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Panaeolus antillarum (Basidiomycota, Psathyrellaceae) from wild elephant dung in Thailand

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Abstract

Panaeolus antillarum is reported from material collected on wild elephant dung in Khao Yai National Park, Thailand. This new distribution report is supported with morphological and molecular sequence (ITS) data, line drawings, colour photographs and a comparison with material from the Antilles.

Key Words – agarics – coprophilous fungi – fungal diversity – taxonomy

Introduction

The agaric genus *Panaeolus* is global in distribution and a common component of the coprophilous mycota. The first report of *Panaeolus* from Thailand was that of Rostrup (1902), wherein G. Massee described as new *P. albellus* Massee, based on material collected on buffalo dung. He noted the species was allied with *P. campanulatus* (L.) Quél., but differing in adnate lamellae and larger basidiospores (ellipsoid, 20 × 10 µm). Apparently, the taxon has been overlooked and not treated since, remaining a *nomen dubium*. In the same publication, Massee reported *P. campanulatus* (= *P. papilionaceus* (Bull.) Quél.) also from buffalo dung. Since then, only four additional species of *Panaeolus* have been reported from Thailand, viz., *P. fimicola* (Pers.) Gillet, *P. retirugis* (Fr.) Gillet (= *P. papilionaceus* var. *retirugis* (Fr.) Gminder), *P. semiovatus* (Sowerby) S. Lundell & Nannf., and *P. sphinctrinus* (Fr.) Quél. (= *P. papilionaceus*) (Soytong 1994, Ruksawong & Flegel 2001, Chandrasrikul et al. 2008). A recent account of coprophilous fungi from Thailand reported only *P. albellus*, *P. campanulatus* and *P.* sp. (Somrithipol 2004). Herein we report *Panaeolus antillarum* (Fr.) Dennis that was collected from wild elephant dung in Khao Yai National Park, supported with morphological and molecular sequence (internal transcribed spacer – ITS) data.

Panaeolus antillarum was first described from material collected on the island of St. Croix in the Greater Antilles (U.S. Virgin Islands). It is considered a pantropical-subtemperate species, reported from both the New World (Caribbean islands, continental US, Central and South America) and Old World (Africa, Australia, China, Europe, India, Southeast Asia, Taiwan), growing commonly on cow, horse and mule dung (Dennis 1961, 1970, Guzman 1973, Pegler 1977, 1983, Gerhardt 1987, 1996, Zhishu et al. 1993, Hausknecht & Krisai-Greilhuber 2003, Rommelaars & Arnolds 2007, Watling & Richardson 2010, Doveri 2011, Hamala et al. 2014, Kaur et al. 2014, Wang & Tzean 2015). Rarely, the species has been reported from elephant dung, from Uganda

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(Pegler 1977) and India (Natarajan & Raaman 1983, Manimohan et al. 2007). The morphology of *P. antillarum* can be quite variable depending on basidiome age and environmental conditions (Bride & Métrod 1950), which has resulted in numerous heterotypic synonyms (see Pegler 1977, 1983, Halama et al. 2014). Indeed, the material reported on here from wild elephant dung from Thailand differs in a number of features from most published descriptions of the species based on specimens from dung of grass-grazing cattle and horses, such that it was initially thought to represent a new species. To aid in identifying the Thai material and expose the morphological differences, recently collected material of *P. antillarum* from the Antilles (Dominican Republic on mule dung) was sequenced (ITS region) and compared. As far as we know, this is the first report of ITS sequences of material from the Antilles, type locality for *P. antillarum*. We report here that despite difference in pileus colouration, basidiospore size and shape, and substrate preference, the ITS sequences of Thai and Antilles specimens are identical.

Materials and Methods

Morphological observations

Macromorphological notes and photographs were obtained from fresh specimens. Colour terms and notations in parentheses are those of Kornerup & Wanscher (1978). Micromorphological analyses were performed using dried material rehydrated in 100% ethanol followed by Melzer's reagent, or Congo Red Solution and 3% KOH and documented using a Nikon Optiphot-2 compound microscope fitted with a drawing tube. Basidiospores were measured in face view and profile, with spore statistics calculated as: x_m, the arithmetic mean of the spore length by the spore width (± standard deviation) from n spores measured in a single specimen; Q, the quotient of spore length and spore width in any one basidiospore indicated as a range of variation in n spores measured; Q_m, the mean of Q-values in a single specimen; n, the number of spores measured per specimen; s, the number of specimens studied. Specimens are deposited in the H.D. Thiers Herbarium at San Francisco State University (SFSU).

Molecular methods

Total genomic DNA was extracted from dried material using the Extract-N-Amp Plant Tissue PCR Kit (Sigma-Aldrich, St. Louis, MO) following the manufacturer instructions. PCR protocols followed those outlined in Perry et al. (2007). The nuclear ribosomal internal transcribed spacer region (ITS) was amplified using primer pairs ITS1-F/ITS4 (Gardes & Bruns 1993; White et al. 1990). Amplification products were cleaned using the ExoSAP-IT PCR Cleanup Reagent (Affymetrix, Santa Clara, CA), and sent to ELIM Biopharmaceuticals (Hayward, CA) for sequencing. Resulting sequencing products were edited, assembled, aligned and compared to top BLAST hits in Geneious 9.0 (Biomatters Ltd., Auckland, New Zealand). Sequences generated as part of this study have been deposited in GenBank (accessions MF497585–MF497586).

Taxonomy

Panaeolus antillarum (Fr.) Dennis, Kew Bull. 15(1): 124. 1961. Figs 1, 2a–f

Basionym: *Agaricus antillarum* Fr., Elench. fung. (Griefswald) 1: 42. 1828.
= *Psilocybe antillarum* (Fr.) Sacc., Syll. fung. (Abellini) 5: 1052. 1887.
= *Annelaria antillarum* (Fr.) Hlaváček, Mykologický Sborník 74(2): 52. 1997.
Heterotypic synonyms: refer to Pegler (1977, 1983) and Halama et al. 2014.
Holotype – Antilles, St. Croix, coll. by P.E. Benzon (material lost).
Mycobank: MB335553; Facesoffungi number: FOF03629

Description of Thai material:

Pileus 20–80 mm diam, obtusely conical, margin incurved to straight, radially rugulose to rugulose-striate; surface subviscid, glabrous, subhygrophanous when young, mottled and radially

streaked; at first dark brown (7F5–8) with paler brown (7E5) or pale brownish grey (6C3) areas, soon disc, spots and radially streaked areas dark brown to brown and elsewhere becoming tan to beige or off-white, streaked and spotted in age; not becoming areolate or scaly. Context 3–5 mm thick, off-white, not staining where bruised. Lamellae narrowly adnexed, close to crowded with 4 series of lamellulae, broad (6–9 mm), pale greyish white (5–6B1), becoming dark grey to black, slightly mottled; lamellar edge white-pruinose; spore deposit black. Stipe 45–110 × 3–7 mm, central, ±terete, cylindrical above, gradually enlarged downward, striatulate above, smooth elsewhere, solid; surface dull, dry, minutely furfuraceous to granulose-pruinose, base with white tomentum; apex off-white, base pale greyish orange (5B3); partial veil absent. Tissues not cyanescent where bruised. Odor and taste mild, not distinctive.

Fig. 1 – Basidiomes of *Panaeolus antillarum* (DED 7874). Scale bars = 10 mm

Basidiospores 11.8–14.4 × 9.6–11.2 (face view) × 8.0–10.2 (profile) µm [x_m = 13.4 ± 0.85 × 10.43 ± 0.59 (face view) × 8.97 ± 0.7 (profile) µm, Q = 1.14–1.37 and Q_m = 1.29 ± 0.06 (face view), n = 25, s = 1], broadly limoniform in face view, asymmetrically ellipsoid in profile, with a broad central germ pore up to 3 µm diam, smooth, dark brown to nearly black, thick-walled (0.7–1.0 µm). Basidia 24–30 × 12.5–14.5 µm, broadly clavate, 4-spored, unclamped. Basidioles broadly clavate. Chrysocystidia 32–45 × 11–14 µm, clavate, seldom with a small papilla, with pale yellow, refractive, oily-glassy contents. Lamellar edge sterile. Cheilocystidia 28–38 × 6.5–12 µm, irregularly fusoid to irregularly ventricose, broadly obtuse, rarely bifid, hyaline, thin-walled. Pileipellis a hymeniform layer on the disc to an epithelium on the margin, of broadly clavate to ampullaceous or subglobose cells 32–48 × 14–34 µm, hyaline, inamyloid, thin-walled, overlaid with a gelatinous film up to 16 µm thick; arising from a subcutis of irregularly-shaped, often short-celled hyphae with diffuse, brown parietal pigments, non-gelatinous. Pileocystidia absent. Pileus trama interwoven; hyphae 5–20 µm diam, irregular in outline, inflated, hyaline, inamyloid, non-gelatinous, thin-walled. Stipitipellis an interrupted trichodermium of erect to repent caulocystidia; cortical hyphae 3.2–6.5 µm diam, cylindrical, hyaline, inamyloid, non-gelatinous, non-incrusted, thin-walled; medullary hyphae similar, up to 13 µm diam. Caulocystidia 28–48 × 6.5–9.5 µm,

irregularly cylindrical to fusoid, broadly obtuse, hyaline, thin-walled. Clamp connections absent in all tissues.

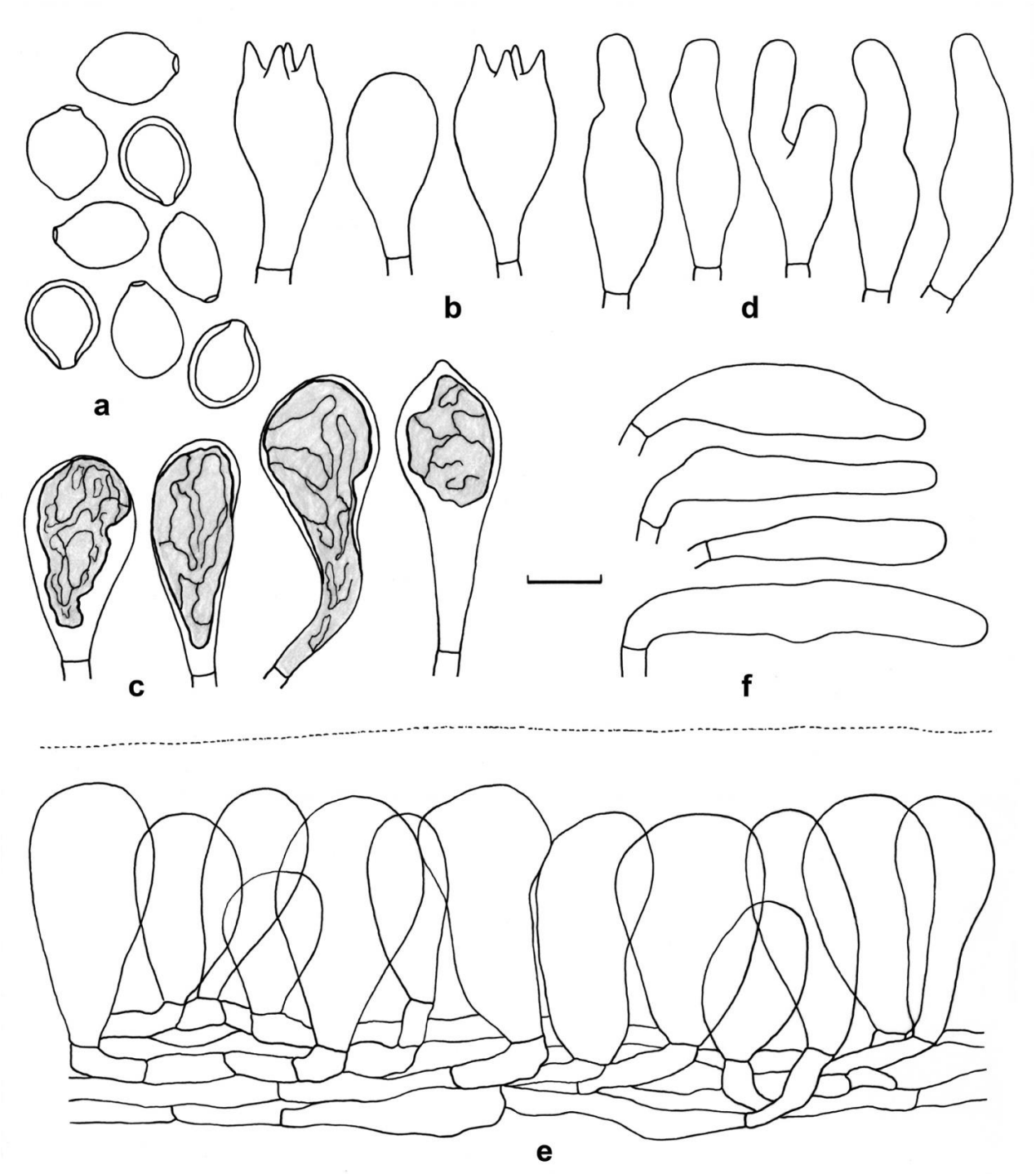

Fig. 2 – Micromorphological features of *Panaeolus antillarum* (DED 7874). a. Basidiospores. b. Basidia and basidiole. c. Chrysocystidia. d. Cheilocystidia. e. Hymeniform pileipellis. Dotted line represents upper limit of gel layer. f. Caulocystidia. Scale bar = 10 µm

Habit and known distribution – Gregarious, coprophilous, with stipe base buried in elephant dung. Thailand.

Material examined – THAILAND. Nakorn Nayok Province, Khao Yai National Park, Princess trail ca 2 km from Visitor's Center, N14˚26.146', E101˚23.080', elev. 750 m, 7 July 2005, coll. by D.E. Desjardin, DED 7874 (MF497585, SFSU). DOMINICAN REPUBLIC. Santiago

Province, Pedregal, on road from Los Montones Convention Center, on mule dung, 15 January 2003, coll. by Lance Lacey, *LL 156* (MF497586, CORT 013830).

Notes – *Panaeolus antillarum* in Thailand is characterized by large basidiomes with the following features: an obtusely conical, brown and off-white, streaked and mottled pileus up to 80 mm diam, that typically does not become areolate or scaly at maturity; a long (up to 110 mm), solid, off-white stipe lacking a partial veil; broadly limoniform (face view) basidiospores 11.8–14.4 × 9.6–11.2 × 8.0–10.2 µm with mean 13.4 × 10.4 × 9 µm; abundant clavate chrysocystidia 11–14 µm diam; irregularly fusoid cheilocystidia; cylindrical caulocystidia; a hymeniform to epithelium-type pileipellis lacking pileocystidia overlaid with a gelatinous exudate; an absence of clamp connections; and growth on wild elephant dung.

In comparison, published descriptions of *P. antillarum* differ in having a convex, pure white to alutaceous cream, non-mottled and non-streaked pileus that often becomes areolate or scaly at maturity; larger subhexagonal (face view) basidiospores 13.3–22 × 9.4–14 × 7.7–11.5 µm with mean range 16.7–18.3 × 11.2–12 × 8.8–9.7 µm; often mucronate chrysocystidia 11–25 µm diam (mean 18 µm); presence of clamp connections; and growth normally on horse and cow dung (Pegler 1977, 1983, Watling and Gregory 1987, Stamets 1996, Hamala et al. 2014, Kaur et al. 2014, Wang and Tzean 2015).

Representative material of *P. antillarum* from the Dominican Republic (Greater Antilles, not too distant from the type locality of St. Croix; CORT 013830!) has the following features: a convex, off-white to tan-brown, non-mottled, non-streaked pileus; subhexagonal (face view) basidiospores 17–20 × 10.3–12.8 × 9.3–10.2 µm with mean 18.3 × 11.5 × 9.7 µm; clavate chrysocystidia 18–25 µm diam (mean 22 µm); absence of clamp connections; and growth on mule dung. Interestingly, material from elephant dung in India showed similar small basidiospores as those from Thailand, measuring 12–16 × 9–11 × 7.5–9 µm (Manimohan et al. 2007).

Prior to this research, the only DNA sequences of *P. antillarum* available from GenBank were based on specimens from Italy (JF908515 – substrate unspecified) and Taiwan (KR998382 – on cow dung). Pairwise comparisons of aligned, overlapping ITS sequences of the Thai material on wild elephant dung, the representative Antilles specimen on mule dung, and the two GenBank sequences determined as *P. antillarum* indicate that they are 97.2 (Italy) to 100% (Antilles, Taiwan & Thailand) similar, differing in 16–0 out of 618 aligned base pairs, respectively. The ITS sequence of *P. antillarum* material from Italy (JF908515) is identical to an ITS sequence of a different collection from Italy identified as *P. fimicola* (Pers.) Gillet, and likely represents the latter taxon. Results from BLAST searches indicate that ITS sequences of two specimens from Sri Lanka (KR867660, KP764810), misdetermined as *P. foenisecii*, are also 100% similar to our sequences from Thailand and the Dominican Republic. If one accepts the primacy of fungal barcode ITS data over morphological variability, these data indicate conspecificity of the Thai material with Antilles *P. antillarum*, and support earlier contentions that *P. antillarum* is a morphologically variable, widespread, pantropical species that grows in cellulose-rich dung of a number of wild and domesticated animals.

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