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PISOLITHUS HYPOGAEUS SP. NOV.: A HYPOGEOUS REPRESENTATIVE OF THE GENUS PISOLITHUS FROM WESTERN AUSTRALIA

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ABSTRACT

Pisolithus hypogaeus, the first record of a *Pisolithus* species with a hypogeous habit, occurs in coastal south-western Australia in association with eucalypt ectomycorrhizal hosts on sandy soils. Ribosomal DNA (rDNA) sequencing shows that *P. hypogaeus* lies within a lineage of other brown- and echinulate-spored *Pisolithus* spp., and is most closely related to two undescribed epigeous *Pisolithus* species from Australia.

KEYWORDS ectomycorrhiza, eucalypt, truffle-like, Sclerodermataceae, sequestrate

INTRODUCTION

Following the systematic arrangement in Kirk *et al.* (2001), as supported by the molecular phylogenetic studies of Binder and Bresinsky (2002), the family Sclerodermataceae includes five genera with species described from Australia: *Calostoma, Favillea, Horakiella, Scleroderma* and *Pisolithus*. *Calostoma* and *Favillea* contain only epigeal species. *Scleroderma* includes ca. 23 species with epigeal habit and up to 11 hypogeous species (Guzman 1970; Beaton and Weste 1982; Sims *et al.* 1995; JM. Trappe, M. Castellano and A. Claridge, unpublished data). The hypogeous genus *Horakiella* has one described species, with a further four undescribed species (Castellano and Trappe 1992; JM. Trappe, M. Castellano and A. Claridge, unpublished data). *Pisolithus* includes at least five validly described species (Kirk *et al.* 2001; Watling *et al.* 1995) but the molecular genetic studies by Cairney *et al.* (1999), Anderson *et al.* (2001) and Martin *et al.* (2002) indicate the presence of up to an additional eight species. Australian *Pisolithus* material has been partly revised and assigned provisional names (MJ. Priest, unpublished data) that have been cited in other works (Bougher and Syme 1998; Anderson *et al.* 2001; Martin *et al.* 2002).

No hypogeous representatives of *Pisolithus* have been reported to date. However, examination of numerous hypogeous Sclerodermataceae from south-western Australia has brought a new hypogeous *Pisolithus* species to our attention. Based upon analysis of rDNA sequences, *P. hypogaeus* sp. nov. is closely related to some epigeous brown-spored *Pisolithus* species.

MATERIALS AND METHODS

Air-dried material was sectioned by hand, rehydrated in 3% KOH and stained with 1 % Congo Red and Melzer's Reagent. Colours are as described in Kornerup and Wanscher (1969). An Olympus BH2 microscope was used for microscopy, and drawings were made using an Olympus LB drawing tube at 1000x magnification. Spore diameters exclude ornamentation. Sequencing of the internal transcribed spacers (ITS) of rDNA was as detailed in Dunstan *et al.* (2000). Genbank (Benson *et al.* 2000) accessions for ITS sequences are AY179746 (MURU5134) and AY179747 (MURU5417). Collections have been deposited in the Western Australia Herbarium (PERTH) and the herbarium of Murdoch University (MURU).

DESCRIPTION

Pisolithus hypogaeus S.R. Thomas, Dell & Trappe sp. nov.

A *Pisolithi* ceteri habitu hypogaeo, statura parvula basidiomatum, penuria basis radicantis, et maturatione simultanea sporarum differt. Holotypus hic designatus: MURU 5417, Australia Occidentalis, Stella Palus, leg. W. A. Dunstan, 9 Iulius 2000.

Basidiomata hypogeous, globose to slightly irregular, 7 – 12 mm, soot brown (near 5F5), with or without a basal depression and few basal hyphae; surface glabrous and dry with shallow pitting (to 1 mm), not rupturing at maturity, drying hard (Figure 1). *Peridium* 0.3 - 0.7 mm thick, wheat to butter yellow (4A5/4B5), not staining with exposure. *Gleba* never exposed, solid, dry, concolorous with the peridium, becoming darker yellow or brown when dried; comprised of irregularly shaped peridioles separated by tramal plates 25-170 µm wide. *Peridioles* persistent, 0.25 x 0.2 – 1.8 x 1 mm, filled with spores at maturity. Matrix frequently present.

Peridium persistent, 250 – 400 µm thick; two layered, suprapellis (120-150 µm thick) of loosely interwoven yellowish-brown hyphae (2 - 4 µm), subpellis (150 - 250 µm thick) of tightly bound interwoven hyaline hyphae (9 - 10 µm dia.) with few intercellular spaces, often constricted at septa (3.6 - 7.3 µm dia.), thin- (to 0.5 µm) or thick-walled (0.7 – 1.5 µm); clamp

Figure 1: Basidiomata of *Pisolithus hypogaeus* (Holotype: MURU5417). Bar = 5 mm

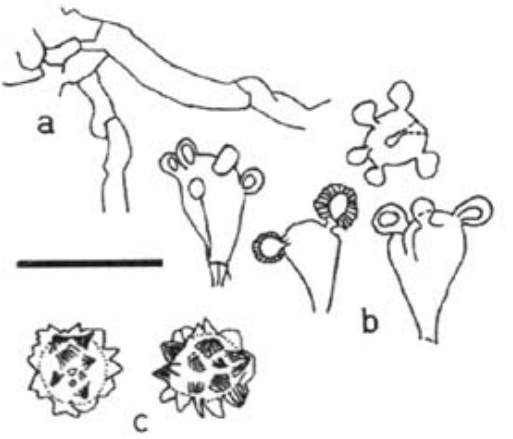

Figure 2: *Pisolithus hypogaeus* (a) tramal hyphae; (b) basidia; and, (c) spores (Holotype: MURU5417). Bar = 20 μm

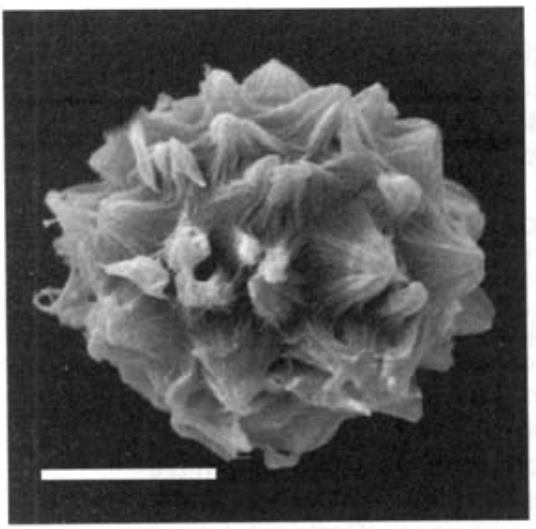

Figure 3: Pisolithus hypogaeus basidiospore (Holotype: MURU5417). Bar = 5 mm

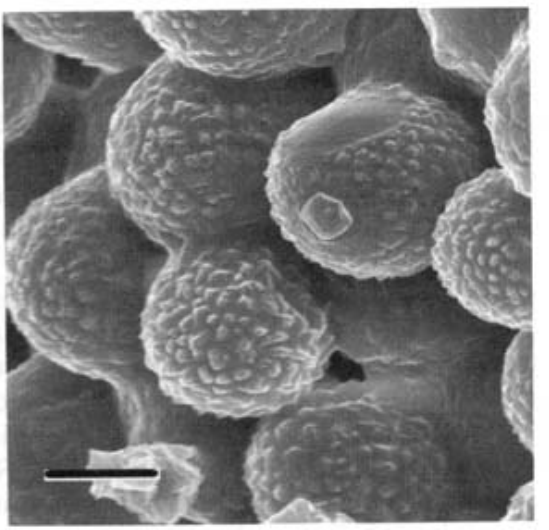

Figure 4: *Pisolithus hypogaeus* basidiospores (Paratype: MURU5023). Bar = 5 μm

connections present. *Trama* of parallel interwoven thin (1.5 - 2.2 µm dia.), hyaline hyphae, with thick (1.8 – 4.4 µm dia.) hyphae in the central layer between peridioles; clamp connections abundant (Figure 2a).
Basidia pyriform, 13.1-18.2 x 7.6 -10.2 µm, thin-walled, 4- (some 6-) spored, basal clamp connections not seen (Figure 2b), sometimes present in peridioles with mature spores. *Spores* in mass raw umber (near 5F8), dark brown in water and KOH, not changing in Melzer's reagent, blue in Cotton Blue; globose, 9.1 µm (6.2 – 12.3 µm), thick walled (1.2 µm), two-layered; hilar appendix inconspicuous. *Ornamentation* of variable sized cones (0.7 - 2.4 µm) comprised of coalesced or connivent echinulae, with ornaments sometimes separated from each other at the base, basal width similar to length (Figures 2c and 3) or reduced and crowded cones concealed within a matrix that forms short ridges between ornaments (Figure 4).

Etymology Growing or remaining below ground.
Distribution and Hosts Found in sandy soil up to 10 cm deep. Known only from *Eucalyptus* spp.-*Banksia* spp. woodland on the mainland (*E. marginata*, *E. gomphocephala* and *Corymbia calophylla*) and *Eucalyptus* spp. revegetated areas on calcareous soils on Rottnest Island (*E. camaldulensis, E. gomphocephala* and *E. platypus*) in coastal south-western Australia.
Collections Examined WESTERN AUSTRALIA: **Holotype** here designated, Star Swamp Reserve, Waterman, W.A. Dunstan, MURU5417, 09.vii.2000, (PERTH06232159); **Paratypes:** Rottnest Island, S.R. Thomas and L. Chapman, MURU5023, 20.vii.1999; S.R. Thomas and T. Thomas, MURU5134, 06.viii. 2000.

DISCUSSION

P. hypogaeus differs from other *Pisolithus* spp. in greatly reduced sporocarp size, hypogeous habit, lack of a rooting base and the manner of sporocarp maturation. In the material so far examined, mature spores are found throughout the sporocarp, in contrast with progressive maturation of spores and disintegration of the peridium and gleba from the apex to the base in other species of *Pisolithus. P. hypogaeus* also lacks the black tar-like matrix present in the immature peridioles of other *Pisolithus* spp. and spores are never dark brown or black at maturity.

Chamberlain (1995) found a high degree of intraspecific variation in basidiospore morphology of some Australian *Pisolithus* species. Examination of spores from a single sporocarp revealed differences in size, density and shape of ornamentation similar to the differences identified between *P. hypogaeus* mainland and Rottnest Island collections. Spores of *P. hypogaeus* from Rottnest Island (Figure 4) have reduced spore ornamentation when compared to the holotype (Figure 3). Rottnest Island has a long history of disturbance (Rottnest Island Authority 2002) and is isolated from the mainland by 18 km of ocean.

sequences of other *Pisolithus* spp. and *Scleroderma* spp. (Martin *et al.* 2002; Y. Chen, WA. Dunstan and B. Dell unpublished data), show that *P. hypogaeus* falls within lineage A1 of *Pisolithus* spp. (Martin *et al.* 2002), and is most closely related to an unnamed epigeal *Pisolithus* species from New South Wales (accession/isolate LJ30 in Anderson *et al.* 2001, Genbank accession AF270774, 93% ungapped sequence homology), and to *P. marmoratus* nom. prov. (Bougher and Syme 1998). Both species have dark brown spores with coalesced spines, similar to *P. hypogaeus*.

At first appearance, sporocarps of *P. hypogaeus* could be mistaken for hypogeous species of *Scleroderma* or *Horakiella*. However, spores in mass of *P. hypogaeus* remain brown at all stages of development, in contrast to most hypogeous *Scleroderma* spp. that have a white gleba in immature specimens and a brown-black to purple black spore mass at maturity (JM. Trappe, M. Castellano and A. Claridge, unpublished data). *Horakiella* spp. can be readily discriminated from *P. hypogaeus* by their spores with thicker walls and ornamentation of shallow lines, wrinkles, reticulation, or minute spines rather than coalesced spines of *P. hypogaeus* (Castellano and Trappe 1992).

Mycophagy is the assumed mode of spore dispersal in hypogeous fungal species, including the Sclerodermataceae. Over several years of research on Rottnest Island there has been evidence of hypogeous *Scleroderma* spp. in the scats of the Quokka (*Setonix brachyurus*; Macropodidae) (SR. Thomas unpublished data). Spores of *P. hypogaeus* have not been identified from Quokka scats. Given the general decline of other mammalian mycophagists within the known range of the fungus, insects are probably important in dispersal of spores of *P. hypogaeus* as adult Coleoptera (beetles) have been observed consuming *P. hypogaeus* basidiomes in the field.

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