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Rhizina Undulata Causing a Rhizina Root Rot on *Larix Cajanderi* in Siberian Taiga Forest of Yakutsk, Russia

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Fruit bodies (YT988134) were analyzed from soil collected under dead *Larix cajanderi* in a Siberian boreal forest site following a fire before one year. The fungal species, *Rhizina undulata*, was identified from morphological examination of the fruit bodies and DNA sequence comparisons. Further phylogenic analysis of the isolate showed clustering with fungi from Korea and Japan, classifying it as an Asian species.

Rhizina undulata, the causative agent of Rhizina root rot, is considered an important soil pathogen in surviving adult trees, as well as in regenerated seedlings of *L. cajanderi* and *Pinus sylvestris*, after fires in Siberian taiga forests.

INTRODUCTION

The taiga forests cover nearly 3 million square km, and contribute to store a very large global carbon (Bonan and Van Cleve, 1992; Schulze *et al.*, 1995). But, forest fires have occurred frequently in the Siberian taiga in the permafrost areas (Dixon and Krankia, 1993). Unfortunately, approximately 30 thousand wildfires arise in this area annually (Valendik, 1996). These huge events may have been causing the great ecological changes on vegetation dynamics and global climate change in response to rising levels of carbon dioxide. Moreover, many scientists consider that forest fires in the boreal zone determine stand structure and dynamics (Isaev and Utkin, 1963; Kurbatskiy, 1964; Roe and Beufait, 1971; Frissel, 1973; Odum, 1975; Scherbakov *et al.*, 1979; Furyaev, 1996; Granstorm, 1996; Godammer and Furyaev, 1996). Therefore, it is a very urgent problem to clearly the effects of forest fires on the biosphere in Siberian taiga forests.

The production of fruit bodies by *Rhizina undulata* Fr. ex Fr. is abundant at burn sites, as the spread of Rhizina root rot is dependent on forest fires (Germmen, 1961; Hargner, 1962; Ginns, 1968; Gibson, 1970). This disease causes the death of adult conifers (Hartig, 1891), including pine (*Pinus*), fir (*Abies*), spruce (*Picea*), hemlock (*Tsuga*), and larch (*Larix*) species (Weir, 1915; Gibson, 1970; Germmen, 1971; Thompson and Tattar, 1973) in Europe and North America (Hartig, 1891, 1892; Weir, 1915; Biraghi, 1963; Gibson, 1970; Germmen, 1971). Similarly, Weir (1915) reported that seedlings raised in nursery environments were attacked by this fungus via damping–off and spread by the formation of fairy rings.

In actual, it is needed an examination to clarify the relationship between forest firs and biodiversity including fungi in Siberian taiga forests. So, we conducted survey to fungal damages of trees after fires, collected fruit bodies near dead *Larix cajanderi* Mayr trees in post–fire site. The morphological characteristics of fruit bodies were described, and compared DNA sequences for species identification. Finally, we conducted phylogenic analyses with other continental isolates of *R. undulata*.

MATERIALS AND METHODS

Study site, sample collection, and identification

The study site was located near the Kenkeme River (62° N, 129° E), about 40 km northwest of Yakutsk, Far East Russia. *Larix cajanderi* was the dominant tree species, followed by *Pinus sylvestris* L.. Three fruit bodies (YT988134), collected in soil under dead *L. cajanderi*, were studied at the macroscopic and microscopic levels for species identification (Fig. 1A, B). The fruit bodies were dried, used for DNA extraction, and stored in the herbarium of the Experimental Forest of Hokkaido University, Japan. Microscopic observations were recorded primarily from dried specimens. Spore sizes and other microstructures were measured in 5% KOH. Symptoms of Rhizina root rot on dead roots of *L. cajanderi* were observed. A Munsell soil color chart (1994) was used to analyze the diagnostic fruit body colors.

DNA extraction and sequencing

Fungal DNA was extracted from 50 to 100 mg of dried fruit body using the DNeasy Plant Mini kit (Qiagen, USA), according to the manufacturer's instructions. The internal transcribed spacer (ITS) region, including the 5.8S ribosomal DNA (rDNA) segment, was amplified using primers specific for the higher fungi ITS1–f (Gardes and Bruns, 1993) and ITS4 (White *et al.*, 1990)

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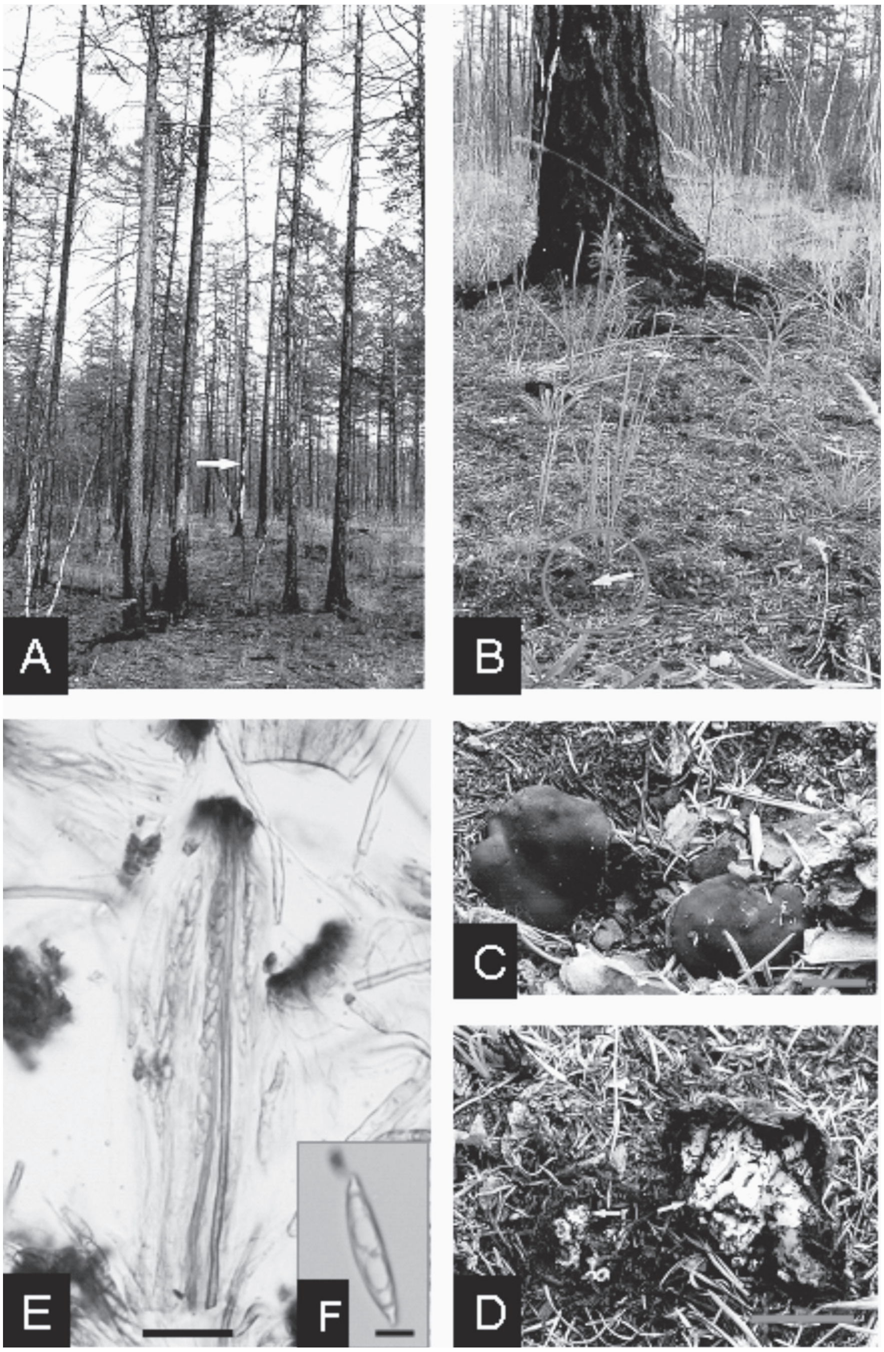

Fig. 1. Dead tree of *L. cajanderi* (A, whitish arrow); Fruit body formation of *R. undulata* on soil near A (B, whitish arrow); Fruit body of *R. undulata* (C); whitish strands formation on soil and beneath of fruit body (D, whitish arrow); Asci eight–spored (E); Spore (F). Bars C and D, 2 cm; E, 100 μm; F, 10 μm.

regions. Each 50–μl amplification reaction mixture contained 5 μl of 10X G–Taq buffer, 4 μl of 2.5 M dNTP, 5 μl of 5X Tuning buffer (Cosmo Genetech, Seoul, Korea), and 0.5 μl G–Tag DNA polymerase (Cosmo Genetech, Seoul, Korea). Amplifications were performed using a 2720 Thermal Cycler (Applied Biosystems). Initial denaturation started at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 48 °C for 1 min, and extension at 72 °C for 1 min; a final extension was performed at 72 °C for 10 min. The PCR products were purified using the LaboPass PCR purification kit (Cosmo Genetech, Seoul, Korea). Sequencing of the purified products was carried out using a BigDye® Terminator v3.1 Cycle Sequencing Kit and the GeneAmp® PCR System 9700. The BigDye® XTerminator™ Purification Kit was used to purify the reaction products. An Applied Biosystems 3730xl DNA Analyzer was used for analysis. All steps were carried out according to the manufacturer's instructions.

Analysis of DNA sequence data

ITS sequences were compared to GenBank database entries of the DNA Data Bank of Japan (DDBJ) using the basic local alignment search tool (BLAST), and the homology for YT988134 was calculated. All sequences used in the analysis, except that of the isolate used in

Table 1. *Rhizina undulat*a collected in this study and best BLAST mach

Species	Origin	Gen Bank accession no.	Similarity (%)
Rhizina undulata	Korea/Gosung	EU339123	100
Rhizina undulata	Korea/Taean	EU346952	100
Rhizina undulata	Japan/Iwate	EU346953	100
Rhizina undulata	Korea/Samcheok	EU346951	99

this study, were GenBank accessions (Table 1).

Phylogenetic analysis was performed for each data set using neighbor–joining (Saitou and Nei, 1987). The ITS sequences were initially aligned using CLUSTAL W in DDBJ. Neighbor–joining analysis was performed using the Tamura–Nei model for estimation of evolutionary distance, and relative support for nodes in resulting trees was generated using 1000 bootstrap replicates (Felsenstein, 1985).

RESULTS

Sample collection and identification

The whitish mycelia under the bark of roots of dead *L. cajanderi* were examined. Three fruit bodies were collected from soil on 1 August 1998 (Fig. 1C, D). Macro– and micro–morphological features of the fruit body (YT988134) were described in detail. The cap of the fruiting body measured between 3.2 and 4.5 cm in diameter, was 1.5–2 cm in thickness, appeared as a flat disk when young, and had an irregular, undulating, and usually dry surface. The color was age–dependent; when young, the fruit bodies were red (2.5YR–4/8), and later became dark reddish brown (2.5YR–3/3) or reddish black (10YR–2.5/1). Their surface was bordered by a pinkish white (5YR–8/2) to white margin (10YR–8/1); their underside was pale yellow (2.5Y–8/3) or very pale brown (10YR–8/2), and was attached to soil by several cord–like strands. The fungus possessed asci that were cylindrical in shape, with thick walls of 450–475 μm in length and 10–17.5 μm in width, and each contained eight spores (Fig. 1E). Cylindrical paraphyses were present, with a slight clavate, and were approximately 450–500 μm length, and 5–7.5 μm thick. Setae were brown, thick–walled, aseptate, 500–652 μm length, and 5–12.5 μm thick. Spores were hyaline, thin–walled, smooth, apiculate, with long, conical appendages, elliptical–fusiform in shape, and ranged from 32.5 to 40 μm in length and 7.5–12.5 μm thick (Fig. 1F). The fungus was gregarious and grew in soil under *L. cajanderi* in burned areas.

DNA sequencing

The YT988134 ITS sequence was 633 base pairs long. A BLAST search using the ITS 1 and 5.8S gene sequences for this isolate against sequences in DDBJ revealed that the DNA sequences of the fungus showed similarities of 99 to 100% to those identified as *R. undulata* (Table 1).

Analysis of DNA sequence data

Ten *R. undulata* DNA sequences, including that of YT988134 (RUSSIA/Yakutsk), formed three main groups in a neighbor–joining tree, albeit with low bootstrap support of 290 and 217. Group I sequences originated from the Netherlands and two unknown regions. Group Ⅱ sequences originated from unidentified areas. Finally, *R. undulata* from Russia in this study was included in group Ⅲ, with sequences collected from Korea and Japan (Fig. 2).

DISCUSSION

YT988134 collected in this study was characterized based on the morphology of fruit bodies. In addition, a BLAST search in DDBJ of the ITS region confirmed the identity of the fungus as *R. undulata*. Cladistics of the *Rhizina* isolates categorized YT988134 as an Asian strain, since it belonged to a group of isolates collected in Korea and Japan. However, the bootstrap support for this continental origin using the ITS region was weak. Thus, further analyses of different regions of ribosomal DNA, such as the intergenic spacer region (IGS), and restriction fragment length polymorphism (RFLP) studies are required to identify phylogenetic relationships among the species.

After damage by a forest fire, symptoms of Rhizina root rot such as whitish hyphal strands on the surface and a hyphal mat were visible under the bark of *L. cajanderi* roots. This is the first report for Rhizina root rot in Cajander larch after fires in boreal forests of Yakutsk, Russia.

In Japan, Rhizina root rot is very common in pine trees on burn sites of costal forests specially. Sato (1971) reported that this fungus occur group dying to pine trees in which the forest stands was without damages of the bonfire or forest fires. Also, *R. undulata* caused this disease to Japanese black pine (*Pinus thunbergii* Parl.) near bonfire sites in costal forests, and to Japanese red pine (*P. densiflora* Sieb. et Zucc.) at burned sites of forest fire in Korea (Lee and Kim, 1990; Lee *et al*., 2005). Although this study site was small areas in the Siberian taiga forests and just a specimen including 3 fruit bodies was used in results, many trees of *L. cajanderi* and *P. sylvestris* surviving from forest fires may be attacked by this fungus and dead, because of the numerous wildfires arise in this area annually (Valendik, 1996).

Fruit body formation of *R. undulata* was reported to be favorable in acidic soil (Jalaluddin, 1967), less so in

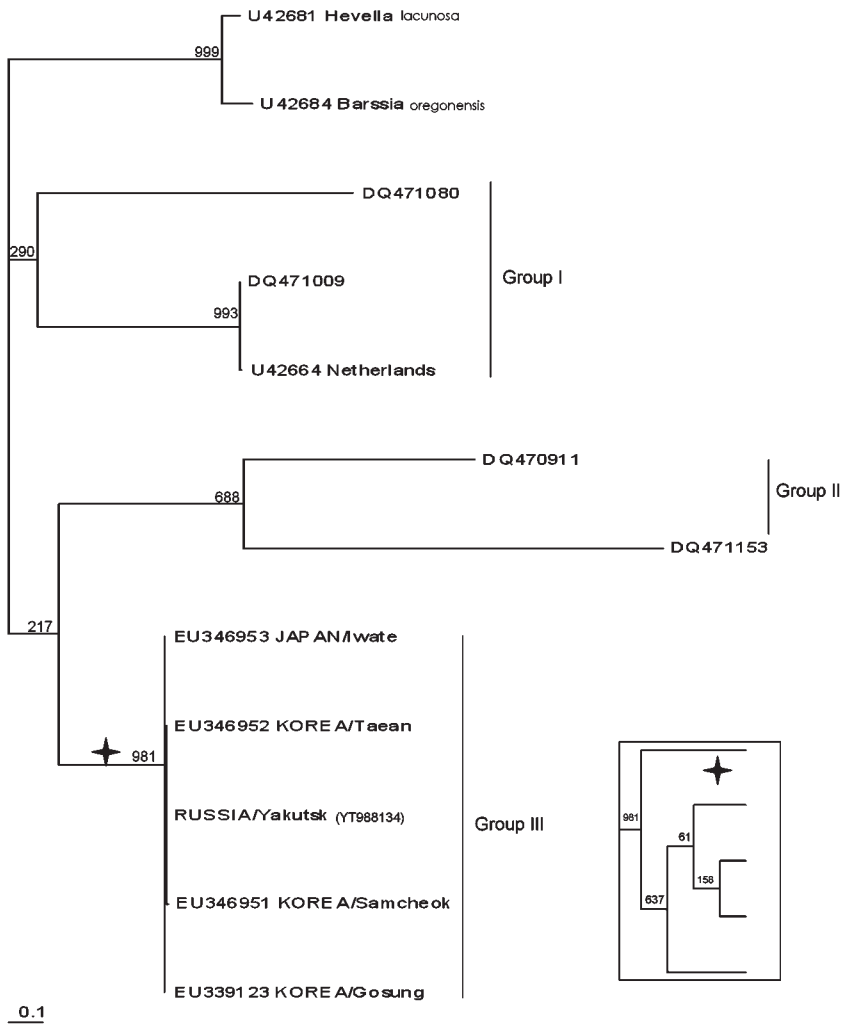

Fig. 2. Phylogram generated from Neighbor–joining analysis of the ITS sequence data used in this study. Bootstrap values are showed on the branch. *Hevella lacunosa* and *Barssia oregonensis* are used as the out group.

neutral soil, and nearly absent in alkaline soil (Sato, 1971). On the other hand, damping–off of Douglas fir, sequoia, and beech seedlings created considerable damage in alkaline soil of pH 8, but was slight in acidic soil (Schonhar, 1955). Other soil–borne diseases, caused by *Rhizoctonia solani* Kuhn and *Fusarium* sp., resulted in damping–off of coniferous seedlings, and were severe on neutral to alkaline soils compared to acidic soil (Tint, 1945; Ito, 1950; Sato and Shoji, 1964). The forest in the Kenkeme area has dry sandy soil, and soil at the burned site was alkaline (Sawamoto *et al.*, 1997). Temperatures in the 35 to 45 °C range are also required for spore germination of *R. undulata* (Jalauddin, 1967).

In the Siberian taiga forests, therefore, it can be concluded that forest fires create favorable environmental conditions for the infection and spread of *R. undulata*. So, any adult trees that survived from the fire will be damaged by *Rhizina* root rot. Moreover, the seedlings bank established after fire will disappear as a result of damping–off due to this fungus. Therefore, the taxonomy and ecology of *R. undulata* for conservation of the Siberian taiga forests will be conducted in detail in the future.

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