

***Pisolithus croceorrhizus* P. Leonard & McMullan-Fisher sp. nov. from Queensland, Australia and New Caledonia**

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Abstract

Pisolithus croceorrhizus P. Leonard & McMullan-Fisher sp. nov. from Australia and New Caledonia is described and illustrated. Analysis of ITS sequence data, and morphological characters, support the distinction of this species from another species with golden rhizoids, *P. aurantioscabrosus* Watling from Malaysia.

Key words: diversity, *Pisolithus*, systematics, Sclerodermatineae, taxonomy.

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Introduction

The genus *Pisolithus* is relatively common in Queensland, at least in coastal heaths and dry sclerophyll habitats, in well drained sandy or stony soils. Until about 1960, all collections would have been given the European name *P. tinctorius* (Pers.) Coker & Couch, the dyeball (Cleland 1935, Aberdeen 1979). Rauschert (1959) clarified the taxonomy and for the next 40 years some collections were deposited as *P. arhizus* (Scop.) Rauschert, the Horse Dung Fungus. These names are also used in some field guides and taxonomic treatments (Fuhrer 2009; Grgurinovic 1997).

After the publication of the Fungi of South Australia (Bougher & Syme 1998), field mycologists began to realise that there were several native Australian species of *Pisolithus*. Martin *et al.* (2002) included a substantial number of Australian sequences in analyses of molecular data, and showed good support for distinguishing at least five native Australian species. Of these, five species have been recorded from Queensland: *Pisolithus albus* (Cooke & Masee) Priest, *P. marmoratus* (Berk.) E. Fisch., *P. microcarpus* (Cooke & Masee) G. Cunn, together with two undescribed species, *Pisolithus* sp. 10 (Martin *et al.* 2002), and *P. sp* 12/13 (Martin *et al.* 2002).

Currently three species of *Pisolithus* are known from New Caledonia. *Pisolithus albus* is apparently fairly widespread, and may include four genetic ecotypes (Hosaka 2009; Jourand *et al.* 2010). The second species is *Polysaccum pusillum* Har. & Pat., found in the rainforest; *Polysaccum* is considered a synonym of *Pisolithus* (Kirk 2012). Watling *et al.* (1995, 1999) described *P. aurantioscabrosus* from Malaysia and discussed how it differed from *P. kisslingi* E. Fisch. from Sumatra and *P. pusillum* from New Caledonia. The final species was distinguished by Hosaka (2009) based on its distinctive ITS sequence, and bright yellow to orange rhizoids. On examination of material from the

Queensland herbarium, a single collection was found that resembled *P. aurantioscabrosus* Watling and the unnamed species from New Caledonia. In this paper we officially describe *Pisolithus croceorrhizus* P. Leonard & McMullan-Fisher sp. nov., distributed in south east Queensland and New Caledonia.

Materials and Methods

Macroscopic features were described from fresh specimens which are held at BRI, of *P. croceorrhizus* and *P. sp* 10a (Martin *et al.* 2002) from Queensland. Colours follow Kornerup & Wanscher (1978). Dried specimens were examined using compound light microscopy, using 5% KOH solution following methods of Largent *et al.* (1973). At least 20 spores were measured for each collection. Spore ranges are given for length and width in microns, followed by arithmetic mean plus or minus standard deviation, and the Q values (length divided by width).

The region of nuclear rDNA data used for analyses included ITS1–5.8S–ITS2. Two novel sequences representing *P. croceorrhizus* sp. nov. and *P. sp* 10a (Martin *et al.* 2002) were obtained. The Blast search in GenBank was used to retrieve close-matching sequences representing a range of species within *Pisolithus*. *Astraeus hygrometricus* (Pers.) Morgan was included as an outgroup (Table 1). Phylogenetic analyses were performed using Bayesian likelihood with the Metropolis coupled Markov chain Monte Carlo (MCMC) search algorithm implemented in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). Each search involved four chains (three heated to 0.2 and one cold) sampled every 1000 generations. A 50% consensus tree was imported to FigTree (Rambaut 2009) and is presented with posterior probabilities.

Results

Molecular analyses

The sequence of *P. croceorrhizus* sp. nov. from Queensland is in a well supported clade (Fig 1.; PP =0.96) that includes sequences from fruit bodies of New Caledonian *Pisolithus* sp. collections (Hosaka 2009), and some environmental sequences from

the rhizosphere of *Tristaniopsis* (Myrtaceae) from New Caledonia (Genbank accessions JN847494, JN847470, JN847462). The sequences from fruit bodies (Queensland and New Caledonia) are identical, while there is only a 2-3 bp difference with sequences from environmental samples. *Pisolithus croceorrhizus* forms a sister relationship with two other clades. One contains specimens referred to as the undescribed

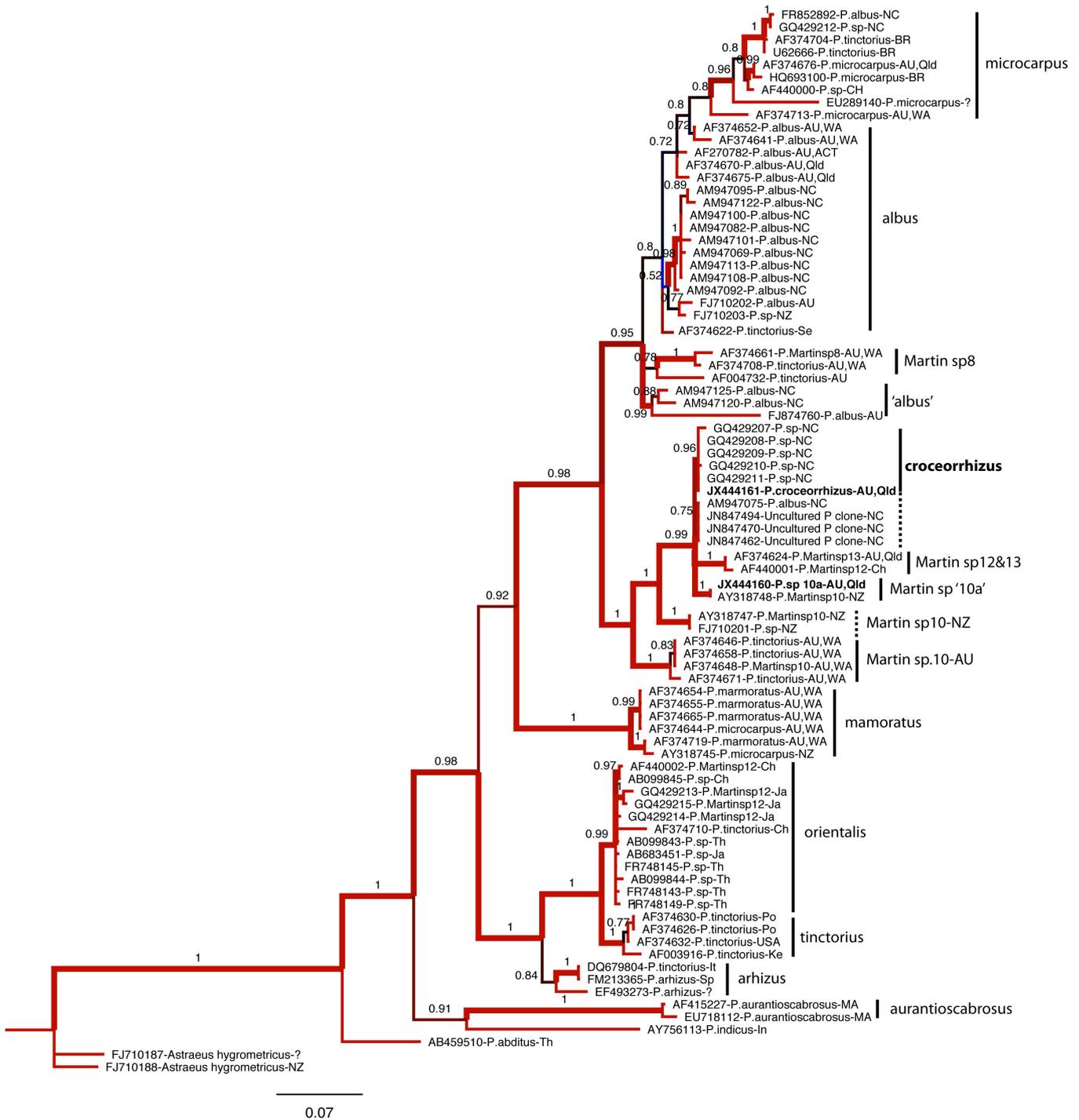


Fig. 1 Bayesian analysis of ITS sequence data for species of *Pisolithus*, presenting 50% consensus tree with posterior probabilities at clades. Clades indicating some species determined by Martin *et al.* (2002) and Genbank numbers of sequences are shown. Sequences indicated by: Genbank# - Species - Country [NC New Caledonia; NZ New Zealand; AU Australia Qld or WA; MA Malaysia; Th Thailand; BR Brazil; Ch China; Se Senegal; Ke Kenya; It Italy; Ja Japan; Po Portugal; In India].

Table 1. Novel *Pisolithus* sequences generated for this study.

Taxon	GenBank No	Geographic locale
<i>Pisolithus croceorrhizus</i> sp. nov.	JX444161	Queensland, Australia
<i>Pisolithus</i> Martin sp.10a	JX444160	Queensland, Australia

Pisolithus sp 12 (Martin *et al.* 2002) from China (associated with introduced *Eucalyptus*), and *P.* sp. 13 from Queensland (Martin *et al.* 2002). The other clade contains specimens referred to as *Pisolithus* sp 10 (Martin *et al.* 2002), including our freshly collected specimen from Queensland which is labelled *Pisolithus* sp 10a (Martin *et al.* 2002).

Taxonomy

***Pisolithus croceorrhizus* P. Leonard & McMullan-Fisher sp. nov.**

(Fig. 2 & 3) MycoBank 802885

Differs from other *Pisolithus* species by its golden rhizoids, that form a pseudostipe; *P. aurantioscabrosus* by small spores ornamented with a partial reticulum at the base of pyramidal spines rather than ridges in a reticulum, and *Pisolithus* sp. 10a (Martin *et al.* 2002) which has verrucose spores.

Holotype: Noosa National Park, Arthur Harrold Reserve, Patrick Leonard and Sapphire JM McMullan-Fisher PL151310, 28 Mar 2010 [BRI AQ796219].

Etymology: from croceus, Latin for saffron yellow, and rhizus, which is Greek for root.



Fig. 2 Fruit body of holotype collection of *Pisolithus croceorrhizus*; note brightly coloured rhizoids (SJM McMullan-Fisher).

Fruiting body: 40 mm high × 35 mm diameter, ovoid, gasteroid; glabrous, but becoming slightly verrucose with age, pale brown (5D7) at apex and paler greyish orange (5B4) below. Gleba of granular, ovoid to irregular cells, initially parchment coloured becoming pale brown (5C4), surrounded by ochraceous tissue contained in a thick walled and dark staining context. Stipe rooting stem part buried to 20 mm; composed of numerous branching rhizoids which combine into a pseudostipe; 60 × 35 mm; bright orange to carrot coloured (5A8, 6B7). Basidia not observed. Basidiospores globose to subglobose; 5.3 – 6.9 × 5.3 – 6.9 μm (mean 6.1 ± 0.45 × 5.9 ± 0.4 μm); Q = 1 – 1.09 (mean Q = 1.05 ± 0.04); thick walled; pale yellow to brown; densely covered in an ornamentation of pyramidal spines (1.2–1.7 μm high) connected basally in a low reticulum.

Habitat: growing in sand in coastal closed forest near *Eucalyptus*, *Corymbia*, *Melaleuca* and *Allocasuarina*. Queensland regional ecosystem 12.2.11: *Corymbia* spp., *Banksia integrifolia*, *Callitris columellaris*, *Acacia* spp. open forest to low closed forest on beach ridges (Queensland Herbarium 2012).

Notes: This species is characterised by its pale brown fruiting body suspended on bright orange-yellow rhizoids forming a pseudostipe, and its spiny-reticulate basidiospores. It does not match any taxa in Cunningham (1942), nor Priest (pers. comm). Analysis of ITS sequences shows this collection to be the same as collections made by Hosaka (2009) in New Caledonia (Fig. 1).



Fig. 3 Spores of *Pisolithus croceorrhizus*, showing spiny reticulum (scale bar 10 μm; PL Leonard).

Discussion

The Queensland specimen was initially thought to be *P. aurantioscabrosus* or a close relative due to the brightly coloured orange rhizoids and general fruit body form (Watling *et al.* 1995). However, *P. croceorrhizus* differs in having much smaller basidiospores with spiny rather than ridged-reticulum, although further work on *P. aurantioscabrosus* (Watling *et al.* 1999) suggests that spore morphology may show variation including conical spines in some specimens. Molecular results put *P. aurantioscabrosus* in a completely different clade to *P. croceorrhizus*. (Fig.1). The host associates of *P. croceorrhizus* in Queensland and New Caledonia both include Myrtaceae; however *P. aurantioscabrosus* is recorded from dipterocarp forests of Malaysia and Thailand.

Hosaka (2009) suggests that there is insufficient variation in the ITS region to confidently separate *Pisolithus* species. However, with the near identical sequences of *P. croceorrhizus* from Queensland and New Caledonia, and strong morphological similarity between collections, we are confident they are one and the same taxon. Providing a name for this taxon also enables those researchers working on environmental sequences to match to herbarium material. *Pisolithus* sp. 10a (Martin *et al.* 2002) is obviously a close relative to *P. croceorrhizus*, however the fruit bodies are more globose with a short but clear stipe present, the rhizoids are yellowish rather than bright orange, and the basidiospores are globose and have verrucose ornamentation rather than a spiny reticulum (Martin *et al.* 2002, Priest pers. comm.).

Two named species of *Pisolithus* recorded from New Caledonia are *Polysaccum pusillum* (Watling *et al.* 1995) and *P. albus* (Hosaka 2009, Jourand *et al.* 2010). The description of *Polysaccum pusillum* (Saccardo & Saccardo 1905) translates as:

“exterior blackish brown to olivaceous brown, sporangial minute, fragile, brown; spherical spores, verrucose 8-12 µm diam. ochre-brown.” This is unlikely to be our species as these spores are larger and are verrucose.

From Genbank searches it appears that most of the sequences of *Pisolithus* from New Caledonia have been called *P. albus* (Hosaka 2009; Jourand *et al.* 2010). All of the *P. albus* ecotype sequences mentioned in Jourand *et al.* (2010) fall within a broadly accepted *P. albus* clade, quite distinct from the *P. croceorrhizus* clade (Fig.1). However, a single *P. albus* sequence from the Jourand *et al.* (2010) study, but not mentioned in the paper, belongs in the *P. croceorrhizus* clade together with three environmental sequences from an unpublished study (Genbank accessions JN847494, JN847470, JN847462,) on ECM diversity of *Tristaniopsis* from New Caledonia (Fig.1). Some of the specimens illustrated in Hosaka (2009) appear to show a change in colour

of the gleba with maturity, from paler parchment in colour to brown. If identification of fruit bodies is based solely upon gleba colour, there could be confusion of *P. croceorrhizus* with *P. albus* in younger fruit bodies. If collections are identified by checking microscopic characters then specimens would be recognizable as *P. albus* by the larger basidiospores with isolated spines and completely lacking any reticulate pattern.

In describing this new species, we hope future collections will show the full extent of its distribution and morphological variation, and help clarify the boundaries of the three closely related species, *P. croceorrhizus*, *P. sp. 10*, and *P. sp.12* (China)/ *13* (Australia) (Martin *et al.* 2002).

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