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Phylogenetic Relationships, Pathogenic Traits, and Wood-Destroying Properties of *Porodaedalea niemelaei* M. Fischer Isolated in the Northern Forest Limit of *Larix gmelinii* Open Woodlands in the Permafrost Area

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The phytopathogenic and wood destroying traits were studied in a basidiomycete fungus, *Porodaedalea niemelaei* M. Fischer, widespread in Siberian permafrost woodlands of Gmelini larch, *Larix gmelinii* (Rupr.) Rupr. Numerous stands of dying out and fallen larch trees with white-rot („corrosion rot“) were found in the study area. Butt rot incidence varied from 63 to 100 % depending on the stand age and raised up to 0.5-1.5 m above root collar on average or up to 9 m maximum. Root rot was also widespread, including larch undergrowth. The biodiversity of xylotrophic fungi was low, with a pronounced dominance of *P. niemelaei*. The main factors of dying out of *L. gmelinii* were infection

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by *P. niemelaei* promoted by mechanical damage of roots by reindeers during migration and climate anomalies. The cultures isolated from the fruiting bodies were identified as *Porodaedalea niemelaei* M. Fischer based on the combination of morphological, culture, and molecular genetic methods. Under laboratory conditions, the strains were characterized as psychrotolerant (temperature limit from 6 to 22 °C) and preferred cultural media based mostly on natural and plant substrates. The most active biodegradation occurred on the broadleaf wood substrates causing up to 50 % of the biomass loss accompanied by active decomposing of the lignocellulosic complex and increasing the amount of water-soluble substances. The phylogenetic analysis demonstrated that *P. niemelaei* is clearly different from other well-studied *Porodaedalea* species, such as *P. chrysoloma*, *P. pini*, and *P. cancriformans*, and is very close to a group of unclassified fungi isolated in Norway and Finland. The phylogenetic analysis included 43 isolates and was based on four genetic markers – ITS, nLSU, rpb2, and tef1, commonly used in fungal phylogenetics.

Keywords: butt and stem rot, genome sequence, ITS, lignocellulosic complex, molecular genetic markers, nLSU, phylogenetics, *Porodaedalea niemelaei*, rpb2, tef1, wood destroying psychrophilic phytopathogenic fungi, white-rot, wood biodegradation.

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Патогенные и дереворазрушающие свойства *Porodaedalea niemelaei* M. Fischer в редколесьях *Larix gmelinii* в районах вечной мерзлоты

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Изучены фитопатогенные и дереворазрушающие свойства базидиального гриба *Porodaedalea niemelaei* M. Fischer, широко распространённого на территории Сибири в районе вечной мерзлоты в редколесьях *Larix gmelinii* (Rupr.) Rupr. В районе исследования обнаружены многочисленные куртины усыхающих деревьев и ветровальные лиственницы с наличием коррозионной гнили. Встречаемость напенной гнили варьирует в пределах 63-100 % в зависимости от возраста древостоя; средняя высота подъема 0,5-1,5 м, максимально до 9 м. Корневая гниль распространена повсеместно, включая подрост лиственницы. Биоразнообразие ксилотрофных грибов скучное с выраженным доминированием *P. niemelaei*. Основным фактором усыхания *Larix gmelinii* является наличие грибной инфекции, способствующими – механическое повреждение корней и климатические аномалии. Культура гриба, изолированная из плодового тела, по совокупности морфолого-культуральных и молекулярно-генетических методов идентифицирована как *Porodaedalea niemelaei* M. Fischer. В лабораторных условиях штамм характеризуется как психротолерантный (температурный предел 6-22 °C), предпочитающий питательные среды на основе натуральных растительных компонентов. Биодеструкция растительных субстратов активнее протекает на лиственной древесине – убыль массы до 50 % на фоне активной биодеградации компонентов лигноцеллюлозного комплекса и увеличения количества водорастворимых веществ. Филогенетический анализ выявил значительные отличия *P. niemelaei* от других хорошо изученных видов *Porodaedalea*, таких как *P. chrysoloma*, *P. pini* и *P. cancriformans*, и близость к группе неклассифицированных грибов, выделенных в Норвегии и Финляндии. Филогенетический анализ включал 43 изолята и базировался на четырех маркеров (ITS, nLSU, rpb2 и tef1), широко применяемых в филогении грибов. Нуклеотидные последовательности всех маркеров были проверены в недавно полностью секвенированном геноме *P. niemelaei*, у изолята, использованного в этом исследовании (http://genome.jgi.doe.gov/PornieStandDraft_FD/PornieStandDraft_FD.info.html).

Ключевые слова: белая гниль, биодеструкция древесины, дереворазрушающие психрофильные фитопатогенные грибы, лигноцеллюлозный комплекс, молекулярно-генетические маркеры, корневая и стволовая гниль, ITS, nLSU, *Porodaedalea niemelaei*, rpb2, tef1.

Introduction

Due to the global climate change, interest in studies of forests growing in permafrost regions with cold climate and a short growing season has greatly increased in recent years. However, wood-destroying fungi that grow in permafrost areas and are an important component of their ecosystem,

ensuring its sustainability, are still insufficiently studied and poorly understood. Extreme growing conditions determine specific regularities and, respectively, special relationships within such ecosystem. Vasilkov (1966, 1971) noted a strong depletion of the fungal species diversity in the Arctic. An extreme example of this depletion is the

complete absence of basidiomycetes of the order Polyporales growing on wood in the subarctic tundra of Western Taymyr (or Taimyr) in northern Siberia (Russia), which was a region of the study presented in this paper. Although, host plants for basidiomycetes – birch and willow – are available there. A similar pattern was observed also by Linder (1947) for the flora of the Canadian Arctic. It is noted that the disappearance of polypores is gradual from the south to the north (Vasilkov, 1966; Parmasto, 1967).

Fungi growing in permafrost areas have unique psychrophilic traits that ensure their survival and reproduction at relatively low temperatures (Farrell et al., 2011). Studies of psychrophilic or psychrotrophic fungi are important for understanding their molecular genetic adaptation to low temperatures, specificity of their enzyme system, and their potential use in biotechnological applications such as effective destruction of lignin and recycling timber and paper industry waste. Fungi use different mechanisms to adapt to low temperature. For instance, basidiomycete snow molds produce extracellular antifreeze proteins. The psychrophilic ascomycete *Sclerotia borealis* has normal mycelial growth under frozen conditions, which is even faster than growth on unfrozen media at a supposedly optimal growth temperature. This fungus does not produce extracellular antifreeze proteins, but osmotic stress tolerance enables the fungus to grow at subzero temperatures (Hoshino et al., 2009). For *Coprinopsis psychromorbidia* (former *Coprinus psychromorbidus*) and *Typhula ishikariensis* the optimal growth temperature is 5–10 °C (Smith, 1987).

The evidence of cold adaptation of cryoconite isolates *Phialophora alba*, *Rhodotorula* sp., *Cryptococcus gilvescens*, and *Articulospora tetracladia* isolated from cryoconite holes of Svalbard in the Arctic can be seen from the fact

that these isolates do not survive at temperatures above 20 °C (Singh and Singh, 2012). For example, *Phoma herbarum* isolated from the soils of Truelove Lowland on Devon Island (75° N) grew better at 2.5° than at 5° C in laboratory culture (Widden and Parkinson, 1978).

The psychrophilic nature of the cellulose decomposition by saprotrophic fungi *Chrysosporium pruinatum*, *Phoma herbarum*, *Cladosporium cladosporioides*, and *Cylindrocarpon magnusianum* was confirmed *in vitro* (Robinson, 2002). Thus, the cellulose decomposition started at -7 °C, and 20 % of the strains showed the optimal rate of decomposition at about 6 °C. At the same time, pectin decomposition started at about 1 °C and reached the optimal rate at or slightly above 18 °C. The rate of growth of all tested strains of *Geomycetes pannorum* extracted from ancient marine sediments and permafrost dated 100–120 thousand years since the end of the Arctic Ocean recession was lower at 26 °C than at 4 °C (Gilichinsky et al., 2005).

The psychrophilic properties corresponded to the definition proposed by Deverall (1968), according to which it is assumed that a psychrophilic organism must have the optimal growth at a temperature of about 10 °C and below. The author highlighted the importance for psychrophilic microorganisms not to be subjected to temperatures above 10 °C during selection, isolation and storage of samples. Otherwise, the loss of psychrophilic properties is expected. However, this was not confirmed in the study of the growth of cultures of two psychrophilic strains of fungi *G. pannorum* isolated from an Arctic cryopeg lens (strain VKM FW-2241) and Central European part of Russia (strain VKM F-3808), respectively (Konova et al., 2009). The adaptive response of cultures of both strains to lowering the temperature to 2 °C was the strengthening of the desaturation processes.

One of the few wood-decomposing fungi growing in high latitudes of the northern forest limit is *Porodaedalea niemelaei* M. Fisch (synonymous to *Phellinus niemelaei* (M. Fisch) Zmitr, Malysheva & Spirin) that belongs to the kingdom Fungi, division Basidiomycota, class Agaricomycetes, order Hymenochaetales, family Hymenochaetaceae, genus *Porodaedalea*. Genus *Porodaedalea* is a complex of morphologically similar species with still unresolved taxonomic problems (Ryvarden and Gilbertson, 1994; Fischer, 1996; Dai, 2010; Tomšovský et al., 2010; Brazee and Lindner, 2013; Tomšovský and Kout, 2013). *P. niemelaei* is characterized by a number of specific morphological traits: dimitic hyphal system, septa without clamp connections, yellowish-brown skeletal hyphae, frequent setae in the hymenium, mostly subulate, rarely ventricose (Niemelä, 2001).

Porodaedalea fungi are among the most important plant pathogens that cause stem rot (trunk rot pathogens) in conifers in the northern hemisphere (Brazee and Lindner, 2013). They cause the greatest economic damage to Scots pine stands, mostly in old-growth forests (Ezhov and Konyushatov, 2001), although serious damage was also observed in the 30-40-year-old forests (Sisto and Luisi, 1991). Trunk rot damage due to *Porodaedalea pini* s.l. is commonly referred to as red ring rot, white pocket rot and red heart (Brazee and Lindner, 2013). In some cases (e.g., in Narol forest district, Poland) damage caused by *P. pini* exceeded 70 % of 40-60-year-old Scots pine trees (Szewczyk et al., 2014). In the old-growth forests of *Larix kaempferi* at the foothill of Mount Fuji 75 % of the study trees were affected by trunk rot. In 40.7 %, this was due to *Porodaedalea chrysoluma* (Ohsawa et al., 1996).

Fungi that cause stem rot, such as *Porodaedalea*, play a fundamental role in forest ecosystems by accelerating stand development through gap formation, altering forest

composition and successional pathways, creating coarse woody debris and wildlife habitat, and recycling organic matter (Hennon, 1995; Hansen and Goheen, 2000; Worrall et al., 2005; Brazee and Lindner, 2013).

One of the main objectives of our study presented in this paper was to confirm the taxonomic status of our samples and to clarify phylogenetic relationships of *P. niemelaei* with other *Porodaedalea*. Sequencing the ITS (internal transcribed spacers 1 and 2), nLSU (28S ribosomal RNA large subunit), the translation elongation factor 1-alpha (*tef1*), and the RNA polymerase II second largest subunit (*rpb2*) gene regions, proved to be a suitable and sufficient method for the differentiation of fungi species, including *Porodaedalea* species (Jeong et al., 2005; Tomšovský et al., 2010; Brazee and Lindner, 2013; Tian et al., 2013; Tomšovský and Kout, 2013; Szewczyk et al., 2014), and we also used these markers in our study.

Materials and methods

Study species

A larch woodland stand in the lower reaches of the Kheta River in Taymyr (northern East Siberia, Russia) was chosen as a study site that has a number of interesting features: withering clumps of trees; a large number of wind-fallen larch trees infested by root rot; no fire for at least 100 years, and possibly the last 450 years; intensive wood decomposition; and absence of old woody debris. The geographical coordinates of the site are N 71°41.97' E 100°34.54'.

Using standard techniques, samples of *P. niemelaei* fruiting bodies (Niemelä, 2001) and the affected wood were collected for the first time at such high latitudes. Among Polyporales fungi only *P. niemelaei* and *Schizophyllum commune* Fr. were present there, but the former one occurred frequently and repeatedly, and the latter one was very rare (Urbonas et al., 1986).

In the most comprehensive directory of fungi in the Russian Arctic (Karatygin et al., 1999) the order Hymenochaetales is presented in the Polar Urals by fungi growing on dead wood of *Larix*, *Salix*, *Alnus*, *Picea*, and *Betula*. *P. chrysoloma* occurring on dead wood of *Picea* was also listed. However, it was recorded in the area of N 66° located more to the south from our sample site (N 71°). To investigate the orographic patterns of *P. niemelaei* distribution and its impact on the larch, a 1 km long transect crossing a ~50 m high hill was set. The depth of the permafrost was 1.5 m on the slopes of southern exposure, 1.7 m at the plateau hill, 1.2 m in the upper part of the slope of northern exposure, and 0.4 m at the bottom in August 2013.

The living ground cover and undergrowth were presented by a limited number of species: herbaceous plants – *Arctagrostis latifolia*, *Arctous alpina*, *Nardosmia frigid*, *Calamagrostis lapponica*, *Carex arctisibirica*, *C. quasivaginata*, *C. redowskiana*, *Rubus chamaemorus*, *Eriophorum brachyantherum*, *E. subholarcticum*, *E. arvense*, *Equisetum pratense*, *E. variegatum*; mosses – *Ptilidium* sp., *Aulacomnium turgidum*, *Hylocomium splendens*, *Tomentypnum nitens*; woody plants and shrubs – *Ledum decumbens*, *L. palustre*, *Betula exilis*, *Vaccinium minus*, *Vaccinium uliginosum* subsp. *microphyllum*, *Salix boganiensis*, *S. glauca*, *S. pulchra*, *S. lanata*, *S. hastata*, *Duschekia fruticosa*, and *Rosa acicularis*.

Several pure cultures were isolated from sterilized surface of the *P. niemelaei* fruiting bodies using the moist-chamber method, followed by passages on solid medium (Bilai, 1982; Bukhalo, 1988). Bacterial microflora was suppressed by adjusting the pH (4.5-5.0) and applying antibiotics (200 u of penicillin and 100 u of streptomycin per ml of medium). 1-3 % a softwood sawdust mixture was added in

the culture medium to stimulate the mycelium growth. Macromorphology was evaluated in Petri dishes on a malt extract agar (MEA) at 6°, 12°, 22°, 26°, 30° ± 2° C during 25 days. The micromorphology was studied using intravital staining and a microchamber for microscopy *in situ* (Bilai, 1982; Singleton et al., 1993) with a microscope Olympus CX41 and a digital camera Nikon D5100, and a scanning electron microscope TM-1000 with magnification of up to 10000-fold and resolution of 35 nm.

To assess the ability of the strains to degrade the lignin-carbohydrate complex, solid-substrate fermentation was done using sawdust of *Larix sibirica* L., *Populus tremula* L. and the vegetative parts of *Helianthus tuberosus* L. (shredded stems and leaves) with substrate humidity of 70 % and temperatures of 12° and 22 ± 2 °C during 30 days in Petri dishes. The chemical composition of the substrates before and after cultivation was studied using standard methods of timber chemistry (Riazanova et al., 2012).

Genetic identification and molecular phylogenetic analysis

For genetic identification two nuclear genome regions in the *ITS* and *nLSU* gene regions were amplified and sequenced using *ITS1/ITS4* and *LROR/LR3* primer pairs, respectively (<http://sites.biology.duke.edu/fungi/mycolab/primers.htm>). The isolated strains were genetically identical based on the used molecular genetic markers; therefore, sequences of the only one strain were used in the phylogenetic analysis and were submitted to the GenBank database (accession numbers KU687403 and KU687404). They were verified using BLAST against the *Porodaedalea niemelaei* genome completely assembled recently by the US DoE JGI (https://genome.jgi.doe.gov/Porniel_2/Porniel_2.info.html; Krutovsky, Kües and Pavlov, unpublished).

The BLAST revealed seven scaffolds (195, 460, 569, 595, 691, 890, 896) containing eight complete and one partial *ITS* loci. The scaffold 195 contained two complete and one partial *ITS* loci. The sequences of the *ITS* loci were identical, except the C/G SNP corresponding to the 587th nucleotide position in the amplified fragment (KU687403). Exactly half of the loci have allele C (in scaffolds 195, 460, 595, and 890) and half – allele G (in scaffolds 195, 569, 691, and 896). The complete genome assembly was used also to locate and extract sequences of other two genetic markers commonly used in the fungi phylogenetic studies – *tef1* and *rpb2* genes. As expected, the genome assembly contained only single copies of these two genes in scaffolds 23 and 12, respectively.

For our comparative phylogenetic study, we used sequences from the most comprehensive published phylogenetic study of *Porodaedalea* performed with the highest number of markers – *ITS*, *nLSU*, *tef1*, and *rpb2* genes (Brazee and Lindner, 2013). The sequences were aligned using the CodonCode Aligner v.4.2.7 software (<http://www.codoncode.com>). The *ITS*, *nLSU*, *tef1*, and *rpb2* gene sequences that represented the same sample were concatenated and used to generate maximum likelihood (ML) and neighbor-joining (NJ) phylogenetic trees using the MEGA7 v. 7.0.18 software (Kumar et al., 2016). Alignment gaps were treated as missing data in all analyses. For ML, the best-fit nucleotide substitution model was chosen using Akaike information criterion (AIC) values generated in JMODELTEST (Guindon and Gascuel, 2003; Posada, 2008), along with log-likelihood ($-\ln L$) scores generated within MEGA. For all individual marker multiple nucleotide alignments, the model that produced the lowest AIC score in JMODELTEST and highest $-\ln L$ score in MEGA was the general time reversible (GTR+I+G) substitution model (Tavaré, 1986). The gamma shape parameter

value was estimated directly from the data within MEGA.

In addition, the bootstrap consensus neighbor-joining (NJ) phylogenetic tree was generated based on the evolutionary distances that were computed using the maximum composite likelihood method (Tamura et al., 2004) and were in the units of the number of base substitutions per site. Confidence for internal branches for both ML and NJ trees was obtained through bootstrap analysis with 1000 replicates (Felsenstein, 1985). Bootstrap support (BS) values >70 % were considered significant in this study. *Onnia tomentosa* was chosen as an outgroup based on previous studies (Wagner and Fischer, 2002; Tomšovský et al., 2010; Brazee and Lindner, 2013).

Results and discussion

Pathogenic properties

The study site was chosen because of the large number of pockets of forest dieback. The absence of large masses of dead wood indicated a high speed of wood decomposition. The predominance among the dead trees of those that died in the last 10-20 years indicated also that this predominance is a new phenomenon. Based on visual analysis, a significant violation of the optimum ratio of destruction-restoration was assumed. It seems that the global warming leads not only to a rapid melting of the permafrost, but also accelerates the destruction of larch forests by root and stem rot pathogens. The absence or reduction in the larch tree canopy fullness will also help to speed up the melting of the permafrost.

The total stem wood stock was 88.8 m³/ha in the stand. The stock of deadwood and greatly weakened trees (that might be lost in the near future) was 45 m³/ha (Fig. 1). This indicates an unfavorable condition of this stand. The relationship between tree diameter and height in this forest stand was obviously linear ($r=0.96$,

$P < 0.0001$; Fig. 2). The maximum height of the trees did not exceed 9 m, diameter – 15 cm. In the southern slope trees with the height of 12 m and diameter of 24 cm occurred sporadically.

It was found in the field, genotypic and laboratory tests that the loss of larch trees and intensive wood decomposition is caused by the fungus *P. niemelaei*. Forest dieback, windrow, and top of the crown dieback were more pronounced on the plateau. The growth and productivity class or quality of the stand was 2.5 (highly weakened). The stand consisted of trees of different ages. The average diameter of the stand on the plateau was 11.2 cm and height – 7.1 m. The maximum age

was 460 years. For the trees of up to 300 years old the frequency of stump rot (at the height of 10 cm) was 63 %; of more than 300 years old – 100 % (Fig. 3). The rot often reached 9 m in tree height, but generally less than 0.5-1.5 m. The trees with a significant spread of rot had typically drying tops and drying large branches. The excavations revealed the widespread presence of root rot, even in the undergrowth larch.

The relationship between the frequency of having a drying top and the diameter in *Larix gmelinii* (Rupr.) Rupr. trees is demonstrated in Fig. 4. The reason for the high dieback of small trees seemed to be due to competition

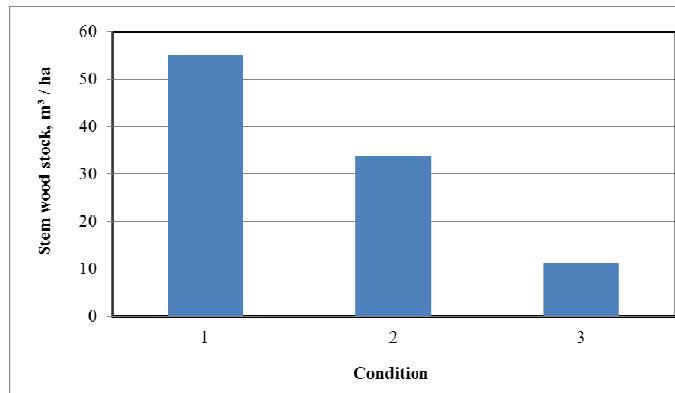


Fig. 1. Stem wood stock of *Larix gmelinii* in the stand studied (1 – healthy and slightly weakened trees; 2 – greatly weakened; 3 – dead wood)

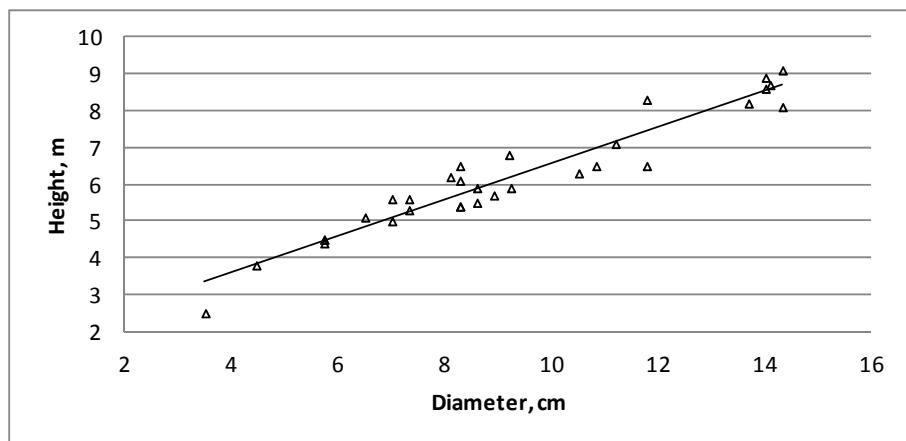


Fig. 2. The relationship between the diameter and height of the *Larix gmelinii* trees

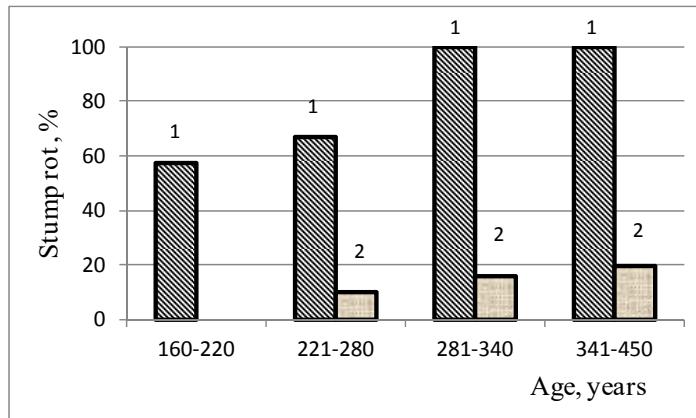


Fig. 3. Frequency of stump rot caused by *Porodaedalea niemelaei* in trees of different ages (1 – test plot; 2 – control with no sign of damage by *P. niemelaei*)

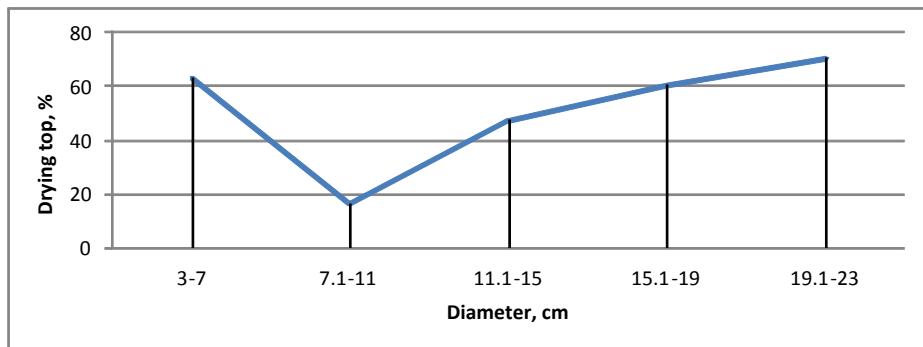


Fig. 4. The relationship between the frequency of having a drying top and the diameter in *Larix gmelinii* trees

with neighboring woody plants, especially with *Duscheckia fruticosa* and genus *Salix*.

The impact of climate change on woody vegetation may act indirectly, through creation of favorable conditions promoting diseases and pests. However, the role of diseases in the ecosystem response to climate change is still poorly understood (Wiedermann, 2007). In northern latitudes with permafrost the effect of existing and projected more intense warming on the vegetation will be enhanced by psychrophilic microorganisms. Perhaps one of the reasons for such a high rate of destruction is also the noted increase in winter precipitation (Fig. 5, 6). Snow cover is one of the most important

factors controlling the microclimate and growing conditions of plants in the Arctic and Alpine ecosystems (Wipf and Rixen, 2010).

Thus, in the experiment with artificial snow retention in the arctic tundra the increase in snow cover stimulated the growth of *Empetrum hermaphroditum* in the first years of study, as expected (Olofsson et al., 2011). In the sixth year of the experiment a parasitic fungus *Arwidssonia empetri* spread quickly among the plant populations and killed the majority of the *E. hermaphroditum* shoots.

One of the reasons for the dying up of larch is certainly climate changes (Fig. 5-8), such as: 1) Reduced rainfall from June to August leading to

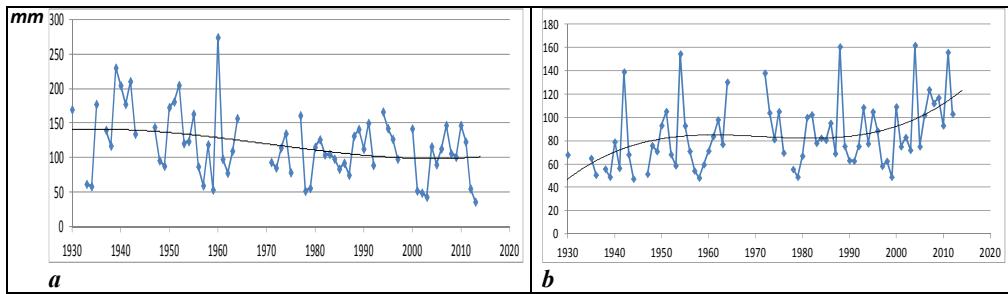


Fig. 5. Precipitation dynamics with trend lines in Khatanga, a rural locality in Taymyrsky Dolgano-Nenetsky Region of Krasnoyarsk Krai, Russia, located on the Khatanga River on the Taymyr Peninsula. It is one of the northernmost inhabited localities in Russia (**a** – June-August, **b** – November-February)

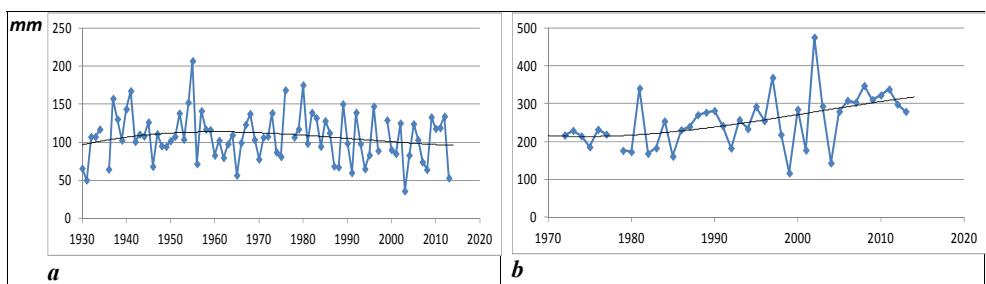


Fig. 6. Precipitation dynamics with trend lines in Dixon, an urban village to the north of the Krasnoyarsk Krai, Russia in Taymyrsky Dolgano-Nenetsky Region. It is the northernmost inhabited locality in Russia (**a** – June-August, **b** – November-February)

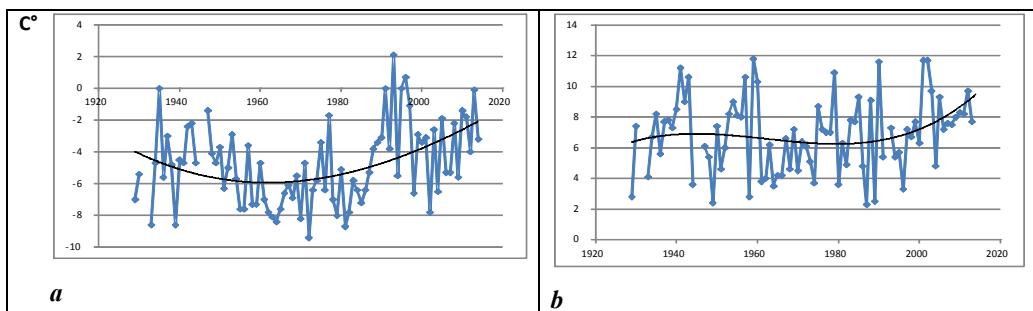


Fig. 7. Mean air temperature dynamics with trend lines in Khatanga (**a** – May, **b** – June)

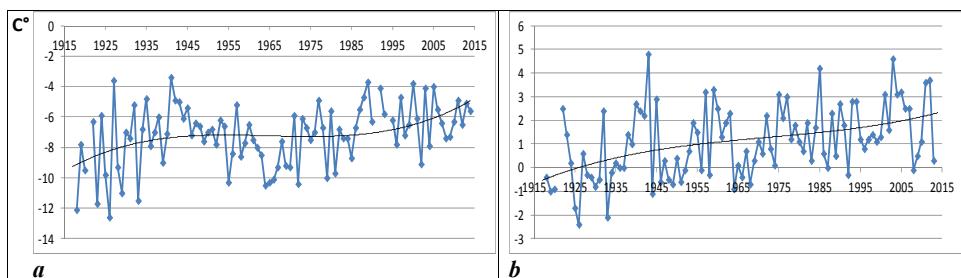


Fig. 8. Mean air temperature dynamics with trend lines in Dixon (**a** – May, **b** – June)

a shortage of moisture for larch. The root system is located mainly in the upper soil layers that dry up quickly reducing resistance to root rot pathogens. In the period from 2000 to 2003 there was a sharp decrease in rainfall accompanied by the growing temperature. 2) Increased winter precipitation in November and February optimizing conditions for overwintering fungi. 3) A pronounced increase in the average temperature in May-June promoting an earlier and higher activity of fungi.

Along with climatic anomalies an equally important reason for the high distribution of fungus is the penetration of infection through mechanical damage to the roots caused by reindeer migrating through larch woodland (personal observations). While the spread of rot does not cause much harm to the stem, it causes a rapid withering of the roots. In such harsh growing conditions even partial root withering leads to lower resistance to climatic anomalies.

At the same time the major factors leading to the drying out are 1) the presence of infection, 2) root damage (predisposing), and 3) climate anomalies (contributing). Despite such a significant pathogenic impact on the larch stands, *P. niemelaei* plays an important role in maintaining a favorable balance of minerals and sustainable functioning of the ecosystem, and not only due to the wood and debris decomposition, but also through the active influence on ecosystem components (regulation of age structure, composition, etc.).

Cultural characteristics and micromorphology of fungal strains isolated from fruiting bodies

P. niemelaei fruiting bodies were found only on fallen trees (Fig. 9A), including those that remained viable due to the still intact roots, often completely covering the bottom of the stem for 0.3-1 m (sometimes up to 3 m and more). The shape varied from flattened (most common)

to three-sided. The cap edges were often quite sharp (sometimes in old specimens of up to 8 cm thick, rounded). The color of the upper surface of the cap varied from light brown to grayish-black with concentric grooves and rigid hairs. The lower surface of young specimens was yellow-brown, in the old specimens – dark brown. They had a dimitic hyphal system, septa without clamp connections, brown skeletal hyphae, brown, narrow, and pointed bristles (Niemelä, 2001).

Infection by the fungus occurs through the roots (Fig. 9B). The fungus causes white (corrosive) rot. In the initial stage of decay reddish-brown spots of various shapes are formed in mature timber area. Dark transverse cracks appear in the wood. In the next step brownish and then white spots and dark brown (black) winding lines appear on the reddish-brown background. In the final stage of decay cavities appear, and wood turns into brown, spongy mass, easily split into fibers (Fig. 9C). Only a few external annual rings remain intact.

Fungal strains were obtained from a *P. niemelaei* fruiting body by accumulation in a moist chamber, followed by passages on agar medium. The strains formed colonies with characteristic morphological features on the MEA (Fig. 9D, E): substrate hyphae (mycelium) of beige and brown color, close fitting to the surface of the agar medium; fluffy aerial mycelium, sometimes with a powdery coating, consisting of a well-developed direct short hyphae, separate hyphae intertwined in different directions; color white, then yellow, ocher to dark brown with distinct concentric zones; the peripheral part of the colony is less dense; undersurface dark brown; a faint mushroom smell. At 22 °C the average radial growth rate was 0.6 mm / day; at 12 °C the growth rate was no more than 0.1 mm / day; at 6 °C and 26 °C the growth stopped. On plant substrates, the strains formed well-developed colonies of white or yellowish color. When aging



Fig. 9. Fruiting bodies *Porodaedalea niemelaei* on the affected wood (A); root rot in the undergrowth *Larix gmelinii* (B); final stage of rot development in *Larix gmelinii* (C); colony *Porodaedalea niemelaei* on malt extract agar (D); beer-wort agar (E); larch sawdust (F); scanning electron microscopy of the vegetative structures *Porodaedalea niemelaei* (x 3000-10000) (G-I)

along the edge of the colony a cortical layer of brown color was developed (Fig. 9F).

The strains had the following micro morphological features on the MEA: dimitic hyphal system; thin hyphae of 1.0-1.5 microns in diameter, larger hyphae – 2.0-3.0 microns;

septate with rounded ends. Generative hyphae actively branched out under direct and acute angles (Fig. 9G) forming with age irregularly rounded circular and oval-shaped terminal structures (Fig. 9H), resembling chlamydospores, and intercalary hyphal thickening structures

(Fig. 9I). There were no clamp connections; the hyphae were capable of anastomosing. In the submerged culture the hyphae was actively branching forming rounded ends; their cytoplasm was vacuolated.

Wood decomposing properties

The *P. niemelaei* strains were able to colonize used plant substrates (sawdust *L. sibirica* and *P. tremula*; vegetative part *H. tuberosus*): the radial growth rate at 22 °C was in the range of 0.93-0.99 mm per day and was higher than on MEA at 12 °C – 0.31-0.52 mm per day (Fig. 10).

The colonization rate was naturally decreasing when the temperature was reducing, but not much – it was almost the same growing on the plant substrates at 22 °C, and only slightly higher than at 12 °C in the media containing the larch sawdust. The chemical composition changes are presented in Table 1. The greatest weight loss was observed for aspen wood and was more than 50 %, which was almost 3.5 times higher than that for the larch wood and almost two times higher than for the *H. tuberosus* vegetative parts. In *H. tuberosus* the hardly hydrolysable polysaccharides and lignin-like

substance were equally affected, but the water extractable substances and easily hydrolyzable polysaccharides remained practically unchanged quantitatively.

The greatest change in the component composition was observed for the aspen wood: the amount of easily hydrolyzable polysaccharides was reduced by 3.2 times, hardly hydrolysable polysaccharides – by 2.5 times, lignin-like residue – by 2.1 times, while the content of water-extractable substances was increased by 2 times. The larch wood was less affected: the polysaccharide content was reduced by 1.5 times and the content of water-extractable substances was also increased by 1.5 times. These strains did not affect much the lignin-like substances in the larch wood; during cultivation the nonhydrolysable lignin-like residue content was reduced only by 2 %, compared to the starting weight of the substance. This behavior does not mean that the strains do not affect the lignin. It is known that lignin is not always completely decomposed, some part of it turns into a highly condensed product, because degradation processes compete with polymerization and polycondensation reactions. Condensed lignin-containing diphenyl

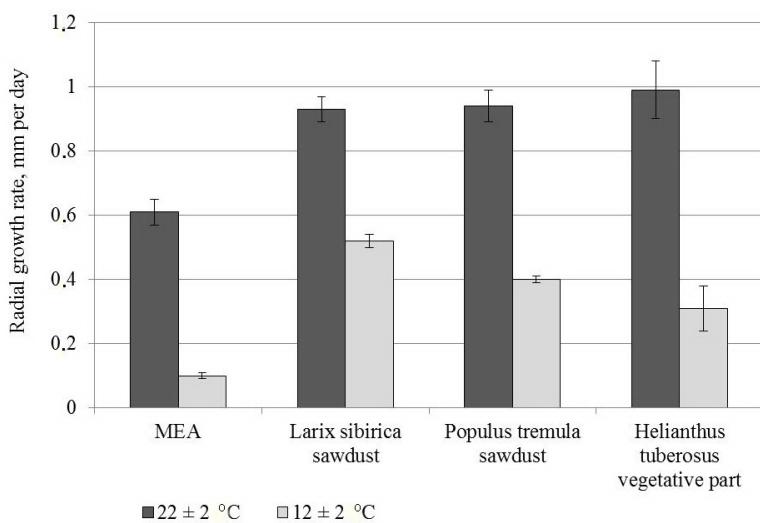


Fig. 10. The radial growth rate of the *Porodaedalea niemelaei* strain on different substrates

Table 1. The chemical composition of plant substrates before and after the treatment by the *Porodaedalea niemelaei* strains, % of dry weight

Substance	<i>Helianthus tuberosus</i> vegetative parts			<i>Larix sibirica</i> sawdust			<i>Populus tremula</i> sawdust		
	before		after	before		after	before		after
	a	b		a	b		a	b	
hot water extractable	25.7	32.1	24.7	12.2	23.3	17.9	2.8	12.3	5.7
easily hydrolysable polysaccharides	13.9	17.4	13.4	24.1	18.3	15.6	23.3	15.6	7.2
hardly hydrolysable polysaccharides	38.3	30.3	23.3	37.2	34.9	26.9	45.1	38.8	17.8
total polysaccharides	52.2	47.7	36.2	61.3	53.2	42.5	68.4	54.4	25.0
non hydrolysable lignin-like residue	17.9	15.1	11.6	24.3	25.2	22.3	23.8	24.8	11.4
weight loss				23.2			15.2		53.8

a and b – without and with correction for the loss of weight, respectively.

bonds exhibit high resistance to fungal enzymes (Dalmova and Arhmedova, 2001).

Molecular phylogenetic analysis

The genetic analysis using four markers commonly used in the phylogenetic analysis of fungi clearly confirmed that this fungus belongs to *Porodaedalea*. With regards to the species, it is clearly different from the well-studied *Porodaedalea* species, such as *P. chrysoloma*, *P. pini*, and *P. cancriformans*. This fungus is very close to the group of unclassified fungi isolated in Norway and Finland (Fig. 11, 12).

Conclusions

On the territory of the Siberian permafrost numerous stands of dying out and fallen *L. gmelinii* trees with a complex of characteristic traits were found. The predominance among dead trees of those that died in the last 10-20 years, lack of accumulation of dead wood mass, signs of biodegradation, and no traces of fires were indicating tree death due to xylotrophic fungi actively destroying wood at a sufficiently high rate. Although the basidiomycete biodiversity was very low in the studied area, the fruiting

bodies of *P. niemelaei* occurred frequently and repeatedly.

It was found that *P. niemelaei* plays a leading role in the pathogenesis of *L. gmelinii*, infecting mechanically damaged roots and causing white (“corrosion”) rot. Butt rot incidence varied from 63 to 100 % depending on the age of the trees and increased for the trees older than 300 years, reaching the maximum height of 9 m above root collar. Root rot was widespread, including larch undergrowth.

The main factor of dying out of *L. gmelinii* was the presence of a *P. niemelaei* infection promoted by mechanical damage to roots by reindeers during migration and facilitated by climatic anomalies characteristic for the study area (a sharp increase in the average air temperature in May and June, decrease in rainfall in June-August, and increase in November-February).

The cultures isolated from the fruiting body were identified as *Porodaedalea niemelaei* M. Fischer based on the combination of morphological, culture, and molecular genetic methods. Under laboratory conditions, the strains were characterized as psychrotolerant (limit temperature of 6-22 °C), slow growing,

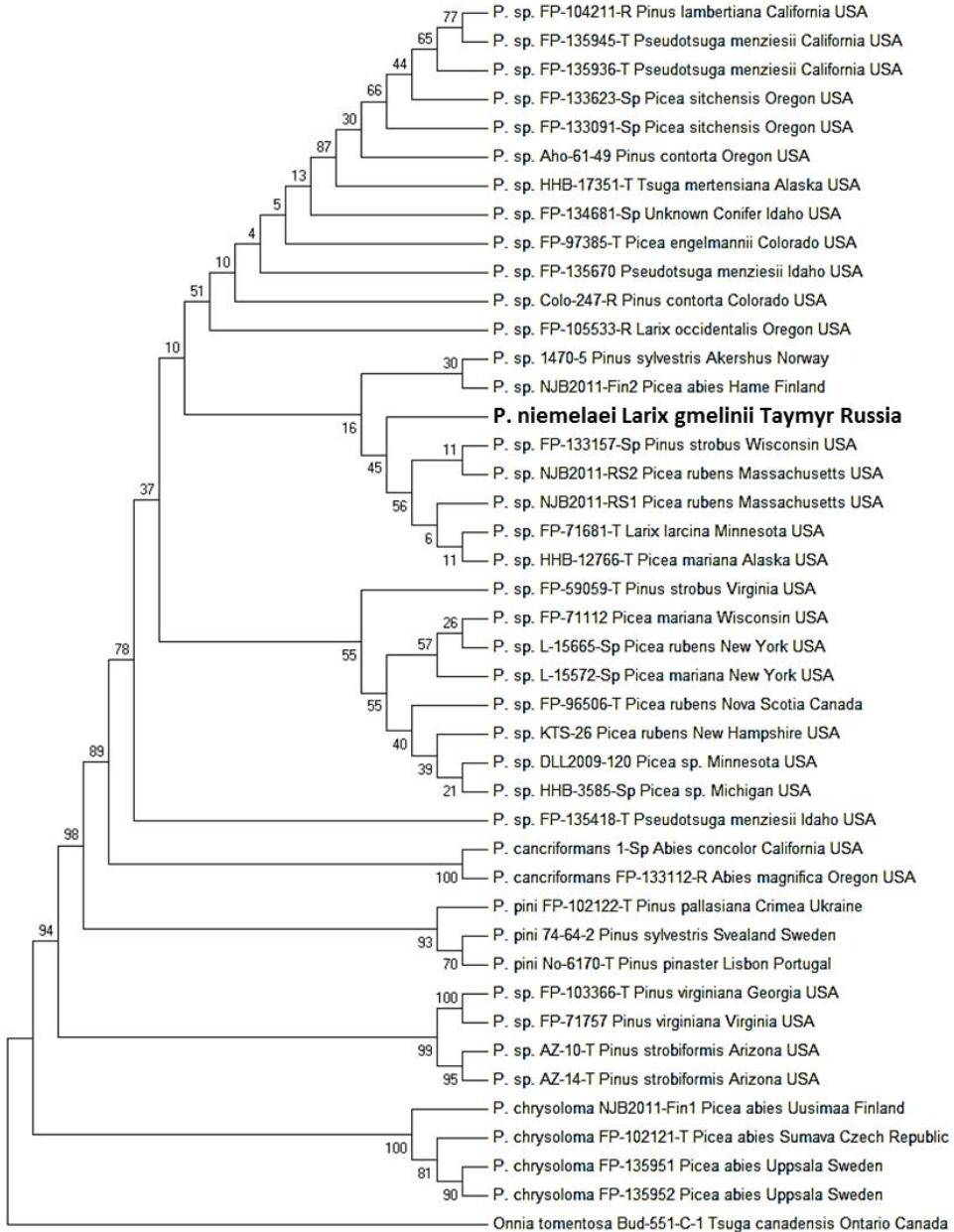


Fig. 11. The bootstrap consensus Maximum Likelihood (ML) phylogenetic tree of 41 *Porodaedalea* isolates published by Brazee and Lindner (2013) together with the *Porodaedalea niemelaei* sample isolated in Taymyr, Russia, and *Onnia tomentosa* used as an outgroup species. First in the labels are given the species names, followed by the isolate IDs and geographic origin. The tree was inferred by using the ML method based on the General Time Reversible model (Nei and Kumar, 2000). The bootstrap values are presented next to the branch nodes as the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test with 1000 replicates (Felsenstein, 1985). The initial tree(s) for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach, and then selecting the topology with a superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.739)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 49.2% sites). All positions containing gaps and missing data were eliminated. There were a total of 2739 positions in the final dataset. The tree was generated using MEGA7 v. 7.0.18 (Kumar et al., 2016).

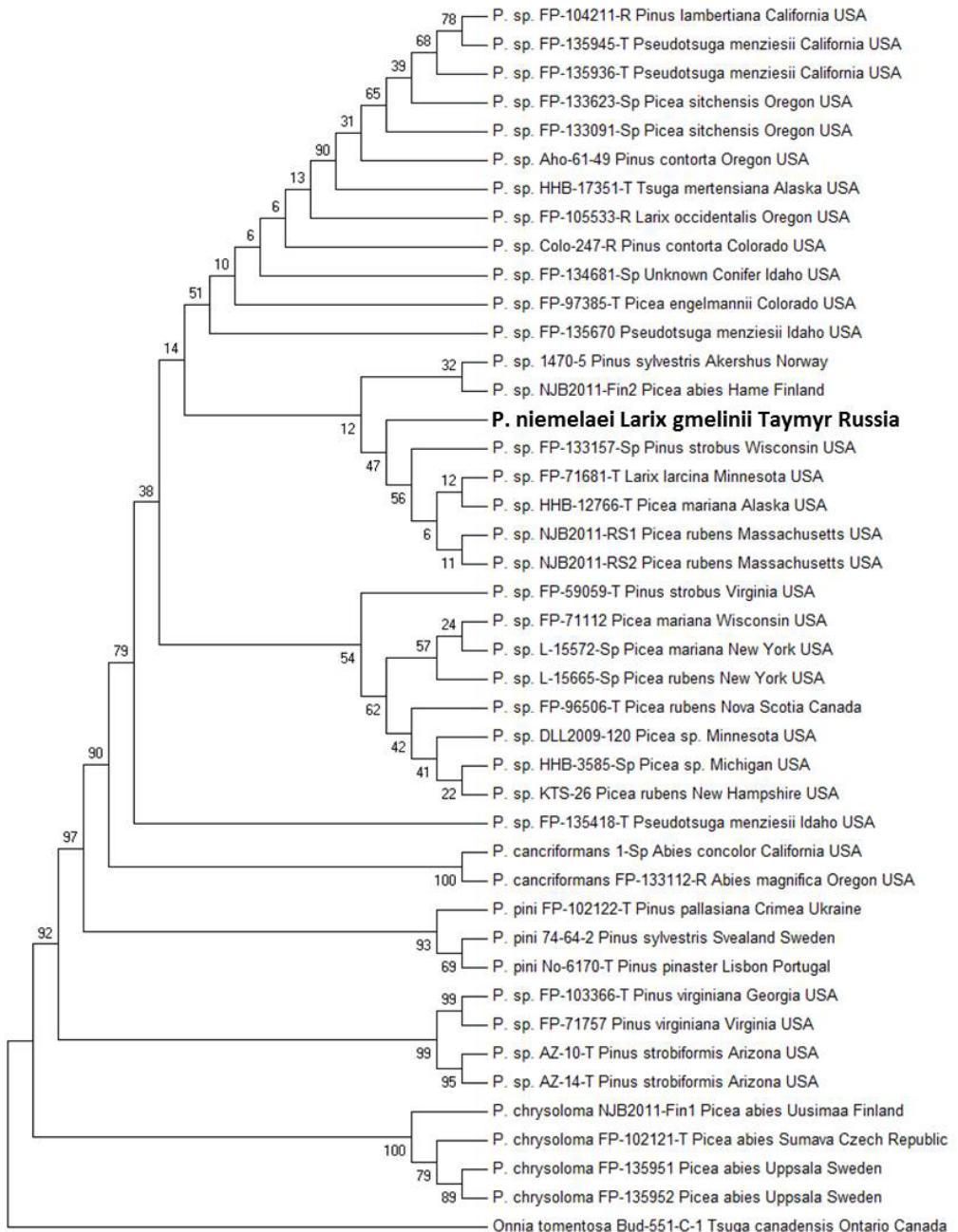


Fig. 12. The bootstrap consensus Neighbor-Joining (NJ) phylogenetic tree of 41 *Porodaedalea* isolates published by Brazee and Lindner (2013) together with the *Porodaedalea niemelaei* sample isolated in Taymyr, Russia and *Onnia tomentosa* used as an outgroup species. First in the labels are given the species names, followed by the isolate IDs and geographic origin. The tree was inferred by using the Neighbor-Joining method (Saitou and Nei, 1987). The bootstrap values are presented next to the branch nodes as the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test with 1000 replicates (Felsenstein, 1985). The evolutionary distances were computed using the maximum composite likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 2739 positions in the final dataset. The tree was generated using MEGA7 v. 7.0.18 (Kumar et al., 2016)

and preferring media consisting of mostly natural substrates. The biodegradation was the most active in the media containing aspen wood substrates with biomass loss of up to 50 %, accompanied by active degradation of the lignocellulosic complex components.

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