

Molecular phylogeny of ectomycorrhizal fungus *Tylopilus* based on nuLSU rDNA in Thailand

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Abstract

Tylopilus is a genus of bolete fungi in Boletaceae. *Tylopilus* species are ectomycorrhizal fungi associated with many plant species and widely distributed across subtropical and tropical regions. *Tylopilus* co-distributes with plant families Dipterocarpaceae, Myrtaceae, Fagaceae and Pinaceae throughout Thailand. Little knowledge of the systematics of this genus based on molecular phylogeny has been obtained. In this study, phylogenetic relationships among *Tylopilus* species were studied based on large subunit ribosomal DNA (nuLSU rDNA). Sequences of thirteen *Tylopilus* local samples were compared with some *Tylopilus* species available in GenBank database. Phylogenetic analysis suggested that at least seven *Tylopilus* species exist in Thailand. Only one *Tylopilus* species corresponded to *Tylopilus ballouii* which was a species complex. The remaining species did not match any previously reported species in GenBank. Therefore, morphological data of these Thai *Tylopilus* species are needed for further study.

Keywords: *Tylopilus*, ectomycorrhiza, phylogenetic relationship, nuLSU rDNA

Introduction and Objective

Tylopilus is a large genus of ectomycorrhizal fungi in family Boletaceae which has at least 75 species[1],[2]. The geographical ranges of these fungi include temperate zone but mainly distributed in tropical[2],[3]. In Thailand, *Tylopilus* species distribute with their host plants families, Dipterocarpaceae, Myrtaceae, Fagaceae and Pinaceae in every parts. Although the diversity of *Tylopilus* is abundant, the knowledge of the systematics of this genus based on molecular phylogeny has never been well investigated. In this study, phylogenetic relationships among *Tylopilus* spp. were studied based on nuclear large subunit ribosomal DNA (nuLSU rDNA).

Materials and Methods

2.1 Taxon sampling

Thirteen specimens in genus *Tylopilus* were collected during July to August 2010. The study sites were Dipterocarp forests, Coniferous forests and *Eucalyptus* plantations in Chiangmai, Phitsanulok, Kanchanaburi and Chaiyaphum Provinces.

2.2 DNA extraction and sequencing

DNA was extracted from dried specimens by CTAB method[4]. A pair of primers used for the amplification of nuclear large subunit (nuLSU) rDNA were LR0R and LR7[5]. All PCR products were sequenced and blasted in GenBank database.

2.3 Phylogenetic analyses

Thirteen sequences of Thai *Tylopilus*, 23 sequences of *Tylopilus* spp. from GenBank database and 2 outgroups were aligned and the dataset were analyzed with Maximum likelihood by using Mega5[6].

Results and Discussion

The molecular phylogenetic tree of *Tylopilus* (Fig.1) indicated that seven *Tylopilus* species were found in this study. One species might be *Tylopilus ballouii* (*Tylopilus* sp.4) which was a species complex. The remaining species did not match any previously reported species in GenBank.

Tylopilus spp. could be separated into 6 clades. Clade I which bootstrap support (BS) was 60% contained a species of Thai *Tylopilus*. All species in this clade associated with Pinaceae

and Fagaceae. Their host plant families also co-distributed with the species in clade II which comprised the other Thai *Tylopilus* species. The largest clade in the tree was clade VI which comprised three Thai species. This clade could be divided into three subclades. Two subclades with very strongly supported were subclade which contained *Tylopilus formosus* and *Tylopilus* sp.5 (BS = 98%) and subclade which comprised *Tylopilus* sp.6 and *Tylopilus* sp.7 (BS = 91%). The first subclade associated with Myrtaceae and other subclade associated with Dipterocarpaceae.

depended on their host plants[7]. Moreover, morphological features of *Tylopilus* spp. in this study need to be investigated for the identification in further study.

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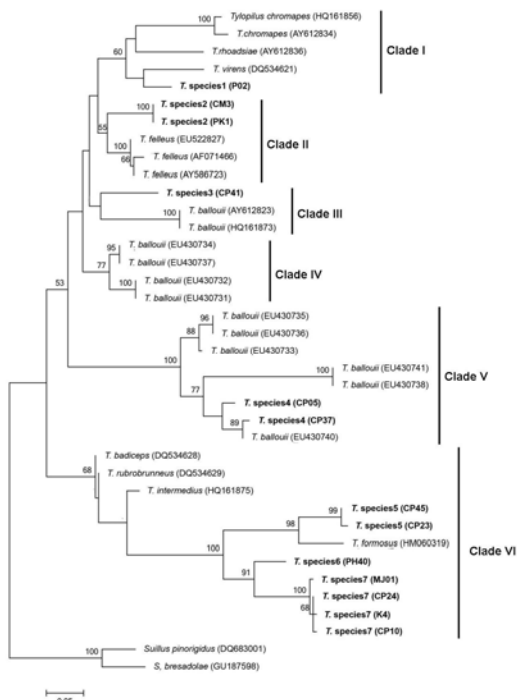


Figure 1. Phylogenetic relationships of *Tylopilus* inferred from the nuLSU-rDNA dataset. Bold taxa represented *Tylopilus* spp. in this study. Bootstrap frequencies > 50% was shown above supported branches.

The relationship among a species complex, *Tylopilus ballouii* was still complicated. This species complex was composed of three clades (clade III, IV and V). *Tylopilus* sp.3 was combined in clade III with poorly supported. The clade V was strongly supported (BS = 100%) and was separated into 3 subclades. Taxa in each subclade also shared the same host plant families.

According to the tree, phylogenetic relationships among *Tylopilus* spp. based on nuLSU rDNA were in accord with the host plants. The phylogenetic relationship in other genus within Boletaceae such as *Leccinum* also