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Two interesting cantharelloids from Nan and Kanchanaburi Provinces, Thailand

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Abstract —Two cantharelloid fungi are recorded and described from Nan and Kanchanaburi Provinces, Thailand. *Cantharellus atratus*, a new record for Thailand, is recombined in *Craterellus* based on molecular studies. Although previously known from Peninsular Malaysia, *Pterygellus polymorphus* var. *minor* represents a new record for Thailand.

Key words — ectomycorrhizal fungi, ecology, dipterocarps, taxonomy

Introduction

Nan Province is situated in northern Thailand where dipterocarp forests predominate. Several excursions over a two-year period were made to compare macromycetes of a woodland in Veingsa District with a study area in Sisawat District, Kanchanaburi Province, in western Thailand. Two cantharelloids collected during this work are described herein.

Materials & methods

Collections

The Nan locality consisted of seasonal dipterocarp forest dominated by *Shorea obtusa* Wall. and *S. siamensis* Miq. accompanied by *Dipterocarpus tuberculatus* Roxb., *D. obtusifolius* Teijsm. ex Miq. and a major understorey of diverse graminoids. The forest overstorey in Kanchanaburi Province is dominated by *S. siamensis* and *D. tuberculatus* and

an understorey primarily composed of *Cycas siamensis* Miq. and diverse graminoids. The collected specimens were dried in silica gel and deposited in Bangkok, Chulalongkorn University, Microbiology Department (BCUM ND1)

DNA analysis

Genomic DNA extracted from fresh basidiomes with cetyltrimethylammonium bromide (CTAB) as described in Zhou et al. (1999). DNA was amplified in a 50 µl reaction mixture that contained 1 µl of DNA template, 1x buffer [16 mM $(NH_4)_2SO_4$, 67 mMTris-HCl (pH 8.8 at 25°C), 0.01% Tween-20], 2.0 mM $MgCl_2$, 250 µM dNTPs (Bioline Ltd, London, UK), 20 pmol ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990), and 2.5 U BIOTAQ polymerase (Bioline Ltd, London, UK). The PCR cycle comprised an initial denaturation step at 94°C for 5 min followed by 38 cycles of 94°C for 1 min, 51°C for 1 min and 72°C for 1 min, and a final extension at 72°C for 5 min on a TP600TaKaRa PCR Thermal Cycler Dice™ (Takara Bio Inc., Japan). The PCR product was sub-cloned with Takara Cloning Kits (Takara Bio Inc., Japan) following the manufacturer's instructions. Plasmid DNA was extracted from transformed cells suspended in 50 µl sterile water in a 1.5 ml tube that was incubated in boiling water for 5 min. The supernatant was PCR amplified with primers ITS1 and ITS4. After confirming fragment insertion on agarose gels, the inserts were sequenced using Thermo Sequenase Pre-mixed Cycle Sequencing Kits (Amersham International plc. Buckinghamshire, England) using primers Texas Red M13F and T7 following the manufacturer's instructions. The sequences obtained, including the complete ITS regions, were registered in DDBJ. A blast homology search was conducted in DDBJ/EMBL/GenBank database using Blastn. Phylogenetic analysis was performed with Neighbor-Joining method using PAUP 4.08b (Swofford 1999).

SEM observation

Fresh specimens were prepared for the scanning electron microscope by fixation in 2.5% glutaraldehyde 0.1 M phosphate buffer at pH 7.2 for 2 hr at 5°C. Specimens were then rinsed twice in phosphate buffer for 10 min and once in distilled water for 10 min. The specimens were dehydrated in a 30, 50, 70 and 95% ethanol series for 10 min each followed by three changes of absolute ethanol for 10 min each. Specimens were dried in a critical point dryer (Balzers model CPD 020). The dried samples were fixed to brass stubs with double sided sticky tape and then coated with gold in a sputter coater (Balzer model SCD 040). Specimens were observed under SEM (JEOL model JSM 5410LV) with an accelerating voltage of 15 kV. Photographs were recorded by computer.

Taxonomy

Craterellus atratus (Corner) Yomyart, Watling, Phosri, Piapukiew & Sihan., **comb. nov.** Fig. 1

MycoBank No. MB 563344

≡ *Cantharellus atratus* Corner, Ann. Bot. Mem. 2: 62, 1966.

Pileus 8–9 mm dark fuliginous black, not perforated but depressed at centre, appearing fibrillose-zoned when fresh, centrally stipitate (although limb broader on one side), margin wavy slightly fimbriate. Stipe 7–9 × 0.75–1 mm greyish buff throughout, darker downwards to slightly swollen at base

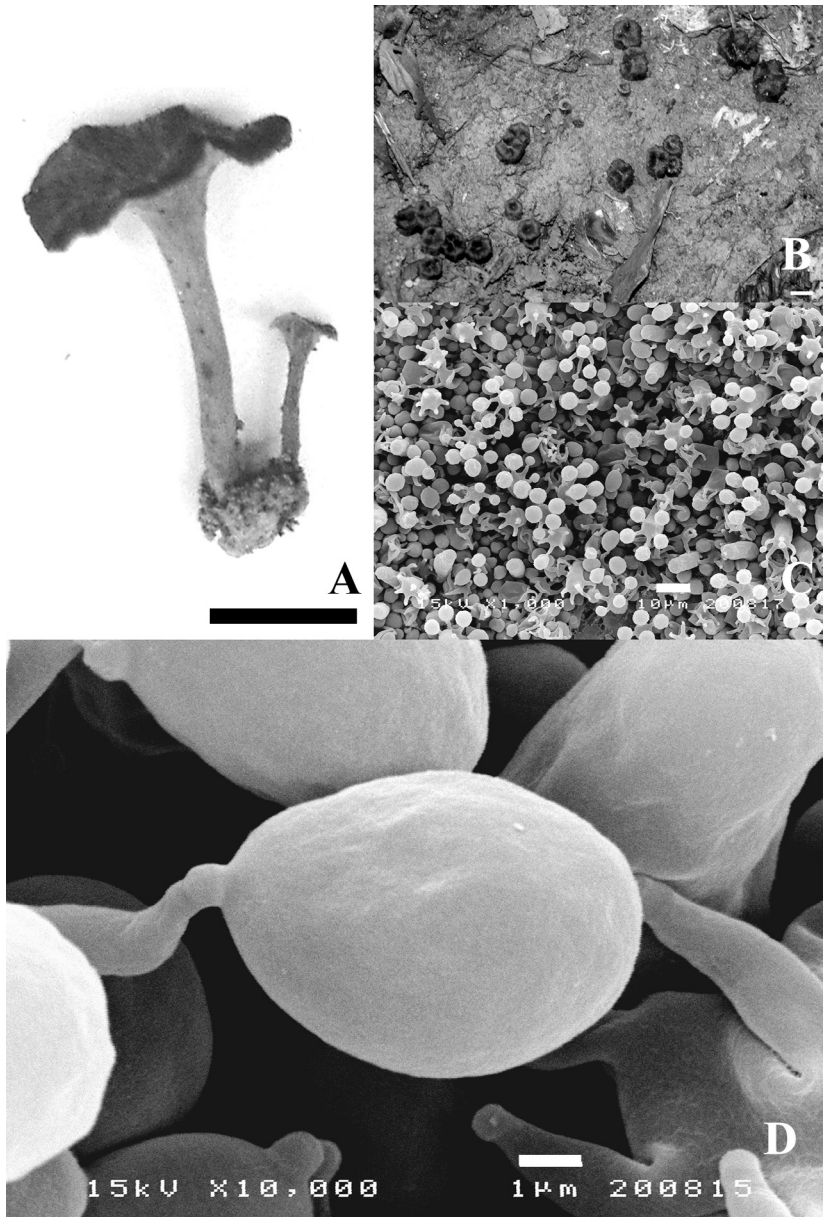
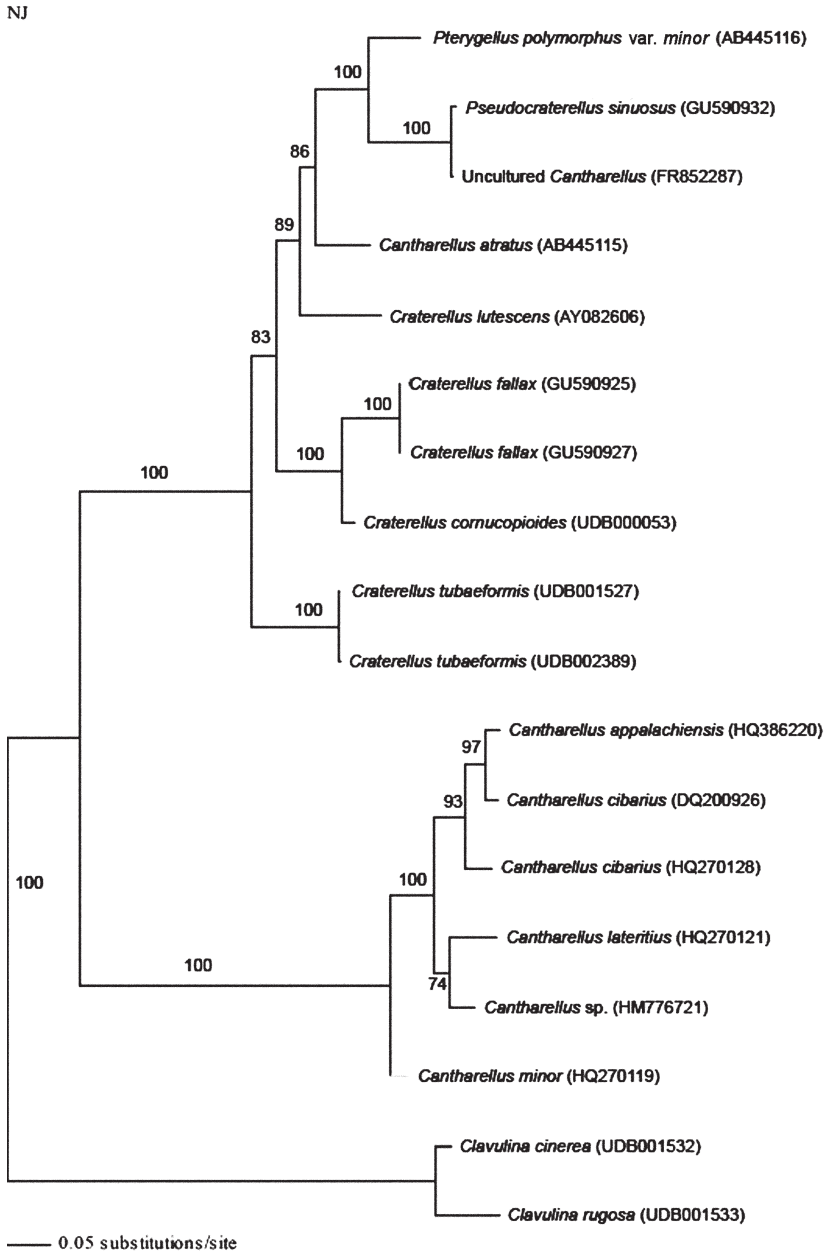


Fig. 1. *Craterellus atratus*. A: Pair of basidiomes. B: Distribution of basidiomes in study area. C: Scanning electron micrograph showing variable number of basidiospores on hymenium. D: High magnification scanning electron micrograph, showing ellipsoid basidiospore shape with smooth wall. Scale bars: A, B = 1 cm; C = 10 µm; D = 1 µm.


NJ
Pterygellus polymorphus var. minor (AB445116)
Pseudocraterellus sinuosus (GU590932)
Uncultured Cantharellus (FR852287)
Cantharellus atratus (AB445115)
Craterellus lutescens (AY082606)
Craterellus fallax (GU590925)
Craterellus fallax (GU590927)
Craterellus cornucopioides (UDB000053)
Craterellus tubaeformis (UDB001527)
Craterellus tubaeformis (UDB002389)
Cantharellus appalachiensis (HQ386220)
Cantharellus cibarius (DQ200926)
Cantharellus cibarius (HQ270128)
Cantharellus lateritius (HQ270121)
Cantharellus sp. (HM776721)
Cantharellus minor (HQ270119)
Clavulina cinerea (UDB001532)
Clavulina rugosa (UDB001533)
100
100
86
100
89
83
100
100
100
100
97
93
100
100
74
0.05 substitutions/site

embedded in sandy, surface passing irregularly into a pale grey, smooth to very faintly wrinkled hymenium which commences uniformly dark greyish buff but then becomes darker. Flesh concolorous.

Basidiospores 9 × 6–7 µm, ellipsoid, smooth, thin-walled with minute guttules, inamyloid. Basidia 4–6-spored. Cystidia absent. Clamp-connections rare.

Specimen examined: **THAILAND, Nan Province,** Veingsa District, locality, on bare ground in dipterocarp forest, 29 July 2006, coll. Sihanonth (BCMU ND1, GenBank AB445115).

Corner (1966), who described this species from the type collection from Rio de Janeiro, Brazil, also recorded it from Pernambuco, Brazil, and Brunei. Our material agrees with the pickled collection held in Edinburgh as a collection sent by Singer from Pernambuco, Brazil. Singer misidentified the Pernambuco collection as the Venezuelan *Craterellus orinocensis* Pat. & Gaillard, a much larger fungus reaching 8 cm high and 2–3 cm wide, allied with *Cr. cornucopioides* (L.) Pers. (Patouillard & Gaillard 1888) and possibly, as Heinemann (1958) suggests, a variety of *Cr. cornucopioides*. Singer's Pernambuco material is a rather small (pileus 2 cm diam) cantharelloid fungus and resembles a minute, smooth version of the widespread temperate *Cr. tubaeformis* (Fr.) Quél. (= *C. infundibuliformis* (Scop.) Fr.). Unlike *Cr. tubaeformis, Cr. atratus* shows no hint of yellow in the stipe. Corner (1966) originally included *C. atratus* in *Cantharellus* subg. *Phaeocantharellus,* but Donk (1969) indicated that the name *Cantharellus* sect. *Leptocantharellus* Peck has priority for this group. Subsequent molecular studies (Feibelman et al. 1997, Dahlman et al. 2000) have shown that core species in this group are more closely allied to *Craterellus* than to the *Cantharellus cibarius* consortium and should therefore be accepted in *Craterellus* (Fig. 2), and we therefore propose the new combination, *Cr. atratus*.

Pterygellus polymorphus var. ***minor*** Corner, Ann. Bot. Mem. 2: 170, 1966. Fig. 3

Pileus 0.5-3 cm plane or plano-convex & simple at first then almost multipileate from deep incisions, rich, clear chrome-yellow but more ochraceous yellow towards centre in older specimens, deeply depressed, minutely scurfy roughened especially towards margin, smoother inwards and minutely fibrillose; margin uniform when young then soon wavy and flanged, becoming even more so with age and almost dichotomously lobed, irregularly

Fig. 2. A neighbor-joining tree showing placement of *Craterellus atratus* (AB445115) and *Pterygellus polymorphus* var. *minor* (AB445116). *Clavulina cinerea* and *Clavulina rugosa* were used as outgroups. Numerical values on branched are the bootstrap values as percentage bootstrap replication from a 1000 replicate analysis. The scale bar indicates 0.05 of the genetic distance between samples.

Fig. 3. *Pterygellus polymorphus* var. *minor*. A: cluster of basidiomes in study area. B: Scanning electron micrograph showing four basidiospores attached to basidia. C: High magnification scanning electron micrograph of basidiospores, showing smooth wall and ellipsoid shape. Scale bars: A = 1 cm; B = 10 µm; C = 5µm.

incised, wavy and toothed with fimbriate extensions. Stipe rich carrot colour to chrome yellow, darkening downwards, quite short compared with extension of hymenial surface. Hymenium smooth, lacking even minute wrinkles except at pileus margin, whitish or ivory as if frosted, quite deep and irregular and fimbriate, yellow towards basal regions. Flesh thin, pale yellow with a possible faint pinkish cast.

Basidiospores 8–9 × 6–7 µm ellipsoid to broadly ellipsoid, smooth, hyaline, thin-walled, non-amyloid. Basidia 4-spored. Cystidia absent. Clamp-connections absent.

Specimen examined: **THAILAND**, **Kanchanburi Province**, Sisawat District, locality, on soil troops in dipterocarp forest, 20 October 2007, coll. Sihanonth (BCMU KD1, GenBank AB445116).

Although agreeing in all microscopic characters with *P. polymorphus* Corner var. *polymorphus*, the small size of the basidiomes in the Kanchanaburi collections places them in *P. polymorphus* var. *minor,* a small fungus described from the Reservoir Jungle, Singapore, and differing from the type variety in its smaller size, being only slightly over 10 mm maximum. Corner (1966) described the typical variety as growing on soil in woodland in Pahang and Kedah, Malaysia. Our material agrees with both figures and coloured illustrations of *P. polymorphus* var. *minor* as well as with an excellent colour photograph in The Mycologist, portraying material collected in New Guinea (Verbeken & Walleyn 1999). Superficially the fungus approaches *Craterellus aureus* Berk. & M.A. Curtis in outward appearance, except that but the pileus is not perforate.

Our material also resembles *Pseudocraterellus luteus* (Pat.) D.A. Reid, a similar species that Corner (1966) suggested might be compared with *P. polymorphus.* Indeed, all our collections are very close to *Ps. luteus* except for the prominent fimbriate pileus margin present in our material. The basidiome colour also differs, being more yellowish than orange when fresh before finally fading to brownish in *Ps. luteus* whilst *P. polymorphus* is an intense yellow-orange (especially in the stipe) when young before fading to an ochraceous yellow. *Pterygellus* is a stereoid-like fungus and differs from *Pseudocraterellus* in the lack of secondary septation and in the thickened hyphal walls. *Pterygellus polymorphus* sequence analysis places the fungus on a branch close to *Pseudocraterellus sinuosus* (Fr.) Corner, as indicated in Fig. 2.

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Literature cited

Corner EJH. 1966. A monograph of the cantharelloid fungi. Annales of Botany Memoirs 2: 1–255.

Dahlman M, Danell E, Spatafora JW. 2000. Molecular systematics of *Craterellus*: cladistic analysis of nuclear LSU rDNA sequence data. Mycol. Res. 104: 388–394. http://dx.doi.org./10.1017/S0953756299001380

Donk MA. 1969. Notes on *Cantharellus* sect. *Leptocantharellus.* Persoonia 5: 265–284.

Feibelman TP, Doudrick RL, Cibula WG, Bennett JW. 1997. Phylogenetic relationships within the *Cantharellaceae* inferred from sequence analysis of the nuclear large subunit rDNA. Mycol. Res. 101(12): 1423–1430. http://dx.doi.org./10.1017/S0953756297004115

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycete applications to the identification of mycorrhizae and rusts. Mol. Ecol. 1: 113–118. http://dx.doi.org./10.1111/j.1365-294X.1993.tb00005.x

Heinemann P. 1958. Champignons recoltres au Congo Belge par Madame M. Goossens-Fontana. III. *Cantharellineae.* Bull. Jardin Bot.de L'Etat, Bruxelles 28(4): 385-438.

Patouillard N, Galliard MA. (1888) Champignons de Vénézuéla et principalement de la région du Haut-Orénoque récoltés par. M.A.Galliard Bulletin Sociéte de Mycologique de France 4: 7-46

Reid DA. 1962. Notes on fungi which have been referred to the *Thelephoraceae* sensu lat. *Persoonia* 2 (2): 109-169. A monograph of stipitate stereoid fungi. Nova Hedwigia Beihefte 18: 1–382.

Swofford DL. 1999. PAUP*. Phylogenetic analysis using parsimony (and other methods), version 4.08b8. Sinauer Associates, Sunderland, MA, USA.

Verbeken A, Walleyn R. 1999. Is *Pterygellus* mycorrhizal with a euphorbia? Mycologist 13: 37.

White TJ, Bruns T, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. 315–322, in: MA Innes et al. (eds). PCR protocols. A guide to methods and applications. San Diego, California: Academic Press, Inc.

Zhou Z, Miwa M, Hogetsu T. 1999. Analysis of genetic structure of a *Suillus grevillei* population in a *Larix kaempferi* stand by polymorphism of inter-simple sequence repeat (ISSR). New Phytol. 144: 55–63.