

Pisolithus albus (Sclerodermataceae), a first record from Egypt

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Abstract. *Pisolithus albus* (Basidiomycota, Sclerodermataceae) is reported here for the first time from Egypt where it was found on the Karaman Island in Sohag Governorate. It was found forming an ectomycorrhizal association with *Eucalyptus occidentalis* and *Vachellia nilotica*. The fungus was also reported near two potentially new hosts: *Paspalum distichum* and *Dodonaea viscosa*; however, the mycorrhizal connections with these two hosts is not proven. Phylogenetic analyses for the combined ITS and LSU rDNA sequences dataset placed the Egyptian specimen within the *Pisolithus albus* complex clade. It is described, illustrated, and phylogenetically analyzed in this article. Various hosts and locations of the taxon are reviewed.

Key words: Basidiomycota, ectomycorrhizal fungi, Middle East, molecular phylogeny, sub-tropical fungi

Introduction

Pisolithus (Basidiomycota, Sclerodermataceae) is one of the most cosmopolitan mutualistic gasteromycete genera in the world (Marx 1977). It was introduced by Albertini and Schweinitz (1805) to accommodate the type species *Pisolithus arenarius*. Members of this genus were recorded from various habitats with regard to temperature, nutrient levels and heavy metal concentrations, and has been recorded from tropical, subtropical and temperate regions (Watling et al. 1999; Martín et al. 2013; Rusevska et al. 2015; Jaouani et al. 2015; Crous et al. 2016; Mifsud & Mifsud 2022). *Pisolithus* species make mycorrhizal associations with different plant species in the families Casuarinaceae, Dipterocarpaceae, Fagaceae, Myrtaceae and Pinaceae (Marx 1977; Malloch & Kuja 1979). *Pisolithus* is widely used in forestry and horticulture due to growth stimulation properties reported in different tree species, including acacias, eucalypts and pines (Garbaye et al. 1988; Duponnois & Bâ 1999).

Because of the heterogeneity of *Pisolithus* in terms of sporocarp, spore and mycelial culture morphology, the genus was considered for a long time as monotypic (Coker & Couch 1928; Cunningham 1942; Pilát 1958), and described species within the genus have been widely regarded as conspecific with *Pisolithus tinctorius* (Chambers & Cairney 1999). In the last two decades, many phylogenetic studies established several species under this

genus (Anderson et al. 1998; Martin et al. 1998; Gomes et al. 2000; Díez et al. 2001; Lebel et al. 2018). These studies revealed significant genetic variation within the species, implying that the *P. tinctorius* group is a complex of several species that cannot be distinguished by morphological studies (Anderson et al. 2001; Martin et al. 2002; Kanchanaprayudh et al. 2003; Moyersoén et al. 2003; Singla et al. 2004). Martin et al. (2002) concluded that there are three distinct lineages of *Pisolithus* with at least 11 different species. The genus currently comprises 20 species (Mycobank 2022). One of the most common species in this genus is *Pisolithus albus*, that was collected from a wide range of geographical locations, e.g., Australia, Brazil, China, India, Italy, Morocco, Malta, New Caledonia, New Zealand, Pakistan, Senegal, Spain, Thailand, Tunisia, and the USA. However, this is the first record of this species from Egypt. Most of the collections of *Pisolithus albus* were in ectomycorrhizal associations with *Eucalyptus*, *Acacia* and *Kunzea*. Hosaka (2009) and Jourand et al. (2010) supposed that *P. albus* may have four genetic ecotypes. These genetic variations and biogeographical patterns may originate from long-distance dispersal, and this may give the species high adaptability to change its mycorrhizal associations (Hosaka 2009).

This work aimed to report the first record of *Pisolithus albus* from Egypt, based on morphological examination and phylogenetic analyses. Hosts and locations of *Pisolithus albus* are also reviewed.

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Materials and methods

Site description

Samples were collected from Karaman Island (26°35' 26.1"N, 31°42'29.6"E – 26°33'48.8"N, 31°42'01.4"E), that is located in the middle of the River Nile at Sohag Governorate, Egypt. It is an uninhabited island, which is characterized by sandy or clay soil with an elevation about 62 m above the sea level, hot desert climate and precipitation less than 2.28 mm per year. The habitats on the island vary from submerged to dry lands. The island vegetation is a mixture of wild plants and urban trees.

Sample collection and morphological description

Basidiomata of *Pisolithus albus* were collected from Karaman Island several times during the period between December 2018 and November 2020. Metadata including date, geographic coordinates, habitat and associated plant communities were recorded on site. Collections were packed in clean sterilized plastic bags and brought to the laboratory for further examination. Microscopic observations and measurements were taken from tissue sections mounted in 5% KOH and examined using an Olympus SZ61 stereomicroscope. Micrographs were taken using an Olympus BX51 compound microscope (Olympus, Tokyo, Japan) equipped with a TouP Tek XCAM1080PHA (TouP Tek, Zhejiang, China) digital imaging system. Dried basidiocarps were deposited in Sohag University Microbial Culture Collection (SUMCC). Basidiospores from the recorded species did not germinate on potato dextrose agar (PDA; Oxoid, Basingstoke, UK) or MMN agar media (Marx 1969).

DNA extraction, sequencing and phylogenetic analyses

Total genomic DNA was extracted directly from the gleba of fruiting bodies using a microbial DNA extraction kit (MOBIO; Mo Bio Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol. The primer pairs ITS1 and ITS4 (White et al. 1990) and LROR and LR7 (Vilgalys & Hester 1990) were used for PCR amplification and sequencing of internal transcribed spacer (ITS) regions and the partial large subunit nuclear rDNA (LSU), respectively. PCR amplification and DNA sequencing were conducted as described by Abdel-Wahab et al. (2009) by MacroGen Inc., South Korea. Sequences were assembled using Sequencher 4.2.2 (Gene Codes Corporation). Newly generated sequences were deposited in the GenBank (Tab. 1). Sequences of ITS-LSU were aligned with other sequences of *Pisolithus albus* recorded from different localities and hosts, other species of *Pisolithus* and the outgroup taxa: *Boletinus merulioides* and *B. exiguus* using Clustal X (Thompson et al. 1997) and optimized manually. The concatenated sequence alignments were obtained from SequenceMatrix v 1.7 (Vaidya et al. 2011). Maximum-parsimony (MP) and maximum-likelihood (ML) phylogenetic analyses were constructed using MEGA X (Kumar et al. 2018).

Bootstrap support (Felsenstein 1985) was obtained by using the Tamura-Nei model. Bayesian inference (BI) analysis was carried out using MrBayes ver. 3.1.2 (Ronquist & Huelsenbeck 2003) with the SYM+G model that was determined by Akaike information criterion (AIC) in MrModeltest 2.2 (Nylander 2004). Four simultaneous Markov chains were run for five million generations and trees were sampled every 100 generations. Trees were visualized using Njplot ver. 2.3 (Perrière & Gouy 1996), and additionally layouts were done in the program of Adobe Illustrator CC (Adobe Systems Inc., CA, USA).

Results

Phylogenetic analysis

The combined ITS and LSU rDNA dataset consisted of 57 taxa, of which 40 belong to the *Pisolithus albus* complex, 15 representatives of other species of *Pisolithus* and 2 species from *Boletinellaceae* used as an outgroup, namely *Boletinus merulioides* and *B. exiguus*. Phylogenetic analyses of individual ITS and LSU rDNA datasets produced similar topology (data not shown). The ITS dataset consisted of 455 characters, which 135 gaps are excluded, 213 constant, 20 variable parsimony-uninformative and 87 parsimony-informative characters. The LSU rDNA dataset consisted of 600 characters, which 49 gaps are excluded, 483 constant, 32 variable parsimony-uninformative characters and 36 parsimony-informative characters.

The Bayesian analysis resulted in 50,000 trees after five million generations. The first 12,500 trees, representing the burn-in phase of the analyses were discarded, while the remaining trees were used for calculating posterior probabilities. The maximum parsimony of combined ITS and LSU rDNA dataset consisted of 1,055 characters that included: 154 gaps (that are excluded), 725 constant, 52 variable parsimony-uninformative and 124 parsimony-informative characters. The most parsimonious tree had a length of 375 steps, a consistency index (CI) of 0.798680, a retention index (RI) of 0.919419 and the composite index is 0.769860. Maximum parsimony tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei & Kumar 2000) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). Bootstrap analysis was performed with 1000 replicates to assess the statistical support for the tree. Maximum likelihood analysis yielded one tree with -ln likelihood score of 3435.47. ML, MP and Bayesian analyses produced trees with similar topologies. The Bayesian phylogenetic tree is shown in Figure 1. Molecular phylogenetic analyses of the combined ITS and LSU rDNA placed our *Pisolithus albus* material within the *P. albus* complex with high statistical support (95/85/99 for MP/ML/BYPP, respectively) (Figure 1). The collection from Egypt is nested in a subclade containing *Pisolithus albus* collections from Australia, Pakistan, New Caledonia, India, Senegal and Thailand in ectomycorrhizal associations with *Acacia*, *Eucalyptus* and unknown hosts.

Table 1. List of species, potential hosts, countries of origin, voucher/culture numbers and GenBank accession numbers of sequences used in this study. The novelties described here are in bold font.

Taxa	Voucher/Culture number	GenBank accession number		Country of origin	Potential host
		ITS	LSU		
<i>Pisolithus albus</i>	SUMCC H-18004	OK184610	OK175781	Egypt	<i>Eucalyptus occidentalis</i> , <i>Vachellia nilotica</i>
<i>Pisolithus albus</i>	CMU52-8	JQ365190	–	Thailand	–
<i>Pisolithus albus</i>	CMU52-9	JQ365191	–	Thailand	–
<i>Pisolithus albus</i>	CMU53-5	JQ365187	–	Thailand	–
<i>Pisolithus albus</i>	PISOLI-12	AJ629887	–	Thailand	<i>Eucalyptus camaldulensis</i>
<i>Pisolithus albus</i>	15PISOLI	FR748123	–	Thailand	<i>Eucalyptus camaldulensis</i>
<i>Pisolithus albus</i>	13PISOLI	FR748121	–	Thailand	<i>Eucalyptus camaldulensis</i>
<i>Pisolithus albus</i>	14PISOLI	FR748122	–	Thailand	<i>Eucalyptus camaldulensis</i>
<i>Pisolithus albus</i>	BBH:28599	FR748127	–	Thailand	–
<i>Pisolithus albus</i>	Pak IF7	MN295477	–	Pakistan	–
<i>Pisolithus albus</i>	KSRF-0007	MF510372	–	India	–
<i>Pisolithus albus</i>	COI-022	AF374690	–	Senegal	<i>Eucalyptus</i> sp.
<i>Pisolithus albus</i>	COI-015	AF374688	–	Senegal	<i>Eucalyptus</i> sp.
<i>Pisolithus albus</i>	COI-024	AF374622	–	Senegal	<i>Eucalyptus</i> sp.
<i>Pisolithus albus</i>	mar02	AF228655	–	Morocco	<i>Eucalyptus</i> sp.
<i>Pisolithus albus</i>	ast05	AF228656	–	Spain	<i>Eucalyptus</i> sp.
<i>Pisolithus albus</i>	NT03	FJ874739	–	Australia	–
<i>Pisolithus albus</i>	NT01	FJ874741	–	Australia	–
<i>Pisolithus albus</i>	NT04	FJ874742	–	Australia	–
<i>Pisolithus albus</i>	CS02	FJ874751	–	Australia	<i>Eucalyptus</i> sp.
<i>Pisolithus albus</i>	PERTH4681	FJ710202	EU718176	Australia	–
<i>Pisolithus albus</i>	QLD04	FJ874745	–	Australia	–
<i>Pisolithus albus</i>	RLB8183	KY689606	KY689577	Australia	<i>Acacia</i> sp., <i>Eucalyptus</i> sp.
<i>Pisolithus albus</i>	PA1	AF440868	–	Australia	<i>Eucalyptus</i> sp.
<i>Pisolithus albus</i>	MH731(CSH4339)	AF374675	–	Australia	<i>Eucalyptus</i> sp.
<i>Pisolithus albus</i>	MDB4536	KY689608	KY689579	Australia	<i>Acacia</i> sp., <i>Eucalyptus</i> sp.
<i>Pisolithus albus</i>	MDB4535	KY689607	KY689578	Australia	<i>Eucalyptus</i> sp.
<i>Pisolithus albus</i>	MDB F151/11	KY689591	KY689569	Australia	<i>Eucalyptus</i> sp.
<i>Pisolithus albus</i>	MDB F36/10	KY689592	KY689570	Australia	<i>Eucalyptus rupestris</i>
<i>Pisolithus albus</i>	CA02	AF270782	–	Australia	<i>Eucalyptus</i> sp.
<i>Pisolithus albus</i>	MEL:2382873	KP012747	–	Australia	–
<i>Pisolithus albus</i>	MD07-004	AM947069	–	New Caledonia	<i>Acacia</i> sp.
<i>Pisolithus albus</i>	MD07-220	AM947117	–	New Caledonia	<i>Acacia spirorbis</i>
<i>Pisolithus albus</i>	MD07-146	AM947100	–	New Caledonia	–
<i>Pisolithus albus</i>	MD07-142	AM947099	–	New Caledonia	–
<i>Pisolithus albus</i>	MD07-027	AM947076	–	New Caledonia	–
<i>Pisolithus albus</i>	PDD77422	KY689594	KY689572	New Zealand	<i>Kunzea</i> sp.
<i>Pisolithus albus</i>	PDD77434	KY689595	KY689573	New Zealand	<i>Kunzea</i> sp.
<i>Pisolithus albus</i>	PDD77430	KY689596	KY689574	New Zealand	<i>Kunzea</i> sp.
<i>Pisolithus albus</i>	PDD77428	KY689599	KY689575	New Zealand	<i>Kunzea</i> sp.
<i>Pisolithus croceorrhizus</i>	MDB F53/07	KY689610	KY689581	Australia	<i>Eucalyptus miniata</i>
<i>Pisolithus croceorrhizus</i>	MDB F21/11	KY689609	KY689580	Australia	<i>Eucalyptus brachyandra</i>
<i>Pisolithus croceorrhizus</i>	PA684WTV01	KY689612	–	Australia	<i>Acacia</i> sp., <i>Eucalyptus</i> sp.
<i>Pisolithus tympanobaculus</i>	MU98/6	AF374646	–	Australia	<i>Eucalyptus</i> sp.
<i>Pisolithus tympanobaculus</i>	MU98/8	AF374648	–	Australia	<i>Eucalyptus</i> sp.
<i>Pisolithus tympanobaculus</i>	MU98/19	AF374658	–	Australia	<i>Eucalyptus</i> sp.
<i>Pisolithus arhizus</i>	92fbisPISOLI	FR748132	–	Italy	Willwood
<i>Pisolithus arhizus</i>	92bbisPISOLI	FR748129	–	Italy	Willwood
<i>Pisolithus tinctorius</i>	ATCC 38054	KC146359	KC146374	USA	–
<i>Pisolithus tinctorius</i>	AWW219	EU718114	EU718148	USA	–
<i>Pisolithus thermaeus</i>	PDD77420	KY689621	–	New Zealand	<i>Kunzea</i> sp.
<i>Pisolithus thermaeus</i>	PDD74168	KY689622	KY689588	New Zealand	<i>Kunzea</i> sp.
<i>Pisolithus thermaeus</i>	PDD100258	KY689625	–	New Zealand	<i>Kunzea</i> sp.
<i>Pisolithus marmoratus</i>	VIC30600	HQ693099	–	Brazil	<i>Eucalyptus</i> sp.
<i>Pisolithus marmoratus</i>	MDB F22/07	KY689589	–	Australia	<i>Eucalyptus miniata</i> , <i>E. tetradonta</i>
<i>Boletinellus merulioides</i>	AFTOL-ID 575	DQ200922	AY684153	USA	–
<i>Boletinellus exiguus</i>	MA-Fungi 47677	AJ419185	–	Brazil	–

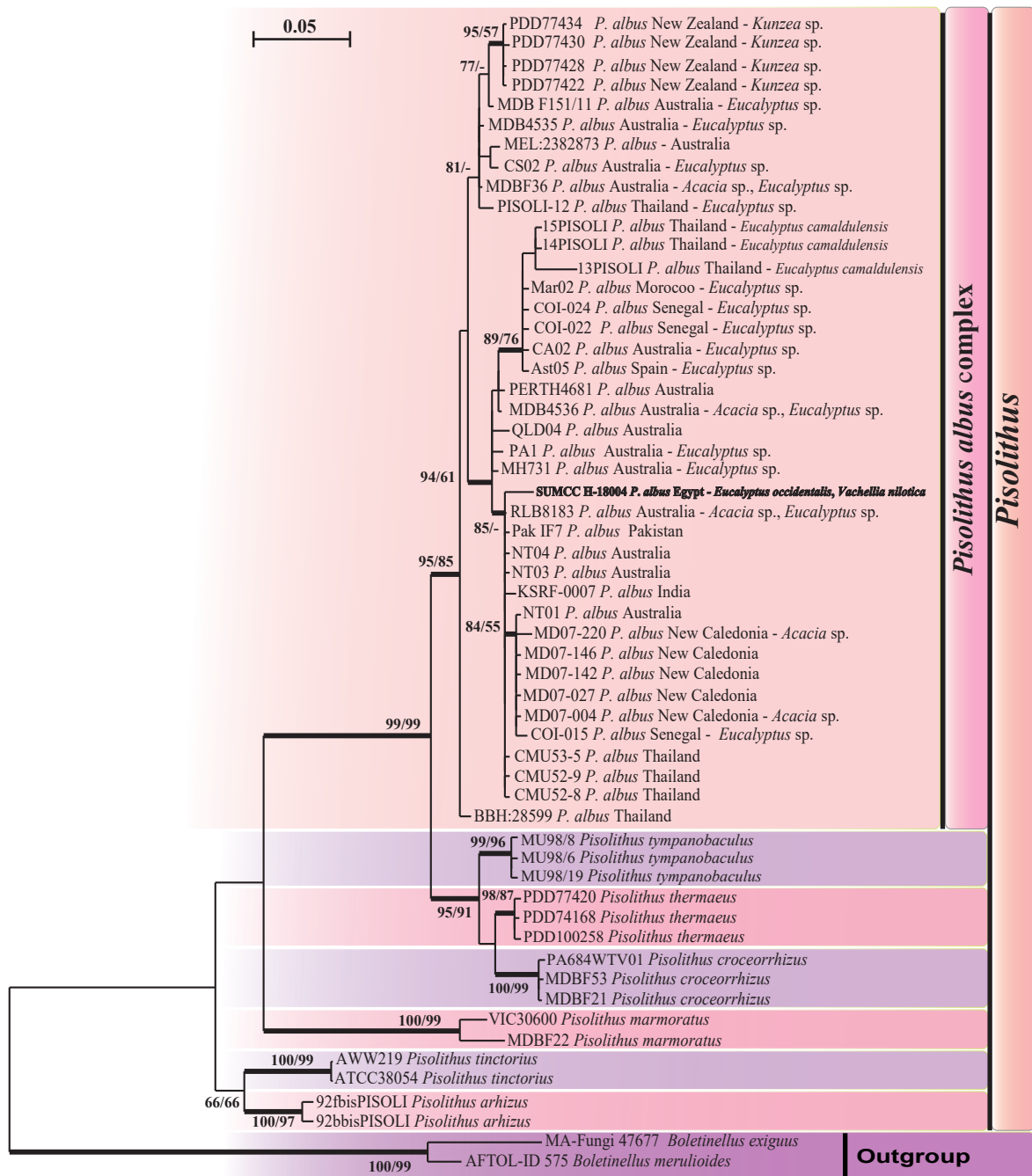


Figure 1. Bayesian inference tree generated from the concatenated ITS and LSU sequences of genus *Pisolithus*. The tree is rooted with *Boletinus merulioides* (AFTOL-ID 575) and *B. exiguus* (MA-Fungi 47677). Bootstrap support on the nodes represents MP and ML $\geq 50\%$. Branches received Bayesian posterior probabilities (BYPP) $\geq 95\%$ are in bold. The sequences generated in this study are in bold letters.

Taxonomy

Pisolithus albus (Cooke & Masee) Priest, in Lebel, Pennycook & Barrett, Phytotaxa 348(3): 167. 2018 (Fig. 2)

\equiv *Polysaccum album* Cooke & Masee, Grevillea 20: 36. 1891.

Description. Basidiomata epigeous, club-shaped, with irregular, subglobose, piriform or capitulate shape, 4.5–15 cm wide \times 6–18 cm high and with a stout deeply rooting base 3–8 \times 4–13 cm in diameter, attached to the substratum by yellowish rhizomorphic strands. Peridium thin, shiny, smooth, membranous, single-layered, leathery

when young, dry ruptured at maturity, white to cream and rarely buff in ripe basidiomata, yellowish around the base of fresh specimens, cracked into irregular segments from the top downwards at maturity, exposing the mature spores. Gleba developing within subglobose to ovoid or polygonal peridioles, 1–5 \times 1–3 mm (av. = 3.1 \times 2 mm, n = 25). Peridioles are larger in the upper parts, while smaller and tightly packed toward the stipe, whitish when young turning into ochre to pulverulent umber at maturity. It is encased by a very thin, yellow-ochre membrane and separated by gelatinous or sticky, carboneous tramal plates. At maturity of the basidiomata, peridioles wall collapse and the gleba turned into powdery mass. *Basidia* 8–10 \times 16–23 μ m (av. = 9 \times 19.5 μ m, n = 10), hyaline,

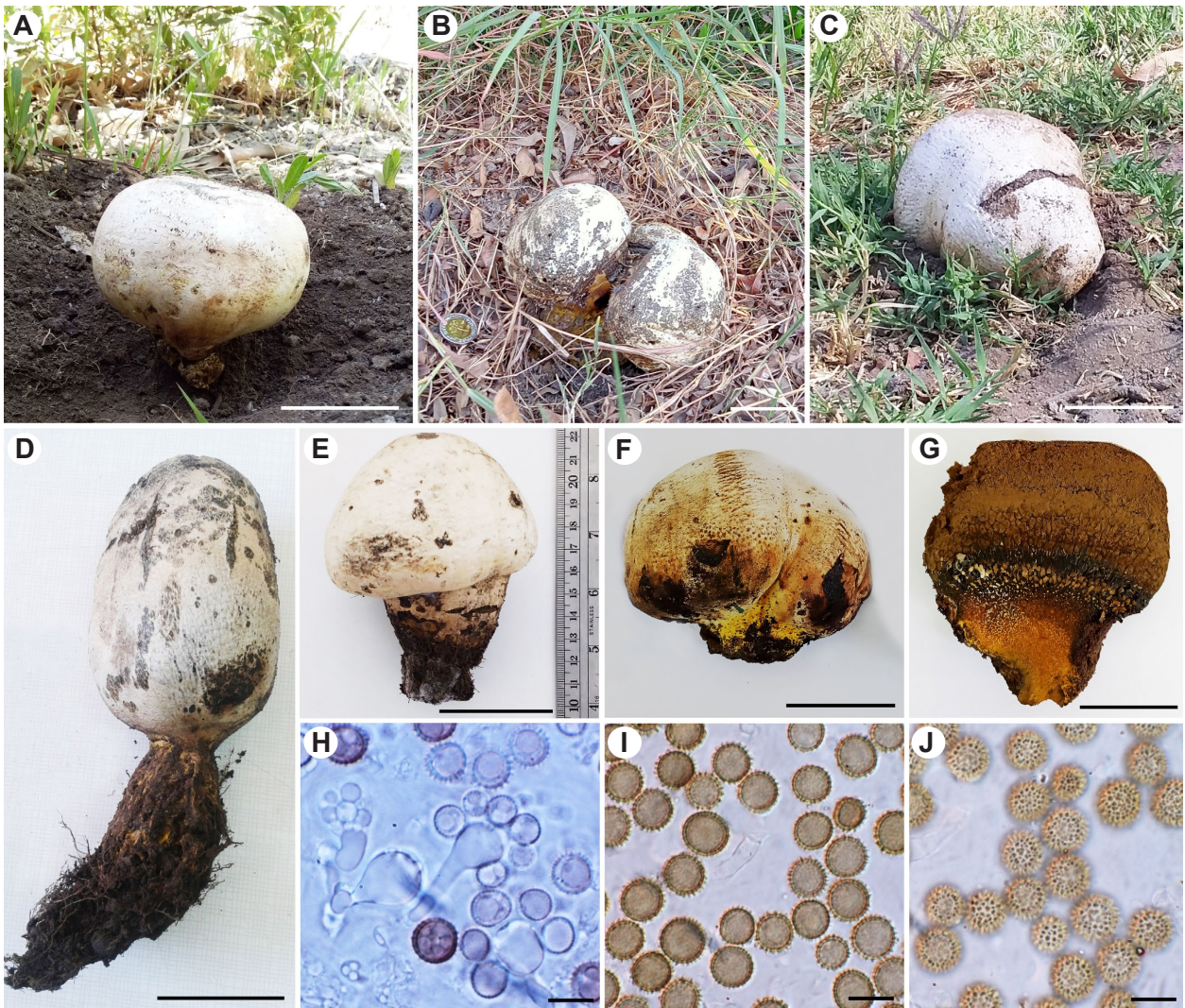


Figure 2. *Pisolithus albus* (SUMCC H-18004). A–G – basidiomata; H – basidium and basidiospores; I–J – basidiospores. Scales: A–F = 5 cm, G = 3 cm, H–J = 10 μ m.

clavate with a swollen apex, carried 5 or 6 basidiospores. Clamp connection present. Basidiospores 7–11 μ m in diameter (av. = 9 μ m, n = 45), globose to subglobose, bright yellow when young turned into reddish-brown at maturity, coarsely ornamented with broadly connate structures that form irregular pyramid-like spines.

Materials examined. EGYPT. Sohag Governorate, Karaman Island (26°34'42.3"N, 31°42'06.5"E), on sandy land near *Eucalyptus occidentalis* (Myrtaceae), 3 December 2018, M. S. Bakhit & A. E. Abdel-Aziz (SUMCC H-18004); Karaman Island (26°34'42.3"N, 31°41'56.1"E) near *Paspalum distichum* (Poaceae) and *Dodonaea viscosa* (Sapindaceae), 5 November 2019, M. S. Bakhit (SUMCC H-19005); Karaman Island (26°34'40.1"N, 31°42'06.8"E), in soil near *Vachellia nilotica* trees (Fabaceae), 11 March 2020, A. E. Abdel-Aziz (SUMCC H-20009).

GenBank accession numbers. ITS: OK184610; LSU: OK175781.

Notes. *Pisolithus albus* was recorded in several consecutive surveys from Karaman Island in autumn and winter 2018, 2019 and 2020. In this period, temperature ranged from 18 to 32°C; relative humidity was from 22 to 45%.

Discussion

Pisolithus albus is one of the most common gasteromyces in the world, which is recorded here for the first time from Egypt to extend our knowledge about its distribution in Africa to four countries, namely: Egypt (this study), Morocco (Yakhlef et al. 2009), Senegal (Duponnois & Bâ 1999) and Tunisia (Jaouani et al. 2015). Morphologically, *Pisolithus albus* specimens from Egypt are slightly different than collections from other localities. Basidiomata in our collection (4.5–15 cm wide \times 6–18 cm high) are slightly bigger than that recorded from Tunisia (3–12 cm in diameter) and smaller than that recorded from Italy (3–20 cm in diameter); peridioles in our collection are subglobose to ovoid or polygonal, while they are elliptic-ovoid to lens shape in the Tunisian collection. Basidia were observed clearly in our collection, which carried 5–6 basidiospores, while they were not observed in other collections recorded from Italy, Tunisia, Morocco and Senegal; basidiospores from the Egyptian collection are slightly smaller than those recorded in the Tunisian collection (7–11 μ m vs 9–12 μ m diameter in Egyptian and Tunisian records, respectively). During this study, *P. albus* was found associated with *Eucalyptus occidentalis* and

Vachellia nilotica (syn. *Acacia nilotica*). This fungus was reported previously in ectomycorrhizal association with *Acacia* and *Eucalyptus* species from different countries (Díez et al. 2001; Founoune et al. 2002; Martin et al. 2002; Kanchanaprayudh et al. 2003; Singla et al. 2004; Jaouani et al. 2015; Gargano et al. 2018; Lebel et al. 2018; Chouhan & Panwar 2021). During the present study, *P. albus* basidiomata were reported for the first time in the vicinity of two potential hosts, *Paspalum distichum* and *Dodonaea viscosa*; however, the mycorrhizal connections with these two hosts were not investigated. Therefore, detailed studies are required to prove mycorrhizal association of these two hosts with the fungus.

Phylogenetic analysis of the combined ITS and LSU sequences placed the Egyptian specimen within *P. albus* complex clade and formed a distinct lineage with *P. arhizus*, *P. croceorrhizus* and *P. tympanobaculus*, which is similar to the results obtained by Martin et al. (2002) and Lebel et al. (2018). Phylogenetically, the Egyptian specimen showed a high level of genetic similarity with collections of *P. albus* from Australia (RLB8183) which forms mycorrhizal associations with *Acacia* sp., *Eucalyptus* sp., and other collections from Australia (NT03, NT04), Pakistan (Pak IF7) and India (KSRF-0007) where its hosts are unknown.

Conclusion

This study documents the first reports of *Pisolithus albus* from Egypt. It was found forming ectomycorrhizal associations with *Eucalyptus occidentalis* and *Vachellia nilotica*. It was also reported near two potentially new hosts, *Paspalum distichum* and *Dodonaea viscosa*. The reported specimens were compared morphologically with other reports of *Pisolithus albus* from different localities and showed slight differences. Phylogenetic analyses of the combined ITS and LSU sequences placed the Egyptian specimen within *P. albus* complex clade.

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