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Full paper

Russula velenovskyi new to Japan, with phylogenetic implications of *Russula* species between Japanese subalpine forests and Northern Europe

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ABSTRACT

To compare morphological characters and phylogenetic placement between Japanese and European *Russula*, 32 specimens of 12 species were collected from Japanese subalpine forests and Northern Europe. Several sequences of nrDNA ITS region (ITS) of these *Russula* species were obtained. High homological similarities were shown between ITS sequences of several *Russula* samples collected from Japanese subalpine forests, Europe and North America. These facts show distribution of the same *Russula* species among these areas. From morphological observations and phylogenetic analyses, two same *Russula* species, *R. velenovskyi,* and *R. decolorans* are found in Japan, Europe and North America. Of these, *R. velenovskyi* collected from Mt. Fuji, Mt. Nyukasa and Mt. Tateshina in mountainous area of central Honshu is reported as a new Japanese record.

1. Introduction

The genus *Russula* Pers. (Russulaceae, Russulales), which is one of the most important symbionts associated with various forest trees in subalpine to alpine zones. Although species diversity of *Russula* in subalpine to alpine zones has intensively been studied in Europe and North America (Aučina et al., 2011; Jamoni, 2008; Miller & Buyck, 2002; Ohenoja & Ohenoja, 2010; Romagnesi, 1985; Sarnari, 2005), there are a few records of *Russula* species in subalpine to alpine forests of Japan (Ikeda, 2013; Murata, 1978). Recently, to enumerate *Russula* species in Japanese subalpine forests, we have carried out samplings of macrofungal specimens around Mt. Fuji (peak altitude, 3776 m; N35.21.38; E138.43.39), Mt. Nyukasa (alt. 1955 m; N35.53.47; E138.10.18) and Mt. Tateshina (alt. 2531 m; N36.06.13; E138.17.42) in the mountainous area of central Honshu and DNA sequencing of several specimens. Among them, we collected fruiting bodies that morphologically match *R. velenovskyi* Melzer & Zvára hitherto not recorded in Japan, in mixed forests dominated by *Betula ermanii* Cham., *Abies veitchii* Lindl. and *Tsuga diversifolia* (Maxim.) Mast. Moreover, to compare morphologies and phylogenetic placement to Japanese specimens, we have conducted fieldworks in Norway and Germany, and several *Russula* species including *R. velenovskyi* have been collected. In this paper, we briefly describe the morphology and habitat of the Japanese collections of *R. velenovskyi*, and examine their phylogenetic placement based on a nuclear ITS rDNA gene dataset. We further discuss the homology of *Russula* species among Japanese subalpine forests, Northern Europe and North America based on their phylogeny. In this paper, infrageneric classification of *Russula* followed Bon (1988).

2. Materials and methods

2.1. Fungal specimens and morphological observations

We collected 32 *Russula* specimens including *R. velenovskyi* in Japan, Norway and Germany by our fieldworks. Fruiting bodies collected for the present study were air-dried after macro- and microscopic observation and DNA extraction. The specimens were deposited in the herbarium of Osaka Museum of Natural History, Japan (OSA; Table 1). Morphologies of the fruiting bodies were recorded according to Shimono, Hiroi, and Takamatsu (2014).

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Table 1
Russula species newly examined in the present study with associated GenBank accession numbers of ITS sequences.

Taxon name	Sampling date	Collector	Locality	Specimen ID	GenBank ID
Section *Tenellae*					
Russula cessans	2015/9/22	Y. Shimono	Germany, Neustreliz	OSA–MY–7811	LC192757
Section *Decolorantes*					
R. decolorans	2012/8/23	Y. Shimono	Norway, Oslo	OSA–MY–7780	LC192758
R. decolorans	2012/8/23	Y. Shimono	Norway, Oslo	OSA–MY–7781	LC192759
R. decolorans	2013/9/7	Y. Shimono	Norway, Oslo, Lillomarka	OSA–MY–7782	LC192760
R. decolorans	2013/9/7	Y. Shimono	Germany, Neustreliz	OSA–MY–7783	LC192761
Section *Viridantes*					
Russula sp.	2015/9/5	Y. Shimono	Japan, Yamanashi, Mt. Fuji	OSA–MY–7784	LC192762
Russula sp.	2015/9/5	Y. Shimono	Japan, Yamanashi, Mt. Fuji	OSA–MY–7785	LC192763
R. aff. *favrei*	2015/9/5	Y. Shimono	Japan, Yamanashi, Mt. Fuji	OSA–MY–7786	LC192764
Section *polychromae*					
R. integra	2015/9/23	Y. Shimono	Germany, Neustreliz	OSA–MY–7787	LC192765
R. integra	2015/9/23	Y. Shimono	Germany, Neustreliz	OSA–MY–7788	LC192766
R. integra	2015/9/23	Y. Shimono	Germany, Neustreliz	OSA–MY–7789	LC192767
R. integra	2015/9/23	Y. Shimono	Germany, Neustreliz	OSA–MY–7790	LC192768
R. integra	2015/9/23	Y. Shimono	Germany, Neustreliz	OSA–MY–7791	LC192769
R. integra	2015/9/23	Y. Shimono	Germany, Neustreliz	OSA–MY–7792	LC192770
Section *Coccineae*					
R. velenovskyi	2012/8/25	Y. Shimono	Norway, Oslo	OSA–MY–7793	LC192771
R. velenovskyi	2012/8/25	Y. Shimono	Norway, Oslo	OSA–MY–7794	LC192772
R. velenovskyi	2015/9/4	Y. Shimono	Japan, Yamanashi, Mt. Fuji	OSA–MY–7795	LC192773
R. velenovskyi	2015/9/5	Y. Shimono	Japan, Yamanashi, Mt. Fuji	OSA–MY–7796	LC192774
R. velenovskyi	2012/8/18	Y. Shimono	Japan, Nagano, Mt. Nyukasa	OSA–MY–7797	LC192775
R. velenovskyi	2012/8/14	Y. Taneyama	Japan, Nagano, Mt. Tateshina	OSA–MY–7798	LC192776
R. paludosa	2012/8/25	Y. Shimono	Norway, Oslo	OSA–MY–7799	LC192777
R. paludosa	2013/9/7	Y. Shimono	Norway, Oslo	OSA–MY–7800	LC192778
R. paludosa	2013/9/11	Y. Shimono	Germany, Neustreliz	OSA–MY–7801	LC192779
Section *Fragiles*					
R. emetica	2015/9/18	Y. Shimono	Norway, Oslo	OSA–MY–7802	LC192780
R. emetica	2015/9/18	Y. Shimono	Norway, Oslo	OSA–MY–7803	LC192781
R. aff. *laccata*	2015/10/24	K. Hashimoto	Japan, Nagano, Sugadaira	OSA–MY–7804	LC192782
Section *Atropurpurinae*					
R. aquosa	2015/9/18	Y. Shimono	Norway, Oslo	OSA–MY–7805	LC192783
R. aff. *aquosa*	2015/9/5	Y. Shimono	Japan, Yamanashi, Mt. Fuji	OSA–MY–7806	LC192784
Section *Sanguineae*					
R. aff. *queletii*	2015/9/5	Y. Shimono	Japan, Yamanashi, Mt. Fuji	OSA–MY–7807	LC192785
R. drimeia	2015/9/18	Y. Shimono	Norway, Oslo	OSA–MY–7808	LC192786
Section *Insidiosae*					
R. aff. *badia*	2015/9/4	Y. Shimono	Japan, Yamanashi, Mt. Fuji	OSA–MY–7809	LC192787
Section *lilaceae*					
R. aff. *lutea*	2015/9/4	Y. Shimono	Japan, Yamanashi, Mt. Fuji	OSA–MY–7810	LC192788

Micromorphological features of specimens were examined using a microscope BH2 (Olympus, Tokyo, Japan) under the phase contrast. Color annotations of fresh materials were determined based on Kornerup and Wanscher (1978). Measurements were made on more than 50 randomly selected basidiospores, and the size average was given in the description. Value of quotient (Q) of length and width average was also calculated to indicate basidiospore shape.

2.2. DNA extraction and molecular phylogenetic analysis

The nucleotide sequences of ITS region including the 5.8 ribosomal RNA gene were sequenced for 32 specimens in this study. Their specimen number and accession numbers were given in Table 1. Additional 20 ITS sequences were retrieved from GenBank and DDBJ databases for phylogenetic analysis. The procedures of DNA extract, polymerase chain reaction amplification (PCR), primer pairs, sequencing, and phylogenetic analysis were described in Shimono, Kato, and Takamatsu (2004, 2014). Only different parts are described here. Fungal DNA was extracted from the lamellae of 32 fresh fruiting bodies using Indicating FTA Cards (Whatman International Ltd., Maidstone, England) based on the manufacturer's protocol for plant samples. PCR amplification of the ITS region was carried out using one prepared FTA disc 2 mm diam, according to the manufacture's instruction. The primer pairs for PCR amplification were ITS1F/ITS4B (Gardes & Bruns, 1993) or ITS1/ITS4 (White, Bruns, Lee, & Taylor, 1990) for ITS region. PCR reactions were performed using KOD FX Neo DNA polymerase (Toyobo, Tokyo, Japan) in 25 mL reaction volumes containing 5 μL of 0.2 mM dNTP, 12.5 μL of PCR buffer, and 0.5 U KOD FX Neo. PCR conditions for ITS was 94 °C for 2 min, followed by 40 cycles at 98 °C for 10 s, 55 °C for 30 s, 68 °C for 1 min, and a final 6 min at 68 °C. The DNA sequencing was performed at SolGent Co. Ltd. (Daejeon, South Korea) using an ABI 3700 automated DNA Sequencer (Applied Biosystems, Waltham, USA).

32 sequences newly generated by the present study were deposited in DNA Data Base of Japan (DDBJ) under the accession number LC192757–LC192788. The final alignment is available from TreeBASE (http://www.treebase.org/) as a NEXUS file under the accession number 20215. According to the Akaike Information criterion value, a general time reversible with gamma distributed rate heterogenetic and a proportion of invariant sites (GTR + G + I) was chosen as the optimal substitution model for the ITS analysis (Akaike, 1974). Phylogenetic analyses of the sequence data were performed in MEGA 6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) with the help of the maximum likelihood (ML) method after testing the best models. For the ML, Clade robustness was assessed using a bootstrap analysis with 1000 replicates (Felsenstein, 1985).

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