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Pisolithus albus, A New Record For Turkish Gastroid Fungi

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Abstract: Fungal specimens were sampled from the Bodrum district of Muğla province / Türkiye on September 21, 2020, and they were scrutinized by performing both morphological and rDNA sequence-based phylogenetic analyses. Considering the micro- and macromorphological data and the high (>99%) sequence similarity between the sampled specimen (ANK Akata & Altuntaş 699) and *Pisolithus albus* (Cooke & Massee) Priest., the collected specimen was identified as *P. albus*. As a result of this study, *P. albus* was reported as a new record for Turkish Gastroid fungi. A brief description of the lately recorded species was stated along with its macro-photograph, and illustration of spores and discussed briefly.

Key words: *Pisolithus albus*, Gastroid fungi, New record, Türkiye

Pisolithus albus, Türkiye Gastroid Mantarları İçin Yeni Bir Kayıt

Öz: 21 Eylül 2020 tarihinde Muğla ili Bodrum ilçesinden mantar örnekleri toplanmış ve hem morfolojik hem de rDNA dizi tabanlı moleküler filogenetik analizler uygulanarak detaylı bir şekilde incelenmiştir. Toplanan örnek (ANK Akata & Altuntaş 699) ile *Pisolithus albus* (Cooke & Massee) Priest. arasındaki yüksek (>%99) dizi benzerliği ve mikro- ve makromorfolojik veriler göz önüne alındığında, toplanan örnek *P. albus* olarak tanımlanmıştır. Bu çalışma sonucunda *P. albus* Türkiye Gastroid mantarları için yeni kayıt olarak rapor edilmiştir. Yeni kaydedilen türün kısa bir tanımı, makro fotoğrafı ve sporların gösterimi ile birlikte sunulmuş ve kısaca tartışılmıştır.

Anahtar kelimeler: *Pisolithus albus*, Gastroid mantarlar, Yeni kayıt, Türkiye

Introduction

Pisolithus is a genus of the gastroid family *Sclerodermataceae* within the order *Boletales* (*Basidiomycota*). Its members have a wide distribution ranging from temperate to tropical regions and they are ectomycorrhizal species associated with some woody plants. The members have been reported in different environments such as forests, damaged areas, eroded soils, plantations, mining, and roadsides. They are also known to be highly effective in promoting plant growth in dry habitats with high soil temperatures (Moyersoen et al. 2004; Lebel et al., 2018).

According to the index fungorum, the genus contains 16 species (*P. kisslingii* E. Fisch., *P. arhizus* (Scop.) Rauschert, *P. aureosericeus* M.P. Martín, Kaewgraj., *P. abditus* Kanch., Sihan., Hogetsu & Watling, *P. hypogaeus* S.R. Thomas, Dell & Trappe, *P. indicus* Natarajan & Senthil., *P. marmoratus* (Berk.) E. Fisch., *P. orientalis* Watling, Phosri & M.P. Martín, Pennycook & Beever, *P. capsulifer* (Sowerby) Watling, Phosri & M.P. Martín, *P. aurantioscabrosus* Watling, Phosri & Watling, *P. microcarpus* (Cooke & Massee) G. Cunn., *P. calongei* M.P. Martín, Phosri & Watling, *P. croceorrhizus* P. Leonard & McMull.-Fish, *P. tympanobaculus* T. Lebel & M.D. Barrett and *P. thermaeus* T. Lebel, *P. albus* (Cooke & Massee) Priest.). forming an ectomycorrhizal association with broadleaved and coniferous trees (Martin et al., 2002).

P. albus is prevalent in dry or dispersed areas such as sandy and gravelly soils, and develops in ectomycorrhizal associations with *Acacia* Wılld., *Corymbia* K. D. Hill & L. A. S. Johnson and native *Eucalyptus* L'Hér. members in Australia, endemic species *Kunzea tenuicaulis* de Lange in New Zealand, native

species *of Acacia, Arillastrum* Pancher ex Baill., *Melaleuca* L., *Sannantha* Peter G. Wilson, Babingtonia Lindl. and *Tristaniopsis* Brongn. & Gris in New Caledonia, plantation *Acacia* and *Eucalyptus* species in China, India, Morocco, Malaysia, Thailand, Spain and Senegal, plantation of *Eucalyptus* members in Burkina Faso, Chad, Tunisia, Niger, Côte d'Ivoire, Morocco, Madagascar and Italy (Gargano et al. 2018, Hosaka, 2009; Jaouani et al., 2015; Lebel et al., 2018).

Considering the current literature on Turkish mycobiota (Sesli and Denchev, 2014; Sesli et al, 2020), the sole member of the genus *Pisolithus* that has thus far been reported from Türkiye is *P. arhizus*, and to the best of our knowledge, there is no record related to *P. albus*.

Material and Method

Morphological study

Pisolithus specimens were sampled from Bodrum (Muğla-Türkiye) amid fieldwork conducted on September 21, 2020. Macroscopic and ecological features of the specimens were noted at their site of collection. Necessary macroscopic and microscopic data were obtained by standard techniques. Identification of the specimens was performed with the guidance of the literature (Gargano et al., 2018; Jaouani et al., 2015; Lebel et al., 2018). Herbarium materials were prepared from the identified specimens and kept at Ankara University Herbarium (ANK).

ITS rDNA Sequence Analyses

The nuclear DNA of ANK AKATA & Altuntaş 699 was enriched by employing the CTAB method as previously described (Rogers and Bendich, 1994). The quality and the quantity of the isolated nuclear DNA were assessed spectrophotometrically (Nanodrop Lite Thermo Scientific) and later it was used as a template in a polymerase chain reaction (PCR) to amplify the Internal Transcribed Spacer (ITS) rDNA regions. In the PCR amplification of the ITS rDNA regions, the ITS1 forward and ITS4 reverse universal oligonucleotide primers were utilized as described elsewhere (Stielow et al, 2015). The PCR amplicons were electrophoretically validated as single and sharp bands on an agarose gel and later they were purified with PureLink™ PCR Purification Kit (Thermo) and sequenced with Sanger dideoxy sequencing method using the ITS1 and ITS4 primers and the BigDye™ Direct Cycle Sequencing Kit (Thermo) in the sequencing PCR. The fragment analyses were performed using ABI Prism 310 Sequencer. Agarose gel electrophoresis and Sanger DNA sequencing were carried out as previously reported (Chen et al., 2014).

Phylogenetic Characteristics of ANK Akata & Altuntaş 699

In molecular phylogenetic analyses, the sequences obtained from the Sanger chromatograms were assembled using Geneious Prime software (Dotmatics) and a similarity index analysis was performed using the NCBI BLASTn online tool. According to this search tool, the in-group and the out-group members were determined and their sequence data were obtained from the NCBI GenBank for the phylogenetic analysis. The assembled sequence and the ITS rDNA sequences of the selected in-group and out-group members were aligned using the MUSCLE algorithm of MEGAX software (Kumar et al., 2018). The evolutionary history of ANK Akata & Altuntaş 669 was estimated from a phylogenetic tree constructed using the Maximum Likelihood method and K2 + G nucleotide substitution model (Kimura, 1980). The bootstrap method was implemented for the accuracy estimation by applying 1000 bootstrap replicates (Felsenstein, 1985).

Result

Fungi

Basidiomycota Whittaker ex R.T. Moore

Boletales E.-J. Gilbert

S*clerodermataceae* Corda

Pisolithus Alb. & Schwein

Pisolithus albus (Cooke & Massee) Priest, in Lebel, Pennycook & Barrett, Phytotaxa 348(3): 167 (2018) (Figure 1).

Basionym: *Polysaccum album* Cooke & Massee, Grevillea 20 (no. 94): 36 (1891).

Obligate synonym: *Pisolithus albus* (Cooke & Massee) Priest, in Bougher & Syme, Fungi of Southern Australia (Nedlands): 122 (1998).

Macroscopic and microscopic features

Basidiomata 30-50 mm in diam., epigeous, claviform, subglobose to pyriform, base mostly deeply rooting. **Peridium** thin, membranous, smooth, one-layered, whitish to grey or cream-colored, brownish in ripe basidiomata. **Gleba** ellipsoid to ovoid, developing within peridioles, **Peridioles** covered by a thin, yellowish to the ochraceous membrane, embedded in and divided by a gelatinous blackish matrix. **Stem** 10-20 mm broad, yellowish to light brown. **Spore mass**: olive-brown. Clamp connection present. **Basidia** not seen. **Spores** 9-11 µm diam (including ornamentation), yellowish brown, globose with erect or curved spines (up to 1µm high).

Examined samples: TÜRKİYE—Muğla: Bodrum, Turgutreis, under eucalyptus, sea level, 37° 01' 12'' N, 27°15' 07'' E, 21.09.2020, ANK Akata & Altuntas 699.

Evolutionary History of ANK Akata & Altuntaş 699

The nuclear ITS rDNA sequence of ANK Akata & Altuntaş 699 revealed by Sanger sequencing was entered to the NCBI GenBank with the accession no: OP363350.1. In a evolutionary history analysis of ANK Akata & Altuntaş 699, considering the results of the nucleotide BLAST analysis conducted using the ITS rDNA sequence of the specimen, some members of the genus *Pisolithus*, the single genus of the family *Pisolithaceae*, were chosen for ingroup sequences and

the nuclear ITS rDNA sequence of *Lycoperdon perlatum* was chosen for the outgroup sequences. As a result of the evolutionary history analysis, four distinct clades aroused in addition to an outgroup (Figure 2). While the clade 4 included some distinct isolates of *Pisolithus albus* and the specimen ANK Akata & Altuntaş 699, the clades 1, 2, and 3 comprised other species from the genus Pisolithus including *P. arhizus*, *P. capsulifer* and *P. croceorrhizus* respectively. On the other hand, *Lycoperdon perlatum* branched separately from the ingroup clades and formed an outgroup as anticipated. The BLAST analysis conducted with the nuclear ITS rDNA sequence of ANK Akata & Altuntaş 699 showed similarity rates above 99% with different isolates of *P. albus*. The phylogenetic analyses conducted herein further confirmed the close identity relationship of this specimen with *P. albus* with a high branch bootstrap rate.

Discussions

Pisolithus members can easily be recognized in the field because of their macroscopic appearance but it is not doable to define the full range of known *Pisolithus* species without using macroscopic and microscopic characteristics and information about mycorrhizal partners (Lebel et al., 2018).

P. albus is an ectomycorrhizal species associated with plants containing the members of *Eucalyptus, Kunzea, Acacia, Tristaniopsis, Arillastrum, Babingtonia, Melaleuca, Sannantha and Corymbia. P. thermaeus* T. Lebel, *P. orientalis* Watling, Phosri & M.P. Martín, Pennycook & Beever, *P. marmoratus* (Berk.) E. Fisch., *P. croceorrhizus* P. Leonard & McMull.-Fish. *P. microcarpus* (Cooke & Massee) G. Cunn., and *P. tympanobaculus* T. Lebel & M.D. Barrett may be confused with *P. albus* in terms of their similar morphology and ectomycorrhizal partners. Like *P. albus, P. croceorrhizus* form ectomycorrhizal associations with *Eucalyptus, Acacia, Corymbia,* and *Kunzea tenuicaulis* and it is characterized by its pale brown basidiomata suspended on golden rhizoids forming a pseudostipe, and its spiny reticulate basidiospores. This species may be confused with former species in young basidiomata but *P. albus* is easily separated from *P.croceorrhizus* by its larger spores with isolated spines.

Although *P. albus* and *P. marmoratus* are associated with similar plants (*Eucalyptus* and *Kunzea tenuicaulis*), the latter species differs from the former by its fragile and darker peridium (brown and amber) with warts, mass tan to sand brown spore mass, globose to subglobose spores (7–12 x 7–9µm in diam.) with echinulate spines. *P. microcarpus* has an ectomycorrhizal relationship with *Eucalyptus* and *Acacia* members.

Despite sharing similar habitats, *P. albus* and *P. microcarpus* can be distinguished from each other by using both microscopic and macroscopic features. While black-warted, golden-brown peridium and short (6–7.5µm diam.) with erect spines are characteristics of *P. microcarpus*, whitish to grey or cream-colored peridium and larger spores (9-12 µm diam.) with spines are of *P. albus. P. orientalis* and *P. tympanobaculus* are associated specifically with *Eucalyptus* species. While *P. orientalis* differs from *P. albus* by its snuff brown to cigar brown peridium and globose spores with isolated groups of connate spines, *P. tympanobaculus* by pale yellow buff peridium with black patches and smaller spores (6-8,5 µm diam.) with ornamentation of robust short spines coalescing into secondary conical warts to 0.6 µm high.*P. thermaeus* has only been recorded to associate with *Kunzea tenuicaulis* which is endemic species in New Zealand. This species could be distinguished from *P. albus* by its pale brown peridium with dark patches (Gargano et al., 2018; Jaouani et al., 2015; Lebel et al., 2018; Mifsud and Mifsud 2022; Phosri et al, 2012).

The genetic diversity of fungal species far exceeds their morphological diversity and therefore for more robust identifications of fungal species, the genetic information is usually exploited along with using conventional methods that rely solely on morphological data. For this purpose, various useful genetic markers including rRNA gene regions such as nrITS, nrSSU, and nrLSU as well as sequences of protein-coding genes are employed for molecular systematics studies for the last several decades (Raja et al., 2017). Among them, ITS is one of the most extensively used genetic markers for members of the kingdom fungi and therefore provides valuable information for molecular taxonomic studies. Hence, we utilized nuclear ITS rDNA sequences for the molecular identification of ANK Akata & Altuntaş 699. nrITS rDNA-based molecular identification revealed more than 99% similarity between *P. albus* and the specimen (GenBank ID: OP363350.1) (Figure 2).

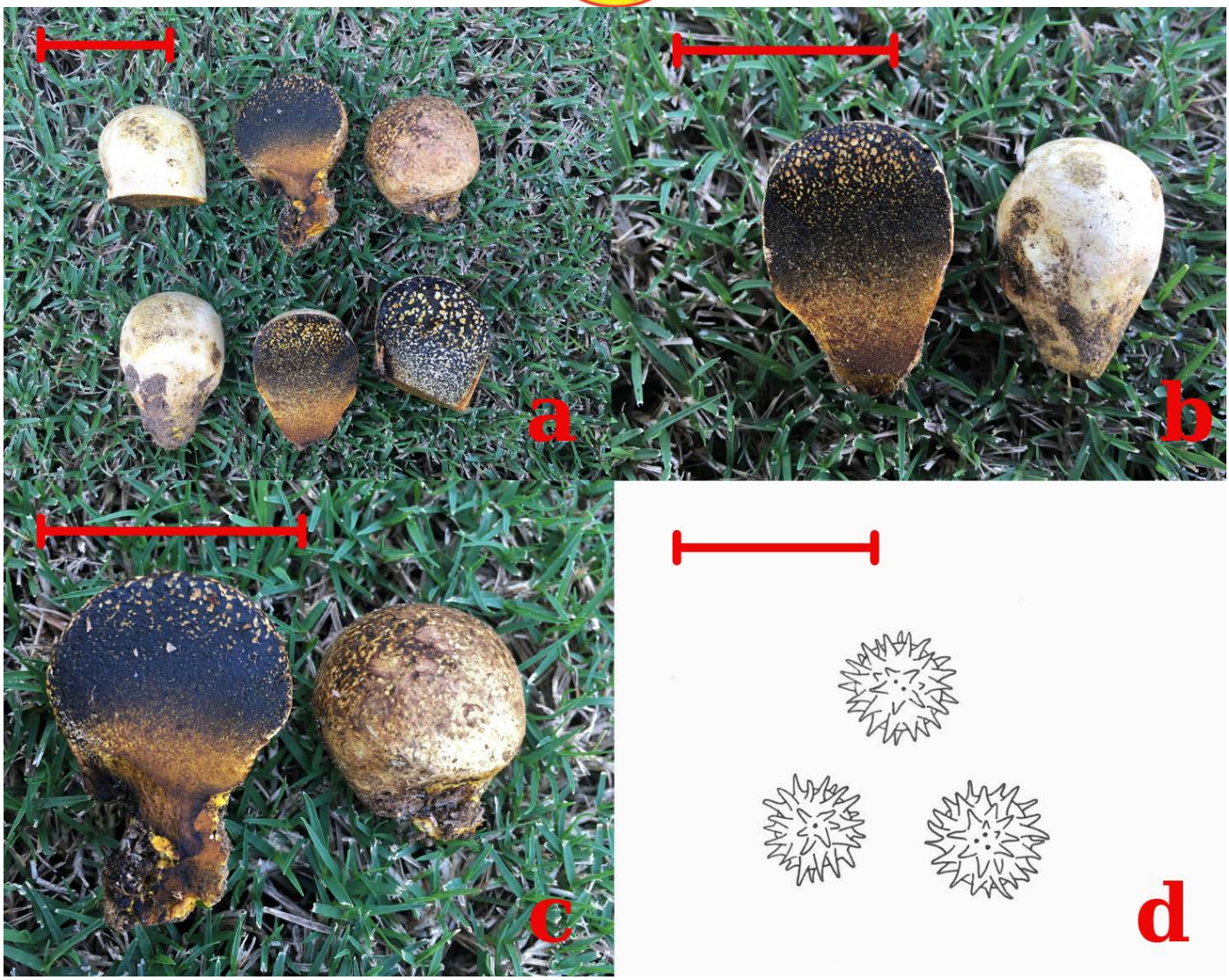

Figure 1. *Pisolithus albus*: **a-c.** basidiomata (bars: 5 cm)**, c.** spores (bar: 20 µm).

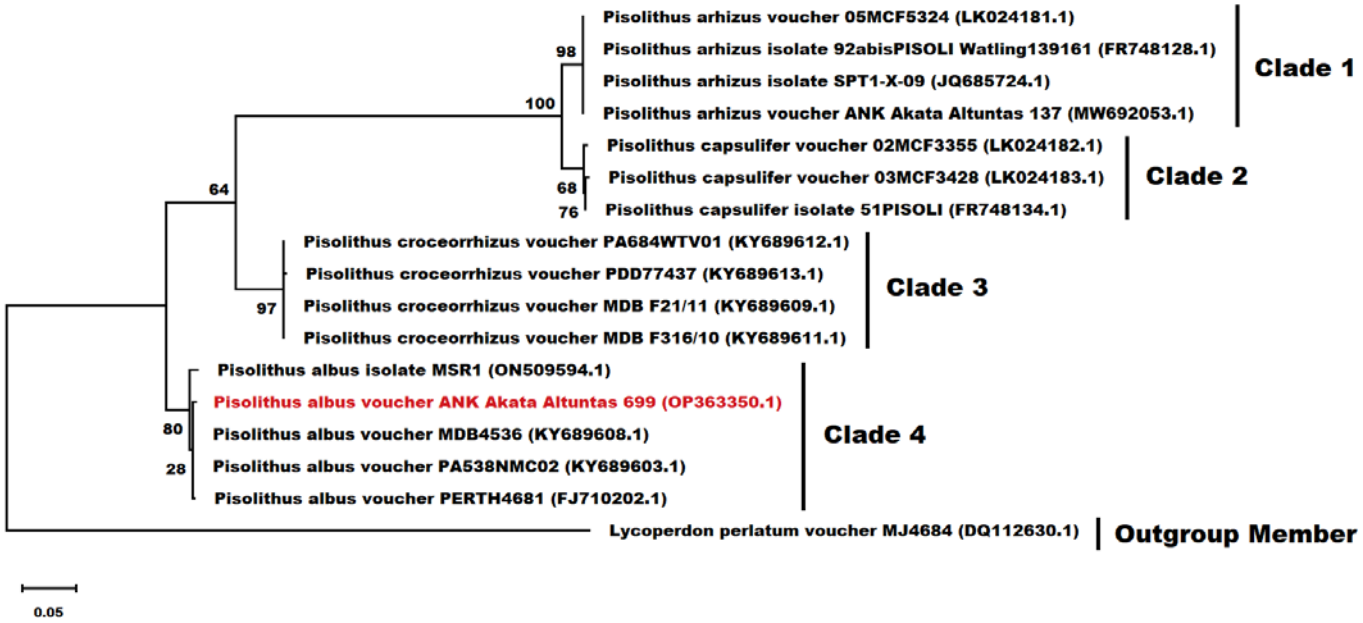

Figure 2. The ML phylogenetic tree showing the evolutionary relatedness of 17 fungal specimens conjectured from the nrITS rDNA region. Bootstrap rates (≥50) were shown for each branch. All of the sequences used to construct the phylogenetic tree were obtained from NCBI GenBank except for ANK Akata & Altuntaş 699. *Lycoperdon perlatum* was used as the outgroup member in the phylogenetic tree. GenBank accession numbers are also provided for each sequence. The scale bar given on the lower left indicates a genetic distance of 0.05.

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