

Phylogeography of the Genus *Pisolithus* Revisited with some Additional Taxa from New Caledonia and Japan

Kentaro Hosaka

Department of Botany, National Museum of Nature and Science,
Amakubo 4-1-1, Tsukuba 305-0005, Japan
E-mail: khosaka@kahaku.go.jp

Abstract Molecular phylogenetic analyses for the genus *Pisolithus* (Basidiomycota, Boletales, Sclerodermatineae) were conducted based on the ITS sequences. More than three hundred sequences were retrieved from the GenBank database. In addition, the ITS sequences from some specimens recently collected from New Caledonia and Japan were newly generated for this study. Taxa from New Caledonia did not form a monophyletic group, suggesting multiple dispersals of the genus to New Caledonia, but they all belong to the same clade which is roughly corresponding to the “Lineage B” designated by the previous study. In most cases, Australian taxa were demonstrated to be sister taxa to New Caledonian taxa, but at least in one clade, taxa from New Zealand and New Caledonia were more closely related to each other. Taxa from Japan did not form a monophyletic group, either. Japanese taxa were demonstrated to be closely related to other Asian taxa from China and Thailand. Biogeographical patterns of *Pisolithus* indicate that long distance dispersal may be the most important factor, and this may be associated with its ability to switch ectomycorrhizal hosts rather frequently.

Key words: Basidiomycota, biogeography, Boletales, ectomycorrhizae, fungi, ITS, phylogeny, systematics.

Introduction

The genus *Pisolithus* (Basidiomycota, Boletales, Sclerodermatineae) is an ectomycorrhizal fungus and reported worldwide (Marx, 1977). It was once thought that one to a few species are distributed worldwide, but because of its morphological, biochemical, and ecological diversity (Burgess *et al.*, 1995; Grand, 1976; Grenville *et al.*, 1985; Kope and Fortin, 1990; Marx, 1977), mycologists have recognized that many biological species exist in this genus.

The genus has been considered closely related to the genus *Scleroderma* (Cunningham, 1944; Jülich, 1981), which was further confirmed by recent molecular studies (Binder and Hibbett, 2006; Hibbett and Binder, 2002; Hibbett and Thorn, 2001; Matheny *et al.*, 2007; Watling, 2006). Some unexpected findings include a close relationship of the genus to *Calostoma* and

Boletinelus (Binder and Hibbett, 2006), which along with *Pisolithus* and *Scleroderma* are included in the suborder Sclerodermatineae. Currently *Pisolithus* is in the order Boletales (Kirk *et al.*, 2008).

Despite morphological, biochemical, and ecological variations, infrageneric taxonomy of *Pisolithus* has not been advanced until recently (Kanchanaprayudh *et al.*, 2003b; Reddy *et al.*, 2005; Thomas *et al.*, 2003; Watling *et al.*, 1995). More recently, large scale phylogenetic studies have been conducted for the genus and some infrageneric classification systems are being established (Martin *et al.*, 2002; Moyersoer *et al.*, 2003; Kanchanaprayudh *et al.*, 2003a). Importantly, these studies revealed a significant genetic diversity of the genus and molecular data clearly demonstrated that there are clear biogeographical patterns.

Martin *et al.* (2002) conducted phylogeograph-

ical study of *Pisolithus* based on worldwide sampling and demonstrated that there are two major lineages within the genus, and clear trends in biogeographical patterns and ectomycorrhizal host affiliations. Based on these findings, they hypothesized an ancient origin of the genus with subsequent vicariance when Pangaea was split into the northern (Laurasia) and southern (Gondwana) continents. Although such an ancient origin of modern ectomycorrhizal taxa has frequently been postulated (Halling, 2001), the exact time of origin for *Pisolithus* has never been investigated. It is noteworthy that recent molecular clock studies consistently indicate that fungi have a very ancient origin (Heckman *et al.*, 2001; Hedges *et al.*, 2004; Taylor and Berbee, 2006) and the origin of modern ectomycorrhizal fungi probably predated that of plant partners (Hibbett and Matheny, 2009).

Although Martin *et al.* (2002) and the studies by subsequent workers (Moyersoen *et al.*, 2003; Kanchanaprayudh *et al.*, 2003a) were extensive and made significant contribution to fungal biogeographical studies, some areas of significance have largely been ignored. Most notably, taxa from New Caledonia, where *Pisolithus* is known to occur, have never been sampled in previous studies. This ignorance of New Caledonian fungal flora is more or less true for fungal biogeographical studies in general. Even though New Caledonia has been a center of interest for studying vicariance biogeography of many plants and

animals (Sanmartín *et al.*, 2004; Swenson *et al.*, 2001), fungal biogeographical studies, including the ones with extensive taxon sampling (Coetzee *et al.*, 2001, 2003; Hibbett, 2001; James *et al.*, 1999, 2001; Matheny *et al.*, 2009; Zervakis *et al.*, 2004) did not include taxa from New Caledonia. Notable exception includes the study by Hosaka *et al.* (2008), but only a few specimens from New Caledonia were included.

Recent fieldwork in New Caledonia and Japan revealed several species of *Pisolithus*. In addition, more and more DNA sequences of *Pisolithus* from New Caledonia and other parts of the world are now deposited in GenBank. These allow us to cover more complete diversity of *Pisolithus*, which in turns allows us to further clarify biogeography of the genus. This study attempts to answer the questions of monophyly and origin(s) of the genus *Pisolithus* in New Caledonia and Japan.

Materials and Methods

Taxon sampling, PCR, and DNA sequencing

Taxa sampled, along with GenBank accession numbers, are listed in Tables 1 and 2. A total of 317 taxa (30 outgroup and 287 ingroup taxa) were sampled for this study. The selection of outgroup taxa was based on the phylogeny of previous studies (Binder and Hibbett, 2006) to cover the diversity of Boletales. Because *Pisolithus* belongs to the suborder Sclerodermatineae and is

Table 1. Specimens used in this study with information of geographical origin, presumable ectomycorrhizal hosts, and GenBank accession numbers

Taxon	Herbarium	Specimen No.	Locality	Presumable ECM hosts	Accession No.
<i>Pisolithus</i> sp.	TNS	KH-NC09-002	New Caledonia, Goro	Myrtaceae/Leguminosae	GQ429210
<i>Pisolithus</i> sp.	TNS	KH-NC09-004	New Caledonia, Goro	Myrtaceae/Leguminosae	GQ429207
<i>Pisolithus</i> sp.	TNS	KH-NC09-005	New Caledonia, Goro	Myrtaceae/Leguminosae	GQ429208
<i>Pisolithus</i> sp.	TNS	KH-NC09-006	New Caledonia, Goro	Myrtaceae/Leguminosae	GQ429209
<i>Pisolithus</i> sp.	TNS	KH-NC09-026	New Caledonia, Goro	Myrtaceae/Leguminosae	GQ429211
<i>Pisolithus</i> sp.	TNS	KH-NC09-059	New Caledonia, Les Bois du Sud	Myrtaceae/Leguminosae	GQ429212
<i>Pisolithus</i> sp.	TNS	F-13374	Japan, Mie	<i>Pinus sieboldii</i>	GQ429214
<i>Pisolithus</i> sp.	TNS	F-2158	Japan, Tokyo	unknown	GQ429215
<i>Pisolithus</i> sp.	TNS	F-12086	Japan, Hyogo	<i>Castanopsis/Quercus</i>	GQ429216
<i>Pisolithus</i> sp.	TNS	F-16874	Japan, Mie	unknown	GQ429213

Table 2. DNA sequences used in this study with information of geographical origin, presumable ectomycorrhizal hosts, and GenBank accession numbers

Genus	Species	Accession No.	Locality	Presumable ECM hosts
[INGROUP]				
<i>Pisolithus</i>	<i>albus</i>	AF 440868	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>albus</i>	FJ 710202	Australia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947069	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947070	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947071	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947072	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947073	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947074	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947075	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947076	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947077	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947078	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947079	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947080	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947081	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947082	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947083	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947084	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947085	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947086	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947087	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947088	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947089	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947090	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947091	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947092	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947093	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947094	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947095	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947096	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947097	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947098	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947099	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947100	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947101	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947102	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947103	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947104	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947105	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947106	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947107	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947108	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947109	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947110	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947111	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947112	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947113	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947114	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947115	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947116	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947117	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947118	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947119	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947120	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947121	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947122	New Caledonia	n/a

Table 2. -(Continued)-

Genus	Species	Accession No.	Locality	Presumable ECM hosts
<i>Pisolithus</i>	<i>albus</i>	AM947123	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947124	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947125	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947126	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947127	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AM947128	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	FN 390950	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	FN 390951	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	FN 390952	New Caledonia	n/a
<i>Pisolithus</i>	<i>albus</i>	AY 318746	New Zealand	<i>Kunzea</i>
<i>Pisolithus</i>	<i>albus</i>	AM084705	n/a	n/a
<i>Pisolithus</i>	<i>arhizus</i>	AF 096976	n/a	n/a
<i>Pisolithus</i>	<i>arhizus</i>	EF 493273	n/a	n/a
<i>Pisolithus</i>	<i>aurantioscabrosus</i>	AF 415226	Malaysia	<i>Shorea</i>
<i>Pisolithus</i>	<i>aurantioscabrosus</i>	AF 415227	Malaysia	<i>Shorea</i>
<i>Pisolithus</i>	<i>aurantioscabrosus</i>	EU 718112	n/a	n/a
<i>Pisolithus</i>	<i>marmoratus</i>	AY 318745	New Zealand	<i>Kunzea</i>
<i>Pisolithus</i>	<i>marmoratus</i>	AF 440866	n/a	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>microcarpus</i>	AF 440867	Australia	n/a
<i>Pisolithus</i>	<i>microcarpus</i>	AM084706	n/a	n/a
<i>Pisolithus</i>	<i>microcarpus</i>	EU 289140	n/a	n/a
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374624	Australia	<i>Acacia</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 004732	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 004735	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374637	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374639	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374640	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374641	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374642	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374643	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374644	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374645	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374646	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374647	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374648	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374649	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374650	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374651	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374652	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374653	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374654	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374655	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374656	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374657	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374658	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374659	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374660	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374661	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374664	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374665	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374667	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374668	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374669	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374670	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374671	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374672	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374674	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374675	Australia	<i>Eucalyptus</i>

Table 2. -(Continued)-

Genus	Species	Accession No.	Locality	Presumable ECM hosts
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374676	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374716	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374718	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374719	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374662	Australia	<i>Eucalyptus/Acacia</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374663	Australia	<i>Eucalyptus/Acacia</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374708	Australia	<i>Eucalyptus/Acacia</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374714	Australia	<i>Eucalyptus/Acacia</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374715	Australia	<i>Eucalyptus/Acacia</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374666	Australia	<i>Eucalyptus/Melaleuca</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374713	Australia	<i>Eucalyptus/Melaleuca</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 004736	Australia	n/a
<i>Pisolithus</i>	<i>tinctorius</i>	AF 004737	Australia	n/a
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374720	Australia	n/a
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374696	Brazil	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374697	Brazil	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374698	Brazil	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374699	Brazil	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374700	Brazil	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374701	Brazil	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374702	Brazil	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374703	Brazil	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374704	Brazil	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374705	Brazil	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374706	Brazil	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	U 62666	Brazil	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374673	China	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374678	China	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374679	China	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374680	China	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374681	China	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374684	China	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374710	China	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374717	China	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374711	China	<i>Pinus/Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374707	France	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374712	France	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 143234	France	n/a
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374683	India	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	DQ 679804	Italy	n/a
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374629	Japan	<i>Pinus/Betula</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 003915	Kenya	<i>Afzelia</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228653	Kenya	<i>Afzelia</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 003914	Kenya	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 003916	Kenya	<i>Pinus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228647	Kenya	<i>Pinus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374638	Malaysia	<i>Acacia</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AY 739178	Mexico	n/a
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374633	Nicaragua	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374636	Portugal	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374677	Portugal	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374695	Portugal	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374626	Portugal	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374630	Portugal	<i>Pinus/Quercus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374622	Senegal	<i>Acacia</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374623	Senegal	<i>Acacia</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374685	Senegal	<i>Eucalyptus*</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374686	Senegal	<i>Eucalyptus*</i>

Table 2. -(Continued)-

Genus	Species	Accession No.	Locality	Presumable ECM hosts
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374687	Senegal	<i>Eucalyptus</i> *
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374688	Senegal	<i>Eucalyptus</i> *
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374689	Senegal	<i>Eucalyptus</i> *
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374690	Senegal	<i>Eucalyptus</i> *
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374691	Senegal	<i>Eucalyptus</i> *
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374692	Senegal	<i>Eucalyptus</i> *
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374693	Senegal	<i>Eucalyptus</i> *
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374694	Senegal	<i>Eucalyptus</i> *
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374682	South Africa	<i>Eucalyptus</i> *
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374627	South Africa	<i>Pinus</i> *
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374628	South Africa	<i>Pinus</i> *
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228641	Spain	<i>Cistus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228642	Spain	<i>Cistus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228643	Spain	<i>Cistus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228644	Spain	<i>Cistus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228648	Spain	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228652	Spain	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228650	Spain	<i>Pinus/Quercus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228649	Spain	<i>Quercus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228651	Spain	<i>Quercus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228645	Spain	<i>Quercus/Cistus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228646	Spain	<i>Quercus/Cistus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374631	Thailand	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374625	Thailand	<i>Pinus/Quercus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 143233	USA	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374632	USA	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374634	USA	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374635	USA	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 374709	USA	<i>Pinus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 140547	n/a	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228654	n/a	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228655	n/a	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228656	n/a	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	AF 228657	n/a	<i>Eucalyptus</i>
<i>Pisolithus</i>	<i>tinctorius</i>	EF 529622	n/a	n/a
<i>Pisolithus</i>	<i>tinctorius</i>	EU 559631	n/a	n/a
<i>Pisolithus</i>	<i>tinctorius</i>	EU 718114	n/a	n/a
<i>Pisolithus</i>	sp.	AF 004733	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 004734	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270771	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270772	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270773	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270774	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270778	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270779	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270780	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270781	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270782	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270783	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270784	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270785	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270786	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 270787	Australia	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 440001	Australia	n/a
<i>Pisolithus</i>	sp.	AF 440865	Australia	n/a
<i>Pisolithus</i>	sp.	AY 179746	Australia	n/a
<i>Pisolithus</i>	sp.	AY 179747	Australia	n/a
<i>Pisolithus</i>	sp.	AF 440000	China	n/a

Table 2. -(Continued)-

Genus	Species	Accession No.	Locality	Presumable ECM hosts
<i>Pisolithus</i>	sp.	AF 416589	India	<i>Eucalyptus</i> *
<i>Pisolithus</i>	sp.	AF 270777	Indonesia	n/a
<i>Pisolithus</i>	sp.	AB 106875	Japan	<i>Pinus</i>
<i>Pisolithus</i>	sp.	AY 318747	New Zealand	<i>Kunzea</i>
<i>Pisolithus</i>	sp.	AY 318748	New Zealand	<i>Kunzea</i>
<i>Pisolithus</i>	sp.	AF 270776	Philippines	n/a
<i>Pisolithus</i>	sp.	AB 099922	Thailand	<i>Dipterocarpaceae</i>
<i>Pisolithus</i>	sp.	AB 099908	Thailand	<i>Eucalyptus</i> *
<i>Pisolithus</i>	sp.	AB 099909	Thailand	<i>Eucalyptus</i> *
<i>Pisolithus</i>	sp.	AB 099910	Thailand	<i>Eucalyptus</i> *
<i>Pisolithus</i>	sp.	AB 099911	Thailand	<i>Eucalyptus</i> *
<i>Pisolithus</i>	sp.	AB 099912	Thailand	<i>Eucalyptus</i> *
<i>Pisolithus</i>	sp.	AB 099913	Thailand	<i>Eucalyptus</i> *
<i>Pisolithus</i>	sp.	AB 099914	Thailand	<i>Eucalyptus</i> *
<i>Pisolithus</i>	sp.	AB 099915	Thailand	<i>Eucalyptus</i> *
<i>Pisolithus</i>	sp.	AB 099916	Thailand	<i>Eucalyptus</i> *
<i>Pisolithus</i>	sp.	AB 099917	Thailand	<i>Eucalyptus</i> *
<i>Pisolithus</i>	sp.	AB 099918	Thailand	<i>Eucalyptus</i> *
<i>Pisolithus</i>	sp.	AB 099843	Thailand	<i>Pinus</i>
<i>Pisolithus</i>	sp.	AB 099844	Thailand	<i>Pinus</i>
<i>Pisolithus</i>	sp.	AB 099845	Thailand	<i>Pinus</i>
<i>Pisolithus</i>	sp.	AB 099846	Thailand	<i>Pinus</i>
<i>Pisolithus</i>	sp.	AB 099847	Thailand	<i>Pinus</i>
<i>Pisolithus</i>	sp.	AB 099902	Thailand	<i>Pinus</i>
<i>Pisolithus</i>	sp.	AB 099903	Thailand	<i>Pinus</i>
<i>Pisolithus</i>	sp.	AB 099904	Thailand	<i>Pinus</i>
<i>Pisolithus</i>	sp.	AB 099905	Thailand	<i>Pinus</i>
<i>Pisolithus</i>	sp.	AB 099906	Thailand	<i>Pinus</i>
<i>Pisolithus</i>	sp.	AB 099907	Thailand	<i>Pinus</i>
<i>Pisolithus</i>	sp.	AB 099919	Thailand	<i>Shorea</i>
<i>Pisolithus</i>	sp.	AB 099920	Thailand	<i>Shorea</i>
<i>Pisolithus</i>	sp.	AB 099921	Thailand	<i>Shorea</i>
<i>Pisolithus</i>	sp.	AJ 629887	Thailand	n/a
<i>Pisolithus</i>	sp.	AF 270775	USA	n/a
<i>Pisolithus</i>	sp.	AF 142991	n/a	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AF 440002	n/a	<i>Eucalyptus</i>
<i>Pisolithus</i>	sp.	AB 106874	n/a	<i>Pinus</i>
<i>Pisolithus</i>	sp.	EF 192104	n/a	n/a
<i>Pisolithus</i>	sp.	EF 192105	n/a	n/a
<i>Pisolithus</i>	sp.	EF 192106	n/a	n/a
<i>Pisolithus</i>	sp.	EF 192107	n/a	n/a
<i>Pisolithus</i>	sp.	EF 192108	n/a	n/a
<i>Pisolithus</i>	sp.	EF 192109	n/a	n/a
<i>Pisolithus</i>	sp.	EF 192110	n/a	n/a
<i>Pisolithus</i>	sp.	EU 718113	n/a	n/a
<i>Pisolithus</i>	sp.	FJ 710201	n/a	n/a
<i>Pisolithus</i>	sp.	FJ 710203	n/a	n/a
[OUTGROUP]				
<i>Boletinellus</i>	<i>exiguus</i>	AJ 419185	Brazil	n/a
<i>Boletinellus</i>	<i>merulioides</i>	DQ 200922	n/a	n/a
<i>Boletinellus</i>	<i>rompelii</i>	AJ 419192	Brazil	n/a
<i>Calostoma</i>	<i>berkeleyi</i>	FJ 710189	n/a	n/a
<i>Calostoma</i>	<i>cinnabarinum</i>	AY 854064	n/a	n/a
<i>Calostoma</i>	<i>fuscum</i>	FJ 710190	n/a	n/a
<i>Calostoma</i>	<i>insignis</i>	EU 718092	n/a	n/a
<i>Calostoma</i>	<i>japonicum</i>	FJ 710191	n/a	n/a
<i>Calostoma</i>	<i>lutescens</i>	FJ 710192	n/a	n/a

Table 2. -(Continued)-

Genus	Species	Accession No.	Locality	Presumable ECM hosts
<i>Calostoma</i>	<i>miniata</i>	FJ 710194	n/a	n/a
<i>Calostoma</i>	<i>orirubrum</i>	FJ 710196	n/a	n/a
<i>Calostoma</i>	<i>ravenelii</i>	FJ 710197	n/a	n/a
<i>Calostoma</i>	<i>retisporum</i>	FJ 807565	Malaysia	n/a
<i>Calostoma</i>	<i>rodwayi</i>	FJ 710198	n/a	n/a
<i>Calostoma</i>	<i>sarasini</i>	FJ 710199	n/a	n/a
<i>Paxillus</i>	<i>involutusp</i>	AF 167700	n/a	<i>Fagus</i>
<i>Scleroderma</i>	<i>areolatum</i>	EU 819518	n/a	Fagaceae
<i>Scleroderma</i>	<i>bovista</i>	EU 819517	n/a	<i>Castanea</i>
<i>Scleroderma</i>	<i>cepa</i>	EU 819439	n/a	<i>Castanea</i>
<i>Scleroderma</i>	<i>citrinum</i>	FJ 824090	n/a	<i>Castanea</i>
<i>Scleroderma</i>	<i>dictyosporum</i>	FJ 840449	Burkina Faso	legume
<i>Scleroderma</i>	<i>laeve</i>	EU 718120	n/a	n/a
<i>Scleroderma</i>	<i>macalpinei</i>	EU 718122	n/a	n/a
<i>Scleroderma</i>	<i>meridionale</i>	EU 718121	n/a	n/a
<i>Scleroderma</i>	<i>michiganense</i>	EU 819441	n/a	<i>Castanea</i>
<i>Scleroderma</i>	<i>polyrhizum</i>	EU 718123	n/a	n/a
<i>Scleroderma</i>	<i>sinnamariense</i>	AB 453030	Thailand	<i>Dipterocarpus</i>
<i>Scleroderma</i>	<i>verrucosum</i>	FJ 840461	n/a	legume
<i>Scleroderma</i>	<i>xanthochroum</i>	EU 718126	n/a	n/a
<i>Suillus</i>	<i>luteus</i>	L 54110	n/a	n/a

n/a=information not available from GenBank database.

*=ectomycorrhizal plants introduced from other continents.

closely related with *Scleroderma*, *Calostoma*, and *Boletinellus* (Binder and Hibbett, 2006), the majority of outgroup taxa were chosen from these genera. Additional outgroup taxa were selected from more distantly related groups within Boletales: *Suillus* in the suborder Suillineae, and *Paxillus* from the suborder Paxillineae.

The vast majority of sequences were retrieved from the studies by Martin *et al.* (2002), Moyer-soen *et al.* (2003), and Kanchanaprayudh *et al.* (2003a), but large numbers of unpublished sequences were also included. Specimens collected in New Caledonia by the authors were deposited at the fungal herbarium of the National Museum of Nature and Science, Tsukuba, Japan (TNS). Four additional specimens of the genus *Pisolithus* from Japan housed at the TNS were also used for this study. Presumable ectomycorrhizal hosts were determined based on field observation by the authors, specimen labels, or the GenBank database. If such information was unavailable, ectomycorrhizal hosts were treated as unknown.

DNA was extracted from glebal tissue of fresh

or dried fruiting bodies. The protocol generally follows that of Doyle and Doyle (1987) but with the following modifications. For fresh materials, immature glebal tissue was soaked in DMSO buffer (Seutin *et al.*, 1991) with an addition of 100 mM Tris-HCl (pH 8.0) and 0.1 M sodium sulfate (Na_2SO_3) under 4°C until extraction. For dried materials, immature glebal tissue was soaked overnight in modified DMSO buffer under room temperature.

Soaked tissue samples were then ground in liquid nitrogen using mortar and pestle. After grinding, samples were immediately transferred to 1.5 mL tubes with 1,000 mL of 2X CTAB buffer (Doyle and Doyle, 1987) with an addition of 0.1 M Na_2SO_3 . Samples were incubated at 65°C for 1 hour, and centrifuged at 12,000 rpm for 5 min. Only the aqueous phase was transferred to a new tube, and precipitated tissue debris was discarded. The equal volume of the mixture of chloroform : isoamylalcohol (24 : 1) was added to the buffer, mixed vigorously for two minutes, and centrifuged at 12,000 rpm for 15 min. The aqueous phase was pipetted out and transferred to a

new tube. This step of using chloroform was conducted only once.

After transferring ca. 300 mL of the aqueous phase, 1,000 mL of 6 M sodium iodine buffer (6 M NaI, 50 mM Tris-HCl (pH 7.4), 10 mM EDTA, 0.1 M Na₂SO₃) was added and mixed gently for 1 minute. Silica mixture was prepared following the protocol of Rogstad (2003), and 25 mL of the mixture was added to the samples. Samples were incubated at 55°C for 1 hour, and centrifuged at full speed for ca. 10 seconds. The supernatant was discarded and 750 mL of washing buffer (10 mL Tris-HCl (pH 7.4), 1 mM EDTA, 100 mM NaCl, 50% EtOH) was added, mixed briefly, and centrifuged at full speed for ca. 5 seconds. This washing step was repeated twice. After finishing the washing step, samples were centrifuged one more time at full speed for 10 seconds, the remaining washing buffer was pipetted out, and precipitated silica was dried at room temperature for 30 min to 1 hour. Final elution was performed by adding 100 mL of ultra pure water, mixed briefly, and incubated at 65°C for 15 min. Samples were centrifuged at 12,000 rpm for 1 min, and supernatant layer was transferred to a new tube and stored at -20°C until PCR was performed.

DNA sequence data were obtained from the internal transcribed spacer regions (ITS) of the nuclear ribosomal DNA. The primer combination of ITS5 and ITS4 (White *et al.*, 1990) was used. PCR reactions were carried out using 20 µl reaction volumes each containing: 1 µl genomic DNA, 1 µl dNTP (4 mM), 1 µl of each primer (8 µM), 0.5 units of taq polymerase (TAKARA, Japan), 2 µl MgCl₂ (25 mM), and 2 µl Bovine Serum Albumin (10 mg/ml). Cycling parameters were 1 cycle of 94°C for 3 min, 30 cycles of 94°C for 1 min, 51°C for 30 sec and 72°C for 1 min, with a final extension at 72°C for 15 min. PCR products were electrophoresed in 1% agarose gels stained with ethidium bromide and visualized under UV light. PCR products were then purified using the ExoSAP-IT (Affymetrix Inc., USA) and directly sequenced using the Big Dye Terminator Cycle Sequencing Kit (Applied

Biosystems Inc., Norwalk, CT, USA), following the manufacturer's instructions. The primers used for cycle sequencing are ITS1 and ITS4 (White *et al.*, 1990).

Phylogenetic analyses

DNA sequences were initially aligned using Muscle v.3.6 (Edgar, 2004a, 2004b), followed by manual alignment in the data editor of BioEdit ver. 7.0.1 (Hall, 1999). Ambiguously aligned regions were excluded from the analyses. The dataset was then analyzed by maximum parsimony (MP) and neighbor-joining (NJ) analyses. MP analyses were conducted under the equally weighted parsimony criterion using PAUP* version 4.0b10 (Swofford, 2002). Due to computational intensity and dense taxon sampling of terminal clades, a two-step search approach was conducted. In the first step, the heuristic search option (with TBR, no Multrees) and 1,000 replicates of random addition sequence were performed, keeping only up to two shortest trees per replicate. In the second step, all of the shortest trees from the first step were used as starting trees for heuristic search option (with TBR and Multrees on) with MAXTREES set to 10,000. Support for the individual nodes was tested with bootstrap (BS) analysis under the equally-weighted parsimony criterion. BS analysis was based on 1,000 BS replicates using the heuristic search option (TBR and Multrees options off), with ten random addition sequences. NJ analysis was conducted with the Kimura-2-parameter model, with a transition/transversion ratio set to 2, and a gamma shape set to 0.5. Support for individual nodes was tested by BS analysis based on 1,000 BS replicates under the same settings.

Results and Discussion

PCR amplification and sequencing

Most DNA samples produced a clear, single band of the ITS region (ca. 650 bp). Direct sequencing of all PCR products resulted in clear peaks without ambiguous base calling, so cloning was not necessary for sequencing

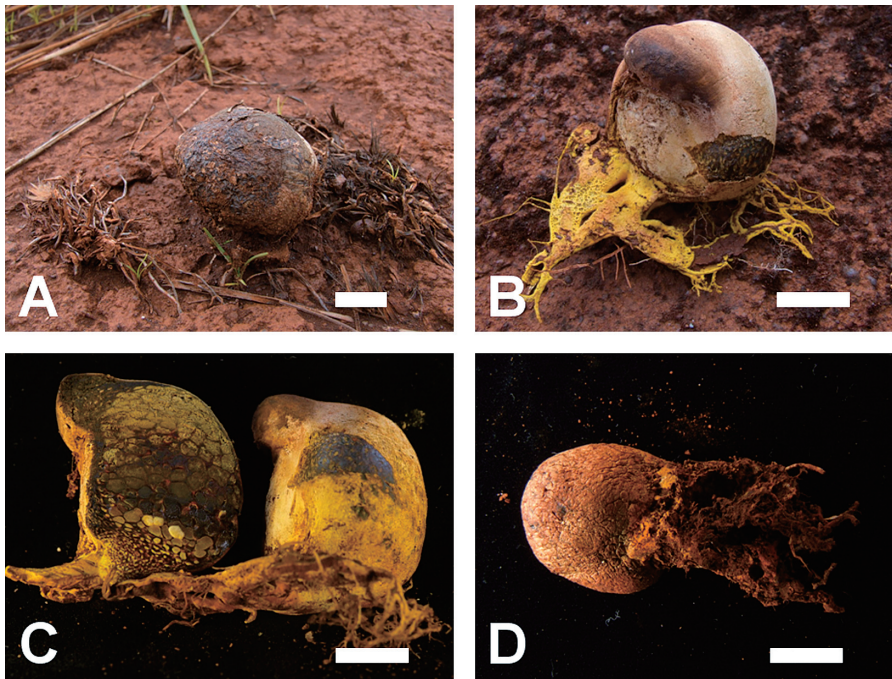


Fig. 1. Basidiomata of *Pisolithus* spp. A. *P.* sp. from New Caledonia (KH-NC09-002). B. *P.* sp. from New Caledonia (KH-NC09-026). Note presence of stalk and thick rhizomorphic base with bright yellow color. C. Longitudinal section of basidiomata of *P.* sp. from New Caledonia (KH-NC09-026). D. *P.* sp. from New Caledonia (KH-NC09-059). Note brownish basidiomata with rhizomorphs without conspicuous yellow tint. Bars=1 cm.



Fig. 2. Typical habitat of *Pisolithus* spp. in New Caledonia. Note bare soil with scattered woody plants. Photo taken in April, 2009 near town of Goro, Southern Province, New Caledonia.

Pisolithus from New Caledonia and Japan used for this study. We have also attempted to amplify ca. 50 additional specimens of *Pisolithus* housed at the TNS herbarium, which have been collected from various locations in New Caledonia and Japan. However, the PCR reactions using the same universal primers (ITS5 and ITS4) failed. Because most of these specimens were collected more than 30 years ago, this failure may be due to degradation of DNA molecules. This needs to be solved by using internal primers to amplify shorter fragments, or by designing taxon specific primers for *Pisolithus*.

Phylogenetic analyses

The aligned dataset consists of 1,408 characters, of which 827 characters were only ambiguously aligned and therefore excluded from the analyses. In addition, 547 characters were parsimony uninformative so the remaining 386 characters were considered parsimony informative and used for the MP analyses.

After the first step of a two-step search approach (see Methods), only two trees with tree length 4,148 were recovered. Those trees were used as starting trees for the second step. The second step quickly recovered the trees with equal tree lengths (4,148) and reached the maximum numbers of trees (10,000). The run was aborted after the number of trees reached 10,000. All trees were saved and the strict consensus was calculated using PAUP*.

The most parsimonious trees had 4,148 steps with a CI of 0.2244, RI of 0.7714, and RC of 0.1731. Although a large number of trees (10,000) were recovered, many nodes were not collapsed when strict consensus was calculated. This might indicate that the large number of trees generated by parsimony analyses was not necessarily due to an insufficient resolution power of the ITS dataset, but that a dense taxon sampling with closely related (or often identical) ITS sequences may be responsible. Nonetheless, bootstrap analyses revealed that almost no deep nodes had significant support. Furthermore, the alignment file consisted of many ambiguously

aligned regions due to difficulty in assessing positional homology. Aligning the ITS regions across all taxa used in this study is an obvious challenge, and therefore more slowly evolving genes with sufficient resolution power, e.g., *atp6* (Kretzer and Bruns, 1999; Hosaka *et al.*, 2006, 2008; Hosaka and Castellano, 2008) are desirable for further analyses.

Both MP and NJ analyses produced similar topology and largely recovered the “Lineage A” and “Lineage B” designated by Martin *et al.* (2002) (Fig. 3). All sequences of New Caledonian taxa belong to the Group 1 (Fig. 3), which roughly corresponds with the Lineage B sensu Martin *et al.* (2002). Based on a global tree of the genus, it is apparent that taxa from New Caledonia (shaded by black in Fig. 3) do not form a monophyletic group, suggesting multiple origins of New Caledonian taxa. Taxa from Japan did not form a monophyletic group, either (shaded by gray in Fig. 3).

Taxonomic implication

This study, along with previous ones (Martin *et al.*, 2002; Moyersoen *et al.*, 2003; Kanchanaprayudh *et al.*, 2003a), revealed serious taxonomic problems for the genus *Pisolithus*. Although it is possible that species names used for GenBank sequences and herbarium specimens were simply misidentification, several “species” appeared in multiple positions in phylogenetic trees. Some examples include *P. tinctorius* and *P. albus*, which appeared many times in very distantly related clades. Applying the name “*P. tinctorius*” is particularly problematic because in many countries, it is virtually the only name used for this genus. Because mycologists have long been aware that the genus has wide variation in macro/microscopic characters, cultural property, biochemical property, and ecology (Burgess *et al.*, 1995; Grand, 1976; Grenville *et al.*, 1985; Kope and Fortin, 1990; Marx, 1977), new phylogenetic information should be integrated into the modern taxonomy of *Pisolithus*.

Even within small countries, such as New Caledonia and Japan, significant diversity of

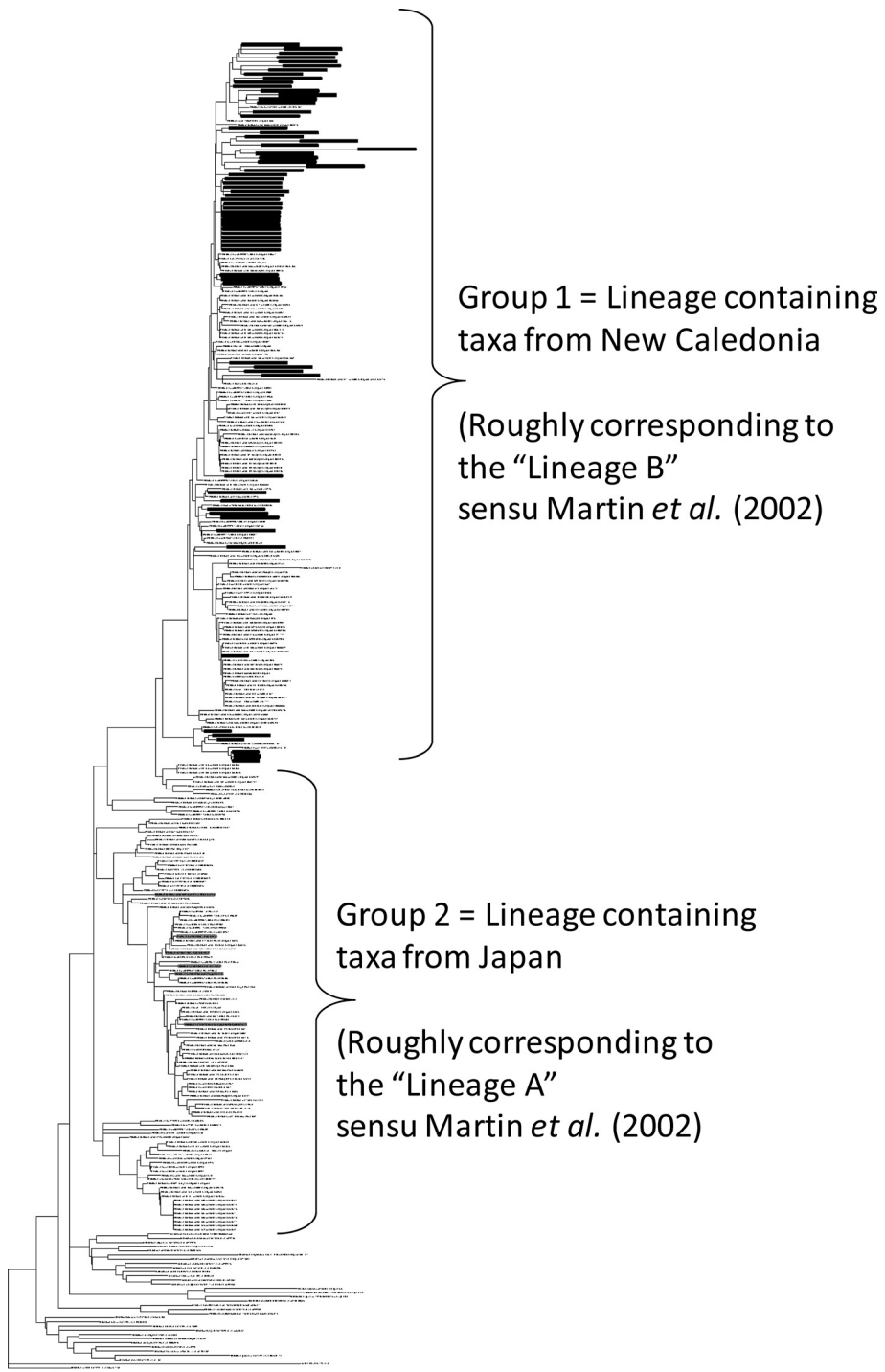


Fig. 3. Phylogenetic tree of *Pisolithus* and related taxa derived from neighbor-joining analysis based on the ITS dataset of 317 taxa. Taxa from New Caledonia are shaded by black, and taxa from Japan are shaded by gray.

Pisolithus species exists. Although we do not have significant statistical support for many nodes in phylogenetic trees, it is apparent that both New Caledonia and Japan have several phylogenetically distinct species (Figs. 3, 4 and 5). We have not conducted detailed morphological examinations for New Caledonian and Japanese specimens used in this study, but such a study will surely confirm distinct features of species in each countries.

Biogeographical implication: New Caledonia

Although not many species of *Pisolithus* is known from New Caledonia, phylogenetic analyses clearly demonstrated a high diversity of genus exists in this area.

One of the intriguing questions regarding the New Caledonian taxa is the origin of these taxa from the other continents. Geological evidence

suggests that New Caledonia was once a part of supercontinent Gondwana, but it was separated with New Zealand from the rest of Gondwana ca. 80 million years ago (McLoughlin, 2001). Whether it was once completely submerged under ocean or significant area of land mass has always existed as part of an ancient continent “Zealandia” is still controversial (Trewick *et al.*, 2007), but the fact that New Caledonia shares geological history with other parts of Gondwana, e.g., present day Australia, New Zealand, and South America (McLoughlin, 2001), makes it attractive system to investigate potential role of vicariance versus dispersal.

As expected, taxa from New Caledonia were shown to be closely related to taxa from Australia (Figs. 3 and 4), which also has a Gondwanan origin (McLoughlin, 2001). Geographically, however, New Caledonia shared its most

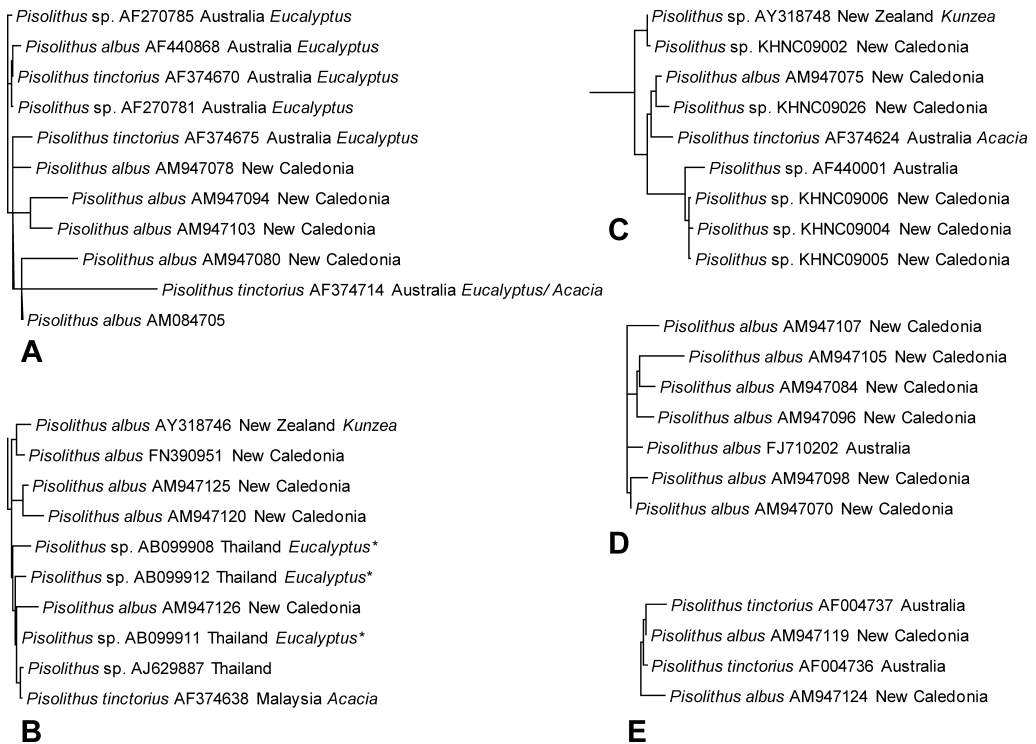


Fig. 4. Some representative clades of *Pisolithus* containing taxa from New Caledonia (A–E). Each clade (A–E) was directly retrieved from NJ trees in Fig. 3. Taxon names followed by GenBank accession numbers, geographical origin of the specimen (if known), and presumable ectomycorrhizal hosts (if known). Asterisk (*) indicates that ectomycorrhizal host plants are introduced from other continents.

recent connection with New Zealand (McLoughlin, 2001; Trewick *et al.*, 2007). Therefore, for vicariance to be a major factor for biogeography of *Pisolithus*, there should be an indication of a sister relationship between New Caledonia and New Zealand taxa. Such a relationship was observed in some parts of the tree (Figs. 4B and C), but it is still premature to draw any conclusions of vicariance origin for these taxa. It is noteworthy, however, that *Pisolithus* spp. in New Zealand were thought to have been dispersed from Australia via west-drift wind (Moyersoen *et al.*, 2003). Such a long-distance dispersal of spores by west drift wind throughout the Southern Hemisphere was documented for plant pathogenic fungi (Watson and de Sousa, 1983). If this is correct, long-distance dispersal, including trans-oceanic dispersal, of macrofungi should happen much more frequently than we currently recognize. A recent trend in biogeographical studies is to re-evaluate the importance of long-distance dispersal (de Queiroz, 2005), and frequent long-distance dispersal was postulated for many

organisms, including some macrofungi (Knapp *et al.*, 2005; Hosaka *et al.*, 2008; Matheny *et al.*, 2009).

Based on field observations of the fruit-body habitat, ectomycorrhizal hosts of New Caledonian fungi could not be identified. It was clear that no *Nothofagus*, which is an important ectomycorrhizal genus in the Southern Hemisphere, was present near collecting sites, but potential ectomycorrhizal partners included Myrtaceae and Leguminosae. However, detailed examination of phylogenetic trees indicates that all taxa from New Caledonia are closely related to species associating with Myrtaceae (mostly *Eucalyptus*) (Fig. 3). Therefore it appears likely that *Pisolithus* in New Caledonia may also be associated with myrtaceous plants.

Biogeographical implication: Japan

Taxa from Japan did not form a monophyletic group (Fig. 3). They are closely related with species from other Asian countries, including China and Thailand (Fig. 5). The fact that they

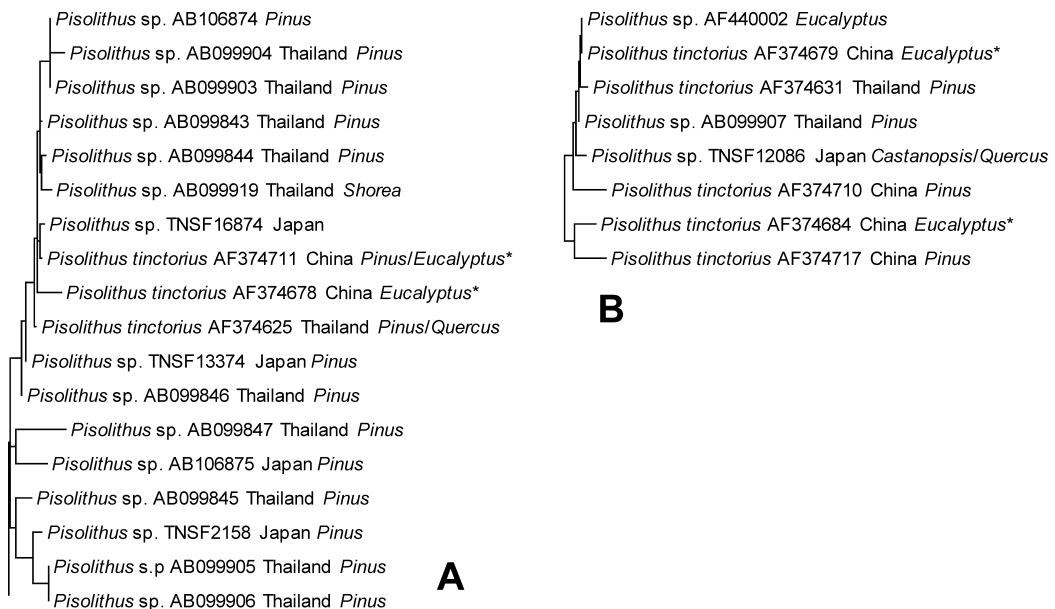


Fig. 5. Some representative clades of *Pisolithus* containing taxa from Japan (A, B). Each clade (A, B) was directly retrieved from NJ trees in Fig. 3. Taxon names followed by GenBank accession numbers, geographical origin of the specimen (if known), and presumable ectomycorrhizal hosts (if known). Asterisk (*) indicates that ectomycorrhizal host plants are introduced from other continents.

did not form a clade with taxa from more distantly located continents, such as Australia, indicates that although long-distance dispersal could be a major factor of biogeography of this genus, such dispersal is more restricted within smaller regions, e.g., Asia.

There seems to be some trends in phylogeny and ectomycorrhizal host association. For example, pine-associated species from Japan (such as AB106875 and TNSF2158) are closely related with pine-associated species from other countries. However, this fungus-plant association appears to be less strict in the Northern Hemisphere. Taxa from Thailand can be associated either with Pinaceae or Dipterocarpaceae, and they are sometimes closely related, if not identical (Fig. 5A). Even more striking is that some taxa collected in China under introduced *Eucalyptus* are closely related to other Asian taxa associated with native plants. This may indicate that those eucalypt-associated taxa are not introduced from Australia, where *Eucalyptus* originally exists, but at some point switched their hosts from native plants to *Eucalyptus*. This kind of dramatic host shift has rarely been documented, and warrants further investigation.

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