



# *Tylocinum* is no longer monotypic: *Tylocinum brevisporum* sp. nov. (Boletales, Boletaceae) from northern Thailand

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## Abstract

### Background

*Tylocinum* Y.C. Li & Zhu L. Yang 2016 is a Boletaceae genus belonging in subfamily Leccinoideae. It was described in 2016 from China and, prior to this study, it contained only one species, *T. griseolum* Y.C. Li & Zhu L. Yang 2016. During our survey of Boletaceae from Thailand, we collected some specimens that could be identified as a *Tylocinum* species, different from *T. griseolum*.

### New information

The bolete specimens, collected in forests dominated by Dipterocarpaceae and Fagaceae in northern Thailand, are described as *Tylocinum brevisporum* Raghoonundon & Raspé sp. nov. Macroscopic and microscopic descriptions with illustrations are provided, as well as a 3-gene phylogeny, which confirms the new taxon's position in *Tylocinum*. *Tylocinum brevisporum* differs from the only other known *Tylocinum* species (*T. griseolum*) by its

brownish-grey colour, greyish-orange to brownish-orange colour change in the hymenophore when bruised, smaller pores ( $\leq 0.5$  mm), longer tubes (up to 6 mm long), shorter and narrower basidiospores, longer and broader basidia and longer pleurocystidia relative to cheilocystidia. *T. brevisporum* is the second species from the genus *Tylocinum* and the only one to be found outside China thus far.

## Keywords

new species, Boletaceae, Leccinoideae, molecular phylogeny, taxonomy, Thailand

## Introduction

*Tylocinum* Y.C. Li & Zhu L. Yang 2016, is a monotypic genus of ectomycorrhizal (ECM) boletes (Boletaceae, Boletales, Agaricomycetes, Basidiomycota, Fungi). Typical characters of the genus are its dark scabrous stipe surface, white to pallid unchanging context in the pileus and stipe, white to pallid hymenophore, trichodermium pileipellis and smooth basidiospores (Wu et al. 2016). The type species *Tylocinum griseolum* Y.C. Li & Zhu L. Yang 2016, was originally described from China and was the only species known from this genus at the time. The phylogenetic analyses by Wu et al. (2016) showed that *Tylocinum* forms a separate clade from all other generic clades in the subfamily Leccinoideae.

The plant family Dipterocarpaceae includes many species of large trees that are often dominant in the tropical and subtropical lowlands of Southeast Asia, where the species diversity of Dipterocarpaceae is highest (Ashton 1982, Hamilton et al. 2019). Many Dipterocarpaceae are well known to be ECM, symbiotically associating with various ECM fungi, including mushroom-forming species (Watling et al. 2002, Yuwa-Amornpitak et al. 2006, Brearley 2012). Several new genera and species of boletes have recently been documented from tropical dipterocarp forest (Desjardin et al. 2009, Neves et al. 2012, Hosen et al. 2013, Halling et al. 2014, Raspé et al. 2016, Wu et al. 2016, Vadthanarat et al. 2019, Chuankid et al. 2019). Members of the Fagaceae, which also form ECM associations, co-occur with dipterocarps in Southeast Asia (Smith et al. 2008), which promotes higher mycodiversity and ECM colonisation in those tropical forest ecosystems (Corrales et al. 2018).

In this study, we describe a new species of *Tylocinum* from dry dipterocarp forests of northern Thailand, with description, illustrations and molecular phylogenetic analyses of a multi-gene DNA sequence dataset (*atp6*, *tef1* and *rpb2*).

## Materials and methods

### Specimens collected

Fresh basidiomata were collected during the rainy season (2019) from Chiang Mai and Chiang Rai Provinces, northern Thailand. The basidiomata were photographed on-site and

wrapped in aluminium foil. The descriptions of the macroscopic features were made on the same day, after which the basidiomata were dried in an electric drier at 45–50°C. Specimens were deposited in the Mae Fah Luang University (MFLU) or CMUB Herbaria.

### Ecological, morphological and taxonomic study

The habitat, locality information and macro-chemical reactions on fresh basidiomata were recorded. Spore prints were taken for each collection. Colour codes were given using Kornerup and Wanscher (1978) as a guide. Microscopic characters were studied in the dried specimens. The following mounting solutions were used to observe the tissues: 10% aqueous potassium hydroxide (KOH) or 28–30% ammonium hydroxide (NH<sub>4</sub>OH) solutions or 1% ammoniacal Congo red solution. The microscopic structures were studied at magnifications of 60× and 100×, photographed with a calibrated Nikon Y-TV55 camera, fitted to a Nikon DIC microscope. A total of 60 basidiospores, 30 basidia, 30 pleurocystidia, 30 cheilocystidia and 30 terminal cells and 30 hyphae for both the pileipellis and stipitipellis were measured. The dimensions of the microscopic features are presented in the following format: (a–) b–c–d (–e), in which c represents the average, b the 5<sup>th</sup> percentile, d the 95<sup>th</sup> percentile and a and e the minimum and maximum values, respectively. Q, the length/width ratio for the spores, is presented in the same format. All microscopic features were drawn by free hand, using a drawing tube. Faces of Fungi (Jayasiri et al. 2015) and MycoBank numbers are provided for the new species.

### DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from CTAB-preserved tissues or dry specimens (ca. 10 mg) using a CTAB isolation procedure, adapted from Doyle and Doyle (1990). The *atp6*, *tef1* and *rpb2* gene regions were amplified by polymerase chain reaction (PCR). For amplification of *atp6*, the primers ATP6-1M40F and ATP6-2M were used (Raspé et al. 2016). EF1-983F and EF1-2218R (Rehner and Buckley 2005) were used to amplify *tef1* and bRPB2-6F and bRPB2-7.1R (Matheny 2005) were used to amplify *rpb2*. The PCR amplification, purification and sequencing of *atp6*, *rpb2* and *tef1* were used following the procedure from Raspé et al. (2016).

### Sequence alignment and phylogenetic analysis

The sequences were assembled using Geneious 8 (Biomatters). The Basic Local Alignment Search Tool (BLAST) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) from GenBank was used to find the closest matches to the sequences. Reference sequences (Table 1) were downloaded and aligned using MAFFT v. 7 (Kato and Standley 2013; <http://mafft.cbrc.jp/alignment/server/>). Then, the concatenated three-gene matrix was prepared.

All analyses were done on the CIPRES Science Gateway (<https://www.phylo.org>; Miller et al. 2012). Maximum Likelihood (ML) phylogenetic tree inference was done using RAxML-HPC2 v.8.2.10 (Stamatakis 2006), using the GTRCAT model of sequence evolution with 25

categories. Three *Lanmaoa* species and three *Baorangia* species were selected as outgroup. Four partitions were defined: *atp6*, *tef1* exons, *rpb2* exons and introns. Statistical support of the clades was obtained using 1,000 rapid bootstrap replicates.

Using jModeltest2 (Darriba et al. 2012) on XSEDE via the CIPRES Science Gateway, the best-fit model of substitution for analysis in MrBayes was estimated for each gene, based on the Bayesian Information Criterion (BIC). GTR + I + G for *atp6* and introns, SYM + I + G for *tef1* exons and K80 + I + G for *rpb2* exons were selected as the best fit models. Partitioned Bayesian analysis was performed with MrBayes 3.2.7a (Ronquist et al. 2012). Two runs of four cold and one heated chains were run for 1,000,000 generations and sampled every 200 generations. The average standard deviation of split frequencies was 0.005106 at the end of the runs. The burn-in phase (25%) was estimated by checking the stationarity in the plot generated by the sump command.

Table 1.

List of collections used for DNA analyses, with origin, GenBank accession numbers and reference(s).

Species	Voucher	Origin	<i>atp 6</i>	<i>tef 1</i>	<i>rpb 2</i>	References
<i>Baorangia major</i>	OR0209	Thailand	<a href="#">MG897421</a>	<a href="#">MG897431</a>	<a href="#">MG897441</a>	Phookamsak et al. (2019)
<i>Baorangia pseudocalopus</i>	HKAS75739	China	–	<a href="#">KJ184570</a>	<a href="#">KM605179</a>	Wu et al. (2015)
<i>Baorangia rufomaculata</i>	BOTH4144	USA	<a href="#">MG897415</a>	<a href="#">MG897425</a>	<a href="#">MG897435</a>	Phookamsak et al. (2019)
<i>Borofutus dhakanus</i>	OR0345	Thailand	<a href="#">MH614660</a>	<a href="#">MH614709</a>	<a href="#">MH614755</a>	Vadthananarat et al. (2018)
<i>Ionosporus longipes</i>	LEE1180	Malaysia	<a href="#">MT085461</a>	<a href="#">MT085471</a>	<a href="#">MH712031</a>	Khmelniitsky et al. (2019)
<i>Lanmaoa asiatica</i>	OR0228	China	<a href="#">MH614682</a>	<a href="#">MH614730</a>	<a href="#">MH614777</a>	Vadthananarat et al. (2019)
<i>Lanmaoa carminipes</i>	BOTH4591	USA	<a href="#">MG897419</a>	<a href="#">MG897429</a>	<a href="#">MG897439</a>	Phookamsak et al. (2019)
<i>Lanmaoa pallidorozea</i>	BOTH4432	USA	<a href="#">MG897417</a>	<a href="#">MG897427</a>	<a href="#">MG897437</a>	Phookamsak et al. (2019)
<i>Leccinum monticola</i>	HKAS76669	China	–	<a href="#">KF112249</a>	<a href="#">KF112723</a>	Wu et al. (2014)
<i>Leccinum quercinum</i>	HKAS63502	China	–	<a href="#">KF112250</a>	<a href="#">KF112724</a>	Wu et al. (2014)
<i>Leccinum scabrum</i>	RW105a	Belgium	<a href="#">KT823979</a>	<a href="#">KT824045</a>	<a href="#">KT824012</a>	Raspé et al. (2016)
<i>Leccinum scabrum</i>	VDKO0938	Belgium	<a href="#">MG212549</a>	<a href="#">MG212593</a>	<a href="#">MG212635</a>	Vadthananarat et al. (2018)
<i>Leccinum schistophilum</i>	VDKO1128	Belgium	<a href="#">KT823989</a>	<a href="#">KT824055</a>	<a href="#">KT824022</a>	Raspé et al. (2016)
<i>Leccinum varicolor</i>	HKAS57758	China	–	<a href="#">KF112251</a>	<a href="#">KF112725</a>	Wu et al. (2014)
<i>Leccinum varicolor</i>	VDKO0844	Belgium	<a href="#">MG212550</a>	<a href="#">MG212594</a>	<a href="#">MG212636</a>	Vadthananarat et al. (2018)

Species	Voucher	Origin	<i>atp 6</i>	<i>tef 1</i>	<i>rpb 2</i>	References
<i>Leccinellum</i> aff. <i>crocipodium</i>	HKAS76658	China	–	<a href="#">KF112252</a>	<a href="#">KF112728</a>	Wu et al. (2014)
<i>Lecinellum</i> cf. <i>intusrubens</i>	OR0082	Thailand	<a href="#">MZ803019</a>	<a href="#">MZ803024</a>	<a href="#">MZ824749</a>	This study
<i>Leccinellum crocipodium</i>	VDKO1006	Belgium	<a href="#">KT823988</a>	<a href="#">KT824054</a>	<a href="#">KT824021</a>	Raspé et al. (2016)
<i>Leccinellum cremeum</i>	HKAS90639	China	–	<a href="#">KT990781</a>	<a href="#">KT990420</a>	Wu et al. (2016)
<i>Leccinellum</i> sp.	HKAS53427	China	–	<a href="#">KF112253</a>	<a href="#">KF112727</a>	Wu et al. (2014)
<i>Leccinellum</i> sp.	OR0711	Thailand	<a href="#">MH614685</a>	<a href="#">MH614733</a>	<a href="#">MH614780</a>	Vadthananarat et al. (2019)
<i>Octaviania hesperi</i>	KPM-NC 17793	Japan	<a href="#">KC552150</a>	<a href="#">JN378422</a>	–	Orihara et al. (2016)
<i>Octaviania japonimontana</i>	KPM-NC 17797	Japan	<a href="#">KC552151</a>	<a href="#">JN378425</a>	–	Orihara et al. (2016)
<i>Octaviania nonae</i>	KPM-NC 17748	Japan	<a href="#">KC552143</a>	<a href="#">JN378403</a>	–	Orihara et al. (2016)
<i>Octaviania tasmanica</i>	MEL 2341996	Australia	<a href="#">KC552156</a>	<a href="#">JN378436</a>	–	Orihara et al. (2012), Orihara et al. (2016)
<i>Octaviania zelleri</i>	MES270	USA	<a href="#">KC552161</a>	<a href="#">JN378440</a>	–	Orihara et al. (2012), Orihara et al. (2016)
<i>Pseudoaustroboletus</i> cf. <i>valens</i>	OR0477	China	<a href="#">MZ803020</a>	<a href="#">MZ803025</a>	<a href="#">MZ824750</a>	This study
<i>Retiboletus brevisbasidiatus</i>	OR0570	Thailand	<a href="#">MT085469</a>	<a href="#">MT085476</a>	<a href="#">MT085479</a>	Chuankid et al. (2021)
<i>Retiboletus brunneolus</i>	HKAS 52680	China	–	<a href="#">KF112179</a>	<a href="#">KF112690</a>	Wu et al. (2014)
<i>Retiboletus fuscus</i>	OR0231	China	<a href="#">MG212556</a>	<a href="#">MG212600</a>	<a href="#">MG212642</a>	Vadthananarat et al. (2018)
<i>Retiboletus fuscus</i>	OR0738	Thailand	<a href="#">MT085462</a>	<a href="#">MT085472</a>	<a href="#">MT085477</a>	Chuankid et al. (2021)
<i>Retiboletus griseus</i>	MB03-079	USA	<a href="#">KT823964</a>	<a href="#">KT824030</a>	<a href="#">KT823997</a>	Raspé et al. (2016)
<i>Retiboletus kauffmanii</i>	OR0278	China	<a href="#">MG212557</a>	<a href="#">MG212601</a>	<a href="#">MG212643</a>	Vadthananarat et al. (2018)
<i>Retiboletus nigrogriseus</i>	BC0179	Thailand	<a href="#">MT085464</a>	<a href="#">MT085474</a>	<a href="#">MT085478</a>	Chuankid et al. (2021)
<i>Retiboletus nigrogriseus</i>	OR049	Thailand	<a href="#">KT823967</a>	<a href="#">KT824000</a>	<a href="#">KT824033</a>	Raspé et al. (2016)
<i>Retiboletus ornatipes</i>	MBsn	USA	<a href="#">MT219514</a>	<a href="#">MT219516</a>	<a href="#">MT219515</a>	Chuankid et al. (2021)
<i>Rhodactina rostratispora</i>	SV170	Thailand	<a href="#">MG212560</a>	<a href="#">MG212605</a>	<a href="#">MG212645</a>	Vadthananarat et al. (2018)
<i>Rossbeevera eucyanea</i>	TUMH-40252	Japan	<a href="#">KC552116</a>	<a href="#">KC552069</a>	–	Orihara et al. (2016)
<i>Rossbeevera griseovelutina</i>	TUMH-40266	Japan	<a href="#">KC552121</a>	<a href="#">KC552073</a>	–	Orihara et al. (2016)

Species	Voucher	Origin	<i>atp 6</i>	<i>tef 1</i>	<i>rpb 2</i>	References
<i>Rossbeevera vittatispora</i>	A.W. Claridge 2137	Australia	<a href="#">KC552105</a>	<a href="#">KC552063</a>	–	Orihara et al. (2016)
<i>Spongiforma thailandica</i>	DED7873	Thailand	<a href="#">MG212563</a>	<a href="#">KF030436</a>	<a href="#">MG212648</a>	Nuhn et al. (2013), Vadthananarat et al. (2018)
<i>Spongispora temasekensis</i>	ACMF5	Singapore	<a href="#">MZ803018</a>	<a href="#">MZ803023</a>	<a href="#">MZ824748</a>	This study
<i>Turmalinea mesomorpha</i> subsp. <i>mesomorpha</i>	KPM-NC 18012	Japan	<a href="#">KC552139</a>	<a href="#">KC552090</a>	–	Orihara et al. (2016)
<i>Turmalinea persicina</i>	KPM-NC 18001	Japan	<a href="#">KC552130</a>	<a href="#">KC552082</a>	–	Orihara et al. (2016)
<i>Turmalinea</i> sp.	Muroi361	USA	<a href="#">DQ218885</a>	<a href="#">DQ219224</a>	<a href="#">DQ219046</a>	Orihara et al. (2016)
<i>Tylocinum griseolum</i>	HKAS50281	China	–	<a href="#">KF112284</a>	<a href="#">KF112730</a>	Wu et al. (2014)
<i>Tylocinum brevisporum</i>	OR622	Thailand	<a href="#">MZ803021</a>	–	<a href="#">MZ824751</a>	This study

## Taxon treatment

### *Tylocinum brevisporum* Raghoonundon & Raspé, sp. nov.

- MycoBank [MB841102](#)
- Faces of fungi number [FoF 10255](#)

#### Materials

##### *Holotype*:

- kingdom: Fungi; phylum: Basidiomycota; class: Agaricomycetes; order: Boletales; family: Boletaceae; taxonRank: species; genus: *Tylocinum*; specificEpithet: *brevisporum*; country: Thailand; stateProvince: Chiang Rai Province, Chang Wat, Doi Pui; verbatimElevation: 730 m; verbatimCoordinates: 19°48'50"N, 99°51'57"E; eventDate: 20 August 2019; identifiedBy: Bhavesh Raghoonundon; institutionID: MFLU 21-0144; institutionCode: Mae Fah Luang University Herbarium; collectionCode: BR137

##### *Other material*:

- kingdom: Fungi; phylum: Basidiomycota; class: Agaricomycetes; order: Boletales; family: Boletaceae; taxonRank: species; genus: *Tylocinum*; specificEpithet: *brevisporum*; country: Thailand; stateProvince: Chiang Mai Province, Mueang District; verbatimElevation: 450 m; verbatimCoordinates: 18°48'40"N, 98°56'31"E; eventDate: 18 May 2015; identifiedBy: Olivier Raspé; institutionID: CMU-B OR622; collectionID: OR622; institutionCode: Chiang Mai University Herbaria

#### Description

**Basidiomata** pileo-stipitate, small to medium-sized (Fig. 1). **Pileus** (1.5–)2.0–2.5 cm in diameter, convex when young, becoming plano-depressed with age; margin deflexed to uplifted, surface finely tomentose, dull and dry, at first brown (7E4) to greyish-brown (8E3–8F4), becoming paler (8D3) near the margin with age; **context** 3–5 mm thick

halfway to the margin, soft and fleshy, off-white, slightly browning on exposure. **Stipe** central, cylindrical, (3.4–)4.9–6.5 cm × 0.6–1.3 cm, surface even, dull and dry, scabrous, covered with granular squamules (dotted-verrucose), brownish-grey (7E2–8E2) when young to reddish-brown (8E5) to dark brown (8F5) with age, no colour change when bruised, basal mycelium off-white; **context** solid, fleshy, off-white, reddish-brown to dark brown near the stipe base (8F7) and in worm wounds, slightly browning on exposure. **Hymenophore** tubulate, subventricose, adnexed, slightly depressed around apex of the stipe, greyish-orange to brownish-orange when bruised.  **Tubes** 3–6 mm long halfway to the margin, off-white, easily separable from one another. **Pores** ≤ 0.5 mm wide at mid-radius, regularly arranged, angular, off-white, turning brown to dark brown (8E5–8F5) when bruised. **Odour** fungoid. **Taste** bitter. **Spore print** not obtained.



Figure 1. [doi](#)

Photograph of *Tylocinum brevisporum* sp. nov. **a, b** Basidioma of specimen OR622; **c** Basidioma of the holotype (BR 137).

**Basidiospores** (6.7–)7.5–10–11.7(–11.8) × (3.1–)3.5–4.7–5.8(–5.9) μm (n = 50) Q = (1.7–) 1.79–2.15–2.5 (–2.61), ellipsoid in central view, oblong to subcylindrical in side view, smooth under light microscope, yellowish to brownish in KOH (Fig. 2). **Basidia** 4-spored, (27–)27–37.4–54(–54) × (9–)9–12.3–19(–19) μm, clavate, yellowish to brownish in KOH, sterigmata up to 3 μm long. **Cheilocystidia** (19–)19.3–25.5–33(–35) × (4–)4.1–6–8.2(–8.5) μm, frequent, fusiform, thin-walled, yellowish to brownish hyaline in KOH and NH<sub>4</sub>OH. **Pleurocystidia** (40–)41–53–69(–70) × (8–)7.4–12–16.6(–17) μm, thin-walled, fusiform to broadly fusiform with a long pedicel and sharp apex,

occasionally containing yellowish inclusions, yellowish to brownish hyaline in KOH and  $\text{NH}_4\text{OH}$ . **Hymenophoral trama** boletoid, elements smooth, cylindrical, hyaline, 5–10  $\mu\text{m}$  wide. **Pileipellis** a trichodermium, hyphae terminations with 3–4 cells that are 5–11  $\mu\text{m}$  wide and terminal cells 31–48  $\mu\text{m} \times 6$ –10  $\mu\text{m}$ , colourless to slightly brownish in KOH. **Pileus trama** composed of interwoven hyaline hyphae 5–9  $\mu\text{m}$  wide. **Stipitipellis** a disrupted hymeniderm with hyphae 3.7–7.4  $\mu\text{m}$  wide, colourless to slightly brownish in KOH and caulocystidia (24–)24.5–35–47(–48)  $\times$  (9–)9.2–12.4–16.9(–17)  $\mu\text{m}$ , thin-walled, clavate to broadly clavate with a sharp apex, yellowish to brownish hyaline in KOH and  $\text{NH}_4\text{OH}$ . **Stipe trama** composed of cylindrical, hyaline, interwoven hyphae 3.7–7.4  $\mu\text{m}$  wide. **Clamp connections** absent.

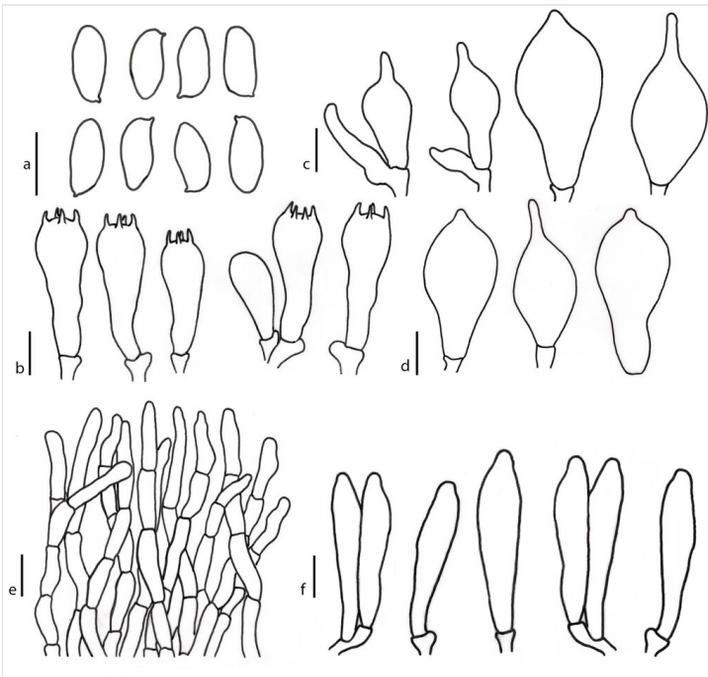


Figure 2. [doi](#)

Microscopic features of *Tylocinum brevisporum*; **a** Basidiospores; **b** Basidia; **c**, **d** Caulocystidia; **e** Pleurocystidia; **f** Cheilocystidia; **g** Pileipellis. Scale bars: a, b, c, d, f = 10  $\mu\text{m}$ , e = 20  $\mu\text{m}$ , g = 50  $\mu\text{m}$ .

## Diagnosis

This species is distinguished from *Tylocinum griseolum* by its greyish-brown colour, greyish-orange to brownish-orange colour change in the hymenophore when bruised, smaller pores ( $\leq 0.5$  mm) and longer tubes (up to 6 mm long). Additionally, the basidiospores are shorter and narrower compared to *T. griseolum* and the basidia are slightly longer and broader. Furthermore, the pleurocystidia of *Tylocinum brevisporum* are longer than its cheilocystidia.

## Etymology

Epithet “*brevisporum*”; from the Latin words *brevi* (short) and *sporae* (spores), referring to the shorter spores of this species compared to *Tylocinum griseolum*.

## Distribution

Thus far known only from northern Thailand.

## Ecology

Solitary, in tropical forest dominated by Dipterocarpaceae (*Dipterocarpus* spp. and *Shorea* spp.), with some Fagaceae (*Quercus* spp., *Lithocarpus* spp. and *Castanopsis calathiformis*).

## Notes

Morphologically, *Tylocinum brevisporum* is similar to *Tylocinum griseolum*, with which it shares the overall grey colour of the basidiomata and dark scabrous stipe surface. However, *Tylocinum brevisporum* is more brownish as compared to the grey *Tylocinum griseolum*. In addition, Wu et al. (2016) mentioned no discolouration in the context of *Tylocinum griseolum*. The context of *Tylocinum brevisporum* becomes slightly brown when bruised. The hymenophore of *T. brevisporum* changes to greyish-orange to brownish-orange when bruised as compared to the unchanging hymenophore of *T. griseolum*. Moreover, *T. griseolum* has relatively larger pores (up to 1.5 mm) than that of *T. brevisporum* (< 0.5 mm). The tubes in *T. griseolum* are also shorter than those of *T. brevisporum*.

The basidiospores of *Tylocinum brevisporum* [(6.7–)7.5–10–11.7(–11.8) × (3.1–)3.5–4.7–5.8(–5.9) μm, Q = (1.7–)1.79–2.15–2.5(–2.61)] are shorter and narrower than those of *Tylocinum griseolum* [(11)12.0–14.5(16) × 4.5–5.5 μm Q = 2.60–3.22] from China. The basidia of *T. brevisporum* [(27–)27–37.4–54(–54) × (9–)9–12.3–19(–19) μm] are also slightly longer and broader than *T. griseolum* [30–45 × 10–12 μm]. Wu et al. (2016) reported that, for *T. griseolum*, the pleurocystidia and cheilocystidia are similarly-sized. In *T. brevisporum*, the pleurocystidia are longer than the cheilocystidia. Phylogenetically, *T. brevisporum* clusters with *T. griseolum*, together forming a well-supported clade (MLB/BPP = 93/1.00) i.e. the genus *Tylocinum*.

## Analysis

### Phylogenetic analysis

The concatenated gene dataset comprised 47 terminals. The final alignment contained 121 sequences (38 for *atp6*, 46 for *tef1*, 37 for *rpb2*) and was 2,676 characters long, including gaps. Both ML and Bayesian analyses produced the same tree topology; thus, only the ML tree is shown with both Maximum Likelihood Bootstrap (MLB) and Bayesian Posterior

Probabilities (BPP) values. In the analyses, the new species *Tylocinum brevisporum* shared a sister relationship with the type species *Tylocinum griseolum* (Fig. 3), providing strong statistical support (MLB = 93 and BPP = 1.00) for the genus *Tylocinum* (Leccinoideae). The *atp6* sequence of the holotype (BR 137) was 100% identical to OR622.

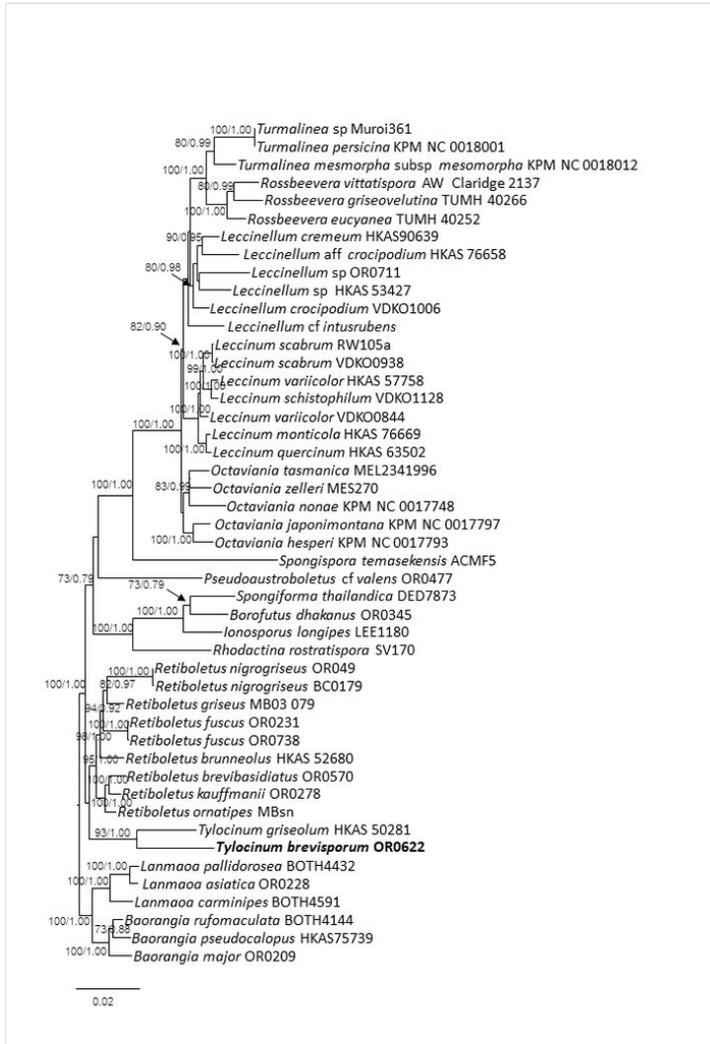


Figure 3. doi

Maximum Likelihood phylogenetic tree inferred from the three-gene dataset (*atp6*, *rpb2*, *tef1*). The three *Lanmaoa* and three *Baorangia* species were used as outgroup taxa. Maximum Likelihood Bootstrap (MLB, left)  $\geq 70\%$  and Bayesian Posterior Probabilities (BPP, right)  $\geq 0.95$  are shown above supported branches. The new species is in bold.

## Discussion

Boletales is a globally-distributed order of fungi, comprising morphologically diverse groups (Binder and Hibbett 2006, Wu et al. 2016), with ECM, ligninolytic, saprobic and mycoparasitic members (Binder and Hibbett 2006, Kirk et al. 2008). Thorough morphological and phylogenetic analyses of the order has led to the discovery of new genera and other taxa (e.g. Binder and Bresinsky 2002, Wu et al. 2014, Zhu et al. 2015, Wu et al. 2016, Orihara et al. 2016, Vadthanarat et al. 2019, Zhang et al. 2019). Boletaceae Chevall. 1826 is a morphologically diverse family currently comprising of 94 genera distributed amongst seven subfamilies (Binder and Hibbett 2006, Wu et al. 2014, Wu et al. 2016). The subfamily Leccinoideae was revealed by the phylogenetic analyses of Wu et al. (2014). Currently, this subfamily comprises fifteen genera, viz. *Binderoboletus* T.W. Henkel & M.E. Sm. 2016, *Borofutus* Hosen & Z.L. Yang 2012, *Chamonixia* Rolland 1899, *Ionosporus* Khmeln. 2018, *Kaziboletus* Iqbal Hosen & Zhu L. Yang 2021, *Leccinum* Gray 1821, *Lecinellum* Bresinsky & Manfr. Binder 2003, *Pseudoaustroboletus* Y.C. Li & Zhu L. Yang 2014, *Octavania*, *Retiboletus* Manfr. Binder & Bresinsky 2002, *Rossbeevera* T. Lebel & Orihara 2012, *Rhodactina* Pegler & T.W.K. Young 1989, *Spongiforma* Desjardin, Manfr. Binder, Roekring & Flegel 2009, *Spongispora* G. Wu, S.M.L. Lee, E. Horak & Z.L. Yang 2018, *Turmalinea* Orihara & N. Maek. 2015 and *Tylocinum*. Only ten of these genera are stipitate-pileate.

Our survey on the diversity of boletes in northern Thailand led to the discovery of a second species of *Tylocinum* (the focus of the present study), being found in tropical forests dominated by Dipterocarpaceae, which have been reported as ECM hosts for Boletaceae (Desjardin et al. 2009, Halling et al. 2014, Wu et al. 2018, Vadthanarat et al. 2019). According to Wu et al. (2016), the white to dirty white hymenophore of *Tylocinum* is similar to that of *Tylopilus* Karst. 1881 when young, while the verrucose stipe surface is similar to *Leccinum*. The stipe surface of *Tylocinum* is dotted-verrucose, which may give a more or less rough touch, but it does not produce markedly projecting scabers like in *Leccinum*. *Tylocinum* is also similar to *Tylopilus*, but there are some morphological differences between the two genera. *Tylopilus* species usually produce larger basidiomata and have minutely and densely tomentose to dotted-tomentose, but never dotted-verrucose, stipitipellis. Moreover, some *Tylopilus* species have reticulate stipe, whereas, in *Tylocinum*, the stipe is at most longitudinally venose near the apex. As the diversity of Boletaceae in Thailand is high and remains understudied (e.g. Vadthanarat et al. 2021), further studies may uncover additional species of *Tylocinum* or related taxa.

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