**Taiwania 60**(4):160–168, 2015
*DOI: 10.6165/tai.2015.60.160*

# Dung-associated, Potentially Hallucinogenic Mushrooms from Taiwan

**Yen-Wen Wang and Shean-Shong Tzean***

*Department of Plant Pathology and Microbiology, National Taiwan University, Taipei 10617, Taiwan.*
**Corresponding author: Tel: 886-2-3366-4595; Email: sst@ntu.edu.tw*

(Manuscript received 2 June 2015; accepted 30 October 2015)

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



臺灣大學學術期刊資料庫

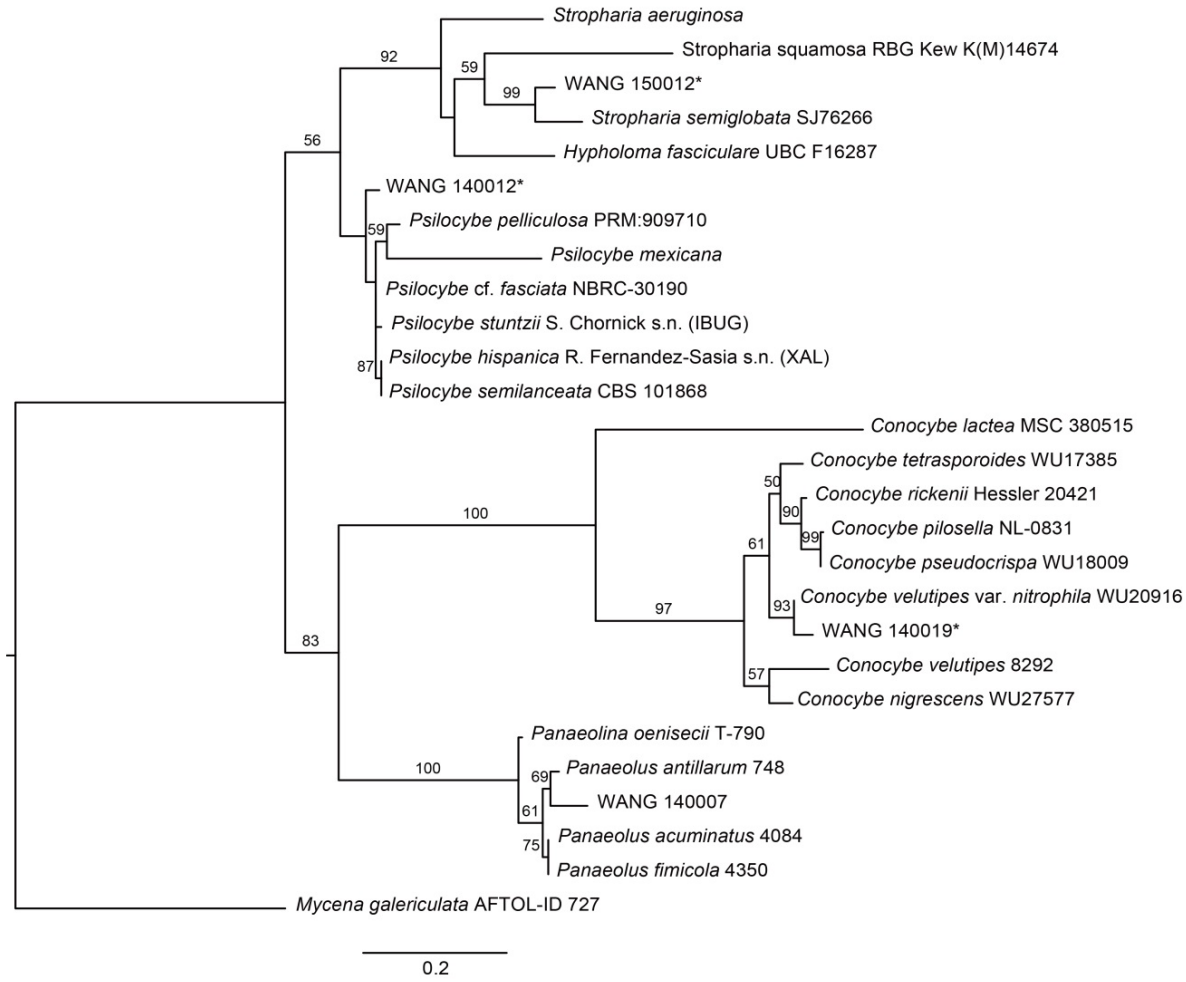



**Fig 1. Maximum likelihood phylogenetic tree of the ITS region of specimens collected in this study (asterisks) and a few allies previously described. Label denotes the bootstrap value (>50).**

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).



臺灣大學學術期刊資料庫

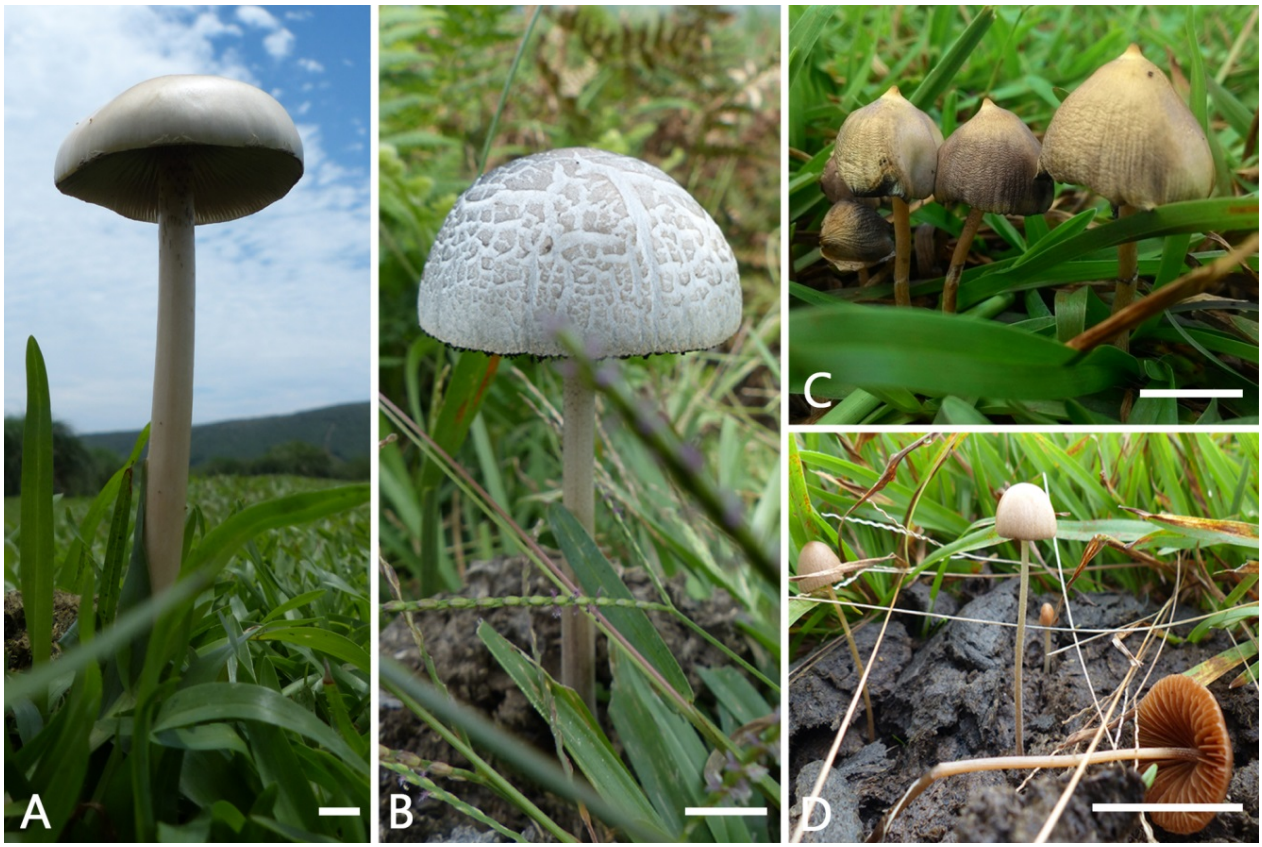



**Fig 2. Habitats of dung-associated mushrooms on Qingtiangang grassland. A, *Protostropharia ovalispora*; B, *Panaeolus antillarum*; C, *Psilocybe angulospora*; D, *Conocybe nitrophila*. Bar = 1 cm.**

## 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

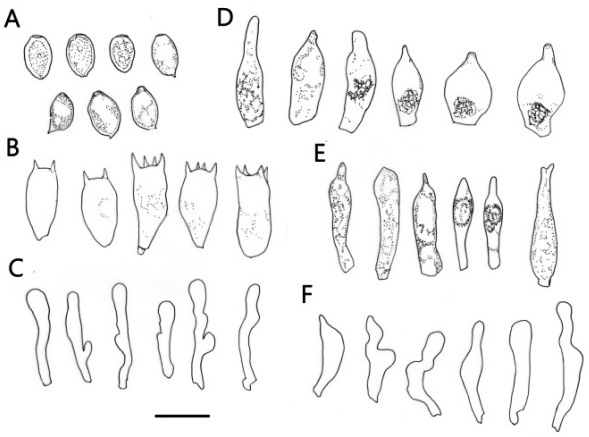



**Fig 3. *Protostropharia ovalispora*. A, Basidiospores; B, Basidia; C, Cheilocystidia; D, Pleurocystidia; E, Chrysocaulocystidia; F, Leptocaulocystidia. Bar = 20 µm.**

(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



臺灣大學學術期刊資料庫

**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 astrocystidia 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



臺灣大學學術期刊資料庫

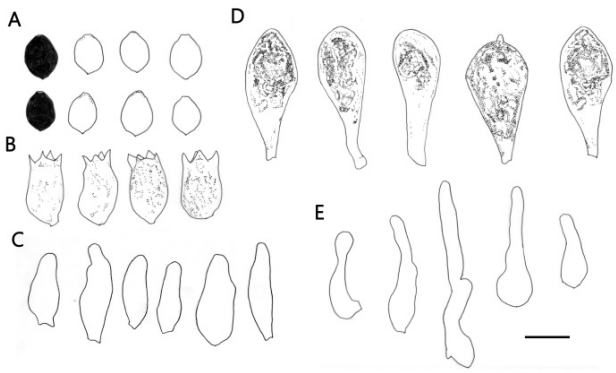



**Fig 4. *Panaeolus antillarum*. A, Basidiospores; B, Basidia; C, Cheilocystidia; D, Pleurocystidia; E, Caulocystidia. Bar = 20 µm.**

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).



臺灣大學學術期刊資料庫

***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.

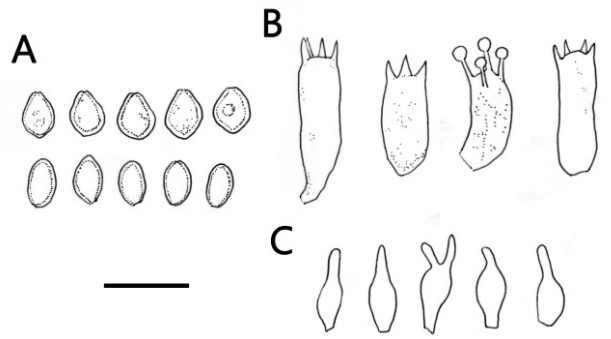



**Fig 5. *Psilocybe angulospora*. A, Basidiospores; B, Basidia; C, Cheilocystidia. Bar = 20 µm.**

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).

臺灣大學學術期刊資料庫

**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.

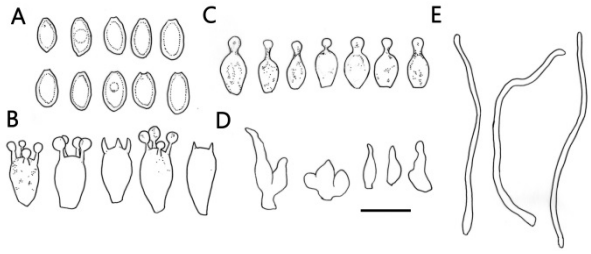



**Fig 6. *Conocybe nitrophila*. A, Basidiospores; B, Basidia; C, Cheilocystidia; D, Caulocystidia, fusiform elements-type; E, Caulocystidia, cylindrical-bent hairs-type. Bar = 20 μm.**

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.



臺灣大學學術期刊資料庫

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).

## ACKNOWLEDGEMENTS

The authors thank S.-T. Shieh and W.-S. Hsiao for providing information regarding the hallucination case; Dr. S.-H. Wu, National Museum of Natural Science, Taiwan for loaning us specimens for the comparative study; and Yangmingshan National Park for the permission of specimen collecition.

## LITERATURE CITED

**Breitenbach, J. and F. Kranzlin.** 1984. Fungi of Switzerland. Vol. 4. Mykologia, Lucerne, Switzerland.

**Cortez, V. G. and R. M. B. da Silveira.** 2008. The agaric genus *Stropharia* (Strophariaceae, Agaricales) in Rio Grande do sul state, Brazil. Fungal Divers. **32:** 31-57.

**Darriba, D., G. L. Taboada, R. Doallo and D. Posada.** 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods **9:** 772-772

**Dennis, R.** 1961. Fungi Venezuelani: IV. Agaricales. Kew Bulletin. 67-ii.

**Dennis, R. W. G.** 1970. Fungus flora of Venezuela and adjacent countries. Royal Botanic Garden, Kew, London, UK.

**Doveri, F.** 2011. Additions to "Fungi Fimicoli Italici": An update on the occurrence of coprophilous basidiomycetes and ascomycetes in Italy with new records and descriptions. Mycosphere **2:** 331-427.

**Doyle, J. J. and J. L. Doyle.** 1990. Isolation of plant DNA from fresh tissue. Focus **12:** 13-15.

**Enderle, M. and H. Hübner.** 1999. *Conocybe-Pholiotina*-studien VIII. Z. Mykol. **65:** 3-22.

**Guindon, S. and O. Gascuel.** 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. **52:** 696-704.

**Guzmán, G. 1973.** Some distributional relationships between Mexican and United States mycofloras. Mycologia **65:** 1319-1330.

**Guzmán, G. 1983.** The genus *Psilocybe*: A systematic revision of the known species including the history, distribution, and chemistry of the hallucinogenic species. Nova Hedwigia, Stuttgart, Germany.

**Guzmán, G., J. W. Allen and J. Gartz.** 1998. A worldwide geographical distribution of the neurotropic fungi, an analysis and discussion. Ann. Mus. civ. Rovereto. Sez.: Arch., St., Sc. nat. **14:** 189-280.

**Halama, M. and B. Kudławiec.** 2014. New localities of *Protostropharia alcis* (Basidiomycota, Agaricales) in Poland. Acta Mycol. **49:** 47-57.

**Halama, M., D. Witkowska, I. Jasicka-Misiak and A. Poliwoda.** 2014. An adventive *Panaeolus antillarum* in Poland (Basidiomycota, Agaricales) with notes on its taxonomy, geographical distribution, and ecology. Cryptogamie Mycol. **35:** 3-22.

**Hausknecht, A.** 1998. Beiträge zur kenntnis der Bolbitiaceae 4. Die sektion *Candidae* und andere hellhütige arten der gattung *Conocybe*. Österr. Z. Pilzk. **7:** 91-121.

**Hausknecht, A.** 2000. Beiträge zur kenntnis der Bolbitiaceae 6. Die *Conocybe tenera*-gruppe in Europa, teil 1. Österr. Z. Pilzk. **9:** 73-109.

**Hausknecht, A.** 2005. Beiträge zur kenntnis der Bolbitiaceae 10. *Conocybe* sektion *Pilosellae*. Österr. Z. Pilzk. **14:** 191-274.

**Hausknecht, A. and I. Krisai-Greilhuber.** 2006. Infrageneric division of the genus *Conocybe*-a classical approach. Österr. Z. Pilzk. **15:** 187-212.

**Hausknecht, A.** 2009. *Conocybe* - *Pholiotina*. Fungi Europaei. Candusso Edizioni, Alassio, Italy.

**Hausknecht, A. and I. Krisai-Greilhuber.** 2009. Two new *Conocybe* species from Europe and corrections to the monograph *Conocybe-Pholiotina*. Österr. Z. Pilzk. **18:** 183-195.

**Hawksworth, D. L.** 1991. The fungal dimension of biodiversity: Magnitude, significance, and conservation. Mycological research **95:** 641-655.

**Kaur, A., N. S. Atri and M. Kaur.** 1918. Diversity of coprophilous species of *Panaeolus* (Psathyrellaceae, Agaricales) from Punjab, India. Mycologia **10:** 31.

**Kornerup, A. and J. H. Wanscher.** 1978. Methuen handbook of colour. 3 edn. Eyre Methuen, London, UK.

**Kytövuori, I.** 1999. The *Stropharia semiglobata* group in NW Europe. Karstenia **39:** 11-32.

**Manimohan, P., K. A. Thomas and V. Nisha.** 2007. Agarics on elephant dung in Kerala state, India. Mycotaxon **99:** 147-158.

**Matheny, P. B., Y. J. Liu, J. F. Ammirati and B. D. Hall.** 2002. Using RPB1 sequences to improve phylogenetic inference among mushrooms (*Inocybe*, Agaricales). Am. J Bot. **89:** 688-698.

**Merlin, M. D. and J. W. Allen.** 1993. Species identification and chemical analysis of psychoactive fungi in the Hawaiian islands. J Ethnopharmacol. **40:** 21-40.

**Moncalvo, J. M., R. Vilgalys, S. A. Redhead, J. E. Johnson, T. Y. James, M. C. Aime, and O. K. Miller, Jr.** 2002. One hundred and seventeen clades of euagarics. Mol. Phylogenet. Evol. **23:** 357-400.

**Noordeloos, M. E.** 2011. Strophariaceae s.l. Fungi Europaei. 13. Edizioni Candusso, Alassio, Italy.

**Pegler, D.** 1968. Studies on African Agaricales: I. Kew Bulletin **21:** 499-533.

**Prydiuk, M. P.** 2007. New records of *Conocybe* species from Ukraine. I. The sections *Mixtae* and *Pilosellae*. Czech Mycol. **59:** 25-38.

臺灣大學學術期刊資料庫

**Ramírez-Cruz, V., G. Guzmán, A. R. Villalobos-Arámbula, A. Rodríguez, P. B. Matheny, M. Sánchez-García and L. Guzmán-Dávalos.** 2013. Phylogenetic inference and trait evolution of the psychedelic mushroom genus *Psilocybe* sensu lato (Agaricales). Botany **91:** 573-591.

**Redhead, S. A.** 2013a. Nomenclatural novelties. Index fungorum **15:** 1-2.

**Redhead, S. A.** 2013b. Nomenclatural novelties. Index fungorum **18:** 1.

**Rommelaars, L. and E. Arnolds.** 2007. Mycology during a heatwave: *Panaeolus antillarum* in the netherlands. Coolia **50:** 145-148.

**Sawada, K.** 1931. Investigation report of fungi from Taiwan. Agricultural Experiment Station, Governor-General of Taiwan, Taiwan.

**Shao, K. T.** 2009. Catalogue of life in taiwan. Web electronic publication, version 2009.

**Stamets, P.** 1996. Psilocybin mushrooms of the world: An identification guide. Berkeley: Ten Speed Press, California, USA.

**Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar.** 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. **30:** 2725-2729.

**Tzean, S. S., W. H. Hsien, T. T. Chang, S. H. Wu and H. M. Ho**. 2015. Mycobiota Taiwanica 3rd ed. Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan.

**White, T. J., T. Bruns, S. Lee and J. Taylor.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M. A. *et al.* (eds.), PCR protocols: A guide to methods and applications, Vol. 18. Academic Press, Inc., New York, USA. pp. 315-322.

**Yeh, K.-W. and Z.-C. Chen.** 1991. Mushroom flora of Lan-Yu island (I). Taiwania **36:** 265-271.

**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**



臺灣大學學術期刊資料庫