinus* (JX110044, JX110046, JX110058) and *Pseudotsuga* (JX110054) in the USA grouped together with *P. laricis* from *Larix* and *Pinus* in Europe and Russia (FJ775562, FJ775563, FJ775565, FJ775566, FJ775567) (Table 2) [7]. Another representative of the *Porodaedalea* Holarctic Group, *i.e.* from *Pseudotsuga* (JX110057), joined *P. gilbertsonii* from *Pseudotsuga* (FJ775560) with significant bootstrap support (BS 74%). *Porodaedalea* sp. from *Pinus lambertiana* Dougl. in the USA (JX110048) was adjacent to these isolates (BS 86%). Two representatives of the *Porodaedalea* Holarctic Group from *Pinus strobiformis* Engelm. in the USA (JX110040, JX110041) created a significantly supported (BS 90%) group. Another two isolates, from *Pinus virginiana* Mill. (JX110038, JX110039) joined the group. Their bootstrap support (73% and 89%) suggests the presence of two different host-specific species. Representatives of the *Porodaedalea* Holarctic Group joined the *P. pini* clade with low boostrap support (BS ≤ 51%). The basal group consisted of four *P. chrysoloma* isolates from *Picea abies* (L.) H. Karst in Europe. *Porodaedalea yamanoi* (Imazeki) Parmasto from *Picea* sp. in Russia (FJ775551) and *Porodaedalea* sp. from *Cedrus atlantica* (Roxburgh) G. Don in Morocco (FJ775550) grouped separately. The latter was proximal to *P. pini*.

Grouping of the Polish isolates of *P. pini* was not related to the geographic range of their host (Figures 1, 2, Table 1). Genetic similarity between isolates did not decrease with increasing geographic separation.

An isolate of *P. pini* from *Pinus pallasiana* D. Don (JX110036) grouped together with *P. pini* from *P. sylvestris* and *Pinus rotundata* Link (FJ775556, FJ775557, J775558).

The nucleotide polymorphism of the Polish isolates of *P. pini* was small (Table 4). When compared with the *P. pini* neotype, the number of nucleotide substitutions in the 517- base-pair length of the ITS1/2 region was 1-7. Sequences from isolates which had larger basidiospores were either identical (5-2011, 43-2011) or had a single nucleotide polymorphism (28-2011) (Table 4). Nucleotide polymorphism was noted more often in isolates from older *P. sylvestris* trees (109-135 years old (7-2010, 10-2011, 24-2011, 27-2011, 46-2011)) than in isolates from younger trees.

4. Discussion

Although *Porodaedalea* is an important pathogen of conifers, it has been poorly studieduntil recently. Current papers have reported on *Porodaedalea* heterogeneity, distribution, possible occurrence of new species, host specificity and pathogenicity, and phylogenetic relationships between species and isolates [3,8,17].

We found limited polymorphism in the ITS 1/2 rDNA region of the *P. pini* isolates collected in Europe. As a consequence, all 40 isolates studied, the neotype of *P. pini* and other *P. pini* isolates from *Larix* and five species of *Pinus* in Europe, formed a significantly supported (BS 69%) monophyletic clade. These results are in accordance with earlier studies of *P. pini* from *Larix* and *Pinus* in Europe [3].

Although there was only limited genetic variation, differentiation of two groups and a one-isolate lineage within the *P. pini* clade was observed. Attempts to correlate groups or isolates of *P. pini* to the host or location were partially successful. Host specificity in *Porodaedalea* has been previously reported [6,16,17], andthree species, *i.e. P. cancriformans* (on *Abies*), *P. chrysoloma* (on *Picea*) and *P. gilbertsonii* (on *Pseudotsuga menziesii* (Mirb.) Franco) are host genus-specific.

Specimen number	Number of basidiospores or setae examined	Basidiospores				Setae			
		Dimension (μm)	Mean length (μm) ± standard deviation	Mean width (μm) ± standard deviation	Length /width (Q)	Dimension (μm)	Mean length (μm) ± standard deviation	Mean width (μm) ± standard deviation	Pore density (mm^{-1})
4-2010	10	5.0–5.5 × 4.0–4.5	5.33±0.19	4.22±0.15	1.11–1.25	30–53 × 6–15	47.0±3.78	9.00±1.35	(3-)2
7-2010	10	5.0–6.0 × 4.0–4.5	5.55±0.24	4.24±0.15	1.11–1.33	37–55 × 7–13	48.0±3.19	9.00±1.16	3-2
8-2010	10	5.5–6.0 × 4.0–4.5	5.73±0.18	4.25±0.15	1.22–1.50	27–54 × 5–15	42.0±4.01	10.0±1.38	(3-)2–1
11-2010	20	5.5–6.0 × 4.0–4.8	5.74±0.18	4.42±0.18	1.14–1.50	28–55 × 8–16	44.0±4.12	12.5±1.43	(3-)2–1
14-2010	10	5.0–6.1 × 3.8–4.8 (-5.0)	5.75±0.25	4.72±0.21	1.00–1.27	52–70 × 10–15	56.0±2.91	12.40±1.10	(3-)2–1
16-2010	20	5.5–6.0 × 4.0–4.8	5.61±0.19	4.23±0.20	1.11–1.37	39–69 × 5–16	52.0±4.27	9.30±1.58	2–1
17-2010	10	5.0–5.5 × 4.0–4.5	5.40±0.17	4.22±0.16	1.11–1.25	30–52 × 6–16	45.0±3.79	10.0±1.55	(3-)2
2-2011	10	5.0–6.1 × 3.8–4.8	5.58±0.24	4.20±0.25	1.10–1.60	51–71 × 10–16	54.0±3.39	12.5±1.24	(3-)2–1
4-2011	20	(5.0-) 5.5–6.2 × 4.3–5.0	5.65±0.28	4.50±0.23	1.11–1.33	38–54 × 7–14	47.0±3.01	10.5±1.44	3–2
5-2011	20	5.5–6.5 × (4.5-) 5.0–5.5	6.03±0.27	5.20±0.18	1.15–1.33	42–53 × 7–16	48.0±2.51	12.2±1.25	(3-)2–1
7-2011	10	(5.0-) 5.5–6.2 × 4.3–5.0	5.80±0.31	4.82±0.16	1.11–1.33	37–53 × 7–13	45.0±2.87	9.80±1.05	(3-)2–1
8-2011	10	5.0–5.5 × 4.0–4.8	5.35±0.19	4.42±0.16	1.04–1.37	30–54 × 8–16	43.0±3.57	12.5±1.43	(3-)2
10-2011	10	5.0–6.1 × 3.8–4.8 (-5.0)	5.28±0.23	4.12±0.22	1.11–1.52	52–70 × 10–15	56.0±3.15	13.5±1.28	(3-)2–1
11-2011	30	5.0–6.0 × 4.0–4.5	5.50±0.22	4.25±0.15	1.11–1.48	37–53 × 7–14	43.0±2.93	10.5±1.35	(3-)2–1
13-2011	20	5.0–6.0 × 3.8–4.8 (-5.0)	5.57±0.23	4.52±0.24	1.11–1.37	52–71 × 10–16	53.0±3.39	13.2±1.26	(3-)2–1
14-2011	10	5.5–6.1 × 4.0–4.5	5.85±0.24	4.42±0.16	1.28–1.50	30–53 × 8–14	42.0±3.87	11.5±1.15	(3-)2–1
15-2011	10	5.5–6.1 × 4.5–4.8	5.93±0.25	4.64±0.12	1.14–1.35	29–55 × 5–15	46.0±3.19	9.50±1.47	(3-)2
16-2011	10	(5.0-) 5.5–6.2 × 4.5–5.0	5.60±0.20	4.72±0.16	1.11–1.33	37–53 × 7–13	45.0±2.89	10.3±1.22	2–1
21-2011	20	5.5–6.1 × 4.0–4.5	5.74±0.24	4.24±0.16	1.28–1.42(-1.50)	28–60 × 5–15	46.0±4.51	9.00±1.50	(3-)2–1
23-2011	30	5.5–6.1 × 4.0–4.5	5.76±0.24	4.42±0.16	1.28–1.42(-1.50)	28–55 × 7–16	46.0±3.94	12.3±1.45	(3-)2–1
24-2011	10	5.5–6.1 × 4.0–4.8	5.81±0.23	4.64±0.20	1.14–1.48	30–55 × 6–15	43.0±3.81	10.8±1.45	2–1
25-2011	10	(5.0-) 5.5–6.2 × 4.3–5.0	5.83±0.18	4.44±0.19	1.11–1.33	38–55 × 7–14	51.0±4.13	9.20±1.42	(3-)2–1

Table 3. Morphological characters of examined sporophores of *P. pini*.

Specimen number	Number of basidiospores or setae examined	Basidiospores				Setae			
		Dimension (μm)	Mean length (μm) ± standard deviation	Mean width (μm) ± standard deviation	Length /width (Q)	Dimension (μm)	Mean length (μm) ± standard deviation	Mean width (μm) ± standard deviation	Pore density (mm^{-1})
26-2011	10	5.5–6.1 × 3.8–4.5	5.80±0.18	4.22±0.17	1.22–1.50	36–55 × 6–16	46.0±3.52	11.2±1.54	(3-)2–1
27-2011	30	5.0–6.5 × (4.5-) 5.0–5.5	6.00±0.28	5.14±0.15	1.10–1.44	42–55 × 7–16	47.0±3.51	11.5±1.45	(3-)2
28-2011	20	5.5–6.5 × 5.0–5.5	6.14±0.24	5.24±0.16	1.10–1.18	37–55 × 7–13	47.0±3.19	10.9±1.33	2–1
29-2011	10	(5.0-) 5.5–6.2 × 4.3–5.0	5.75±0.22	4.42±0.18	1.11–1.33	40–53 × 7–13	43.0±2.68	10.1±1.32	(3-)2–1
30-2011	10	5.5–6.1 × 3.8–4.5	5.71±0.18	4.34±0.18	1.22–1.50	30–54 × 8–16	43.0±3.68	12.4±1.44	(3-)2–1
31-2011	10	(5.0-) 5.5–6.0 × 4.8–5.0	5.75±0.17	4.72±0.10	1.10–1.25	37–60 × 8–13	48.0±3.41	10.8±1.02	(3-)2–1
32-2011	10	(5.0-) 5.5–6.2 × 4.3–5.0	5.98±0.18	4.44±0.18	1.11–1.33	37–53 × 7–13	46.0±3.09	10.2±1.22	3–2
34-2011	10	5.5–6.2 × 4.0–4.8	5.62±0.18	4.43±0.20	1.15–1.50	30–55 × 5–15	43.0±4.02	10.2±1.52	(3-)2
36-2011	30	5.5–6.0 × 4.0–5.5	5.70±0.17	4.48±0.16	1.10–1.50	30–60 × 7–13	45.0±4.81	8.50±1.34	2–1
37-2011	20	6.0–6.5 × 5.0–5.5	6.37±0.18	5.22±0.15	1.10–1.30	40–55 × 5–13	49.0±2.87	10.3±1.55	2–1
39-2011	30	5.0–6.1 × 3.8—5.0	5.53±0.25	4.62±0.25	1.10–1.52	52–69 × 10–15	58.0±3.34	10.0±1.06	(3-)2
43-2011	20	6.0–6.5 × (4.8-) 5.0–5.5	6.25±0.17	5.30±0.17	1.11–1.35	44–55 × 7–15	45.0±2.72	10.3±1.32	(3-)2–1
44-2011	10	5.0–6.1 × 3.8—5.0	5.54±0.26	4.48±0.24	1.00–1.52	51–70 × 9–15	57.0±3.14	10.8±1.19	2–1
45-2011	10	5.0–6.0 × 4.0—5.0	5.56±0.27	4.66±0.23	1.00–1.50	50–69 × 8–15	55.0±3.17	10.2±1.29	(3-)2
46-2011	10	5.5–6.2 × 4.0–4.5	5.75±0.19	4.39±0.14	1.22–1.52	30–55 × 5–15	45.0±3.99	8.20±1.57	(3-)2–1
47-2011	10	5.0–5.5 × 4.0–4.5	5.25±0.18	4.24±0.14	1.11–1.25	30–50 × 6–15	40.0±3.32	8.50±1.56	(3-)2–1
51-2011	10	5.0–5.5 × 4.0–4.5	5.25±0.18	4.12±0.16	1.11–1.25	30–55 × 6–16	47.0±4.05.544–1.42(-spores or seaten n be the new speciestwo different species.	9.30±2.00	2–1
52-2011	20	5.5–6.2 × 4.0–4.8 (-5.0)	5.65±0.22	4.72±0.24	1.11–1.50	39–69 × 8–16	55.0±4.28	13.0±1.25	2–1

continued **Table 3.** Morphological characters of examined sporophores of *P. pini*.

Figure 2. Phylogram obtained by neighbor-joining analysis of ITS1/2 rDNA sequences of 59 *Porodaedalea* taxa. Gaps and missing data were excluded from the analysis. Bootstrap values (1,000 replicates) greater than 50% are listed above the branches. Codes in bold indicate the Polish specimens of *Porodaedalea*. GenBank accession numbers are listed in Table 1. The tree was rooted with the outgroup *Onnia tomentosa*. Scale bar at the bottom indicates the percentage of difference between sequences.

Specimen	33	34	37	75	83	91	111	116	124	151	153	190	196	214	240	253	259	264	271	391	395	406	408	409	410	411	419	422	428	435	436	443	449	475	477	480	483	487	502	511	515	516
JX110037	A	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	C	T	S	T	G	G	T	G	G	A	T
7-2010	A	C	G	T	C	G	C	A	C	G	A	G	A	G	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	C	T	C	T	G	G	T	G	G	A	T
11-2010 [1]	A	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	C	T	C	T	G	G	T	G	G	A	T
14-2010	A	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	T	T	G	C	C	C	T	G	T	T	G	T	G	G	A	T
16-2010	A	C	G	C	C	A	G	G	A	A	A	G	A	A	T	G	A	G	G	T	T	T	C	T	C	G	G	T	G	C	T	C	T	C	T	G	G	T	G	G	A	T
2-2011	A	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	C	T	C	T	G	G	T	G	G	T	A
8-2011	A	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	G	T	G	T	G	G	T	G	G	A	T
10-2011	A	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	A	A	C	C	T	T	C	T	C	G	G	A	A	T	C	C	T	G	T	G	C	T	G	G	A	T
11-2011	A	C	G	C	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	T	T	T	C	T	C	G	G	T	G	C	C	C	T	C	T	G	G	T	G	G	A	T
13-2011	A	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	C	T	C	T	G	G	T	G	G	A	T
15-2011 [2]	A	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	C	T	G	T	G	G	T	G	G	A	T
16-2011	A	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	C	T	C	A	G	G	T	G	G	A	T
24-2011	A	C	G	T	C	G	G	G	A	A	A	G	A	A	G	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	C	T	C	T	G	G	A	A	G	T	A
26-2011	A	C	G	T	C	G	G	G	A	A	T	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	A	T	G	C	C	C	T	G	T	G	G	T	G	G	A	T
27-2011	C	C	G	T	C	G	G	G	A	A	A	G	C	A	T	C	A	G	C	C	T	T	C	T	C	G	G	T	G	C	C	C	A	C	A	G	G	T	C	G	A	T
28-2011	C	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	C	T	C	T	G	G	T	G	G	A	T
30-2011	A	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	C	T	C	T	G	G	T	A	G	A	T
34-2011	C	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	C	T	G	T	G	G	T	G	G	A	T
44-2011 [3]	A	C	G	C	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	C	T	C	T	G	G	T	G	G	A	T
46-2011	A	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	C	G	G	C	T	A	G	C	T	C	G	T	G	C	C	C	T	C	T	G	G	T	G	G	A	T
47-2011	A	C	G	T	C	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	G	T	C	T	G	G	T	G	A	A	T
51-2011	A	C	G	T	C	G	G	G	A	A	A	A	A	A	T	G	A	G	G	C	A	T	C	T	C	G	G	T	G	C	C	C	T	G	T	G	G	T	G	G	A	T
52-2011	C	T	T	T	T	G	G	G	A	A	A	G	A	A	T	G	A	G	G	C	T	T	C	T	C	G	G	T	G	C	C	C	T	C	T	G	G	T	G	G	A	T

Table 4. Nucleotide polymorphism in ITS1/2 rDNA sequences of *Porodaedalea pini* compared with *P. pini* neotype (JX110037).

[1] *Identical to 4-2010, 7-2011, 14-2011, 21-2011, 23-2011, 25-2011, 31-2011, 32-2011, 45-2011*
[2] *Identical to 17-2010, 4-2011, 5-2011, 29-2011, 36-2011, 37-2011, 39-2011, 43-2011*
[3] *Identical to 8-2010*

Porodaedalea pini occurs on *Larix* and *Pinus.* Comparison of *P. pini* from *Larix* with *P. pini* from *Pinus* shows a moderate level of genetic variation in the former. Our analysis, however, included only a single ITS 1/2 rDNA sequence of *P. pini* from *Larix* available at EMBL. Genetic variation of the *P. pini* studied was not related to geographical separation. Similar to this study, absent-to-moderate levels of geographical specificity in *P. pini* in areas greater than ours (approximately 100 000 km^2), *i.e.* in Europe or in North America, has also been reported [3,16,17]. These results suggest that host specificity, rather than geographical separation, operates as a speciation mechanism within *P. pini*.

The lack of geographical specificity and a high degree of genetic homogeneity in most of the *P. pini* isolates studied agree with the theory of genetic structure of the pathogen's population, which is typically composed of a range of many different physiological races, with a few dominants [24]. The number of races, their diversity, the degree of domination as well as their pathogenicity result from environmental conditions and may differ markedly, both locally and seasonally. This could result from a long-lasting and permanent host effect. Forests of northern Poland are dominated by *P. sylvestris* monocultures and the *P. pini* population is rarely exposed to alternative hosts, which generally causes relaxation or shifts in selection pressure, resulting in greater genetic diversity [25,26]. Genetic homogeneity of *P. pini* can additionally result from its limited ability for interspecific and intraspecific mating (resulting from incompatibility of certain isolates and universal compatibility of others) [16,27], high intrasporophore mating, homothallic mode of reproduction and bipolar (one-locus) mating pattern [28].

The phylogenetic study included two Fennoscandian representatives of the *Porodaedalea* Holarctic Group and six isolates from the Atlantic-Boreal region, from the Interior and from the Pacific region of the USA. Six isolates grouped with *P. laricis* from Europe and one with *P. gilbertsonii* from the USA (BS 74%). Another isolate was adjacent to *P. gilbertsonii* (BS 89%). This suggests that some of the unidentified *Porodaedalea* species belong to *P. laricis* or *P. gilbertsonii.* Our findings are in accordance with earlier studies [17], which suggested that the *Porodaedalea* Holarctic Group in the USA may include *P. laricis* and *P. gilbertsonii.* The former species was so far unrecognized and unknown in the USA. Grouping of the American unidentified *Porodaedalea* isolates with *P. laricis* or *P. gilbertsonii* and failure to group with *P. pini* suggests the absence of *P. pini* (in a form recognized in Europe) from North America. This possibility was earlier suggested by Brazee and Lindner [17].

One of the most distal clades of the phylogram consisted of four representatives of the *Porodaedalea* Holarctic Group from *P. virginiana* (JX110038, JX110039) and *P. strobiformis* (JX110040, JX110041). The topology of the clade suggests the presence of at least two different host-specific species, agreeing with earlier suggestions [17]. *Porodaedalea* sp. from *C. atlantica* in Morocco (FJ775550) created a separate lineage with significant bootstrap support (69%), suggesting a new species.

Morphological and phylogenetic species recognition proved the occurrence of *P. pini* on *P. banksiana* in Poland. This is the first record of *P. pini* on a living jack pine tree in Poland.

The method used in the presented study, *i.e.* sequencing of the ITS rDNA with Basidiomycota-specific primers, is widely used in studies of Basidiomycota and *Porodaedalea* [3,19]. The internal transcribed spacer (ITS), between the SSU and large subunit genes, is variable and can be used to explore relationships at both the species and subspecies levels [29,30]. The Basidiomycota-specific primer ITS4-B, when paired with the fungal-specific primer ITS1-F, efficiently amplifies DNA from Basidiomycota and discriminates against other fungi [19].

We studied the phylogeny of *Porodeadalea* with a distance-method (neighbor-joining), and character-based methods (maximum likelihood and maximum parsimony). Results from the neighbor-joining method were presented as we considered this method to be superior to the maximum likelihood and maximum parsimony methods. We found the neighbor-joining method to befast and highly accurate, partly because of the small amount of substitution among lineages [31,32]. The topologies of phylograms obtained by neighbor-joining analysis, maximum likelihood and maximum parsimony were similar. However, only the neighbor-joining method differentiated sequences with single nucleotide substitution.

The topologies of our phylogram, obtained by neighbor-joining analysis with sequences from one locus (ITS1/2 rDNA), and Brazee and Lindner's phylogram, obtained by maximum parsimony analysis with sequences from four loci (ITS1/2 rDNA+nLSU+*rpb2*+*tef1*) [17], were similar. They have similar group location and composition; in both phylograms, the basal group includes *P. chrysoloma*, above which are clades of *P. pini, P. cancriformans* and *P. laricis* conspecific with the *Porodaedalea* Holarctic Group. This supports that the results of our distance-base method using sequences from ITS 1/2 rDNA can be compared with results of the character-based methods using sequences from ITS1/2 rDNA+nLSU+*rpb2*+*tef1*. Sequencing of the ITS 1/2 rDNA region with ITS1-F and ITS4-B primers therefore seems to be a sufficient method for differentiation of species within *Porodaedalea.*

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Conflict of interest

None conflict of interest is declared.

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