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Rhizopogon pumilionum (Ade) Bataille; a new false truffle (Basidiomycota) record for Turkish mycota

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Abstract

In this study, *Rhizopogon pumilionum* (Ade) Bataille is recorded from Turkey for the first time. The new record is described and illustrated. New record was proved by utilizing from its rDNA ITS data. Also, phylogenetic position of *R. pumilionum* was detected in the phylogenetic tree constructed using the sequences of rDNA ITS

Key words: biodiversity, rDNA ITS, *Rhizopogon*, Sivas, Turkey

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***Rhizopogon pumilionum* (Ade) Bataille; (Basidiomycota) Türkiye mikotası için yeni bir yalancı trüf kaydı**

Özet

Bu çalışmada, *Rhizopogon pumilionum* (Ade) Bataille Türkiye'den ilk defa kaydedilmiştir. Yeni kayıt tanımlanmış ve fotoğrafları verilmiştir. rDNA ITS verilerinden yararlanılarak yeni kayıt kanıtlanmıştır. Ayrıca, *R. pumilionum*'un filogenetik konumu, rDNA ITS dizilerinden yararlanılarak oluşturulan filogenetik ağaçta tespit edilmiştir.

Anahtar kelimeler: biyoçeşitlilik, rDNA ITS, *Rhizopogon*, Sivas, Türkiye

1. Introduction

Sivas province falls in Irano-Turanien floristic region and has a high endemism ratio [1]. Karalar village (Yıldızeli) has important forests series. The forest and shrub vegetation of the Karalar village is especially *Pinus sylvestris* L., *Juniperus* L. sp., *Quercus* L. sp., *Populus* L. spp., *Salix* L sp., *Prunus* L sp., *Cistus* L sp., *Crataegus* Tourn. ex L. sp., *Pyracantha* M.Roem. sp., *Cupressus* L. sp., *Pyrus* L. sp. and *Corylus* L. sp. Despite Sivas has a continental climate, studied region has some trees unique for Black Sea climate, such as the *Corylus* grows wildly in the region. With this feature, the region is like a transition zone from the Black Sea climate to the continental climate. This situation reflects the climatic characteristics of the region and capable of hosting a large number of fungal species [2]. In the studies carried out between Akdağmadeni (Yozgat) and Gemerek (Sivas), including our study area there were 66 taxa belonging to 23 families were reported [3]. Also Güngör [4], Işık [5], Işık and Türkekul [6] reported some new macrofungal records for Turkey from studied area and nearby areas.

Turkish *Rhizopogon* Fr. taxa are *R. abietis* A.H. Sm., *R. luteolus* Fr., *R. ochraceorubens* A.H. Sm., *R. rocabrunae* M.P. Martín, *R. roseolus* (Corda) Th. Fr., *R. obtextus* (Spreng.) R. Rauschert and *R. marchii* (Bres.) Zeller & C.W. Dodge [7, 8]

There is no information about the edibility of the newly recorded species mentioned in the literature or by the local people. The specimen is not used by local people for any purpose. But fungus is known as "Pembe patates mantarı" in the region by local people.

Studies on the determination of mycobiota of Turkey [9, 10] will be pioneer in the protection of biodiversity. The aim of this study is to contribute to Turkish fungal biodiversity by adding molecularly proved a new fungal record.

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2. Materials and method

2.1 Field study and identification of samples

Fungal sample presented in study was collected in 2016 during routine field trips from Karalar village region. During field studies morphological and ecological characteristics of the specimens were recorded. Specimens were sectioned with a lanset and sections and sizes of the specimens were measured and it was photographed. For molecular studies some of the samples were stored at - 80 C°. Remaining specimens were dried in the shade at a temperature lower than 40 C°. Sterile razor blades were used to take samples for microscopic studies. Photographs related to micromorphology of the samples were taken by Leica DFC 295 trinocular microscope. Specimen was identified after consultation with the relevant literature [11, 12, 13, 14]. Dried samples were stored in the author's own collection.

2.2 DNA extraction, PCR amplification and Sequencing

Total genomic DNA was extracted using the DNeasy Plant mini kit (Qiagen). ITS1 Forward (TCCGTAGGTGAACCTGCGG) and ITS4 Reverse (TCCTCCGCTTATTGATATGC) primers were used for PCR (to amplify 18S, ITS1, 5.8S, ITS2 and 28S region) and sequencing [15]. Amplifications were conducted using an Applied Biosystems (ABI) veriti 96 well thermocycler using the following program: 1 cycle of 5 min at 95 °C; 35 cycles of 45 s at 94 °C, 45 s at 60 °C, and 2 min at 72 °C; followed by 1 cycle of 10 min. at 72 °C for final extension. Gel electrophoresis in 0.8% agarose gel run in TBE buffer was used to size-fractionate amplicons. Subsequently gels were stained with ethidium bromide and visualized over a UV trans-illuminator. PCR products were sequenced with the primers used to amplify ITS region by Source Bioscience inc. (Nottingham, United Kingdom). Specimen's rDNA ITS sequences were uploaded to GenBank (accession number: OP169016)

2.3 Sequence alignment and phylogenetic analysis

rDNA ITS Sequences of *R. pumilionum* obtained via this study and additional rDNA ITS sequences of *R. abietis* (MH819341.1), *R. luteolus* (EU784397.1), *R. ochraceorubens* (AF071440.1), *R. rocabrunae* (JF908761.1), *R. roseolus* (AJ810073.1) retrieved from GenBank. Genbank accession numbers for each species are given in parentheses. ITS sequences of *R. obtextus* and *R. marchii* which are another Turkish species not yet uploaded to GenBank. Closely related taxon *Alpova alpestris* P.-A. Moreau & F. Rich. (NR_132847.1) retrieved from Genbank and used as outgroup.

ITS sequence were manually/visually checked by using the Bioedit Version 7.1.9 software and aligned via ClustalW alignment software [16]. Ends of the alignment were trimmed to make all the sequences of equal length. MEGA 11 (Molecular Evolutionary Genetics Analysis) [17] phylogenetic analysis programme was used to obtain phylogenetic trees and determine relationships between species.

Phylogenetic trees were constructed using the Neighbor-Joining method [18]. The percentage values of the Bootstrap test, in which each branch of the 1000 replicated pedigrees were evaluated statistically, are shown next to the related combined taxa branches [19]. Evolutionary distances were calculated using the Maximum Composite Likelihood method [17]. Bootstrap value is between 0% and 100%.

3. Results

3.1 Macroscopic and microscopic characters

After investigations in the laboratory *Rhizopogon pumilionum* was identified. According to current literature on Turkish macrofungi [7, 8] *R. pumilionum* is a new record for the Turkish mycota.

3.1.1 Boletales

3.1.1.1 Rhizopogonaceae Gäum. & C.W.Dodge

3.1.1.1.1 Rhizopogon pumilionum (Ade) Bataille (Figure 1)

Fruitingbody cespitose to gregarious, irregular globose to globose, 1-3 cm in diameter, semi-epigeous to epigeous, solid and durable. Surface typically verrucose, rhizomorphs only at the base. Surface salmon orange to cinnamon or orange-reddish. Sorghum-brown patches of the outer peridial layer easily separable from this layer fissures. Peridium thick, claret-brown, 440-1000 µm, with tightly interwoven hyphae. Vinaceous-russet and loosely woven hyphae on outer portion of peridium becoming hyaline and more compact towards gleba. Gleba greyish, olivaceous, cavities averaging 5-6 per mm., subglobose to irregular, empty. Spores 6.1- 9.6 × 2.2-3.5 µm, slender, cylindric to subfusoid with truncate base, hyaline, guttulate. Basidia 13-17 × 4.4 -5 µm, 4-spored. Cystidia rare.

Sivas, Yıldızeli, Karalar village, Armutlu region, in *Pinus sylvestris* forest, 1618 m., N 39°43'17" E 36°09'898'' 26.05.2016, H 1234; Karalar village, Eşek Sırtı region, in *P. sylvestris* forest, N 39°43'14" E 36°12'944'' 26.05.2016, H 1220.

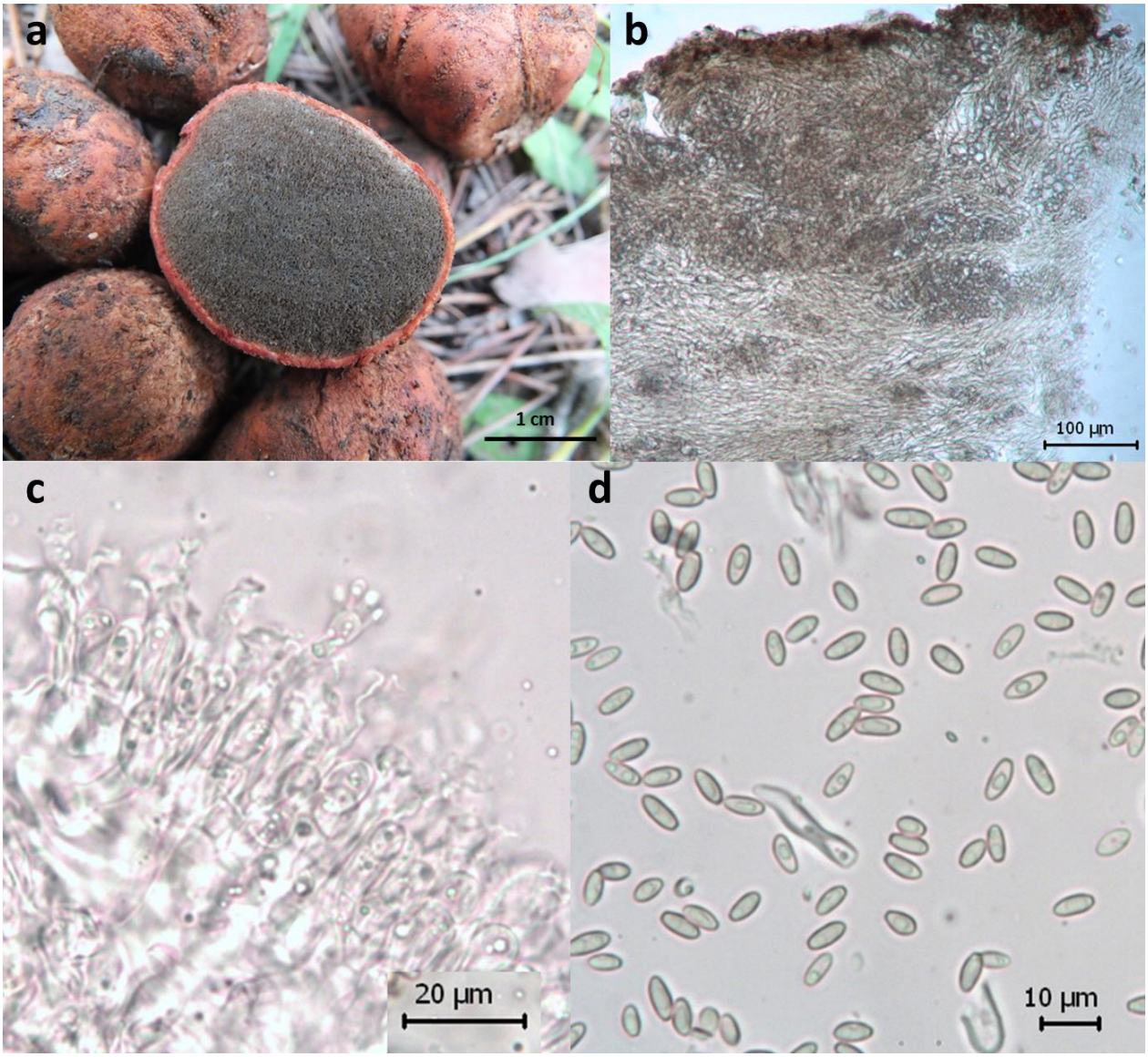

Figure 1. *Rhizopogon pumilionum*. a. fruitingbody, b. Peridium, c. basidium, d. spores

3.2 Molecular phylogeny

GenBank researches showed that ITS sequences of *R. pumilionum* have a 95.7% similarity to morphologically closely related species *R. rocabrunae*. rDNA ITS sequences of seven species used in this study aligned and nearly 710 bp length sequences obtained. Neighbor-Joining tree was given and discussed. Bootstrap values were also given in the tree (Figure 2).

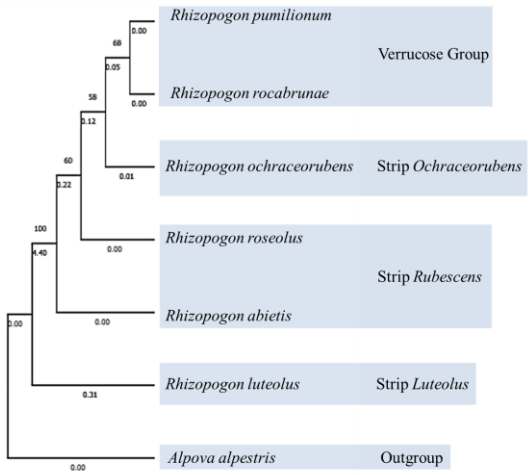

Figure 2. The NJ phylogenetic tree based on rDNA ITS data of Turkish *Rhizopogon* species

4. Conclusions and discussion

Genus *Rhizopogon* is taxonomically problematic. The genus has morphologically similar species. Molecular studies have a potential to solve this taxonomical problem. In this study we used both morphological and molecular data to get correct and reliable results for identification and separate closely related *Rhizopogon* species.

As seen in tree a good divergence has obtained for Turkish *Rhizopogo*n species by utilizing rDNA ITS data. Morphologically closely related granulose, verrucose species (*R. rocabrunae* and *R. pumilionum*) were separated from *Rhizopogon* species which have smooth surface. *R. roseolus* and *R. abietis* which are assessed in strip *Rubesbens* by Smith and Zeller [11] is also phylogenetically close. A phylogenetic study including multiple *Rhizopogon* species should be performed to further comment on sections, stips and series.

R. pumilionum was firstly described by Ade [20] as "globose, reaching the size of a hazelnut with appearance and colours reminding of strawberries". *R. roseolus* and *R. pumilionum* are related species but *R. roseolus* have wider and bigger spores and thin peridium than *R. pumilionum* [13].

Rhizopogon pannosus Zeller & C.W. Dodge, *R. pumilionum* and *R. rocabrunae* are closely related, because of their granulose, slightly verrucose surface. *R. pumilionum* and *R. pannosus* have same typical characters like rhizomorphs only at the base, salmon orange to cinnamon or orange-reddish colours of the peridium and the truncate spores. But *R. pumilionum* has narrower spores than *R. pannosus* and in the colour of the gleba being olivaceous in *R. pumilionum* and buffy to ochraceous in *R. pannosus* [13].

R. pumilionum and *R. rocabrunae* easily distinguished other Turkish *Rhizopogon* taxa with distinctly granulose surface and with the colour of basidiomata. But *R. pumilionum* distinguished from *R. rocabrunae* with a more granulose, verrucose peridium and less gelified hyphae of the tramal plate [14]. *R. rocabrunae* seems strictly associated to *Abies* Mill sp. [14]. Colour of the gleba in *R. pumilionum* greenish and yellowish to brownish in *R. rocabrunae* [21]. Peridial thickness is 440-1000 µm in *R. pumilionum* and 300-600 µm in *R. rocabrunae* [14]. Also our GenBank searches showed that *R. pumilionum* has different ITS sequences from Osmundson's [22] *R. rocabrunae* (nearly 4.5%). As seen Figure 2 both of them are close species than other examined Turkish species and have a good bootstrap value. Also molecular studies conducted on 28S rDNA region of *R. rocabrunae*, *R. pannosus* and *R. pumilionum* showed that these are closely related but separate species [23].

We constructed an identification key for the Turkish *Rhizopogon* taxa, excluding *R. obtextum*, with the relevant literature [11, 21]. *R. obtextum* is accepted by many authors as a synonym of *R. luteolus* [24].

1a. Peridium staining red when injured	2
1b. Peridium not staining red when injured but it may stain some other colour	4
2a. Basidiocarp lacking yellow colours at any time in its development.	*R. roseolus*
2b. Basidiocarp showing yellow at some stage	3
3a. Gleba white at first, then pale olive and finally dark olive	*R. abietis*
3b. Gleba white or yellowish white at first, then reddish brown or brownish ochre	*R. marchii*
4a. Peridium granulose, verrucose	5
4b. Peridium smooth	6
5a. Gleba greenish, olivaceous. Peridium granulose, more verrucose.	*R. pumilionum*
5b. Gleba yellowish to brownish. Peridium granulose, slightly verrucose	*R. rocabrunae*
6a. Peridium firstly yellow-ochre, then golden-yellow or golden-tawny. Surface fibrillose and covered over all by appressed rhizomorphs.	*R. luteolus*

6b. Peridial ground at first lemon yellow to ochraceous, soon cinnamon to russet and covered with brown rhizomorphs *R. ochraceorubescens*

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