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Dung-associated, Potentially Hallucinogenic Mushrooms from Taiwan

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ABSTRACT: To identify potentially hallucinogenic mushrooms, dung-associated mushrooms collected from Qingtiangang, Yangmingshan National Park were subjected to a detailed morphological investigation and phylogentic analysis. The investigation identified four taxa: a recorded species (*Panaeolus antillarum*); a new combination (*Conocybe nitrophila*); and two new species (*Psilocybe angulospora*, *Protostropharia ovalispora*). Morphological and molecular characteristics of the collected mushrooms were compared with allied fungal taxa.

KEY WORDS: Coprophilous, Hallucinogenic, *Protostropharia*, *Panaeolus*, *Psilocybe*, *Conocybe*, Fungal diversity, Taxonomy.

INTRODUCTION

Fungal diversity is rich but has not been thoroughly investigated in Taiwan. Currently, 6,323 fungal species names have been recorded in Taibnet (Shao, 2009). However, it is estimated that there could be more than 25,000 fungal species in Taiwan according to the vascular plant species described and the fungi/vascular plants (6/1) ratio hypothesis suggested by Hawksworth (1991). Accordingly, there may be numerous additional species that remain undiscovered in Taiwan and needed to be further characterized (Tzean *et al.*, 2015).

In May 2014, a case report of hallucinogenic poisoning due to the ingestion of unknown coprophilous mushrooms on Qingtiangang grassland was reported and drew considerable public concern regarding biosafety. Therefore, to identify the species that may have caused the mushroom poisoning, we initiated a biodiversity survey attempting to identify the dung-associated, potential hallucinogenic mushrooms on Qingtiangang and also investigated the potential psychoactive mushroom species described in previous relevant reports. Herein, we report and describe a recorded species (*Panaeolus antillarum*), a new combination (*Conocybe nitrophila*), and two new species (*Psilocybe angulospora*, *Protostropharia ovalispora*) of mushroom.

MATERIAL AND METHODS

Specimen examination and deposition

The specimens were collected at Qingtiangang (25°9'37.4"–10'4.9"N, 121°34'25.7"–43.0"E) in the summers of 2014 and 2015 with the permission of Yangmingshan National Park (document number 20140921), and are deposited in the herbarium of National Museum of Natural Science, ROC (TNM). Other voucher specimens for comparative studies were borrowed from TNM. The color-code designation for the collected mushrooms follow the color standard published by Kornerup and Wanscher (1978). The holotype and ex-type specimens were deposited in National Taichung Science Museum.

Microscopic morphological examination

Fungal tissues or gills from the mushrooms were excised with a sharp scalpel, stained with Meltzer's reagent, mounted on slides and gently tipped onto coverslips. The slides were examined under an Olympus BX51 microscope and measured and imaged with an Olympus DP72 camera and DP2-TWAIN software (Olympus, Tokyo, Japan).

The terminology was defined as follows: Q = spore length/width; Q average = the average Q for every mushroom; Qm = the average Q in a single mushroom; Qm average = the average Qm for every mushroom; Q front = the Q of the front view; and Q side = the Q of the side view; pleurocystidia, the cystidia on the gill side; cheilocystidia, the cystidia on the gill edges; caulocystidia, the cystidia on the stipe; pileocystidia, the cystidia on the pileus; chrysocystidia, cystidia with yellow content; leptocystidia, translucent cystidia; ixocutis, pellis with mucilage and repent hyphae; and hymeniderm, pellis with spherical to polygonal elements.

DNA extraction and amplification

The tissue of fruiting bodies was cut from the stipe for DNA extraction. Genomic DNA was extracted using Doyle and Doyle's cetyl trimethylammonium bromide (CTAB) extraction method (Doyle and Doyle, 1990).

For amplification of rDNA ITS1–5.8S–ITS2, each PCR tube contained 2.5 µl of 10X PCR reaction buffer, 0.5 µl of 10 mM dNTP, 1 µl of each primer (10 µM), 0.3 µl of Prime Taq Polymerase, and template DNA in a total volume of 25 µl. The primers used for the internal transcribed spacers ITS region were ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3'), and the thermal cycle procedure started at a denaturation

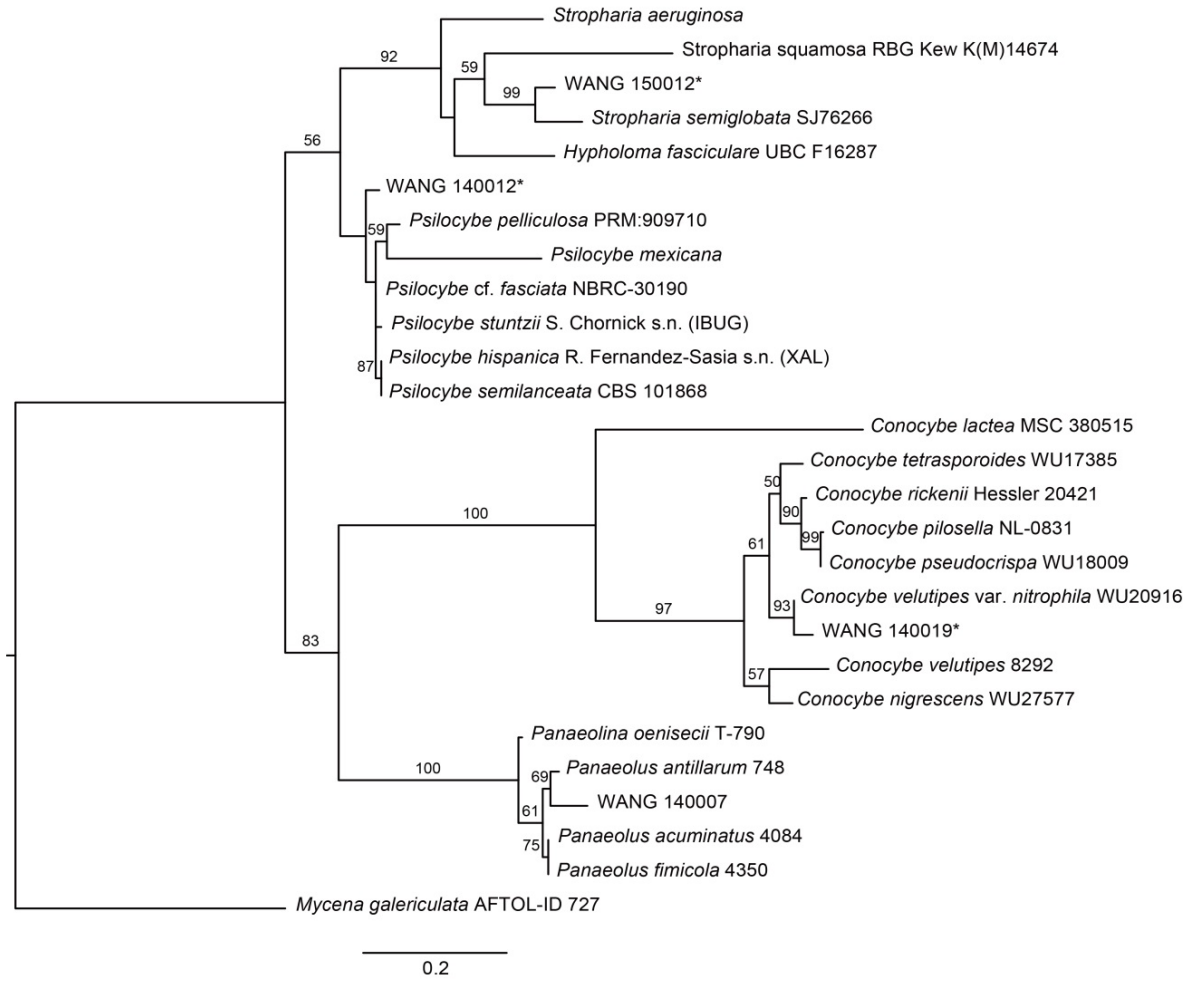

Fig 1. Maximum likelihood phylogenetic tree of the ITS region of specimens collected in this study (asterisks) and a few allies previously described. Label denotes the bootstrap value (>50).

step at 94°C for 5 min, followed by 30 cycles of 30 sec at 94°C, 30 sec at 55°C and 1 min at 72°C, and a final extension step at 72°C for 7 min (White *et al.*, 1990). For RPB1, the gRPB1-Af (5'-GAKTGTCCKGGWCATTTTGG-3') and fRPB1-Cr (5'-CNGCDATNTCRTTRTCCATRTA-3') primer pair was chosen for amplification. Thermal cycles started at 95°C for 5 min, followed by 7 cycles of 40 sec at 95°C, annealing temperature stepdowns of 1°C every cycle from 60°C to 53°C and 2 min at 72°C, and then 36 cycles of 45 sec at 94°C, 90 sec at 53°C and 2 min at 72°C, and a final extension step at 72°C for 10 min (Matheny *et al.*, 2002). The PCR products were confirmed with electrophoresis and sequenced using the ABI 3730XL system. The sequences were uploaded to NCBI databases with accession numbers KR998380–KR998384.

Phylogeny analysis

ITS sequences from this study was aligned with same regions of their allies in different genera from GenBank, NCBI, through MUSCLE in MEGA6 (Tamura *et al.*, 2013) (Appendix 1). Maximum likelihood phylogenetic tree was constructed with PhyML 3.1 (Guindon and Gascuel, 2003.) using TPM2uf+I+G model chosen with jModelTest 2.1.7 (Darriba *et al.*, 2012). Also, bootstrap of 100 replicates was analyzed to determine the confidence of the phylogenetic tree.

Phylogenetic tree construction

Total 27 represent sequences were selected and analyzed by the maximum likelihood in construction of phylogenetic tree. Except genus *Stropharia*, all of the genera were clustered together and most of the clades were supported by high bootstrap value, indicating the affinity and clarify their correlated taxonomic status. The four sequences obtained in this study were located in different genera. WANG 150012, WANG 140007 and WANG 140019 were clustered with *Stropharia semiglobata* SJ76266, *Panaeolus antillarum* 748 and *Conocybe velutipes* var. *nitrophila* WU20916, respectively. However, with a relatively low bootstrap value, WANG 140012 seemed to be a basal group of *Psilocybe* genus. (Fig. 1).

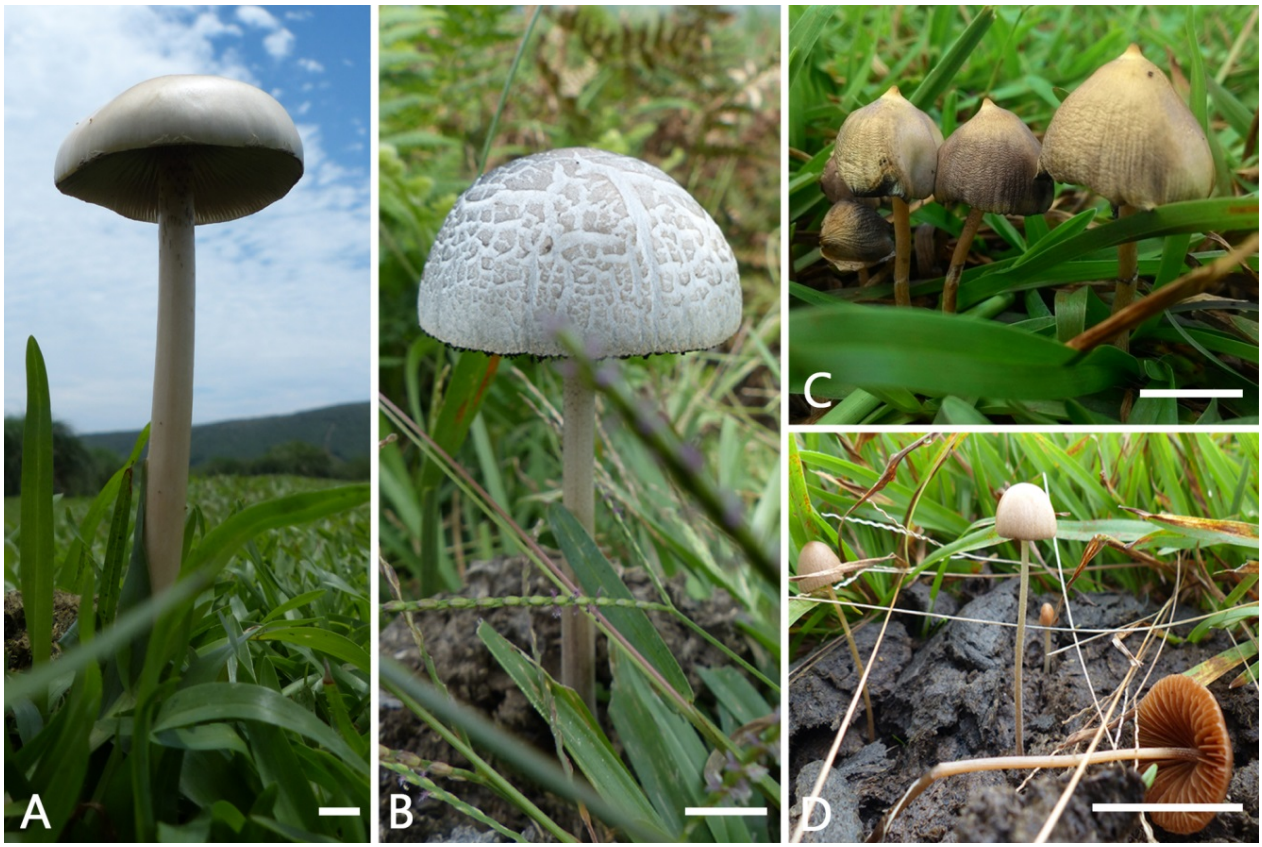

Fig 2. Habitats of dung-associated mushrooms on Qingtiangang grassland. A, *Protostropharia ovalispora*; B, *Panaeolus antillarum*; C, *Psilocybe angulospora*; D, *Conocybe nitrophila*. Bar = 1 cm.

TAXONOMIC TREATMENT

Protostropharia ovalispora Wang & Tzean, ***sp. nov.***
卵孢原球蓋菇 Figs. 2A, 3

Type: **TAIWAN**: Taipei: Qingtiangang, on cow dung, May 24, 2015, *YW Wang 150012* (TNM).

MycoBank: MB812762

Etymology: refers to the oval shape of basidiospores.

Pileus: 2–6 cm in diameter, light orange (5A5) in the middle, white at the margin and apricot yellow (5B6) to dust (5D2) at aged, campanulate to convex, with smooth straight margin, surface covered with thick glutinous veil, viscid in fresh samples, glabrous and slightly fibrous when dry, context fleshy, white. **Stipe:** 5–11 cm × 3–6 mm, orange white (5A2) at the upper part and pale red (7A3) at base, light orange (5A4) to grayish red (7B3) at aged, cylindrical but slightly clavate at base, centric, surface mucous and glabrous to slightly fibrous, sometimes with a faint dusty ring of spores, context fleshy, white, hollow. **Annulus:** not seen. **Volva:** not seen. **Lamella:** eye brown (7F6), brownish gray (7E2) when young and paler at the margin, closely aggregate and thin, with three or seven short lamellulae between two lamellae, with smooth margin, adnate, seceding at aged. **Spore print:** brownish gray

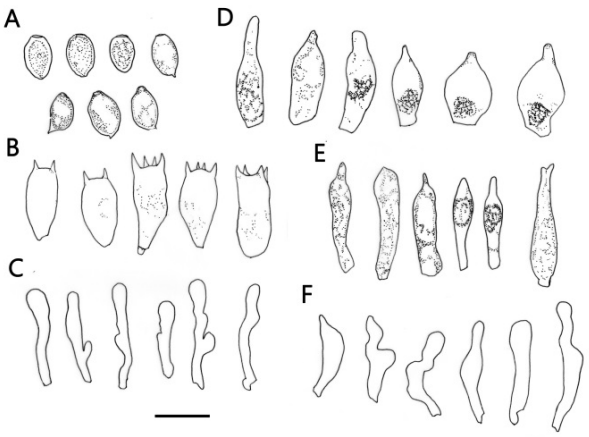

Fig 3. *Protostropharia ovalispora*. A, Basidiospores; B, Basidia; C, Cheilocystidia; D, Pleurocystidia; E, Chrysocaulocystidia; F, Leptocaulocystidia. Bar = 20 µm.

(11D2) to dark magenta (13F3) or black, reddish brown (8E4) when in a large mass. **Basidiospores:** 12.0–15.5 (–15.6) × 7.2–10.7 µm, average 13.6 × 8.9 µm, Q = 1.26–1.86, Q average = 1.53, Qm = 1.46–1.63, Qm average = 1.53 (51, 5), brownish orange (7C4) to reddish brown (9E5), brick red (7D7) in Meltzer's reagent, grayish orange (6B3) when young, broadly ellipsoidal to oval, smooth, thick-walled, often with

Table 1. Comparison of spores Q and the presence of several types of cystidia between *Protostropharia ovalispora* and *Pr. alcis* varieties in different studies.

Species and varieties	Spores Q (Qm)	Cheilochrysocystidia	Caulochrysocystidia	Reference
ovalispora	1.26–1.86 (1.46–1.63)	Occasionally	Present	This study
alcis* var. *alcis	1.68–2.03 (1.74–1.93)	Not seen	Not seen	Kytövuori, 1999
	1.8–2.0	Not seen	Not seen	Halama and Kudławiec, 2014
	1.6–1.9	?	?	Noordeloos, 2011
alcis* var. *austrobrasiliensis	1.44–2.11(1.73)	Not seen	Not seen	Cortez and da Silveira, 2008

various-sized vacuoles, with a large apical centric germ pore. **Basidia**: 23.9–31.4 × (9.6–) 10.8–13.7 µm (19, 3), 4-spored, clavate. **Pleurocystidia:** chrysocystidia, 22.7–36.9 × 9.6–13.4 (–16.0) µm (25, 3), broadly cylindrical to clavate, usually with a broadly mucronate apex, with golden yellow inclusions in Meltzer's reagent, occasionally found on gill edges. **Cheilocystidia:** leptocystidia, 19.3–35.5 × (4.1–)4.4–7.3 µm (31, 3), cylindrical to narrowly spathulate, hyaline, clustered, abundant on gill edges. **Pileipellis:** ixocutis type, consisting of gelatinized, filamentous hyphae, 2.3–2.8(–3.4) µm in diameter. **Caulocystidia:** two types: Leptocaulocystidia 23.7–48.0 × 5.1–9.0 (17, 3) fusiform to narrowly spathulate, sometimes curved, hyaline, clustered, similar to cheilocystidia; Chrysocaulocystidia 26.7–45.2 (–53.5) × 6.7–10.2 µm (18, 2), broadly cylindrical to fusiform, with a broadly mucronate apex, with golden yellow inclusions in Meltzer's reagent, similar to pleurocystidia, only on the top of stipe. **Stipitipellis:** ixocutis type, consisting of gelatinized, filamentous, parallel hyphae, 1.8–3.0 µm in diameter. **Acanthocytes:** absent. **Clamp connection:** present.

Habitat: on cow dung, single or scattered, occasionally clustered.

Additional specimen examined: **TAIWAN**: Taipei: Qingtiangang, on cow dung, May 4, 2014, coll. CC Chen; GC 1405-32 (TNM F0009747).

Notes: The specimen WANG 150012 is morphologically similar to *Stropharia alcis*, first described in Finland by Kytövuori (1999), most of which were found on elk dung in Europe (Halama and Kudławiec, 2014; Noordeloos, 2011). In 2007, a new variety, *S. alcis* var. *austrobrasiliensis* Cortez and da Silveira (2008), was reported in Brazil mainly due to the difference of the habitat, which is commonly observed on cow dung or manured soil. Although sharing a similar morphology with *S. alcis* and having the same habitat with *S. alcis* var. *austrobrasiliensis*, WANG 150012 differs in having more globoid spores, shorter pleurocystidia, and the appearance of chrysocystidia on the gill edges and the top of the stipe (Table 1). Therefore, we suggest it as a new species *ovalispora* and use *Protostropharia* as its genus name based on the absence of acanthocytes and possessing a slimy stipe (Moncalvo *et al.*, 2002; Redhead, 2013a, b). Additionally, we suggest that *Stropharia alcis* var. *austrobrasiliensis* should be transferred to *Protostropharia* genus to match with its species name *Protostropharia alcis.*

Searching the NCBI databases with ITS1 and ITS2 regions, in addition to uncultured fungus clones, *Stropharia semiglobata* shared the most analogous ITS1 and ITS2 sequence of *Protostropharia ovalispora* in the databases with an identity of 94% (289/306) (AY129368) and 95% (293/308) (EU029943). Compared with the whole ITS region of *Protostropharia semiglobata* F0021935, which was sequenced in this study, they also shared a 98% (818/833) identity. In phylogeny study, WANG 150012 also clustered with *Protostropharia semiglobata* SJ76266 (Bootstrap = 99), fully supported the close relativeness (Fig. 1).

In Taiwan, a few specimens of species allied to *Protostropharia ovalispora* have been deposited in National Museum of Natural Science. One specimen, F0027807, collected by C.C. Chen was described as *Psilocybe* sp., having a spore size of 11.4–14.4 × 7.9–9.9 µm, Q = 1.28–1.74, which agrees with our specimen, and the ITS sequence also shares an identity of 99% with WANG 150012. Therefore, the *Psilocybe* sp. is misidentified and considered to be identical to the currently described *Protostropharia ovalispora*.

Panaeolus antillarum (Fr.) Dennis, Kew Bulletin 15 (1): 124 (1961) 安地列斯斑褶菇 Figs. 2B, 4

Agaricus antillarum Fr., Elenchus Fungorum 1: 42 (1828)
Psilocybe antillarum (Fr.) Sacc., Sylloge Fungorum 5: 1052 (1887)
Anellaria antillarum (Fr.) Hlavácek, Mykologický Sbomík: 52 (1997)

Pileus: 1–4 cm in diameter, grayish orange (5B5) to white at the middle and white at the margin, birch gray (5C2) at aged and sometimes stained black, hemispheric to campanulate, with smooth straight margin, surface subviscid and a little glabrous in fresh samples, crackling at aged, context fleshy, white. **Stipe:** 4–13 cm × 2–4 mm, orange white (5A2) at the upper part and honey yellow (5D6) at base, cylindrical, slightly clavate at base, centric, surface mostly smooth but with indistinct small scales, context fleshy, white, solid. **Annulus:** not seen. **Volva:** not seen. **Lamella:** brownish gray (6F2) at aged, brownish orange (6C3) when young, slightly paler at the margin, closely aggregate and thin, with one or three short lamellulae between two lamellae, with smooth margin, narrowly

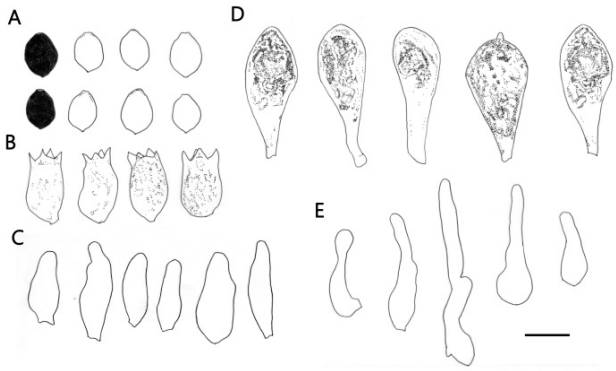

Fig 4. *Panaeolus antillarum*. A, Basidiospores; B, Basidia; C, Cheilocystidia; D, Pleurocystidia; E, Caulocystidia. Bar = 20 µm.

adnate. **Spore print:** brownish gray (7D2) to black. **Basidiospores:** 14.5–20.1 × 9.5–13.0(–14.6) µm, average 17.0 × 11.6 µm, Q = 1.18–1.86, Q average = 1.47, Qm = 1.35–1.62 µm, Qm average = 1.47 (59, 6), caput mortuum (8F7) to black, dark brown (8F5) to black in Meltzer's reagent, brownish orange (6C4) when young, broadly ellipsoidal to oval and a little limoniform, smooth, thick-walled, sometimes with vacuoles, with a centric apical germ pore. **Basidia:** 20.7–32.0(–39.3) × 13.1–17.8 µm (20, 4), 4-spored, broadly fusiform to clavate. **Pleurocystidia:** chrysocystidia, 38.8–60.1(–76.2) × 18.5–23.6(–26.1) µm (15, 4), broadly clavate, sometimes with an obtuse apex, occasionally with broadly mucronate apex, with golden yellow inclusions in Meltzer's reagent. **Cheilocystidia:** leptocystidia, 30.0–49.9 × 6.6–13.9 µm (21, 3), fusiform to narrowly spathulate, hyaline, clustered, abundant on gill edges, rarely observed at aged. Pileocystidia: not seen. Pileipellis: hymeniderm type, consisting of inflated pyriform cells, 6.9–14.2 µm wide. **Caulocystidia:** leptocystidia, 38.4–70.3 µm in length, 4.7–9.3(–13.9) µm at apex, (7.1–) 8.0–11.7(–14.9) µm at base (20, 4), lageniform to slightly lecithiform, hyaline, somewhat similar to cheilocystidia, hyaline, clustered, present on the whole stipe. **Stipitipellis:** consisting of filamentous, rather thick-walled, parallel hypha, 19.4–44.5 µm in diameter. **Clamp connection:** present.

Habitat: on cow dung, single or scattered.

Specimen examined: **TAIWAN**: Taipei: Qingtiangang, on cow dung, Aug. 14, 2014, *YW Wang 140007*; Aug. 16, 2014, *YW Wang 140009*.

Notes: This species is cosmopolitan and has been recorded in several countries, including India, Europe, Africa, continental USA and Hawaii, Mexico, Venezuela, etc. (Dennis, 1961; Dennis, 1970; Doveri, 2011; Guzmán, 1973; Halama *et al.*, 2014; Kaur *et al.*, 1918; Manimohan *et al.*, 2007; Merlin and Allen, 1993; Pegler, 1968; Rommelaars and Arnolds, 2007). The specimen examined in this study appears similar to the descriptions from Rommelaars and Pelger, despite having smaller pilei, but remains acceptable (Pegler, 1968; Rommelaars and Arnolds, 2007).

In Taiwan, there is no previous record of *Pa. antillarum*; however, several binomial names that are considered synonymous to *Pa. antillarum* have been described. The synonym first used in Taiwan is *Panaeolus solidipes* (Sawada, 1931; Shao, 2009). The descriptions are very similar. However, the pleurocystidia of specimens examined by Sawada appears not as chrysocystidia. Another synonym, *Anellaria sepulchralis*, has been described from the outlying island of Taiwan, Lan-Yu, in 1991 (Shao, 2009; Yeh and Chen, 1991). In addition to the size, the only difference can be noted through the descriptions of Yeh's specimens and ours, which is that Yeh's specimens lack cheilocystidia. However, in our examination, we found that the older the specimen is, the more difficult it is to access the cheilocystidia. Therefore, we believe that Yeh's specimens were too old for the cheilocystidia to be found. Additionally, there are two other specimens of *Pa. antillarum* deposited in the National Museum of Natural Science, which were collected in China, under the synonyms *Pa. semiovatus* (F0021899) and *A. semiovata* (F0021940).

The top hit of the blasting of the whole ITS region was the *Panaeolus antillarum* ITS region (JF908515) with an identity of 97% (602/618). The second to fourth top hits were the ITS regions of *Pa. fimicola* (JF908514), *Pa. acuminatus* (JF908518) and *Pa. foenisecii* (syn. *Panaeolina foenisecii*) (KC176293), with identities of 97% (592/608), 97% (597/618) and 96% (593/618), respectively. Phylogeny also showed that *Panaeolus antillarum* 748 (JF908515) was claded with our specimen, supporting that they belonged to a same species (Fig. 1).

Compared with other allied species that shared a high identity of the ITS region, *Pa. antillarum* and *Pa. fimicola* have darker pilei, pileipellis composed of pileocystidia and smaller spores. *Panaeolina foenisecii* also has a brown pileus and roughened spores that easily separate it from other species (Breitenbach and Kranzlin, 1984).

The genus *Panaeolus* consists of a large set of psychoactive mushrooms, including *Pa. fimicola*, *Pa. olivaceus*, *Pa. papilionaceus*, etc. (Guzmán *et al.*, 1998). However, *Pa. antillarum* was recorded as a non-psychoactive mushroom species in most reports (Stamets, 1996).

At least six *Panaeolus* and three *Anellaria* species have been recorded in Taiwan (Shao, 2009). *Pa. acuminatus*, *Pa. fimicola*, *Pa. olivaceus*, *Pa. papilionaceus* and *Pa. subbalteatus* have pileipellis composed of pileocystidia, and the pilei of all of them except *Pa. papilionaceus* are rather dark (Breitenbach and Kranzlin, 1984; Kaur *et al.*, 1918). Both *A. ochroleuca* and *A. planiuscula* have annuli on their stipes (Sawada, 1931).

Psilocybe angulospora Wang & Tzean, ***sp. nov.***

角孢裸蓋菇 Figs. 2C, 5

Type: **TAIWAN**: Taipei: Qingtiangang, on heavily manured soil, Aug. 21, 2014, *YW Wang 140012* (TNM).

MycoBank: MB812763

Etymology: refers to the slightly angular basidiospores.

Pileus: 0.8–2 cm in diameter, light brown (6D5) to yellowish white (4A2), brown (7E5) at aged, eye gray (23D2) to dark blue (23F5) when bruised, hygrophanous, campanulate, often with an acute papilla, with striate and a slight incurve to straight margin, surface glabrous and slightly fibrous, context sturdy, grayish orange (5B3) to yellowish white (4A2). **Stipe:** 4–6 cm × 1–2 mm, orange white (5A2) to birch bark gray (6B2), eye gray (23D2) to dark blue (23F5) when bruised, cylindrical, centric, surface smooth, glabrous and slightly fibrous, context sturdy, golden blonde (5C4) to orange white (5A2), hollow at top but filled with white fibrous pith under position comparable to annulus. **Annulus:** absent, but partial veil often leaving appressed, fragile, grayish brown (11F4) to black fibrils at nearly the middle of the stipe. **Volva:** not seen. **Lamella:** violet brown (11F6), brownish gray (11D2) when younger, closely aggregate and thin, with one or three short lamellulae between two lamellae, with smooth margin, narrowly adnate. **Spore print:** not studied.

Basidiospores: 7.6–10.2(–11.5) × 5.8–8.1 × 4.7–7.1 µm, average 9.3 × 6.8 × 5.8 µm, Q front = 1.08–1.64(–1.77), average Q front = 1.37, Qm front = 1.33–1.41, average Qm front = 1.37, Q side = 1.27–1.94(–2.04), average Q side = 1.61, Qm side = 1.51–1.63(–1.81), average Qm side = 1.62 (104, 5), reddish gray (12C2), grayish orange (6B4) to cinnamon brown (6D6) in Meltzer's reagent, subrhomboid at front, ellipsoidal to oval at side, smooth, thick-walled, sometimes with vacuoles, with an eccentric large germ pore that appears centric in the front view. **Basidia:** 20.9–27.2(–32.2) × 6.1–10.4 µm (19, 4), 4-spored, broadly fusiform to broadly clavate. **Pleurocystidia:** not seen. **Cheilocystidia:** leptocystidia, 16.4–26.3(–29.2) µm in length, (1.6–)1.8–3.0(–3.6) µm wide at apex, (3.6–)4.5–7.1 µm wide at base (37, 4), fusiform to lageniform, sometimes bifurcate, hyaline, clustered, abundant on gill edges. **Pileipellis:** gelatinized hyphae not seen. **Hypodermium:** consisting of inflated, filamentous hyphae, 6.3–17.0 µm in diameter. **Caulocystidia:** not seen. **Stipitipellis:** consisting of short-segmented, inflated, filamentous, rather thick-walled, parallel hyphae, 9.3–22.3 µm in diameter. **Clamp connection:** present.

Habitat: on heavily manured soil, scattered.

Additional specimen examined: **TAIWAN**: Taipei: Qingtiangang, on cow dung, Aug. 28, 2014, *YW Wang 140015*.

Notes: Using the key proposed by Guzmán (1983), we found that the specimen was likely to be a species of *Psilocybe* genus in *Mexicanae* section due to the shape of the spores. The absence of pleurocystidia, the size of spores, and the shape of cheilocystidia and pileus indicate that it is somewhat more similar to *Ps. mexicana* than other species. However, the ITS region of our specimen is substantially more similar to *Ps. semilanceata* (98%) than *Ps. mexicana* (93%). Comparing our specimen to *Ps. semilanceata*, our specimen has slightly angled spores that are in agreement with the description of *Sect. Mexicanae*. However, the extraordinary acute papilla, described by Guzmán as "spear-like pileus", indicates that it is yet similar to *Ps. semilanceata*. Additionally, we sequenced the RPB1 gene and blasted the NCBI database. The top hit was the RPB1 sequence of *Ps. stunzii*, which is phylogenetically close to *Ps. semilanceata*, with an identity of 98% (818/833). Ramírez-Cruz reported that evolution of spore shape in *Psilocybe* and *Deconica* is phylogenetically diverse (Ramírez-Cruz *et al.*, 2013). This fact might explain our observations. That is, our specimen is more similar to the oval-spored *Ps. semilanceata* and *Ps. stuntzii*, but is a new species in which the spore has evolved into an angled shape.

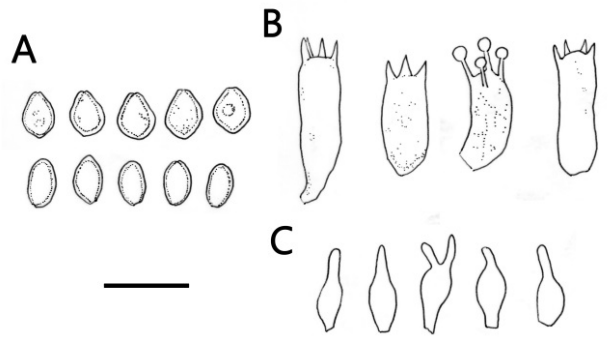

Fig 5. *Psilocybe angulospora*. A, Basidiospores; B, Basidia; C, Cheilocystidia. Bar = 20 µm.

The ITS region of *Psilocybe angulospora* shares the highest identity, 98% (624/639), with *Ps. semilanceata* (HF912359). Another sequence of different species that shares the same identity is the ITS region of *Ps. fasciata* (DQ001401). However, there is a large difference between this sequence and the other strain of *Ps. fasciata* (AB158635). In the ML tree we constructed, it was clustered with *Psilocybe* genus and seemed to be a rather old lineage among these species, but needed multiple DNA markers included to support the phylogenetic analysis (Fig. 1).

In Taiwan, only five species of *Psilocybe* have been described (Shao, 2009; Tzean *et al.*, 2015), but none shares a similar morphology with our specimen. While *Ps. coprophila* can be easily distinguished by not have have significantly larger pileus, *Ps. subcaerulipes* has smaller and thinner-walled spores, and *Ps. ericaea* has been excluded from *Psilocybe* and is being transferred into *Hypholoma* (Breitenbach and Kranzlin, 1984; Guzmán, 1983).

Table 2. Comparisons of spore size and pileocystidia shape between *Conocybe velutipes* and *C. nitrophila* in different studies.

Species	Spores size (μm)	Pileocystidia	Reference
Conocybe nitrophila	11.8–16.0 × 7.4–9.9	not seen	This study
	11–15.5 × 6.5–9.5	not seen	Hausknecht, 2009
Conocybe velutipes	9.5–11.5 × 6–8	hair-like	Prydiuk, 2007
	9.5–13 × 5.5–8.5	hair-like	Hausknecht, 2005

Conocybe nitrophila (Hauskn.) Wang & Tzean, ***comb. nov.*** 嗜氮錐蓋傘 Figs. 2D, 6

MycoBank: MB812764

Conocybe velutipes var. *nitrophila* Hauskn., Conocybe-Pholiotina. Fungi Europaei 11: 377 (2009)

Pileus: 0.8–1.5 cm in diameter, orange white (5A2) to light orange (5A4), raw sienna (6D7) at aged and moist, hygrophanous, hemispherical to a little convex, slightly striate and straight at the margin, surface smooth, context sturdy, pale orange (5A3). **Stipe:** 6–8 cm × 1 mm, grayish orange (6B5) to orange white (5A2), paler at the base, cylindrical, centric, surface smooth but with delicate and fine hair at the top, context sturdy, grayish orange (6B4) to pale orange (5A3), hollow. **Annulus:** absent. **Volva:** not seen. **Lamella:** golden yellow (5B7) to brownish yellow (5C8), closely aggregate and thin, with three short lamellulae between two lamellae, with smooth margin, adnate. **Spore print:** flesh orange (6B3) to brownish orange (7C5).

Basidiospores: 11.8–16.0 (–19.9) × 7.4–9.9 (–11.3) μm, average 14.3 × 8.5 μm, Q front = (1.35–) 1.43–1.92 (–2.04), average Q = 1.70, Qm = 1.57–1.80, average Qm = 1.69 (58, 5), reddish golden (6C7), cadmium orange (5A8) to brownish orange (6C8) in Meltzer's reagent, broadly ellipsoid to oval, smooth, thick-walled, usually with vacuoles, with a large centric germ pore. **Basidia:** 17.3–28.5 × 9.5–13.2 μm (25, 3), 4-spored, broadly clavate. **Pleurocystidia:** not seen. **Cheilocystidia:** leptocystidia, 15.6–23.9 (–28.3) μm in length, (2.2–) 2.8–4.5 (–5.1) μm wide at apex, 5.9–10.0 (–10.5) μm wide at base (31, 3), lecithiform, hyaline, clustered, abundant on gill edges. **Pseudoparaphyses:** not seen. **Pileocystidia:** not seen. **Pileipellis:** hymeniderm type, consisting of spheropedunculate cells, 16.7–39.5 μm wide. **Caulocystidia:** two types: Cylindrical-bent hairs, 41.5–87.1 (–125.9) × 2.7–3.1 (–3.5) μm (21, 3), filamentous and curved, hyaline, scattered, present on the upper part of stipe; Fusiform elements, 5.1–27.1 × 2.3–6.9 μm (27, 3), irregular to narrowly spathulate or ellipsoid, sometimes furcated, hyaline, clustered, occasionally present on the upper part of stipe; lecythiform caulocystidia not seen. **Stipitipellis:** consisting of inflated, filamentous, parallel hyphae, 12.8–31.0 μm in diameter. **Clamp connection:** present.

Habitat: on cow dung, scattered.

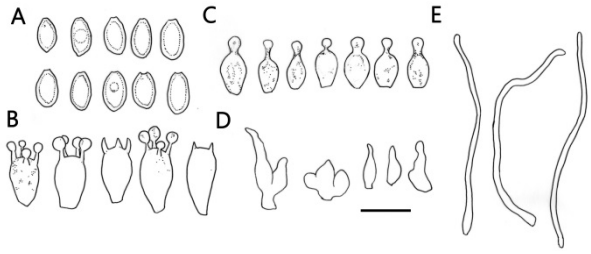

Fig 6. *Conocybe nitrophila*. A, Basidiospores; B, Basidia; C, Cheilocystidia; D, Caulocystidia, fusiform elements-type; E, Caulocystidia, cylindrical-bent hairs-type. Bar = 20 μm.

Specimen examined: **TAIWAN**: Taipei: Qingtiangang, on cow dung, Aug. 26, 2014, *YW Wang 140019*; Oct. 23, 2014, *YW Wang 140019*.

Notes: Morphologically, the currently described *Conocybe nitrophila combination nova* was originally described as *C. velutipes* var. *nitrophila* by Hausknecht and differed from the nominotypical variety by having bigger spores and living in a nitrogen-rich habitat. The descriptions of *C. velutipes* var. *nitrophila* are identical to our specimen. Additionally, we did not find pileocystidia, which was absent in the Hausknecht record but can be found in *C. velutipes*, supporting that this characteristic might be another difference between these two taxa (Table 2) (Hausknecht, 2005, 2009; Prydiuk, 2007).

In the molecular aspect, the blasting result also indicates that our specimen shares a high identity (99%) with *C. velutipes* var. *nitrophila* strain WU20916, which again confirms the morphological identification. In contrast, the ITS region of *C. velutipes* only shares an identity of 91% to 92% with our specimen. The phylogenetic tree also suggested that *C. velutipes* and *C. velutipes* var. *nitrophila* were polyphyletic group (Fig. 1). This result indicates that the specimen we found and the *C. velutipes* var. *nitrophila* strain WU20916 should be distantly related to *C. velutipes*. Summing up the molecular and morphological evidence, we suggest that *C. velutipes* var. *nitrophila* should be emended and treated as an independent species, with *C. nitrophila* belonging to section *Pilosellae* (Hausknecht and Krisai-Greilhuber, 2006). Blasting the ITS sequence also revealed several closely related species, such as *C. pseudocrispa*, *C. pilosella* and *C. tetrasporoides*, of which, their ITS sequences share 96% (603/629), 96% (602/629) and 95% (599/629) identities, respectively.

The genus *Conocybe* also consists of several hallucinogenic species, such as *C. kuehneriana*, *C. siligineoides*, *C. cyanopus*, and *C. smithii* (Guzmán *et al.*, 1998). However, the psychoactivity of *C. nitrophila* has not been previously described and needs to be verified. In Taiwan, three *Conocybe* species have been recorded (Shao, 2009). *C. albipes* has a conical, cream-white pileus and belongs to section *Candidae*, which has pseudoparaphyses (Hausknecht, 1998; Hausknecht and Krisai-Greilhuber, 2006). *C. incarnata* is wine-red and has smaller basidia and basidiospores (Hausknecht, 2005; Hausknecht and Krisai-Greilhuber, 2006, 2009). However, *C. tenera* has smaller basidiospores and belongs to section *Conocybe*, of which caulocystidia is mainly lecythiform (Breitenbach and Kranzlin, 1984; Enderle and Hübner, 1999; Hausknecht, 2000; Hausknecht and Krisai-Greilhuber, 2006).

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Appendix 1. rDNA nucleotide sequences used in phylogenetic analysis and their accession numbers.

Species	Specimen/Strains*	Accession No.
Conocybe lactea	MSC 380515	AY213998
Conocybe nigrescens	WU27577	JX968234
***Conocybe nitrophila*, comb. nov.**	WU20916	JX968233
***Conocybe nitrophila*, comb. nov.**	WANG 140019*	KR998384
Conocybe pilosella	NL-0831	JX968231
Conocybe pseudocrispa	WU18009	JX968230
Conocybe rickenii	Hessler 20421	AY194541
Conocybe tetrasporoides	WU17385	JX968232
Conocybe velutipes	8292	JF907832
Hypholoma fasciculare	UBC F16287	EU486442
Mycena galericulata	AFTOL-ID 727	DQ404392
Panaeolina foenisecii	T-790	KC176293
Panaeolus acuminatus	4084	JF908518
Panaeolus antillarum	748	JF908515
Panaeolus antillarum	WANG 140007*	KR998382
Panaeolus fimicola	4350	JF908519
***Protostropharia ovalispora*, sp. nov.**	WANG 150012*	KR998381
Protostropharia semiglobata	SJ76266	EU029943
***Psilocybe angulospora*, sp. nov.**	WANG 140012*	KR998383
Psilocybe* cf. *fasciata	NBRC-30190	AB158635
Psilocybe hispanica	R. Fernandez-Sasia s.n. (XAL)	KC669289
Psilocybe mexicana	-	HM035083
Psilocybe pelliculosa	PRM:909710	HF912358
Psilocybe semilanceata	CBS 101868	HM035080
Psilocybe stuntzii	S. Chornick s.n. (IBUG)	KC669295
Stropharia aeruginosa	-	JF961355
Stropharia squamosa	RBG Kew K(M)14674	EU784419

***Sequenced in this study**