Türler ve Habitatlar

(2020) 1(2): 45–52
www.turvehab.com
e-ISSN: 2717-770X

Research Article

Confirmation of the existence of *Russula praetervisa* in Turkey

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Received: 15.05.2020 Accepted: 01.07.2020 Published Online: 01.12.2020

Abstract
Russula praetervisa is a species whose presence in Turkey is still unproven. It is included in the table of contents of the *Macrofungi of Turkey Checklist*, but there is no data that specimens have been collected. As a result of the identification of the specimens collected from Datça County of Muğla Province, it became definite that this species is found in Turkey. The macroscopic, ecological and microscopic characters of the species have been given with photographs. Also, the Internal Transcribed Spacer (ITS) gene of the specimen was sequenced, analyzed and a phylogenetic tree illustrating closely related taxa has been presented. The findings have been discussed within the scope of the relevant literature.
Keywords: ITS, biodiversity, *Russula*, taxonomy, Turkey

Russula praetervisa'nın Türkiye'deki varlığının teyidi

Özet
Russula praetervisa, Türkiye'de varlığı henüz kanıtlanmamış bir türdür. *Macrofungi of Turkey Checklist*'te içindekiler kısmında yer almakta fakat örneğin toplandığına dair herhangi bir veri bulunmamaktadır. Muğla ilinin Datça ilçesinden toplanan örneklerin teşhisi sonucu bu türün ülkemizde bulunduğu kesinlik kazanmıştır. Türün makroskobik, ekolojik ve mikroskobik karakterleri fotoğraflarıyla birlikte verilmiştir. Ayrıca örneğin ITS gen dizisi belirlenmiş, analiz edilerek en yakın türleri de gösteren filogenetik ağaç çizilip sunulmuştur. Bulgular uygun literatür kapsamında tartışılmıştır.
Anahtar kelimeler: ITS, biyoçeşitlilik, *Russula*, taksonomi, Türkiye

INTRODUCTION

Russula Pers. is an ectomycorrhizal genus comprising about 750 species in the world (Kirk et al. 2008), generally characterized by brightly colored caps, white to dark yellow-ochre spore print and amyloid ornamented spores. There are six subgenera named as *Compactae* (Fr.) Bon, *Heterophyllidia* Romagn., *Amoenula* Sarnari, *Ingratula* Romagn., *Incrustatula* Romagn. and *Russula* Pers. belonging to this genus (Sarnari 1998). The taxonomic studies related to the genus are ongoing in the world (Dutta et al. 2015; Li et al. 2015; Melera et al. 2017) and in Turkey (Keleş et al. 2014; Doğan & Öztürk 2015; Işık & Türkekul 2017; Çolak et al. 2018).

Russula praetervisa Sarnari is only found in the table of contents of the *Macrofungi of Turkey Checklist Vol. II*, but is not included in the text (Solak et al. 2015). In fact, we presented a poster with the specimens of this species that we found in Datça, Turkey, at the Biodiversity Congress (Bölük et al. 2013). The fact that this poster presentation was not mentioned in the checklist caused

Suggested Citation:
Tırpan, E., Allı, H. & Çöl, B. (2020). Confirmation of the existence of *Russula praetervisa* in Turkey. *Türler ve Habitatlar* 1(2): 45–52.

confusion. In this study, it was confirmed that *Russula praetervisa* exists in Turkey as a result of the identification of specimens collected from Datça County of Muğla Province.

Russula praetervisa belongs to the subsections *Foetentinae* (Melzer & Zvára) Singer of the subgenus *Ingratula*. Subsection *Foetentinae* is characterized by a fetid odor, tuberculate-striate pileus margin, and articulated and branched hair cuticles (Shaffer 1972; Romagnesi 1985; Sarnari 1998). Previously, six species belonging to this subsection, were recorded in Turkey: *Russula amoenolens* Romagn. (Uzun et al. 2006), *R. foetens* Pers. (Sesli 1993; Akata et al. 2009; Demirel et al. 2010; Uzun 2010), *R. grata* Britzelm. (Uzun 2010), *R. pseudoaffinis* Migl. & Nicolaj (Çolak et. al. 2015), *R. sororia* (Fr.) Romell. (Sesli & Denchev 2014) and *R. subfoetens* W.G.Sm. (Watling & Gregory 1977; Sesli & Baydar 1995; Afyon et al. 2004). Identification and accurate classification of these fungi has proven challenging, because of significant similarities in morphology, particularly among the fruiting bodies (Lee et al. 2017). Therefore, analyzing the phenotypic characters as well as taking advantage of the molecular analyzes will eliminate the potential confusion for mycologists and provide more reliable results.

MATERIAL AND METHOD

The main material of the study, the fungal specimens, were collected on 27 December 2012 on the Datça Peninsula of Muğla Province during routine field studies. This specimen was identified and discussed by using classical taxonomy and phylogenetic relationships based on the ITS sequences.

Macroscopic and microscopic observations

The morphological features and habitat of the specimens were photographed and recorded. Microscopic observations were made after applying Congo red for the pileipellis elements and cystidia, and Melzer's reagent was used to observe the amyloidity of spores and tissues. Spores, basidia and cystidia were measured with a Leica DM750 microscope and noted. The literature was used for the identification (Sarnari 1998; Roux 2006; Socha et al. 2011; Kibby 2012; Knudsen & Vesterholt 2012). The specimens were stored as fungarium material at the Muğla Sıtkı Koçman University.

DNA extraction, PCR amplification, and sequence analysis

The mechanical fractionation of the dry material was carried out with liquid nitrogen. Genomic DNA was obtained using the DNeasy Plant Mini Kit (No: 69106, Qiagen, Germany). The amount of template sample to be used in the PCR analysis was determined based on the agarose gel image result of the DNA isolation. The internal transcribed spacer (ITS) was amplified using forward primers ITS1F and reverse primers ITS4 (Gardes & Bruns 1993; Park et al. 2013). DNA Sanger sequencing was performed by Macrogen (Netherlands). The BioEdit software program (Hall 1999) was used for sequence analysis. The BlastN program in the National Center for Biotechnology Information (NCBI) database was used and sequences of close species were found (Table 1). Then, the MEGA6 program was used for multi-alignment and a phylogenetic tree was constructed by the Neighbor-joining method (Kimura 1980; Tamura 2013).

RESULTS AND DISCUSSION

Basidiomycota R.T. Moore

Agaricomycetes Doweld
Russulales Kreisel ex P.M.Kirk, P.F. Cannon & J.C.David
Russulaceae Lotsy
Russula Pers.

Russula praetervisa Sarnari, Monografia Illustrata del Genere Russula in Europa 1: 463 (1998). **Holotype.** Italy. Grosseto, Porto Ercole, Pineta di Feniglia, under *Pinus* and *Quercus*, 10.11.1997, Merlini s.n. (IB 1997/0182).
Description. Pileus 4−6.5 cm, flattened and center depressed, viscid and shiny, margin striate-pectinate, ocher-brown (Figure 1a). Lamellae crowded, whitish-cream. Stipe cylindrical, firm, white but base reddish colored (Figure 1b). Taste mild. Odor like earthy. Basidiospores 6.8−8.5 × 5.4−7 µm, ornamented, amyloid (Figure 1c). Hymenial cystidia mucronate to lageniform (Figures 1d−e). Radial section through pileipellis (Figures 2a−f).
Habitat. *Pinus brutia* Ten. and *Liquidambar orientalis* Mill. mixed forest, sandy soil.

Table 1. Taxa, their Genbank accession numbers, geographic origins and references.

Taxon	ITS Genbank Acces. No.	Geographic origin	References
Russula praetervisa	MK327978	Turkey	**Present paper**
R. praetervisa	KF303597	Italy	Melera et al. 2017
R. praetervisa	KF303598	Italy	Melera et al. 2017
R. praetervisa	KJ530860	Italy	Melera et al. 2017
R. praetervisa	KJ530749	Morocco	Melera et al. 2017
R. pectinatoides	KF245514	USA	Unpublished
R. pectinatoides	EU819493	USA	Palmer et al. 2008
R. amoenolens	GQ166870	USA	Unpublished
R. pectinatoides	KX574696	Korea	Unpublished
R. pectinatoides	KX574694	Korea	Unpublished
R. pectinatoides	JX434670	–	Unpublished
R. pectinatoides	KM052566	Korea	Unpublished
R. recondita	KJ530752	Switzerland	Melera et al. 2017
R. recondita	KJ834611	Switzerland	Melera et al. 2017
R. pectinatoides	JF908639	Italy	Osmundson et al. 2013
R. pectinatoides	DQ422026	–	Unpublished
R. recondita	KJ530757	Spain	Melera et al. 2017
R. recondita	KJ530756	Switzerland	Melera et al. 2017
R. recondita	KF318063	Switzerland	Melera et al. 2017
R. recondita	NR147635	Switzerland	Melera et al. 2017
R. foetens	AF418613	European	Eberhardt 2002
R. subfoetens	KY681430	China	Unpublished
R. subfoetens	JF908672	Italy	Osmundson et al. 2013
R. foetens	JF908679	–	Osmundson et al. 2013
R. juniperina	MH999871	Turkey	Unpublished

Figure 1. *Russula praetervisa*. a-b. Basidiocarps, c. Basidiospores (in Melzer), d-e. Hymenial cystidia (in Congo red).

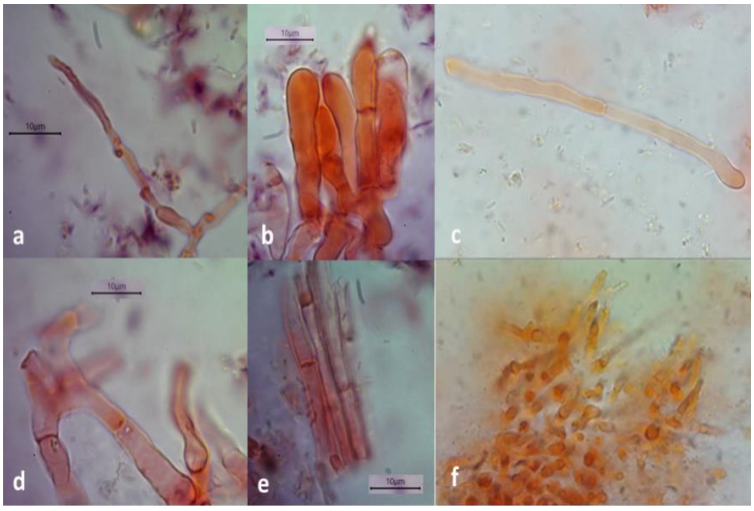

Figure 2. *Russula praetervisa*. a-f. Elements of pileipellis (in Congo red).

Taxonomic notes

According to Çolak and Işıloğlu (2016), although some taxa can be easily distinguished, the multiplicity of species, the extreme similarity between each specimen and the necessity of microscopic detail for identification, make it difficult to distinguish the *Russula* genus species. Some mycologists also emphasize that the *Russula* genus is difficult to identify and that European species are more complex (Bazzicalupo et al. 2017).

Russula praetervisa and *R. pectinatoides* Peck are species close to each other (Melera et al. 2017). Sarnari (1998) took an important step in the right direction by describing *Russula praetervisa* as a species corresponding to nearly identical specimens having distinctly subreticulate spores, the dominant taxon in the Mediterranean area. Sarnari (1998) bases his decision to separate the European concept of *Russula pectinatoides* from the North American concept mainly on spore ornamentation. He observed that the North American material is characterized by spores with

isolated warts, while the Mediterranean material was different in that it had thin connections between the warts. It should be mentioned, however, that Shaffer (1972) shows connections between the warts in one of the drawings of the spores of *Russula pectinatoides* from North America (Bazzicalupo 2018).

In cases where such classical taxonomy can lead to confusion, the use of molecular analyzes and phylogenetic relationships will be useful in achieving a reliable result. Molecular analyzes are also used for the systematic confusions encountered in Turkey (Çöl et al. 2017). The ITS sequence enabled identification of *Russula* section *Foetentinae* at the species level (Lee et al. 2017). As a result of molecular analysis, we observe that our species is *Russula praetervisa* and that it is closely related to *R. pectinatoides* (Figure 3).

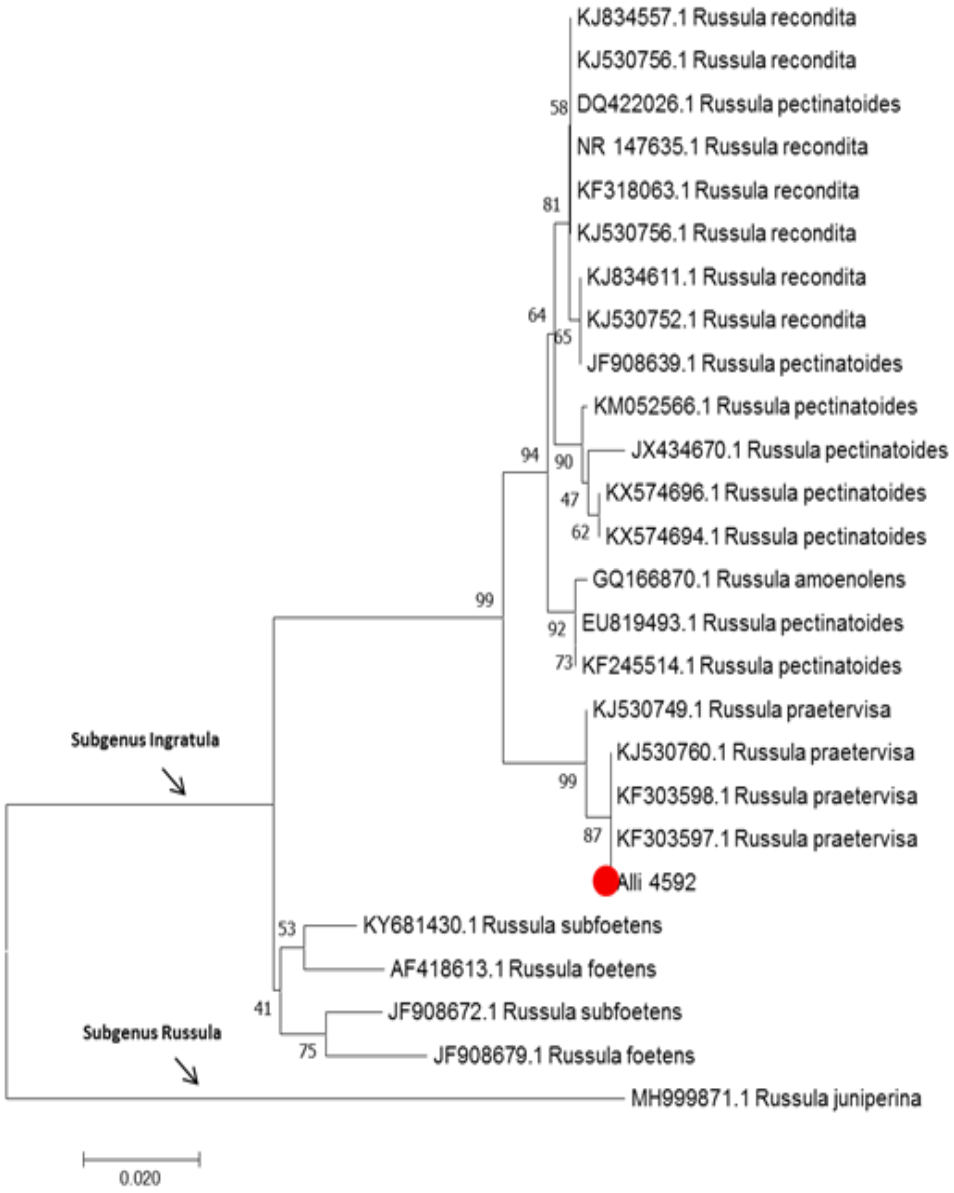

Figure 3. A neighbor-joining tree of the ITS genes of the *Russula praetervisa* and related taxa.

Consulting the current mycobiota checklist (Sesli & Denchev 2014) and the recent contributions regarding species of *Russula* in Turkey (Doğan & Öztürk 2015; Işık & Türkekul 2017; Çolak et al. 2018), we have concluded that *Russula praetervisa* is a species whose existence in Turkey has not been proven previously in detail. The existence of *R. praetervisa* was confirmed by this study. Thus, it is known that the number of *Russula* species found in Turkey is 133.

Specimens examined

Russula praetervisa. Turkey. Muğla: 13. km of Marmaris-Datça road, near İçmeler stream, 27.12.2012, Allı 4592 (Muğla Sıtkı Koçman University).

ACKNOWLEDGMENTS

This study was financially supported by the Muğla Sıtkı Koçman University Scientific Research Projects (BAP 2011-26).

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