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Mycena jingyinga, *Mycena luguensis*, and *Mycena venus*: three new species of bioluminescent fungi from Taiwan

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ABSTRACT: Three new species of bioluminescent fungi - *Mycena jingyinga*, *M. luguensis*, and *M. venus* - are proposed based on phylogenetic and morphological analyses. Of the three new species, the luminescence only admits from the mycelia instead of the fruiting bodies. The DNA sequences of *RNA polymerase II second largest subunit* (*rpb2*) and nuc rDNA ITS1-5.8S-ITS2 (ITS) were used for phylogenetic analyses. The three new species are characterized by small size, white or brown, adnate to subdecurrent lamellae, pubescent stipe with fibrils at bases, four-spored basidia, amyloid spores, cheilocystidia with various shapes, and pileipellis hyphae with short, simple, or diverticulate excrescences. These characteristics are congruent with those of *Mycena* section *Fragilipedes*. The three new species bring the total number of bioluminescent fungi to 108 and 15 species in the world and Taiwan, respectively.

KEY WORDS: Basidiomycetes, Fragilipedes, ITS, phylogeny, *rpb2*, new taxa.

INTRODUCTION

The luminous fungi can emit green light from their fruiting bodies, mycelia, or both. The fungal bioluminescence results from chemical reactions among luciferin, oxygen, and fungal luciferase (Kaskova *et al*., 2017). Due to this fascinating quality, bioluminescent mushrooms in the natural forests have become a new tourist attraction in Taiwan. Besides, the fungal bioluminescent system has great potential in applications to scientific research as a reporter gene (Kaskova *et al*., 2017).

In recent years, the world's known species of bioluminescent fungi (order Agaricales**)** has dramatically increased. Desjardin *et al*. (2008) established a list of 64 species of bioluminescent fungi. Chew *et al*. (2015) revised the list to 81 species. Mihail (2015) discovered five known *Armillaria* species which were bioluminescent. Desjardin *et al*. (2016) reported two new and two known species of bioluminescent mushrooms from Brazil. Terashima *et al*. (2016) reported eight new species of bioluminescent mushrooms from Japan. Cortés-Pérez *et al*. (2019) reported seven species bioluminescent mushrooms from Mexico. Until now, 105 species of bioluminescent fungi have been reported, accounting for about 0.1% of 100,000 described species of fungi (Kirk *et al*., 2008).

Bioluminescent fungi have been classified into four lineages based on molecular phylogeny. The four lineages include *Omphalotus*, *Armillaria*, Mycenoid, and Lucentipes (Desjardin *et al*., 2008; Desjardin *et al*., 2010; Chew *et al*., 2015). Until now, 12 species of bioluminescent fungi have been reported in Taiwan, including *Armillaria mellea* (Vahl) P. Kumm., *Mycena chlorophos* (Berk. & M.A. Curtis) Sacc., *M. haematopus* (Pers.) P. Kumm., *M. inclinata* (Fr.) Quél., *M. kentingensis*, Y.-S. Shih, C.-Y. Chen, W.-W. Lin & H.-W. Kao, *M. manipularis* (Berk.) Sacc., *M. polygramma* (Bull.) Gray, *M. pura* (Pers.) P. Kumm., *M. sanguinolenta* (Alb. & Schwein.) P. Kumm., *M. stylobates* (Pers.) P. Kumm., *Panellus stypticus* (Bull.) P. Karst., and *P. pusillus* (Pers. ex Lév.) Burds. & O.K. Mill (Haneda, 1955; Chang, 1996; Tschen *et al*., 1999; Tschen *et al*., 2000; Tschen *et al*., 2002; Chou and Chang, 2005; Tzean *et al*., 2009; Shih *et al*., 2014). Except for *A. mellea*, which belongs to the *Armillaria* lineage, the other 11 species belong to the Mycenoid lineage.

However, the bioluminescence of these Taiwanese species remains largely unknown. Most of the references only reported the occurrence and distribution, except *M. chlorophos* and *M. kentingensis* (Chou and Chang, 2005; Mori *et al*., 2010; Shih *et al*., 2014). This paper describes three new species of bioluminescent fungi which only mycelia is luminescent from Taiwan.

MATERIALS AND METHODS

Sample collection and isolation of mycelium

Fruiting bodies were collected from decaying tree or bamboo branches in the mountains of Central Taiwan. Pure cultures were obtained from spores or pieces of tissue that were cultured on PDA. We confirmed that the isolated mycelia and fruiting bodies represent the same

species by comparing their ITS sequences. Because the bioluminescence from the pure culture on PDA was weak and not easy to be observed, we conducted an inoculation assay. The isolated mycelia were inoculated into sterile dead branches of *Albizia lebbeck* which kept in a 250 ml sterilized glass bottles with 70 ml distilled water. The bottles were incubated in a growth chamber at 24℃ for two months. Photos of the sterile dead branches before and after the inoculation were compared and taken by a Nikon 3100 in the dark under 128000 IOS Sensitivity with 30 seconds of exposure and light under auto-mode.

Morphological study

Micromorphological features were examined from fresh or rehydrated specimens, followed by 5% potassium hydroxide (KOH), and stained with 1% Congo red. Dextrinoid and amyloid reactions were examined with Melzer's reagent. The measurements of morphological features were conducted by using ImageJ (Abramoff *et al*., 2004). Spore statistics included x_m: the arithmetic mean of the spore length divided by spore width; x_{mr}: the range of spore means; x_{mm}: the mean of spore means (±standard deviation) of specimens; Q: the quotient of the length and width in any one spore; Q_m: the mean of Q values in a single specimen; Q_{mr}: the range of Q_m values; Q_{mm}: the mean of Q_m values when two or more specimens examined. N: number of spores measured per specimen. S: number of specimens measured in a species. Specimens are deposited at the herbarium of the National Museum of Natural Science of Taiwan (TNM).

DNA extraction and sequencing

Total DNA was extracted from cultured mycelia by Genomic DNA Purification Kit (Gentra Systipes, Minneapolis, MN, USA). The DNA sequences of *RNA polymerase II second largest subunit*, *rpb2*) were amplified by polymerase chain reaction (PCR) using the primer pair *rpb2*-83 (5' AAGAYGTRTAYCGRTAYYTDC 3') and *rpb2*-1095 (5' RCADATVCCRAGRATCATRCIT 3') or the primer pair f*rpb2*-5F (5' GAYGAYMGWGATCAYTTYGG 3') (Liu *et al*., 1999) and b*rpb2*-7.1R (5' CCCATRGCYTGYTTMCCCATDGC 3') (Matheny, 2005; Matheny *et al*., 2007). The DNA sequences of the nuc rDNA ITS1-5.8S-ITS2 (ITS) were amplified using the primer pair ITS4 (5' TCCTCCGCTTATTGATATGC 3') and SR6R (5' AAGWAAAAGTCGTAACAAGG 3') (White *et al*., 1990). PCR was carried out in a 50 µl reaction mixture consisting of 5 µl 10× reaction buffer, 4 µl 2.5 mM dNTP, 1µl of each 10 pmole primer, 2 µl of DNA template and 1 µl ProTaq^{TM} DNA polymerase. The reaction was started by a denaturation step at 94 C for 4 minutes, followed by 35 cycles of denaturation at 94 C for 1 min, annealing at 50-55 C for 1 minute, elongation at 72 C for 1 minute. The final elongation was at 72 C for 10 minutes. The PCR products were purified using Micro-Elute DNA Clean/Extraction kit (GeneMark, Taichung City, Taiwan) and sent to the Instrument Center in National Chung Hsing University for sequencing. The *rpb2* sequences of the three new species were submitted to DNA Data Bank of Japan (DDBJ) with accession numbers of LC406707, LC406709, LC406710, LC406726, LC406730, LC406731, LC406732, and LC406734. The ITS sequences of the three new species were submitted to GenBank with accession numbers of MG324361 to MG324370.

Phylogenetic analyses

For phylogenetic analyses, the *rpb2* and ITS sequences of the three new species were compared to existing sequences in the GenBank of the National Center for Biotechnology Information (NCBI) using nucleotide megablast program (Altschul *et al*., 1997). Because there are few *rpb2* sequences of *Mycena* species deposited in GenBank, we downloaded all the *rpb2* sequences of *Mycena* species with species names and query coverage more than 92%. In addition, the *rpb2* sequence of *Hemimycena gracilis* was downloaded and used as the outgroup. In the analyses of ITS sequences, the blasted sequences in GenBank with the species name, query cover and identity more than 92%, were downloaded. In addition, the ITS sequences of *Mycena chlorophos* and *M. kentingensis* were also downloaded for analyses because they are common bioluminescent mushrooms and a newly discovered species (Mori *et al*., 2010; Shih *et al*. 2014) in Taiwan. Furthermore, the ITS sequences of *Hemimycena ochrogaleata* and *Hemimycena tortuosa* were downloaded and used as the outgroup. The accession numbers of the *rpb2* and ITS sequences are shown in Figure 1. Sequences were aligned using the program clustalw implemented in bioedit v.7.0.5.3 (Hall, 1999). Phylogenetic trees were constructed by Bayesian inference (BI) and maximum likelihood (ML) methods using mrbayes 3.2 (Huelsenbeck and Ronquist, 2001) and phyml 3.0 (Guindon *et al*., 2010), respectively. JModelTest (Posada, 2008) were used to determine the best-fit model for both *rpb2* and ITS datasets based on the Akaike information criterion (AIC). The best-fit model of HKY+I+G with the gamma substitution parameter (G = 1.851) and proportion of invariable sites (I = 0.515) was selected for *rpb2* datasets. The best model of GTR+G with G = 0.6070 was selected for ITS sequences. mrbayes was run with 10^6 generations of the MCMC chain. Trees were saved every 100 generations and the first 2500 trees (25%) were discarded as burn-in. Statistical support for the nodes was estimated from 1000 pseudoreplicates and posterior probability for ML and BI analyses, respectively. All sequence alignments and phylogenetic trees were submitted to TreeBase with the study accession URL of: http://purl.org/phylo/treebase/phylows/study/TB2:S22401.

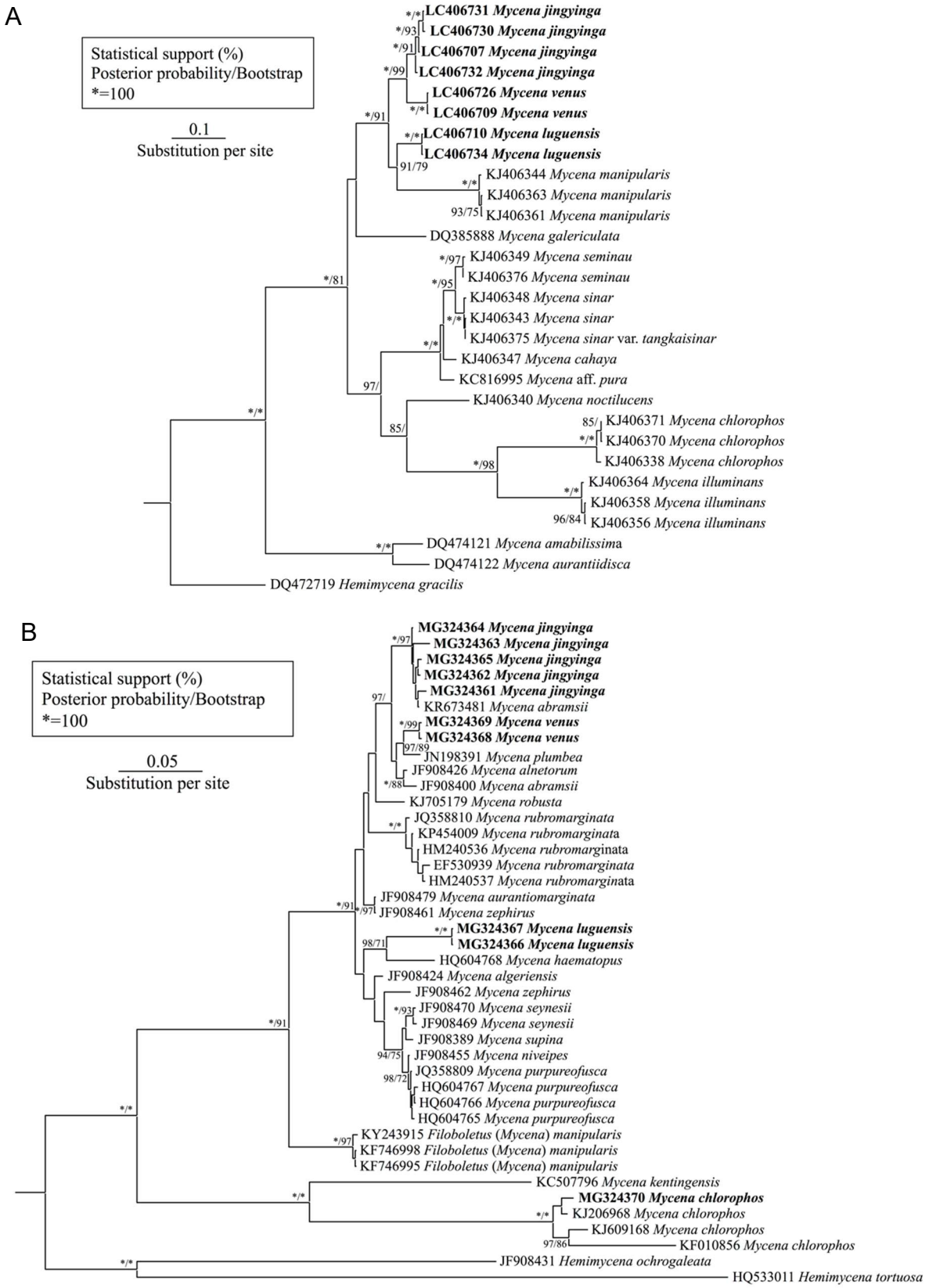

Fig. 1. Phylogenetic tree inferred from Bayesian and maximum likelihood algorithms based on *rpb2* sequences (**A**) and ITS sequences (**B**). Statistical supports are shown on branches with posterior probabilities and bootstrap values larger than 70%. Sequences from this study are shown in boldface.

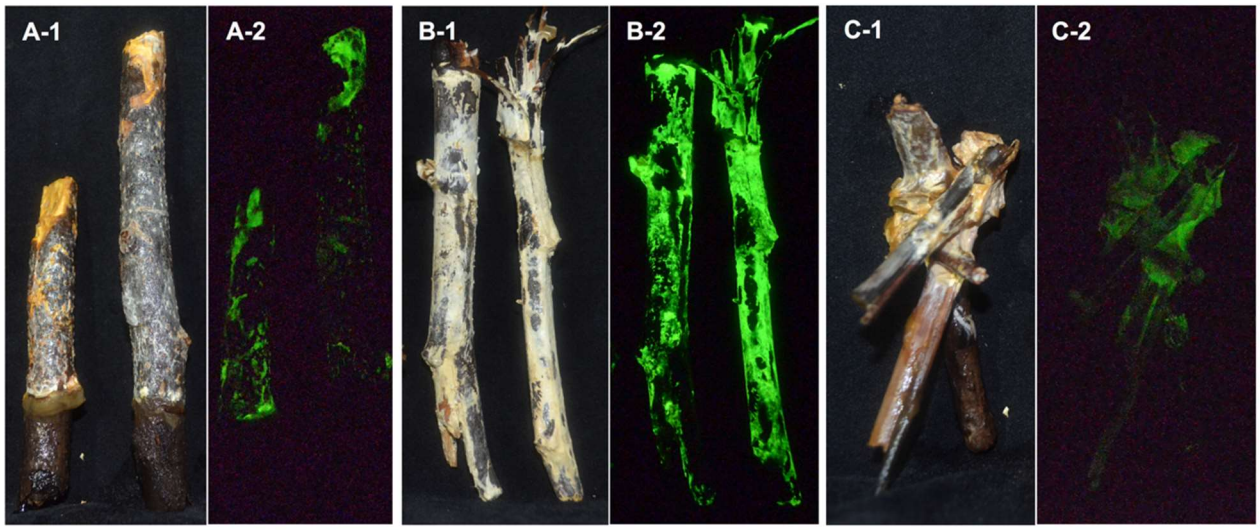

Fig. 2. Mycelia of the three new bioluminescent species. **A**. ***Mycena jingyinga***. **B**. ***Mycena luguensis***. **C**. ***Mycena venus***. Photographs were taken in light (1) and dark (2)..

RESULT

Bioluminescence detection

The sterile branches did not emit bioluminescence before inoculation (Fig. S1) but did emit bioluminescence after inoculation. Thus, it is confirmed that the bioluminescence on the inoculated branches did come from mycelia of the inoculation.

Blast searches and phylogenetic analyses

The *rpb2* and ITS sequences of the three new species were searched in GenBank. The hits of the maximum scores were *rpb2* sequences of *Mycena* (*Filoboletus*) *manipularis* (accession number KJ406337) with 85% identity and ITS sequences of three unrecognized species (accession numbers JF908426, LC314114, and LC314114) with 99% identity to that of the three new species, respectively. For the ITS sequences associated with Latin binominal names in the GenBank, the hits of maximum scores were the sequences of *Mycena alnetorum* (JF908426), *Mycena aurantiomarginata* (JF908479), and *Mycena alnetorum* (JF908426). The sequence identities between species were 98% (between *M. alnetorum* and *M. venus*), 93% (between *M. aurantiomarginata* and *M. luguensis*), and 97% (between *M. alnetorum* and *M. jingyinga*).

Phylogenetic analyses showed that *M. venus* and *M. luguensis* each formed a monophyletic group with high statistical support (more than 99%) (Fig. 1). The ITS sequences of *M. jingyinga* clustered with one of the downloaded sequences of *M. abramsii* (KR673481) to form a monophyletic group with high statistical support (more than 97%). While the other downloaded sequences of *M. abrambii* (JF908400) clustered with *M. alnetorum* (JF908426) to form the other monophyletic group with high statistical support in BI analysis (100%) and moderate support in ML analysis (88%). *Mycena venus*, *M. luguensis*, and *M. jingyinga* had the closest phylogenetic relationships to *M. plumbea, M. haematopus*, and *M. abrambii*, respectively (Fig. 1B).

TAXONOMY TREATMENTS

Mycena jingyinga C.-C. Chang, C.-Y. Chen, W.-W. Lin & H.-W. Kao, ***sp. nov.***

晶瑩小菇 Figs. 2A & 3

Mycobank: 824043

Holotype: TAIWAN. Nantou County: Lugu Township, Xitou, 23°40′N, 120°47′E, ca. 1030 m a.s.l., scattered on decaying bamboo branch, 15 Dec. 2015, *C.-C. Chang*, *CT151215* (TNM F0032411).

Diagnosis: *Mycena jingyinga* is characterized by having white, bell shaped to convex, sulcate pileus with polished surface, white and puberulous stipe, lageniform and smooth cheilocystidia, and lack of pleurocystidia.

Pileus 2–7 mm diameter, convex to bell shaped, slightly umbonate, surface glabrous, translucent-striate to sulcate, margin crenulate, white, grayish brown in the center, with light grayish brown striate, fading to margin. Context thin. Lamellae 10–13 reaching the stipe, adnate to subdecurrent, edge entire, concolorous, white. Stipe 4.8–8.6 × 0.2–0.5 mm, central, equal, cylindrical, slightly swollen in the base, the lower 2/3 covered with white pubescence, denser in the base. Odor none. Basidia 23.8–37.7 × 6.1–8 µm, clavate, 4-spored, colorless, thin-walled. Basidiospores (6–) 8.7–9.2 (–11.4) × (3.5–) 4.3–4.6 (–6.3) µm [x_{mr} = 8.7-9.3 × 4.5-4.8 µm, x_{mm} = 8.95±0.29 × 4.47±0.12 µm, Q = 1.5-2.5, Q_{mr} = 1.9-2, Q_{mm} = 1.94±0.06, n = 30-91, s = 3], ellipsoid, smooth, amyloid, thin-walled. Basidioles 22.6–37.2 × 5.2–8 µm, cylindrical, smooth, thin-walled. Cheilocystidia 36–60.8 × 7.3–14.6 µm, fusoid to lageniform, smooth, rarely

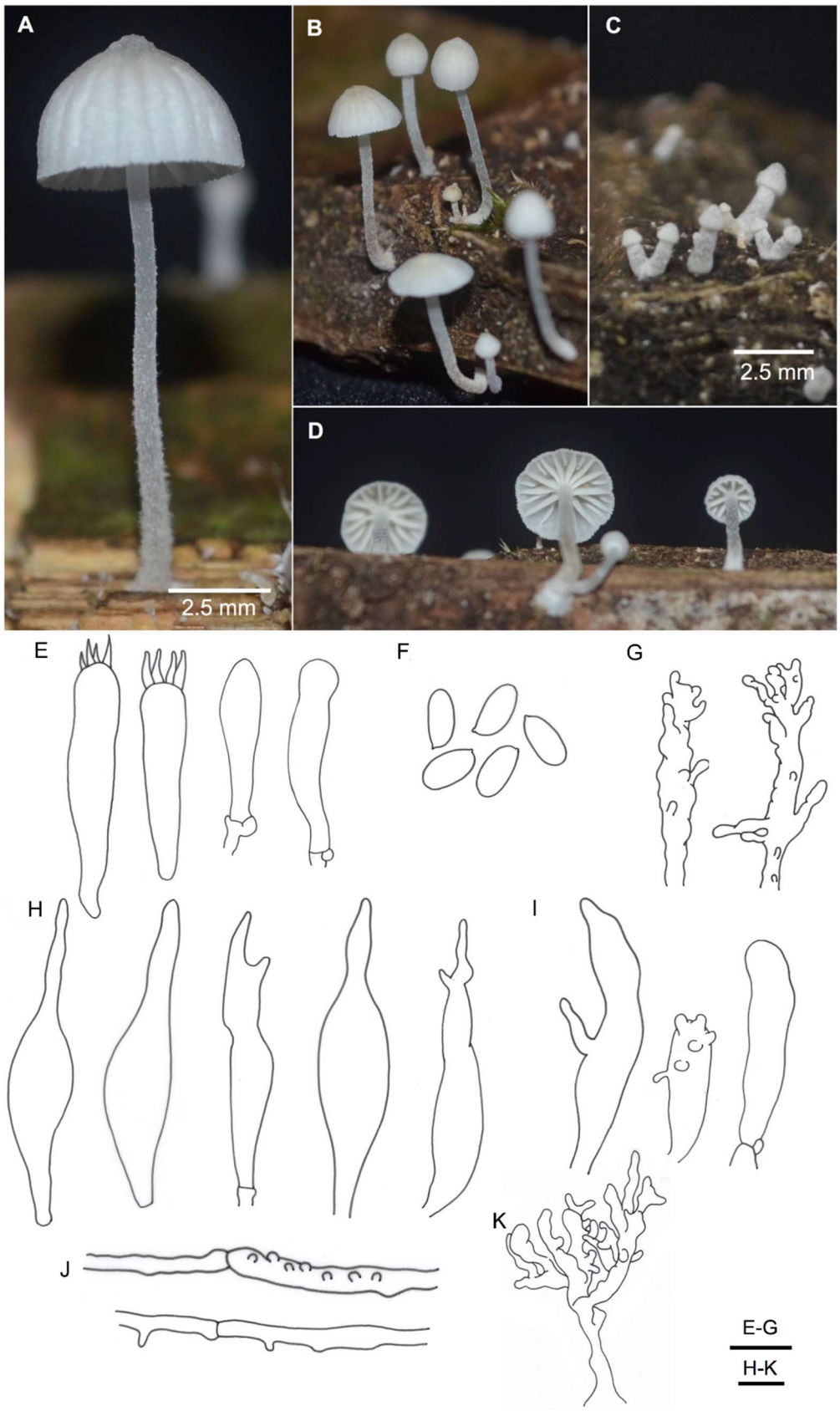

Fig. 3. ***Mycena jingyinga***. **A**, **B**. Fruiting bodies. **C**. Immature fruiting bodies. **D**. Lamellae. **E**. Basidia and basidioles. **F**. Basidiospores. **G**. Terminal cells of pileipellis. **H**. Cheilocystidia. **I**. Caulocystidia. **J**. Pileipellis cells. **K**. Diverticulate excrescence cells of pileipellis. Bars = 10 μm.

apically diverticulate, thin-walled, colorless. Pleurocystidia absent. Lamellar trama hyphae 9.2–30.2 µm, cylindrical, inflated, parallel, thin-walled, dextrinoid. Pileipellis hyphae 3.2–6.1 µm diameter, cylindrical, colorless, covered with sparse, short and simple (2–5 × 2–4.5 µm), or branched and longer (26.6–45 × 2.4–3.5 µm) excrescences. Hypodermium and pileus trama hyphae 40–47.6 µm, inflated, thin-walled, dextrinoid or rarely weakly dextrinoid. Stipitipellis hyphae 2.4–9.7 µm, smooth, cylindrical, parallel, somewhat inflated in terminal, 5.2–7.3 µm diameter, covered with irregular knob-like excrescences, thin-walled. Caulocystidia 30–57.1 × 5–10.5 µm, cylindrical to clavate, apex simple or diverticulate, surface smooth, colorless, thin-walled. Clamp connections common in all tissues. Luminescence observed in mycelium only.

Etymology: Jingying (Chinese) refers to shining.

Additional specimen examined: TAIWAN. Nantou County: Lugu Township, Xitou, 23°40′N, 120°47′E, ca. 1030 m a.s.l., scattered on decaying bamboo branches, 4 Dec 2015, C.-C. Chang, *CT151204* (NCHU); Fonghuanggu, 23°43′N, 120°47′E, ca. 960 m a.s.l., on decaying branch of angiosperm, 30 Mar 2016, S.-F. Li, *FH160330* (TNM F0032412); Taichung City, Heping District, Tangmadanshan, 24°09′N, 120°58′E, ca. 1260 m a.s.l., on decaying Arecaceae branches, 21 Aug 2016, S.-F. Li, *KK160821* (TNM F0032413); Henglingshan trail, 24°14′N, 120°56′E, ca. 1000 m a.s.l., on branch of angiosperm, 7 May 2017, C.-C. Chen, *SS17050702* (NCHU).

Remarks: *M. jingyinga* is micromorphologically similar to *M. abramsii*. However, *M. abramsii* has larger basidiospores (9–12.5 (–13.8) × 4.5–6 (–6.5) µm (Mass Geesteranus, 1988a), pleurocystidia, few stiptipellis terminal cells, and without caulocystidium. In macromorphology, *M. abramsii* has larger pileus 10–40 mm (Aronsen and Læssøe, 2016), 12–30 mm (Mass Geesteranus, 1988a). Pileus colors are grayish brown to dark brown, more lamellae 15–27 (Aronsen and Læssøe, 2016), 19–27 (Mass Geesteranus, 1988a), larger stipe 30–90 × 1-2 (4) mm (Aronsen and Læssøe, 2016); 50–90 × 1–2.5 mm (Mass Geesteranus, 1988a), stipe brown, surface glabrous below, with long, coarse and whitish fibrils covering the base (Aronsen and Læssøe, 2016). *M. abramsii* mostly grows on woody debris, moss-covered trunks of living trees and coniferous debris, but is not found on decaying bamboo branches. Moreover, *M. abramsii* has not been reported to be luminescent.

ITS sequences of *M. jingyinga* have the closest phylogenetic relationship to that of species annotated as *M. abramsii* (accession number KR673481) in the GenBank. However, the species was final or provisional identified as *M.* cf. *abramsii* (Kim *et al*., 2015). In addition, the other ITS sequences of *M. abramsii* (JF908400) in the GenBank clustered with *M. alnetorum* to form a monophyletic group (Fig. 1). Together with the morphological differences mentioned above, we propose that the species with the accession number of KR673481 in the Genbank was incorrectly annotated. While incorrectly annotated, this sequence also clearly represents the *Mycena jingyinga* collection from South Korea.

Mycena luguensis C.-C. Chang, C.-Y. Chen, W.-W. Lin & H.-W. Kao, ***sp. nov.***

鹿谷小菇 Figs. 2B & 4

Mycobank: 824047

Holotype: TAIWAN. Nantou County: Lugu Township, Xitou, 23°40′N, 120°47′E, ca. 1030 m a.s.l., solitary growth on decaying conifer branches, 22 Feb 2016, C.-C. Chang, *CT160222* (TNM F0032409).

Diagnosis: *Mycena luguensis* is characterized by having convex pileus with polished, brown, and sulcate surface, puberulous and white stipe, clavate cheilocystidia with numerous cylindrical excrescences at apex, and clavate pleurocystidia with numerous knob-like excrescence on top.

Pileus 10–14 mm diameter, broadly convex to slightly umbonate, margin flexuous; surface dull, glabrous, sulcate, dark brown in the center, fading to brownish white toward margin. Context thin. Lamellae 13–17 reaching the stipe, adnate, white; edge entire, concolorous. Odor none. Stipe 15.4–35.2 × 1.3–1.4 mm, cylindrical, central, equal; surface puberulous, glabrescent, base with sparse, short, white fibrils. Basidia 17.9–29 × 5.5–7.1 µm, clavate, 4-spored, colorless, thin-walled. Basidiospores (4.6–) 5.2–7.1 (–7.7) × (3.1–) 3.6–3.7 (–5) µm. [x_{mr} = 6–6.3 × 3.7–3.8 µm, x_{mm} = 6.15±0.98 × 3.67±0.07 µm, Q = 1.36–2.12, Q_{mr} = 1.64–1.65, Q_{mm} = 1.64±0.05, n = 35–42, s = 2], ellipsoid, smooth, amyloid, thin-walled. Basidioles 17.8–21.6 × 5.2–7.1 µm, subclavate, colorless, thin-walled. Cheilocystidia 18.8–28.9 × 5.6–8.7 µm, abundant, clavate, with numerous knob-like or irregular excrescences on apex, 2.4–6.2 × 1.6–3.0 µm colorless, thin-walled. Pleurocystidia 15.7–26.3 × 3.9–6.6 µm, abundant, clavate, colorless, smooth, thin-wall, numerous long cylindrical excrescences on the apex, 4.9–21.5 × 0.9–1.5 µm, smooth, colorless. Lamellar trama hyphae 8.6–25.5 µm diameter, smooth, inflated, dextrinoid, thin-wall. Pileipellis hyphae 2–4.6 µm diameter, cylindrical, colorless, thin-wall, covered with scattered, short, simple excrescences, 2.1–5.2 × 1.5–1.9 µm. Hypodermium and pileus trama hyphae 10–28 µm diameter, inflated, thin-walled, with brown pigmented in water. Stipitipelles hyphae 3.1–5.9 µm, covered with sparse, short, knob-like excrescences, colorless, thin-wall, terminal cell 4.1–6.7 µm width, slightly unevenly inflated, smooth, with scattered short excrescences on apex, somewhat furcate. Caulocystidia absent. Clamp connections common in all tissues. Luminescence mycelium only.

Etymology: Lugu (Chinese) refers to deer valley (township name) where the holotype was collected.

Additional specimen examined: TAIWAN. Nantou County: Lugu Township, Xitou, 23°40′N, 120°47′E, ca. 1030 m a.s.l., on decaying bamboo branch, 22 Apr 2016, C.-C. Chang, *CT160422* (TNM F0032411).

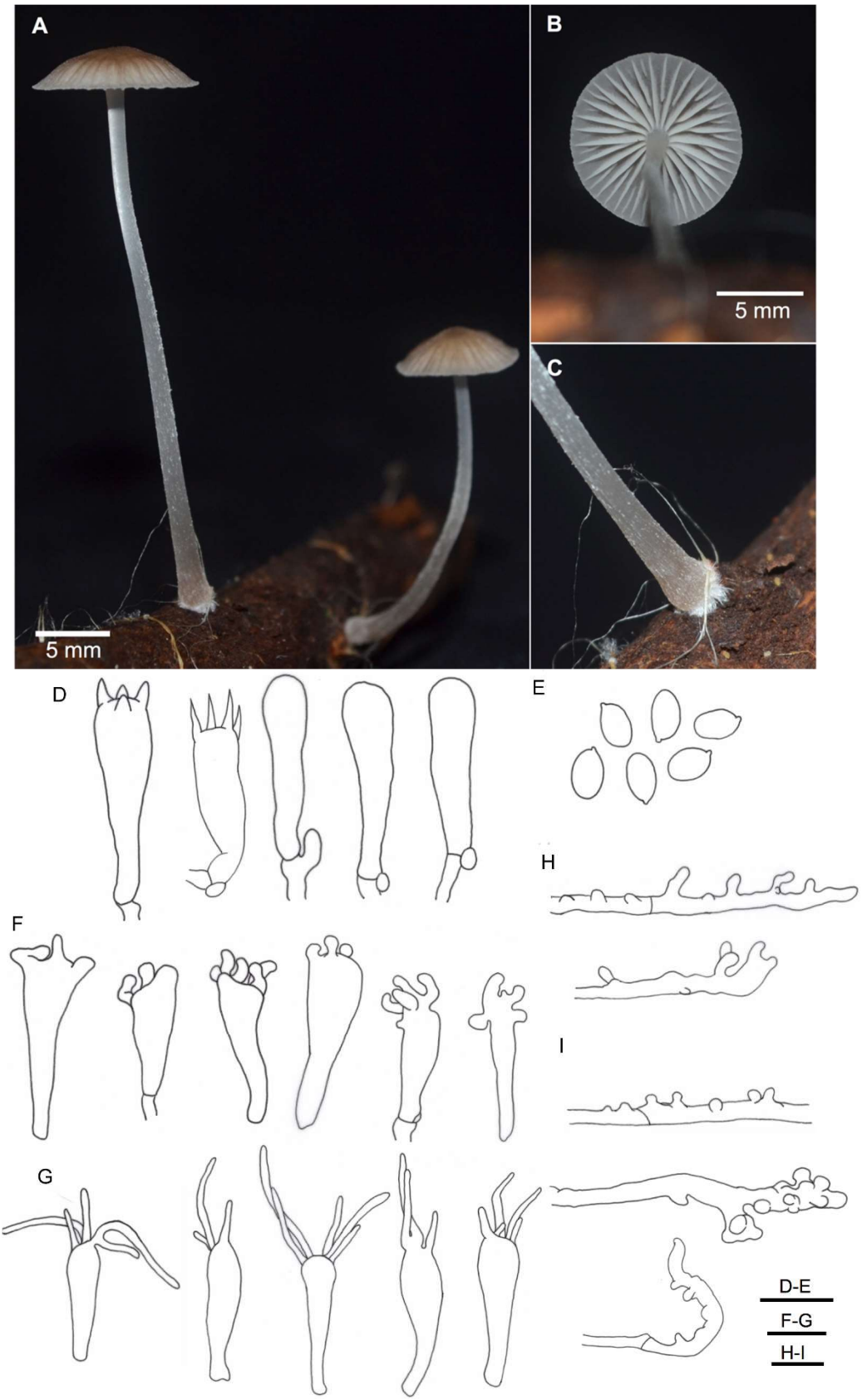

Fig. 4. ***Mycena luguensis***. **A**. Fruiting bodies. **B**. Lamellae. **C**. Base of stipe. **D**. Basidia and basidioles. **E**. Basidiospores. **F**. Cheilocystidia. **G**. Pleurocystidia. **H**. Pileipellis cells. **I**. Stiptipellis hyphae and stiptipellis terminal cells. Bars = 10 μm.

Remarks: *M. luguensis* is similar to *M. semivestipes* and *M. polygramma* f. *pumila* in pileus colors (gray, brown or black), pileipellis and stiptipellis with excrescences, cheilocystidia with three or more excrescences on the apex, presence of pleurocystidia, and spore up to 6 μm. However, *M. semivestipes* has larger cheilocystidia and pleurocystidia (23–32 × 7–11 μm). The shapes of cheilocystidia and pleurocystidia are clavate, fusiform, or irregularly shaped. Pileipellis is embedded in gelatinous matter and has 2-spored basidia (Maas Geesteranus, 1988c; Robich, 2006). *M. polygramma* f. *pumila* has diverticulate pileipellis embedded in gelatinous matter, slightly larger basidiospores 7–8.8 × 6–7 μm (Robich, 2006). Besides, *M. semivestipes* and *M. polygramma* f. *pumila* have not been reported to be luminescent. These morphological differences support that *M. luguensis* is a species different from *M.* semivestipes and *M. polygramma* f. *Pumila*.

The ITS sequences of *M. luguensis* had the highest BLAST score to that of *M. aurantiomarginata*. However, *M. lugenensis* and *M. aurantiomarginata* did not form a monophyletic group. In addition, *M. aurantiomarginata* has not been reported as a bioluminescent mushroom. In the phylogenetic analysis, *M. luguensis* had the closest relationship to *M. haematopus.* However, they are different in many morphological characteristics. For examples, *M. haematopus* has brownish red to pink pileus and crenulate pileus margin. The fruiting bodies bleed when cut. Cheilocystidia and pleurocystidia are fusoid, smooth, and somewhat contain brownish-red pigment. Basidiospores are larger and broader (8–9.5 × 5–6.5 μm) and there is the presence of caulocystidia (Aronsen and Læssøe, 2016).

Mycena venus C.-C. Chang, C.-Y. Chen, W.-W. Lin & H.-W. Kao, ***sp. nov.***

金星小菇 Figs. 2C & 5

MycoBank: 824046

Holotype: TAIWAN. Nantou County: Lugu Township, Xitou, 23°40′N, 120°47′E, ca. 1030 m a.s.l., solitary or cespitose growth on decaying conifer branches, 27 Jan. 2016, C.-C. Chang, *CT160127* (TNM F0032428).

Diagnosis: *Mycena venus* is characterized by having bell shape pileus with brown sulcate and crenulate, white margin, puberulous brown stipe with white dense fiber at the base, and pyriform cheilocystidia with simple to coral-like cylindrical branches at apex.

Pileus 4–7 mm diameter, hemispherical, dull, pruinose, glabrous, sulcate, translucent-striate, grayish-brown on top, fading to pale-white toward the margin, margin crenulate. Context thin. Lamellae 12–15 reaching the stipe, subdecurrent, thin, white; edge entire, concolorous. Odor none. Stipe 15.3–19.4 × 0.7–1 mm, cylindrical, central, equal, surface puberulous, base with dense, short, and white fibrils. Basidia 23.9–31.6 × 6.9–9 μm, clavate, 4-spored, colorless, thin-walled. Basidiospores (4.8–) 6.8–7.7 (–8.7) × (3.2–) 3.8–4.8 (–5.4) μm [x_{mr} = 6.9–7.5 × 3.9–4.7 μm, x_{mm} = 7.21±0.46 × 4.29±0.53 μm, Q = 1.31–2.10, Q_{mr} = 1.62–1.76, Q_{mm} = 1.69±0.01, n = 26, s = 2], ellipsoid, smooth, amyloid, thin-walled. Basidioles 17.8–30.5 × 5.4–8.7 μm, clavate, colorless, thin-walled. Cheilocystidia 15–34.1 × 5.8–14.3 μm, clavate to pyriform, somewhat irregular shape, rarely cylindrical, with few simple or coral-like branched excrescences on the apex, 4.2–21.7 × 1.2–2.2 μm, smooth, thin-walled. Pleurocystidia absent. Lamellar trama hyphae 15.3–22.6 μm diameter, smooth, inflated, colorless, dextrinoid, thin-walled. Pileipellis hyphae 3.3–7 μm diameter, cylindrical, colorless, thin-walled, covered with scattered, short and simple excrescences 1.1–1.8 × 0.7–1.7 μm. Hypodermium and pileus trama hyphae 17.3–37.9 μm diameter, inflated, smooth, colorless, dextrinoid, thin-walled. Stipitipellis hyphae 2.2–4.9 μm diameter, cylindrical, parallel, covering with scattering, short, cylindrical excrescences, terminal cells slightly swollen, 2.4–4.9 μm diameter, diverticulate. Caulocystidia absent. Clamp connections present. Luminescence observed in mycelium only.

Note: Though some obvious white ornamentation, what look like caulocystidia, appears on the base of stipe of *M. venus* (Fig. 5C). However, we have confirmed that the ornamentation is not caulocystidia after making a detailed observation of the structure under a microscope (Fig. S2).

Etymology: Venus (Latin) refers to the metaphor of brightness and beauty, which refers to the elegance of the fruiting bodies, and the brightest star of the sky in the dawn and evening of Taiwan.

Additional specimen examined: TAIWAN. Nantou County: Lugu Township, Xitou, 23°40′N, 120°47′E, ca. 1030 m a.s.l., solitary or cespitose growth on decaying conifer branches, 27 Jan 2016, C.-C. Chang, *CT160127* (TNM F0032428).

Remarks: *Mycena venus* is similar to *M. avellanea*, *M. obtecta* and *M. pectinata* in having basidiospores less than 10 μm in length, brown or gray pileus with avellaneous tints, a lack of pleurocystidia, cheilocystidia apex with two, three, or more excrescences, and hyphae of pileipellis and stiptipellis diverticulate. However, *M. avellanea* has larger basidiospores 8.5–8.8 × 5.5–6.3 μm (Robich, 2006) and larger cheilocystidia 16–54 × 7–22.5 μm (Mass Geesteranus, 1988a); *M. obtecta* has larger basidiospores 8.8–9.4 × 5.8–6.6 μm and longer cheilocystidia 70–30 μm; *M. pectinata* has larger basidiospores 9.0–9.8 × 5.4–6.3 μm (Mass Geesteranus, 1988b); 8–9 × 7 μm (Robich, 2006), longer stipe 4 cm, and larger pileus 2–3 cm (Mass Geesteranus, 1988b; Robich, 2006). In addition, *M. avellanea*, *M. obtecta*, and *M. pectinata* have not been reported to be luminescent.

In the analyses of ITS DNA sequences, *M. venus* had the highest BLAST score to that of *M. alnetorum* (JF908426). However, the phylogenetic analysis supported that *M. venus* is different from *M. alnetorum* because *M. venus* and *M. alnetorum* did not form a monophyletic group (Fig. 1). *M. venus* had the closest

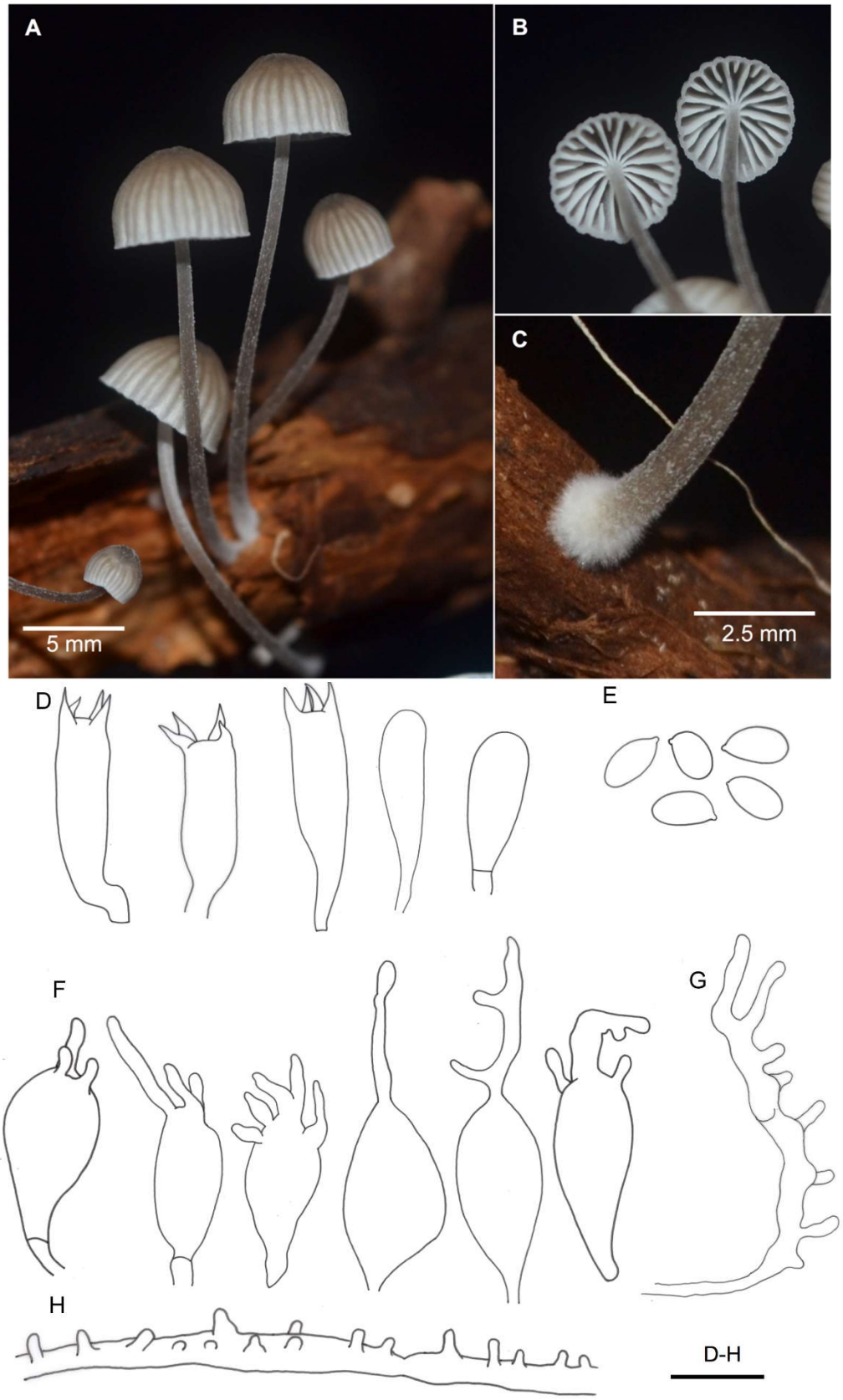

Fig. 5. ***Mycena venus*** (*CT160127*). **A**. Fruiting bodies. **B**. Lamellae. **C**. Base of stipe. **D**. Basidia and basidioles. **E**. Basidiospores. **F**. Cheilocystidia. **G**. Pileipellis cell. **H**. Stiptipellis terminal cell. Bar = 10 μm.

phylogenetic relationship to *M. plumbea.* However, *M. plumbea* has smaller 1–3 cm and darker (dark bluish black to drab gray) pileus, more lamellae reaching the stipe 23–28, larger basidiospores 9–11 × 5–6.5 µm, pileus trama hyphae with brownish contents, mucronate cheilocystidia, and the presence of pleurocystidia (Smith, 1947). Furthermore, *M. plumbea* belongs to section *Filipedes* (Smith, 1947) and has not been reported as a bioluminescent mushroom.

DISCUSSION

In this study, we described and illustrated three new species of bioluminescent fungi - *Mycena jinyinga*, *M. luguensis*, and *M. venus* - in Taiwan, based on morphological and molecular evidences. They are characterized by small size, white or brown, adnate to subdecurrent lamellae, pubescent stipe with fibrils at bases, four-spored basidia, amyloid spores, cheilocystidia with various shapes, and pileipellis hyphae with short, simple, or diverticulate excrescences. These characteristics are congruent with those of *Mycena* section *Fragilipedes* (Maas Geesteranus, 1980; Mass Geesteranus, 1988a; Mass Geesteranus, 1992).

The three new species bring the total of bioluminescent fungi to 15 species in Taiwan and 108 in the world, respectively.

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