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First record of *Albatrellus* (Russulales, Albatrellaceae) from Thailand

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ABSTRACT

The genus *Albatrellus*, mostly known from temperate zones in North America, Europe and some Asian countries, is unusual among Russulales in having a poroid hymenophore, as other Albatrellaceae. *Albatrellus* is reported here for the first time from Thailand, with macro- and micromorphological descriptions and supporting illustrations. Molecular analyses of the internal transcribed spacer (ITS) confirmed that our collection is closely related to *Albatrellus subrubescens*, but genetic distances suggest that it could be a new species. More collections are needed from Thailand or neighboring countries to firmly establish its taxonomic identity. DNA sequencing of ectomycorrhized root tips, with both ITS (to identify the fungal partner) and psbA-trnH (to identify the plant partner) demonstrated that *Pinus kesiya* was the ectomycorrhizal host of the Thai collection of *Albatrellus*.

Keywords: distribution range, ectomycorrhizae, *Pinus kesiya*

Introduction

The genus *Albatrellus*, established by Gray in 1821, belongs to the order Russulales, family Albatrellaceae, which comprises 10 genera, namely *Albatrellopsis* Teixeira, *Albatrellus*, *Cremeogaster* Mattir., *Jahnoporus* Nuss, *Leucogaster* R. Hesse, *Mycolevis* A.H. Smith, *Polyporoletus* Snell and *Scutiger* Paulet (http://www.MycoBank.org). Up to now, approximately 25 species in the genus *Albatrellus* have been reported from temperate zones in North America, Europe and Asia (Gilbertson & Ryvarden 1986–1987; Ryvarden & Gilbertson 1993; Ginns 1997; Zheng & Liu 2006, 2008; Cui *et al*. 2008; Ryman *et al*. 2003; Zheng *et al*. 2006). *Albatrellus* species typically grow on soil in forests and form ectomycorrhizal associations with trees (Agerer *et al*. 1996; Ginns 1997; Kropp & Trappe 1982; Ryman *et al*. 2003; Zheng & Liu 2008). The fruit bodies are medium to large, fleshy, stipitate, with poroid hymenophore and a glabrous to scaly pileus surface. Microscopically, *Albatrellus* species show hyphae with simple septa or septa with clamp connections, smooth, broadly ellipsoid to subglobose basidiospores that are inamyloid to amyloid. Some species are edible (America and China) and/or contain some useful compounds that have special biological activities (e.g., Chaumont & Simeray 1982; Ding *et al*. 2001; Hellwig *et al*. 2003; Nukata *et al*. 2002; Yang *et al*. 2003). According to the Checklist of Mushrooms (Basidiomycetes) of Thailand (Chandrasrikul *et al*. 2011), and to the best of our knowledge, until now, none of the 10 genera in the family Albatrellaceae has been reported from Thailand.

Materials & methods

Specimen collecting

The specimen was photographed and collected from Doi Suthep-Pui national park, Chiang Mai province, Thailand during the rainy season in 2015. The specimen was wrapped with aluminium foil, brought back to the laboratory, and described the same day. A tissue sample was also taken and preserved in CTAB buffer for molecular analyses. The basidiomes were then dried in a ventilated incubator at 45–50°C. The specimen has been deposited in the herbarium

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of the Research Laboratory for Excellence in Sustainable Development of Biological Resource, Faculty of Science, Chiang Mai University, Thailand (SDBR–CMU), with a duplicate deposited in BR.

In order to identify the host tree species, approximately 200 mL of soil containing ectomycorrhized roots were collected in 2016, at the precise location where the specimen was collected in 2015. The roots were washed under flowing water and ectomycorrhized roots were selected, photographed under a stereomicroscope and kept separately in CTAB for molecular identification.

Morphological study

Macroscopic descriptions were based on detailed notes made from fresh basidiomata and photographs of basidiomata. Colours were named and coded following Kornerup & Wanscher (1978). Microscopic structures were observed from dried specimens, rehydrated in 5% potassium hydroxide (KOH) or 1% ammoniacal Congo Red, using an Olympus CX-31 microscope. A minimum of 50 basidiospores and 20 basidia were randomly selected from the specimens and measured in KOH. Dimensions of microscopic structures are presented in the following format: (*a*–) *b*–*c*–*d* (–*e*), in which *c* represents the average, $b = c–1.96 * SD$ and $d = c + 1.96 * SD$, and *a* and *e* extreme values. *Q* is the length to width ratio. Section of the pileus surface were cut radially, vertically at halfway between center and margin of pileus. Sections of stipitipellis were taken from the middle of the stipe. All microscopic features were drawn by free hand, using a drawing tube.

DNA extraction, PCR and sequencing

Genomic DNA was extracted from CTAB-preserved tissue and ectomycorrhizae, using a CTAB-based procedure modified from Doyle & Doyle (1990). The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was amplified by PCR using the primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) under the following thermal conditions: 95 °C for 2 min, 30 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min, and 72 °C for 10 min on a Gene Amp 9700 thermal cycler (Applied Biosystems, USA). PCR products were purified by adding 1 U of Exonuclease I and 0.5 U FastAP Alkaline Phosphatase (Thermo Scientific, St Leon-Rot, Germany) and incubating at 37 °C for 1 hour, followed by inactivation at 80 °C for 15 min. Sequencing was performed by Macrogen Europe (The Netherlands) with PCR primers. The sequence has been deposited in GenBank (accession number KT947121).

The fungal partner of ectomycorrhizae was identified by amplification of the ITS region using and sequencing using ITS-OF-T and LB-W primers (Tedersoo *et al.* 2011) and sequenced with primers ITS1-F and ITS4 as above. The host tree was identified by amplification and sequencing of the chloroplast psbA-trnH region, using psbA-3'f (Sang *et al.* 1997) and trnHf (Tate & Simpson 2003).

DNA sequence analyses

DNA sequences were assembled in Geneious Pro v. 6.0.6 (Biomatters). The ITS sequence of OR996, along with 42 sequences of Albatrellaceae and *Hericium* (Hericiaceae) retrieved from GenBank, were aligned using MAFFT ver. 7 (Katoh & Standley 2013) on the server accessed at http://mafft.cbrc.jp/alignment/server/. ITS1 and ITS2 were delimited by comparison with the sequence AY789078 (*Albatrellus higanensis*, isolate AFTOL-ID 774), which is fully annotated in GenBank. Maximum Likelihood (ML) phylogenetic tree inference was performed using RAxML ver 8.2.3 (Stamatakis 2014) on the CIPRES web server (RAxML-HPC2 on XSEDE; Miller *et al*. 2009). Exactly identical sequences were removed from the data matrix. The analysis was performed with two partitions, one including ITS1 and ITS2, the other including coding sequences (SSU, 5.8S, and LSU), 500 bootstrap replicates, using the GTRCAT model with 25 categories, and the four *Hericium* species as outgroup.

Pairwise genetic distances (proportion of variable sites) within and among *Albatrellus* species sensu stricto (Audet 2010) and OR996 were computed using MEGA ver. 4 (Tumar *et al*. 2007), with pairwise deletion of gaps and missing data.

Results

DNA sequence analyses

In the phylogenetic tree inferred from ITS sequences (Fig. 1), all sequences from *Albatrellus* sensu Audet (2010) formed a well-supported clade (Bootstrap Support, BS = 84 %). The sequence from the specimen collected in Thailand (OR996) was part of a well-supported clade (BS = 99 %) including all *Albatrellus subrubescens* sequences retrieved

from GenBank. The genetic distance between OR996 and *Albatrellus subrubescens* sequences retrieved from GenBank was relatively high (between 0.029 and 0.035), which is higher than the distance observed between some accepted species (see Table 2).

Two ectomycorrhized root samples yielded a fungal ITS sequence 100% identical to the ITS sequence obtained from the specimen OR996. The psbA-trnH sequence from those two root samples was blasted against GenBank database and showed 100% similarity and 100% query coverage with *Pinus kesiya* (JN854191) and *Pinus yunnanensis* (HQ849865).

TABLE 1. List of collections used for DNA analyses, with origin, GenBank accession numbers, and references

Species	Voucher	Origin	ITS	Reference
Albatrellus aff. *subrubescens*	OR996	Thailand	KT947121	This study
Albatrellus subrubescens	voucher GO 2000 b2	Mexico	KC152061	Garibay Orijel *et al.*, unpubl.
Albatrellus subrubescens	voucher GO 2000 b1	Mexico	KC152062	Garibay Orijel *et al.*, unpubl.
Albatrellus subrubescens	PV154 95 ITS2	Czechoslovakia	AF506395	Larsson *et al.* 2004
Albatrellus subrubescens	UPS F007381 (Ryman 6085)	Sweden	AY198208	Ryman *et al.* 2003
Albatrellus subrubescens	UPS F015548 (Muskos 97-180)	Sweden	AY198207	Ryman *et al.* 2003
Albatrellus subrubescens	UPS F012775	Sweden	AY198206	Ryman *et al.* 2003
Albatrellus subrubescens	Vampola PEC154	Czechoslovakia	AY198205	Ryman *et al.* 2003
Albatrellus subrubescens	Jaederfeldt 11/10 1995	Sweden	AY198204	Ryman *et al.* 2003
Albatrellus avellaneus	p820i	USA	EU669394	Gordon and Zych, unpubl.
Albatrellus avellaneus	p817i	USA	EU669393	Gordon and Zych, unpubl.
Albatrellus piceiphilus	Cui2221	China	DQ789397	Dai *et al.*, unpubl.
Albatrellus piceiphilus	Cui2220	China	DQ789396	Dai *et al.*, unpubl.
Albatrellus ovinus	UPS F015553 (Danell 3/8 2000)	Sweden	AY198199	Ryman *et al.* 2003
Albatrellus ovinus	QFB 7990	France	FJ439515	Audet 2009
Albatrellus ovinus	UPS F015550 (Fransson 1)	Sweden	AY198200	Ryman *et al.* 2003
Albatrellus ovinus	UPS F015554 (Danell 11/8 00)	Sweden	AY198203	Ryman *et al.* 2003
Albatrellus ovinus	UPS F015545 (Ryman 9132)	Sweden	AY198202	Ryman *et al.* 2003
Albatrellus ovinus	UPS F015552 (Fransson 3)	Sweden	AY198201	Ryman *et al.* 2003
Albatrellus ovinus	UPS F015551 (Fransson 2)	Sweden	AY198198	Ryman *et al.* 2003
Albatrellus citrinus	KM121397	Sweden	GQ981491	Bidartondo and Doring, unpubl.
Albatrellus citrinus	Lundqvist 21071	Sweden	AY198189	Ryman *et al.* 2003
Albatrellus citrinus	UPS F015546 (Ryman 8002)	Sweden	AY198191	Ryman *et al.* 2003
Albatrellus citrinus	Muskos 850928	Sweden	AY198190	Ryman *et al.* 2003
Albatrellus citrinus	UPS F015549 (Muskos 00-043)	Sweden	AY198197	Ryman *et al.* 2003
Albatrellus citrinus	Wasstorp 0981004	Sweden	AY198195	Ryman *et al.* 2003
Albatrellus citrinus	UPS F015604 (Ryman 8299)	Sweden	AY198193	Ryman *et al.* 2003
Albatrellus citrinus	UPS F007387 (Ryman 6061)	Sweden	AY198192	Ryman *et al.* 2003
Albatrellus cf. *flettii*	GO 2009 412	Mexico	KC152059	Garibay Orijel *et al.*, unpubl.
Albatrellus flettii	DAVFP27659	-	JF899544	Guichon *et al.*, unpubl.
Albatrellus flettii	AHS 82164	USA	AY621802	Albee-Scott 2007
Albatrellus flettii	398IF62	North America	AY061738	Miller and Buyck 2002
Albatrellus caeruleoporus	OSC 66097	USA	KC985126	Audet and Luther 2013
Albatrellus caeruleoporus	K. A. Harrison 8825	Canada	AY963565	Albee-Scott 2007
Albatrellus cf. *ellisii*	GO 2002 1062	Mexico	KC152058	Garibay Orijel *et al.*, unpubl.
Albatrellus ellisii	JLF1838	USA	JX415333	Frank, unpbl.
Albatrellus ellisii	States J WYEF 22 Aug 1998	USA	AY621803	Albee-Scott 2007
Albatrellus ellisii	PKSR12	India	KJ411956	Khaund and Joshi 2014
Albatrellus skamanius	WTU 9750	-	EU669253	Gordon and Zych, unpubl.
Hericium erinaceum	strain CBS449 85	Czech Republic	DQ185928	Ko *et al.*, unpubl.
Hericium abietis	strain CBS243 48	Canada	AY534579	Park *et al.* 2004
Hericium coralloides	strain ATCC52480	Canada	AY534584	Park *et al.* 2004
Hericium americanum	strain CBS493 63	Canada	AY534581	Park *et al.* 2004

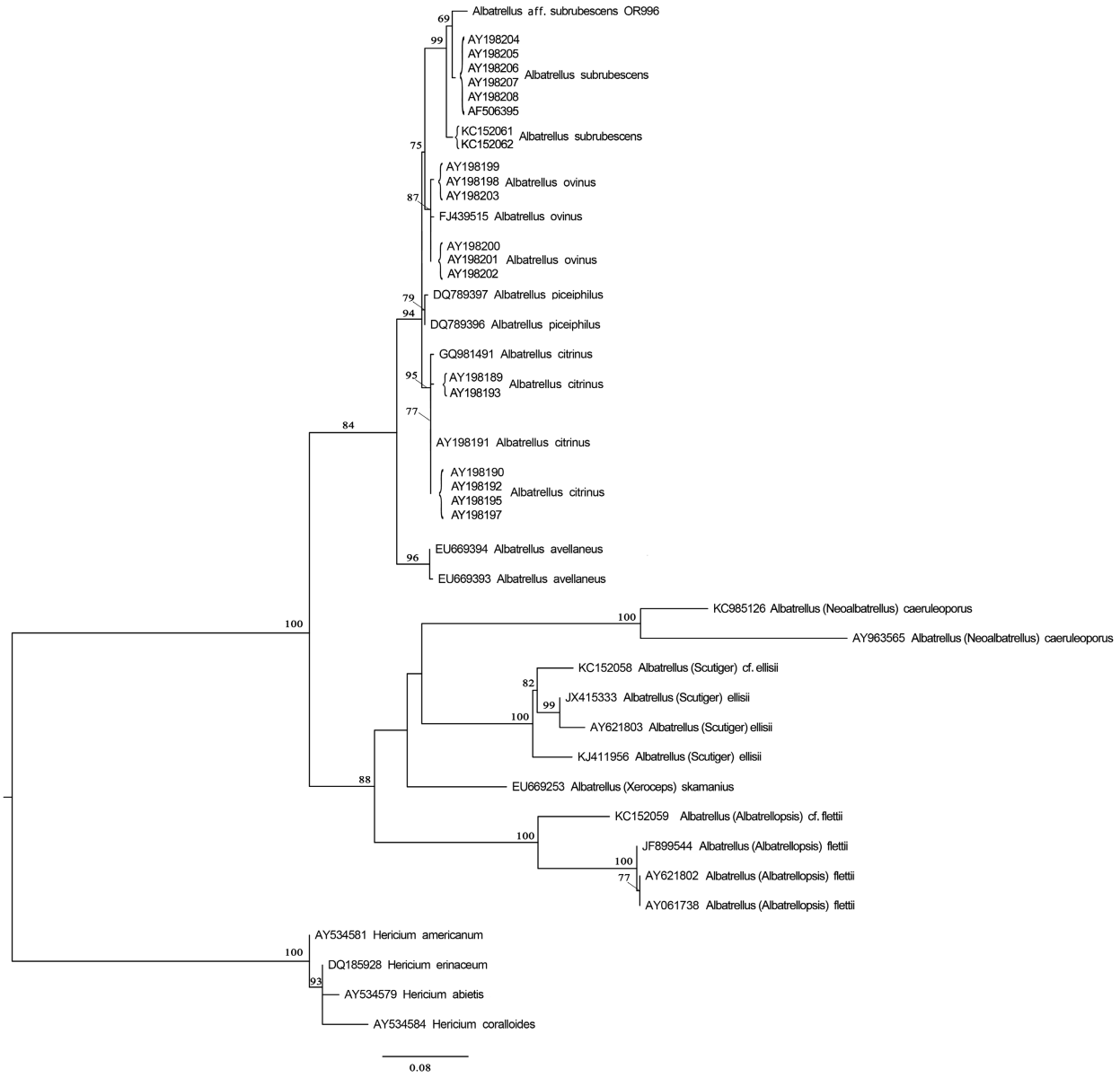

FIGURE 1. Phylogenetic tree inferred by maximum likelihood from ITS sequences.

TABLE 2. Genetic distances within (shaded cells) and between *Albatrellus* sensu stricto (Audet 2010) species and OR996. 1 = OR996; 2 = *Albatrellus subrubescens* from Europe; 3 = *Albatrellus subrubescens* from Mexico; 4 = *Albatrellus ovinus*; 5 = *Albatrellus citrinus*; 6 = *Albatrellus piceiphilus*; 7 = *Albatrellus avellaneus*. Where more than 2 sequences are available, genetic distance is given as minimum-***mean***-maximum.

	1	2 (*N* = 5)	3 (*N* = 2)	4 (*N* = 7)	5 (*N* = 8)	6 (*N* = 2)	7 (*N* = 4)
1	–						
2	0.030-***0.0300***-0.030	0.000					
3	0.029-***0.0320***-0.035	0.012-***0.0135***-0.015	0.000				
4	0.054-***0.0546***-0.055	0.039-***0.0396***-0.040	0.032-***0.0338***-0.036	0.000-***0.0024***-0.005			
5	0.057-***0.0580***-0.060	0.042-***0.0433***-0.045	0.038-***0.0389***-0.041	0.023-***0.0254***-0.032	0.000-***0.0012***-0.005		
6	0.051-***0.0515***-0.052	0.036-***0.0370***-0.038	0.027-***0.0300***-0.033	0.018-***0.0202***-0.022	0.018-***0.0198***-0.022	0.003	
7	0.081-***0.0815***-0.082	0.074-***0.0743***-0.075	0.068-***0.0690***-0.070	0.055-***0.0575***-0.059	0.050-***0.0534***-0.056	0.050-***0.0520***-0.054	0.000-***0.0015***-0.003

Taxonomy

Albatrellus Gray, Nat. Arr. Brit. Pl. (London) 1: 645 (1821)
Albatrellus* aff. *subrubescens (Murrill) Pouzar, Česká Mykol. 26: 196 (1972)

Basidiomes medium-sized, stipitate (Fig. 2). *Pileus* 35–50 mm in diameter; surface off-white to pale ochraceous, with faint pinkish hues at places when fresh, discoloring yellow when bruised, dull, dry, minutely and densely felted; margin inflexed becoming reflexed, irregular at places, with 1–3 clefts. *Pileus context* 7–8 mm thick at the centre of pileus, 3–4 mm thick half-way to the margin, slightly thinner at the margin, unchanging when bruised. *Stipe* excentric, cylindrical, 25–30 × 4–7 mm, white to yellowish white, with white basal tomentum and rhizoids. Surface even, dull, dry, minutely and densely tomentose, slightly orange when bruised. *Stipe context* solid. *Tubes* 0.5–1 mm long, deeply decurrent at least on one side of the stipe, yellowish white. *Pores* irregular, 0.3–1.5 mm in diameter, regularly arranged, white, dry. *Odor* aromatic; *taste* not recorded.

FIGURE 2. Basidiomata of *Albatrellus* aff. *subrubescens* (OR996). Scale bar = 1 cm. (Photograph by O. Raspé).

Basidiospores (Fig. 3) (3.5–)3.7–4–4.4(–4.5) × (3–)2.7–3.1–3.5(–3.5) µm, Q = (1.14–)1.15–1.3–1.45(–1.5) {N = 55}, broadly ellipsoid, smooth, thin-walled, mostly with one large drop, hyaline in 5% KOH, amyloid (bluish grey in Melzer reagent). *Basidia* 4–spored, (18–)16.7–20.8–24.9(–27) × (6–)5.7–6.3–6.9(–7) µm {N = 20}, clavate, hyaline in 5% KOH, yellowish in Melzer reagent, without basal clamp connection; sterigmata 3–4 µm long, slightly curved. *Pleurocystidia* and *cheilocystidia* absent. *Hymenophoral trama* subregular, 35–65 µm wide. *Pileipellis* (Fig. 3) 45–65 µm thick, with repent hyphae composed of slightly thick-walled and slightly amyloid, cylindrical to irregular, 6–54 × 3–7 µm elements, with very small incrustations on the walls, and yellowish brown content in Melzer reagent. *Pileus context* composed of slightly thick-walled, 4–14 µm wide hyphae with scattered, very small incrustations on the walls. *Stipitipellis* 65–85 µm thick; terminal cells 8–45 × 3.5–9 µm, slender to slightly swollen, with roundish apex; cell wall 0.5–1.5 µm thick, amyloid, with scattered incrustations; content yellowish brown in Melzer reagent; caulocystidia not seen. *Stipe context* composed of slightly thick-walled and slightly amyloid, 5–15 µm wide hyphae. *Clamp connections* not seen in any tissue.

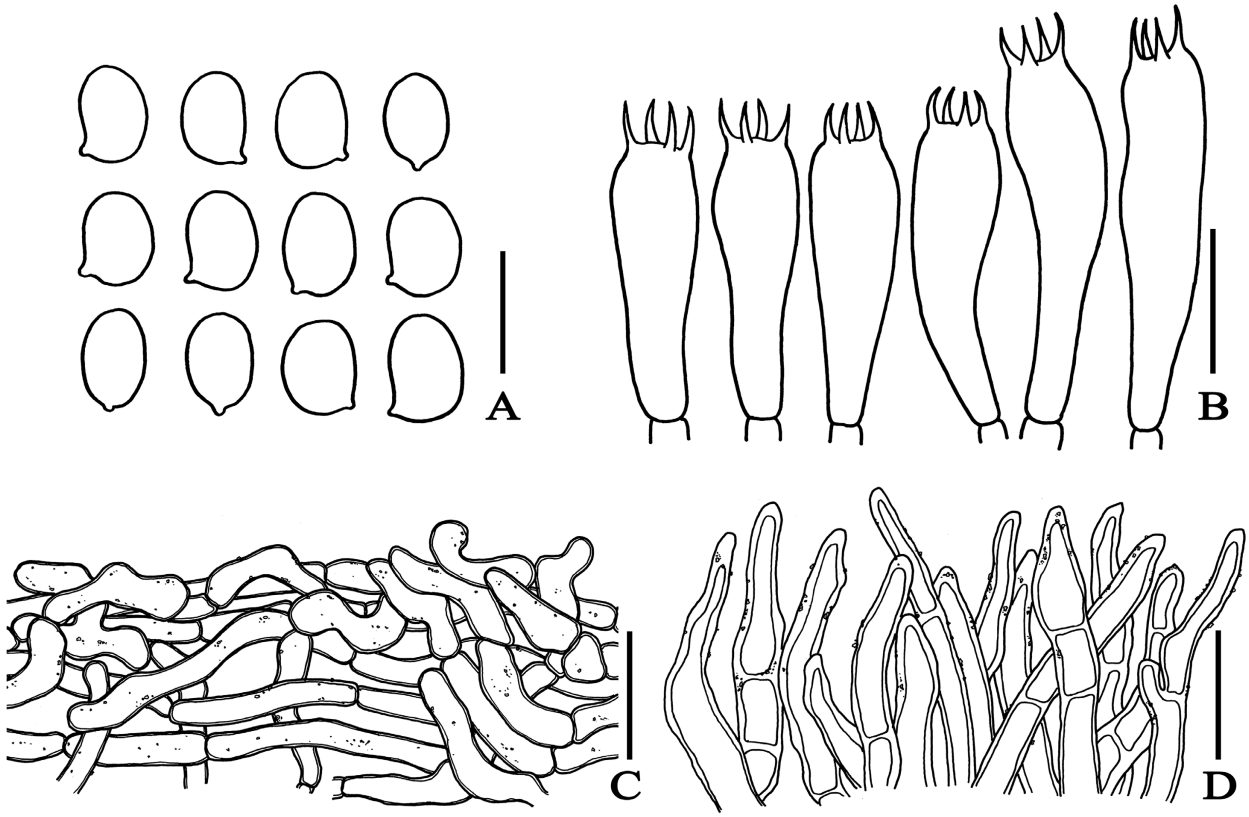

FIGURE 3. Microscopic features of *Albatrellus* aff. *subrubescens* (OR996). A. Basidiospore; B. Basidia; C. Pileipellis; D. Stipitipellis. Scale bars: A, 5 µm; B, 10 µm; C–D, 20 µm. (Drawings by S. Vadthanarat).

Ectomycorrhizae (Fig. 4) development dichotomous, with either only a few ramifications or many (coralloid development); axes ca. 0.2–0.35 mm across, mostly black to dark brown, covered with short hairs; extremities sometimes covered by a white layer, which may represent the initiation of basidiomes.

Ecology: Road verge in mixed forest with *Pinus kesiya*, *Carpinus poilanei*, *Betula alnoides* and *Lithocarpus* spp. Ectomycorrhizal with *P. kesiya*.

Material examined: THAILAND, Chiang Mai Province: Meuang District, Doi Suthep-Pui National Park, elev. 1,590 m, N 18°49'00"–E 098°53'36", 16 July 2015, Olivier Raspé 996 (SDBR-CMU OR996, BR5020187498846).

Remarks: Following the keys provided by Zheng & Liu (2008) and Audet (2010), our material was identified as *Albatrellus subrubescens*. However, the protologue of *A. subrubescens* (Murrill 1940) present a number of discrepancies with our material. Basidiomes are larger (pileus 6–9 cm across, stipe 1–2 cm in diameter), and the pileus is said to be decorated with erect, dark-colored, tufts of fibrillose squamules that are conspicuous on the disk. Moreover, the pores are described as 'circular to slightly angular', and very small (4–5 per mm). Unfortunately, neither Zheng & Liu (2008) nor Audet (2010) provided a detailed description of the species. The description given by Ginns (1997) of North American material, also mentioned larger basidiomes ('pileus to 10 cm in diameter), and 'some [pileus] covered with blackish grey to purple-grey fibrils', but only 2–3 pores per mm. Ginns (1997) also mentioned 'faint rosy buff tints centrally, and yellow stains where handled', which we observed in our material. However, Ryman *et al.* (2003) used the yellow discoloring of the pileus surface as a diagnostic characteristic to distinguish *A. citrinus* from *A. subrubescens*, the latter being said to stain orange. Therefore, it is unclear whether the discrepancies between the protologue of *A. subrubescens* and our material are compatible with within-species variation or if the variation described by various authors results from the inclusion of material of different species.

The morphology of *Pinus kesiya* / *Albatrellus* aff. *subrubescens* ectomycorrhizae we observed can be compared to the *Picea abies* / *Albatrellus ovinus* ectomycorrhizae described by Agerer *et al.* (1996). Both are similar in color, but also show marked differences. While the latter show monopodial growth and abundant rhizomorphs, dichotomous growth and only rare or no rhizomorphs were observed in the former. However, rhizomorphs may have been lost in the cleaning process.

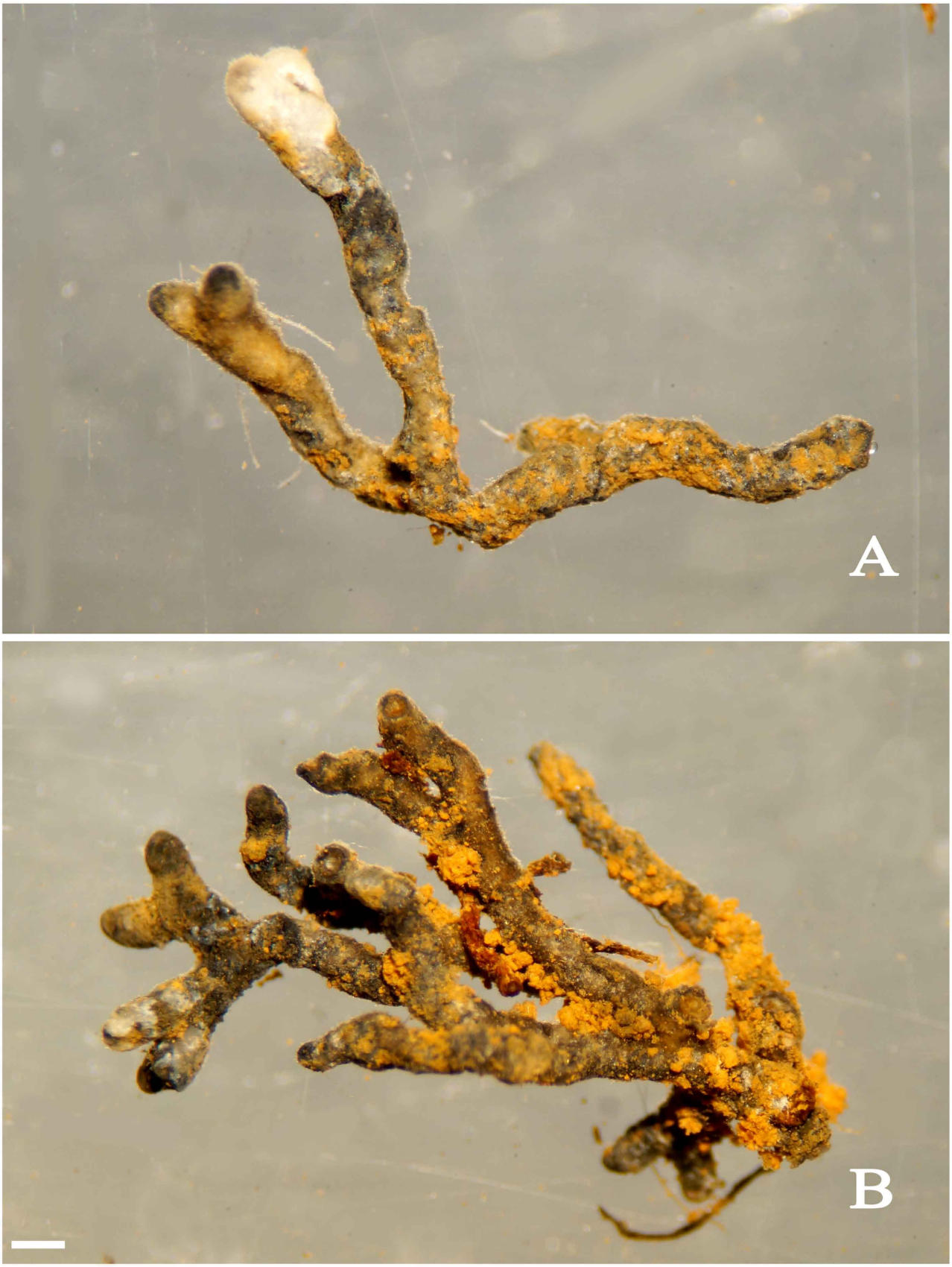

FIGURE 4. The two sequenced ectomycorrhized root tips of *Pinus keziya* / *Albatrellus* aff. *subrubescens*. A. Ectomycorrhiza showing white layer on one root tip. B. Ectomycorrhiza showing brown rhizomorphs. Scale bar: 0.2 mm. (Photographs by S. Vadthanarat).

Discussion

The genus *Albatrellus* is mostly distributed in temperate areas (Cui *et al*. 2008; Gilbertson & Ryvarden 1986; Ginns 1997; Núñez & Ryvarden 2001; Ryman *et al*. 2003; Ryvarden & Gilbertson 1993; Zheng & Liu 2008). In tropical or subtropical Southeast Asia, two species have been reported, only from high elevations in Malaysia, namely *A. borneensis* Corner and *A. cochleariformis* (Cooke) Ryvarden (Lee 2012). A number of species have, however, been also reported from Yunnan, China (Zheng & Liu 2008). Our collection represents the first report of *Albatrellus*, and, to the best of our knowledge, of Albatrellaceae from Thailand. Micro- and macroscopic characteristics of our Thai collection showed that it is closely related to *A. subrubescens*, which is usually found in *Picea-Pinus* forests in temperate zones (Ginns 1997; Ryman *et al*. 2003; Ryvarden & Gilbertson 1993; Zheng & Liu 2008). Coniferous forests are relatively rare in Thailand, although more common in Northern Thailand, which, together with the tropical climate, may explain why *Albatrellus* is rare and has not previously been reported from Thailand. More collections from Thailand, as well as careful revision and sequencing of *A. subrubescens* from different parts of the world are needed to estimate morphological and genetic variation of *A. subrubescens* and to establish whether collections from Thailand deserve to be described as a new species or not.

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