MYCOBIOLOGY
2022, VOL. 50, NO. 5, 294–301
https://doi.org/10.1080/12298093.2022.2132001

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Mycena subpiligera sp. nov., a Symbiotic Species from China Associated with the Seed Germination of *Gastrodia elata*

Li-na Liu[a,b], Guo-ying Zhou[b], Ai-rong Shen[a], Bao-ming Shen[a], Yun Tan[a] and Zhu-ming Tan[a]

[a]Hunan Academy of Forestry, Changsha, PR China; [b]Faculty of Life Science and Technology, Central South University of Forestry and Technology, Changsha, PR China

ABSTRACT

Mycena subpiligera, a new taxon in sect. *Fragilipedes* that can strongly enhance the germination efficiency of *Gastrodia elata* seeds, was discovered in subtropical areas of China. As revealed by a morphological comparison with related *Mycena* species as well as maximum likelihood (ML) and Bayesian phylogenetic analyses based on sequences of the internal transcribed spacer (ITS) and the large subunit (LSU) regions of nuclear ribosomal RNA, the new taxon can be distinguished from phenotypically similar and phylogenetically related species. Optimal cultural conditions for *M. subpiligera* basidiomata are reported, and the germination rate of the new species is compared with that of *M. citrinomarginata*.

ARTICLE HISTORY
Received 21 January 2022
Revised 16 September 2022
Accepted 29 September 2022

KEYWORDS
Gastrodia elata; germination; *Mycena*; new taxon; basidiomata formation

1. Introduction

Mycena (Pers.) Roussel is a large genus in Agaricales with up to 600 species worldwide [1]. Persoon was the first mycologist who used the name *Mycena* as a section in *Agaricus* L. [2], and this section is characterized by pileus membranous and convex, with sulcate margin and stipe glabrescent. Roussel later raised sect. *Mycena* to generic rank [3]. It has been widely accepted now and many species in *Mycena* are found and several monographs have been published [4–8].

The orchid *Gastrodia elata* Blume, known as "Tianma" in traditional Chinese medicine, has been used in Asia for centuries to treat many human diseases, such as headache, vertigo, blackout, hemiplegia, and epileptic convulsions [9,10]. This species also has strong potential for treating Alzheimer's and Parkinson's diseases [11]. The seeds of *G. elata* are minute, and most contain an undifferentiated embryo that lacks a well-defined endosperm [12]. The few-celled embryo contains small amounts of proteins and lipids and very little sugar [13,14]. Because of this absence of nutritional reserves, the seed germination of *G. elata* in nature completely depends on mycorrhizal fungi [15,16].

Extensive research has shown that several *Mycena* species are essential for stimulating germination and the early stages of protocorm development in *G. elata* [17–23]. In the cited studies, most mycorrhizal *Mycena* were isolated from various Orchidaceae members or protocorms of *G. elata*. Species in *Mycena* with tiny basidiomata are abundant, which complicates the identification without basidiomata solely based on the few reliable DNA sequences in GenBank. Many researchers have focused on the mycorrhizal *Mycena* of *G. elata* and they intended to culture and identify them in the lab, however, only four species (*M. osmundicola* J.E. Lange, *M. orchidicola* Fan et Guo, *M. dendrobii* Fan et Guo, and *M. anoectochila* Fan et Guo) are able to form basidiomata in cultivation and have thus been successfully identified [17,19,20,24].

During field investigations in subtropical areas of China, we discovered a species of *Mycena* in Hunan Province and successfully obtained this strain by tissue isolation. According to our morphological observations, phylogenetic analyses based on nuclear ribosomal DNA internal transcribed spacer (ITS) and large subunit (LSU) sequences, and germination experiments, the isolated taxon is new to science and can strongly improve the germination rates of *G. elata* seeds.

2. Materials and methods

2.1. Sampling and morphological observations

Two specimens (HUIF50007 and HUIF50036) collected from Hunan Province, China, were dried in silica gel and preserved at the herbarium of Hunan Institute of Forestry (HUIF). The strain of the new taxon was isolated from basidiomata of the type specimen (HUIFS50007). Macroscopic characteristics of

CONTACT Zhu-ming Tan tzm100@aliyun.com; Guo-ying Zhou zgyingqq@163.com

fresh specimens were recorded with colors described according to Kornerup and Wanscher [25]. To obtain microscopic characteristics, dried specimens were examined by light microscopy (Olympus BX51, Olympus Cooperation, Tokyo, Japan). Microscopic characterization and examination of chemical reactions were carried out in 5% KOH or 1% Congo Red solution (in distilled water). Basidiospores, cheilocystidia, pileipellis, stipitipellis, and tramas were tested for their chemical reaction to Melzer's reagent [26]. The spore quotient (Q) = length (L)/breadth (B) was calculated from measurements of 30 basidiospores per collection. And more than 20 individuals for other structures were also measured in each collection.

2.2. DNA extraction, PCR amplification, and sequencing

Total genomic DNAs from the two dried specimens and related strains (8103, TMMFJ, MFXG, and SHXG from the Institute of Applied Mycology of Huazhong Agricultural University and MFJ from the professional cooperative of lv-zhou *Gastrodia elata* in Sui-ning county which are widely used in China) were extracted using a NuClean Plant Genomic DNA kit (CWBIO, Norcross, GA). ITS and LSU regions of the extracted DNA were, respectively, amplified using primer pairs ITS4/ITS5 and LROR/LR7 [27] according to the PCR cycling conditions described in Liu and Bau [28]. The resulting products were sequenced by the Tsingke Biotechnology Company (Changsha, Hunan, China).

2.3. Data analyses

A total of 88 ITS and LSU sequences of *Mycena*, including four sequences from the new taxon, were subjected to molecular phylogenetic analyses. The analyzed sequences included those selected according to the results of a GenBank BLAST search as well as previously reported sequences of unidentified symbiotic *Mycena* of *Gastrodia* spp. or *Dendrobium* sp. [22,23,29,30]. *Xeromphalina campanella* (Batsch) Kühner & Maire was used as an outgroup. Detailed information on the sequences analyzed is provided in Table 1. Sequences were aligned using MUSCLE version 3.8.31 (Mill Valley, CA) [31]. After selection of the optimal evolutionary model in MrModeltest version 2.3 Uppsala (Sweden) [32], the aligned dataset was analyzed by Bayesian inference (BI) and maximum likelihood (ML). The BI analysis was performed with MrBayes version 3.2.6 (Uppsala, Sweden) [33]. The ML analysis was performed in RAxMLGUIversion 1.5b1 (Heidelberg, Germany) [34] using a rapid bootstrapping algorithm and 1000 replicates, followed by a ML tree search. The resulting tree was visualized in Figtree version 1.4.3 (Edinburgh, UK) [35].

2.4. Cultivation

The isolated tissues were cultured on potato dextrose agar (PDA) plates containing 50 ppm ampicillin. The plates were incubated in the dark at 25 °C until hyphae were visible. Hyphal tips were transferred to fresh PDA and then serially transferred until pure cultures were obtained. Decayed leaves of *Castanea mollissima* Blume or *Pinus massoniana* Lamb. were then added to PDA to optimize the medium.

2.5. Symbiotic seed germination and protocorm development of G. elata

Mycena citrinomarginata Gillet strains 8103, TMMFJ, MFXG, and SHXG from the Institute of Applied Mycology of Huazhong Agricultural University and MFJ from the professional cooperative of lv-zhou *Gastrodia elata* in Sui-ning county were also selected for use in comparisons. Mature un-dehisced fruits of *G. elata* were collected from the professional cooperative of lv-zhou *Gastrodia elata* in Sui-ning county. After collection, fruits were sterilized using 75% ethanol, washed three times using distilled water, and dried on sterile filter paper. *Mycena* strains were placed on water agar medium (0.1% agar), and *G. elata* seeds were then scattered around the mycelium. The MFJ strain, which is widely used for seed germination of *G. elata* in Hunan, was selected as a positive control. After incubation of seeds at 25 °C in the dark for 20 d, the number of seeds was determined under a dissecting stereomicroscope every 5 d for 60 d. Six replicates of each plate were counted. And we did this experiment three times.

3. Results

3.1. Phylogenetic analyses

BI and ML phylogenetic trees based on ITS and LSU sequences had similar topologies. As shown in the BI tree in Figure 1, the analyzed taxa were divided into two distinct groups. The two accessions of the new taxon and its isolated strain grouped together (Bayesian posterior probability = 1.00/ML bootstrap = 100%) in Clade 3. The new taxon is thus clearly separate from allied *Mycena* species.

3.2. Taxonomy

***Mycena subpiligera* L.N. Liu, sp. nov.**

Mycobank: MB842467

Table 1. Sequences of basidiomata and strains for phylogenetic analyses used in this study.

Species	Voucher	GenBank Accession no.		Basidiomata/ Strain	Country
		ITS	LSU		
Mycena abramsii	HMJAU43606	MH396629	MK629355	Basidiomata	China
M. abramsii	HUIFS50004	OM228756	OM228764	Strain	China
M. abramsii	HMJAU43282	MH396626	MK629348	Basidiomata	China
M. abramsi	HMJAU43523	MH396628	MK629350	Basidiomata	China
M. adenxa	HMJAU43338	MK733289	MK722344	Basidiomata	China
M. adenxa	HMJAU43691	MK733293	MK722346	Basidiomata	China
M. albiceps	F27622	MZ303026	–	Basidiomata	America
M. albiceps	RA705-6	MK234177	–	Basidiomata	America
M. algeriensis	HMJAU43798	MK733295	MK722347	Basidiomata	China
M. arcangeliana	252b	JF908401	–	Basidiomata	Spain
M. arcangeliana	252f	JF908402	–	Basidiomata	Spain
M. cf. *cinerella*	173	MF926553	–	Basidiomata	–
M. cinerella	Aronsen051014	KT900146	–	Basidiomata	Norway
M. citrinomarginata	SHXG	OM228755	OM228763	Strain	China
M. citrinomarginata	MFJ	OM228754	OM228762	Strain	China
M. citrinomarginata	8103	OM228752	OM228760	Strain	China
M. citrinomarginata	MFXG	OM228753	OM228761	Strain	China
M. citrinomarginata	TMMFJ	OM228751	–	Strain	China
M. citrinomarginata	HMJAU43563	MG654739	MK629351	Basidiomata	China
M. deeptha	DM334g (K(M)178333)	JX481737	–	Basidiomata	India
M. entolomoides	HMJAU43126	MG654738	MK722349	Basidiomata	China
M. galericulata	TENN-F-014675h1	MN088380	–	Basidiomata	America
M. galericulata	TENN-F-069380ss1	MN088383	–	Basidiomata	America
M. galericulata	TENN-F-069380	MN088382	–	Basidiomata	America
M. haematopus	HMJAU43494	MK733296	MK722351	Basidiomata	China
M. hyalinostipitata	HMJAU43693	MH136828	MK629361	Basidiomata	China
M. inclinata	S.D.Russell MycoMap 4978	MK532829	–	Basidiomata	America
M. inclinata	iNat:35919741	MN764198	–	Basidiomata	America
M. laevigata	4747	MH930175	–	Basidiomata	Russia
M. laevigata	HMJAU43187	MK733302	–	Basidiomata	China
M. laevigata	HMJAU43604	MK733303	MK722354	Basidiomata	China
M. laevigata	HMJAU43618	MK733304	MK722355	Basidiomata	China
M. meliigena	39d	JF908429	–	Basidiomata	Italy
M. meliigena	39	JF908423	–	Basidiomata	Italy
M. metata	HMJAU43625	MH396636	–	Basidiomata	China
M. pearsoniana	HMJAU43826	MK733305	MK722356	Basidiomata	China
M. pura	TENN65043	JN182202	–	Basidiomata	America
M. pura	HMJAU43121	MK309793	–	Basidiomata	China
M. purpureofusca	HMJAU43624	MG654740	MK629356	Basidiomata	China
M. rosea	Champ-21	KX449424	–	Basidiomata	–
M. seminau	KLU:M 1223	KF537250	KJ206952	Basidiomata	Malaysia
M. seminau	KLU:M 1226	KF537252	NG070530	Basidiomata	Malaysia
M. semivestipes	HMJAU43825	MK733308	MK722358	Basidiomata	China
Mycena sp.	F69	LC314115	–	Strain	Japan
Mycena sp.	taxon:1916079	LC314114	–	Strain	Japan
Mycena sp.	NIFOS101	KY449288	–	Strain	–
Mycena sp.	taxon:660929	FJ544251	–	Strain	China
Mycena sp.	KFRI1212	HQ662845	–	Strain	Korea
Mycena sp.	KFRI2121	HQ662846	–	Strain	Korea
M. subpiligera	HUIF50007	OM228757	OM228765	Basidiomata	China
M. subpiligera	HUIF50036	OM228758	–	Basidiomata	China
M. subpiligera	HUIFS50007	OM228759	–	Strain	China
M. substylobates	HMJAU43418	MH216189	–	Basidiomata	China
M. supina	128a	JF908388	–	Basidiomata	Italy
M. tenax	OSC 113728	EU669224	EU669275	Basidiomata	America
M. tenax	OSC 113746	EU846251	–	Basidiomata	America
M. tenerrima	HMJAU43816	MK309796	MK629364	Basidiomata	China
M. vulgaris	447h	JF908435	–	Basidiomata	Italy
M. vulgaris	3781	KJ705177	–	Basidiomata	America
Xeromphalina campanella	TENN-F-053583A	KM024575		Basidiomata	Sweden
X. campanella	TENN:F-069178	KP835678	–	Basidiomata	Czech Republic

Diagnosis. Pileus convex, with umbilicate or depressed center

Lamellae short decurrent to decurrent, often stained with yellow-brown to orange-brown spots (Figures 2 and 3). Cheilocystidia fusoid or cylindrical, thick walled. Pileipellis branched, anastomosing, smooth with scattered, cylindrical excrescences. Caulocystidia piliform.

Etymology. Referring to its piliform caulocystidia and it also be closed to *M. piligera* in morphology.

Type. CHINA. Hunan Province, Shaoyang city, Sui-ning County, Hunan Huangsang National Nature Reserve, April 26 2021, Lina Liu, HUIF50007 (Holotype!).

Pileus 0.5–1.9 cm diameter, convex, somewhat flattened, umbilicate or depressed centrally, translucently striate, pale brown (6D7) to brown (6F7) when young, pale yellow-brown (6C6) to brownish-white (6A3) with age, whitish (6A2–6A1) in the peripheral regions, often stained with yellow-brown (6A7) to orange-brown

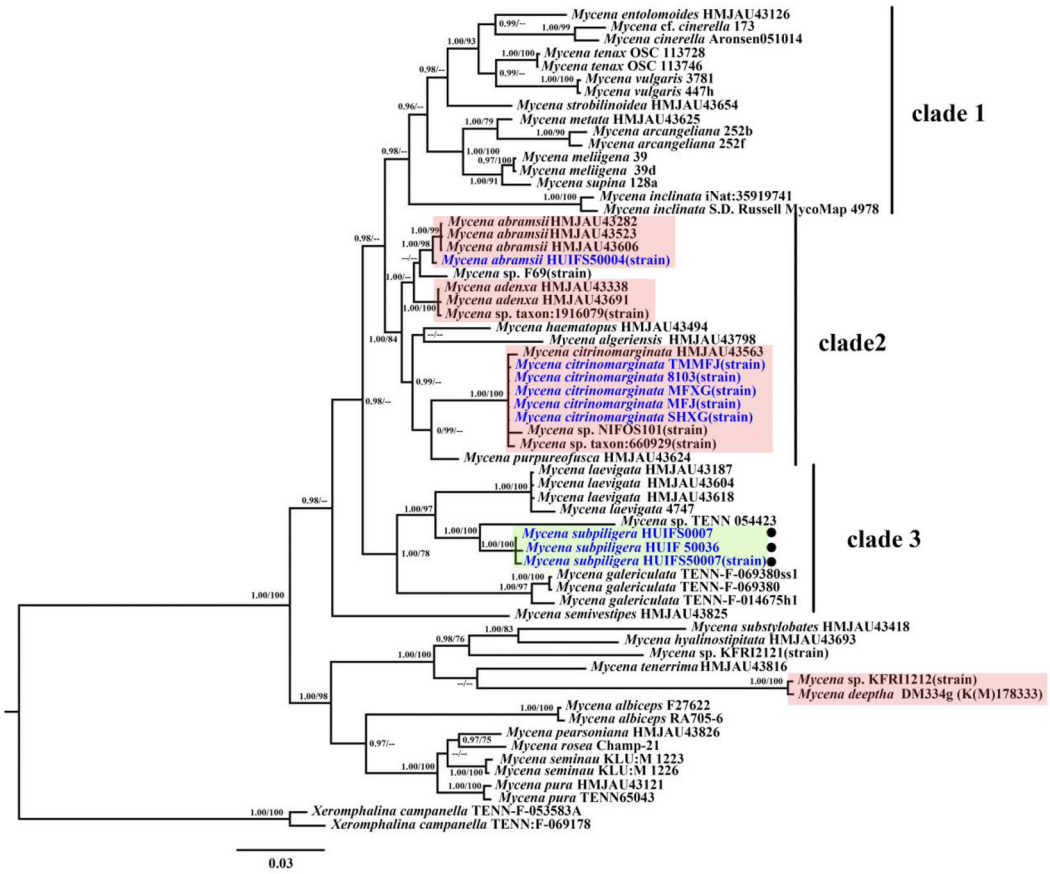

Figure 1. Bayesian tree inferred from partial ITS and LSU sequences showing phylogenetic relationships of *Mycena sudpiligera*. Bayesian posterior probability (≥0.95) and maximum likelihood support values (≥75) are shown (BPP/ML). New species is marked by ●.

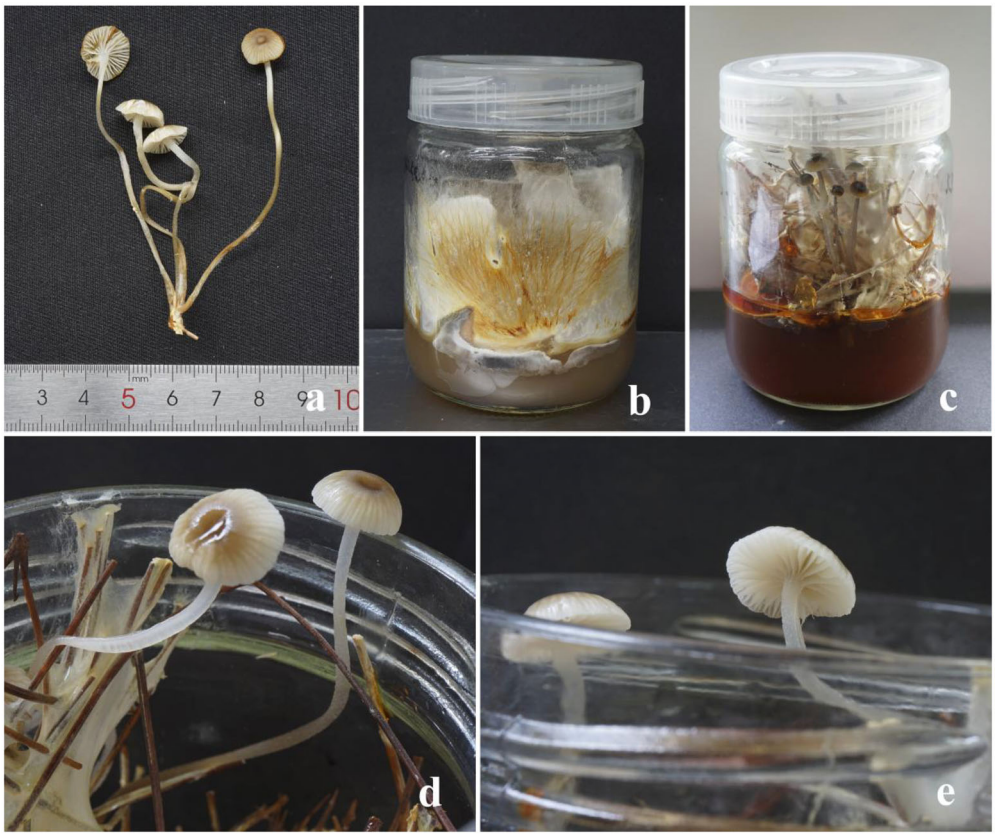

Figure 2. Basidiomata and mycelium of *Mycena subpiligera*. a. Basidomata of *M. subpiligera* in the field (HUIF50007); b. Mycelium of *M. subpiligera* cultured on the media mixed with leaves of *Castanea mollissima*; c–e. *M. subpiligera* cultured on the media mixed with leaves of *Pinus massonina*.

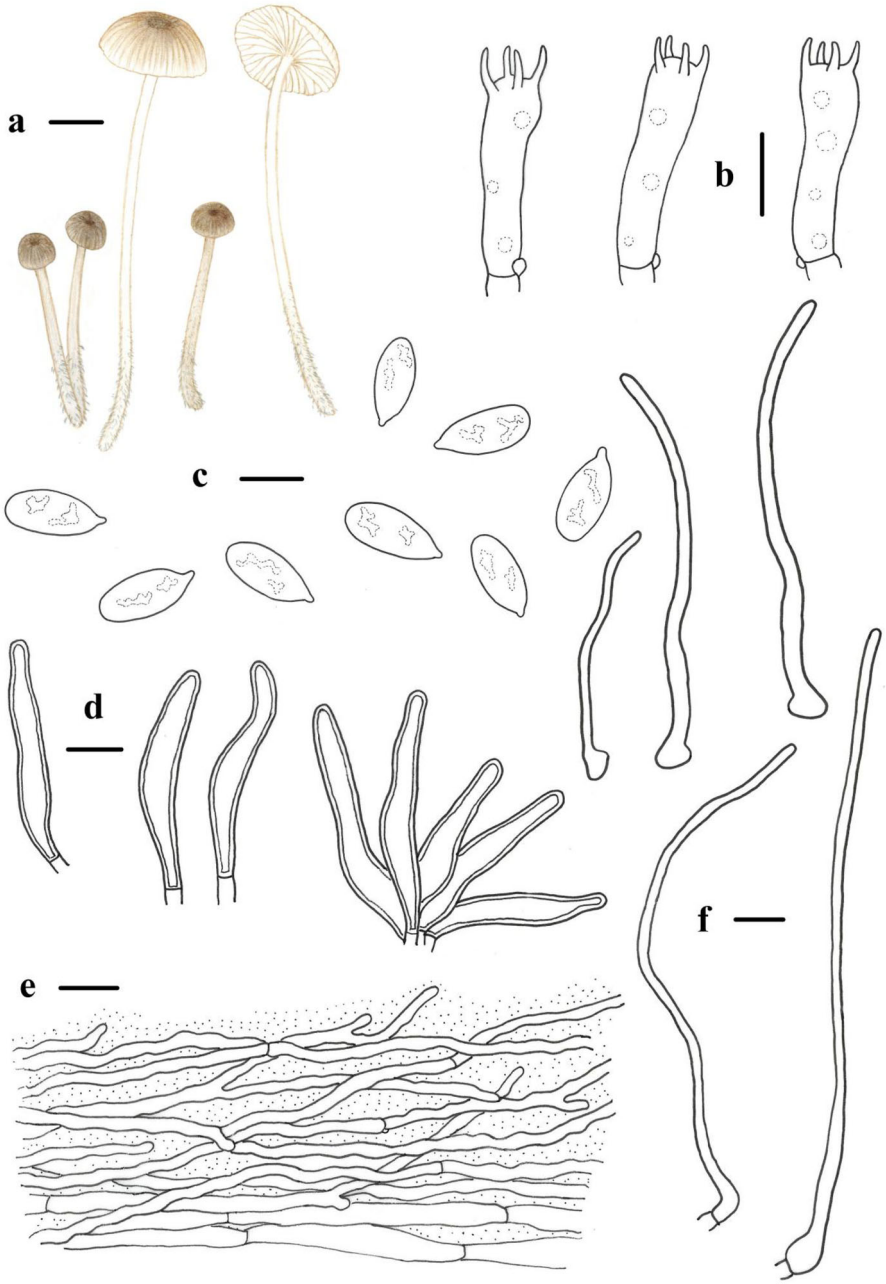

Figure 3. Features of *Mycena subpiligera*. a. Basidiomata; b. Basidia; c. Basidiospores; d. Cheilocystidia; e. Hyphae of Pileipellis; f. Caulocystidia. Scar Bars a = 1 cm; c = 5 μm; b, d, e, f = 10 μm. All drawings from holotype by Lina Liu.

(6C8) spots. Context translucent-white, thin, fragile. Lamellae short decurrent to decurrent, white, edges concolourous, often stained with yellow-brown (6A7) to orange-brown (6C8) spots. Stipe 2.1–6.5 × 0.2–0.4 cm, cylindrical, strait to somewhat flexuous, hollow, pruinose at apex, dense white-pubescent at base, white to brownish-white (6A3). Odorless and with a mild taste.

Basidiospores 6.2–7.4 × 3.1–3.8 μm, $Q = 1.8–2.0$, oblong to subcylindrical, hyaline, guttulate, thin walled, amyloid. Basidia 15–19 × 5–7 μm, four-spored, clavate. Cheilocystidia 15–32 × 3–6 μm, fusoid or lageniform, thick-walled, and hyaline. Pleurocystidia absent. Lamellar trama dextrinoid. Hyphae of the Pileipellis glutinosus, 2–4 μm wide, branched, anastomosing, smooth seldomly with scattered, and cylindrical excrescences. Hyphae of the stipitipellis 2–6 μm wide, smooth. Caulocystidia 84–215 × 4–7 μm, piliform, long fusoid or cylindrical, smooth, and thin to slightly thick-walled. Clamp-connections present in all tissues.

Habitat. Fasciculate or solitary on vegetable debris under forests which mainly composed by *P. massoniana* and *Fagus* sp.

Known distribution. Hunan Province, China.

Additional material examined. CHINA. Hunan Province, Xiangxi Tujia-Miao Autonomous Prefecture, Longshan County, the institute of crop sciences of Longshan, May 27 2021, Lina Liu, HUIF50036.

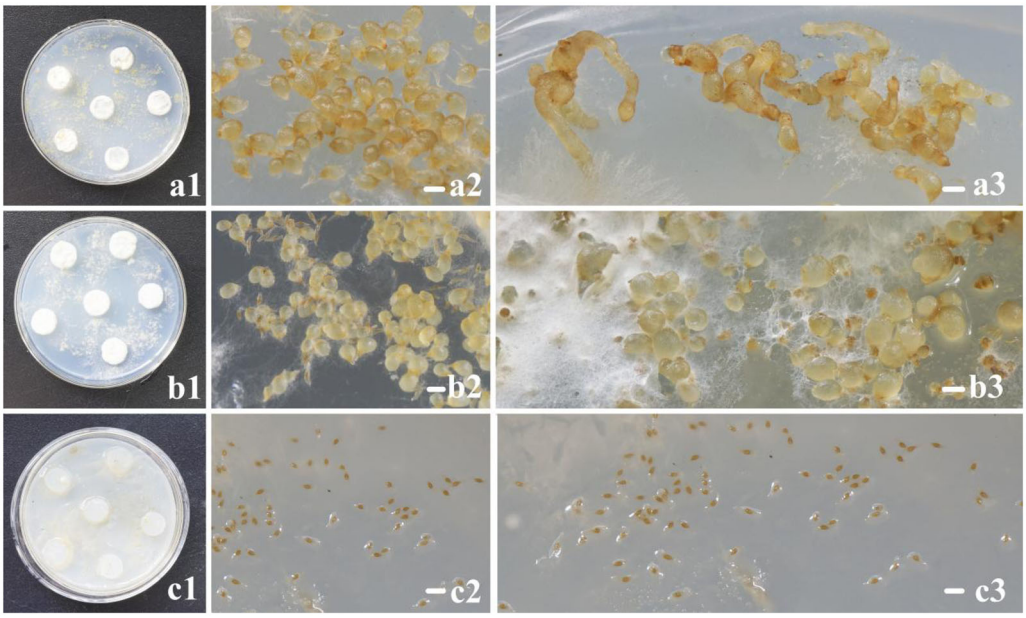

Figure 4. Symbiotic germination of *Gastrodia elata* seeds by *Mycena subpiligera* and *M. citrinomarginata*. a1–a3. plates within *M. subpiligera* (HUIFS50007) mycelium; b1–b3. plates within *M. citrinomarginata* (MFJ) mycelium; c1–c3. blank control; a2–c2. seeds cultured for 30 d; a3–c3. seeds cultured for 60 d. Scar bar = 1 mm.

3.3. Cultivation

We found that basidiomata only formed when tissues were cultivated on PDA mixed with decayed leaves of *P. massoniana* for 80–86 d.

3.4. Symbiotic seed germination and protocorm development of G. elata

Both strains (HUIFS50007 and MFJ) were able to stimulate seeds sprouting of *G. elata* (Figure 4), but the germination rate in the presence of HUIFS50007 (81%) was slightly higher than that of MFJ (74%). In addition, *G. elata* protocorms seem to develop faster with HUIFS50007 than with MFJ mycelium.

4. Discussion

Mycena subpiligera is characterized by the presence of a brown pileus, white lamellae, amyloid spores, fusoid or lageniform cheilocystidia, a smooth pileipellis, and piliform caulocystidia. The new species can be assigned to sect. *Fragilipedes* (Fr.) Quél. on the basis of its white lamellae, amyloid spores, fusoid or lageniform cheilocystidia, and smooth pileipellis [36,37]. Five other species in this section, namely, *M. piligera* Robich, *M. pilosella* Maas Geest., *M. scirpicola* M. Villarreal, Heykoop, Esteve-Rav. & Maas Geest., *M. pruinatipes* Robich, and *M. villicaulis* Maas Geest., superficially resemble the new species in having piliform caulocystidia. Our new taxon can be easily confused with *M. piligera* on account of its similar macroscopic characters and piliform caulocystidia, but *M. piligera* has larger, differently shaped basidiospores (7.5–10 × 4.5–6 µm), thin-walled cheilocystidia, and a diverticulate pileipellis [5]. Although some variation exists within *M. pilosella*, this species complex can be distinguished from *M. subpiligera* by the presence of a conical or hemispheric cap without a depressed center, a diverticulate pileipellis not embedded in gelatinous matter, and a diverticulate stipitipellis [4,36]. Compared with *M. scirpicola*, it has a conical to conical-campanulate cap, adnate lamellae, and a different pileipellis and stipitipellis [7]. *Mycena pruinatipes* differs from our new taxon in possessing a smaller conical cap, pleurocystidia, and a diverticulate pileipellis and stipitipellis [5]. The fact that basidiomata of our newly described taxon only formed on the medium mixed with *P. massoniana* is clearly reminiscent of *M. villicaulis* with a similar habitat, but *M. villicaulis* differs in having a differently shaped cap, a diverticulate pileipellis, a diverticulate stipitipellis, and obvious thick-walled caulocystidia [4].

In our BI and ML phylogenetic trees based on ITS and LSU sequences, the two collected accessions grouped together with high support with one strain of *M. subpiligera* in Clade 3, which in turn was sister to an unidentified *Mycena* (voucher no. TENN054423). This latter voucher was selected for analysis because this sample was the most similar *Mycena* according to the results of a GenBank BLAST search. Although we do not know the morphological characteristics of the unidentified *Mycena*, which was collected in Argentina, it can clearly be distinguished phylogenetically.

To better understand the diversity of *Mycena* species able to enhance the germination efficiency of *G. elata* seeds, we sequenced six *Mycena* strains (including an unpublished one, HUIFS50004)

symbiotic on *G. elata* seeds in China as well as six unidentified symbiotic *Mycena* of *Gastrodia* spp. or *Dendrobium* sp. reported from various studies [22,23,29,30]. Ten strains were successfully identified on the basis of their ITS and LSU sequences. Interestingly, *M. abramsii* (Murrill) Murrill (HUIFS50004), *M. adnexa* T. Bau & Q. Na (taxon 1916079), and *M. citrinomarginata* (TMMFJ, 8103, MFXG, MFJ, SHXG, NIFOS101, and taxon 660929) together with our new taxon can all be assigned to sect. *Fragilipedes* [36]. In contrast, *M. deeptha* Aravind. & Manim. (KFR11212) is a bioluminescent fungus in sect. *Exornatae* Maas Geest. [38], whereas symbiotic *Mycena* identified in previous studies (i.e., *M. osmundicola*, *M. orchidicola*, *M. dendrobii*, and *M. anoectochila*) are all in sect. *Sacchariferae* Kühner ex Singer [17,19,20,24]. In this study, all analyzed strains of *Mycena* spp. were able to stimulate the germination of *G. elata* seeds to some extent. Determining which members of *Mycena* and related genera can stimulate the germination of *G. elata* or other orchids is an interesting research problem that we plan to address in future work.

Acknowledgments

The authors sincerely thank Dr. Xin Chen in Huazhong Agricultural University, Dr. Liang-bin Zeng in Chinese Academy of Agricultural Sciences, Mrs Kai-lin Tan, Mr shu-yuan Tang in the professional cooperative of lv-zhou *Gastrodia elata* in Sui-ning county, and Mr Zu-heng Xiang, Mr. Peng Zhang in Forestry Bureau of Long-shan County for the help in the experimental process. We also thank the kind editors and two anonymous reviewers for their corrections and suggestions to improve our work.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by two projects from the Forestry Department of Hunan Province which named “Study on key techniques of reintroduction of rare orchids” and “Comparative evaluation and cultivation demonstration of different genotypes of rare orchids”.

ORCID

Li-na Liu http://orcid.org/0000-0003-3241-4016

Data availability statement

This data can be opened available from GenBank.

References

[1] He MQ, Zhao RL, Hyde KD, et al. Notes, outline and divergence times of Basidiomycota. Fung Divers. 2019;99(1):105–367.
[2] Persoon CH. Tentamen dispositionis methodicae fungorum in classes, ordines genera et familias. Kessinger Legacy Reprints; 1797.
[3] de Roussel HFA. Flore du calvades et des terreins adjacens. 2nd ed. Caen; 1806.
[4] Robich G. *Mycena* d'Europa. Trento, Italy: Associazione Micologica Bresadola; 2003. p. 1–728.
[5] Robich G. *Mycena* d'Europa volume 2. Trento, Italy: Associazione Micologica Bresadola; 2016. p. 1–796.
[6] Grgurinovic CA. The genus *Mycena* in South-Eastern Australia. Hong Kong: Fungal Diversity Press; 2003. p. 1–329.
[7] Aronsen A, Laessøe T. The genus *Mycena* s.l. Fungi of Northern Europe. Vol. 5. Gylling, Denmark: Narayana Press; 2016. p. 1–376.
[8] Bau T, Na Q, Liu LN. A monograph of Mycenaceae (Agaricales) in China. Beijing, China: Science Press; 2021. p. 1–326.
[9] Huang ZL. Pharmacologic studies and clinical applications of *Gastrodia elata* Bl. J Modern Develop Trad Media. 1985;5:251–254.
[10] Chang HM, But PH. Pharmacology and application of Chinese materia medica. Vol. I. Singapore: World Scientific; 1986. p. 1–185.
[11] Manavalan A, Feng L, Sze SK, et al. New insights into the brain protein metabolism of *Gastrodia elata*-treated rats by quantitative proteomics. J Proteomics. 2012;75(8):2468–2479.
[12] Arditti J. Fundamentals of orchid biology. New York (NY): Wiley; 1992. p. 1–691.
[13] Harrison CR. Ultrastructural and histochemical changes during germination of *Cattleya aurantica* (Orchidaceae). Bot Gaz. 1977;138(1):41–45.
[14] Arditti J, Ghani AKA. Numerical and physical properties of orchid seeds and their biological implications. New Phytol. 2000;146:569.
[15] Arditti J. Factors affecting the germination of orchid seeds. Bot Rev. 1967;33(1):1–97.
[16] Dearnaley JDW. Further advances in orchid mycorrhizal research. Mycorrhiza. 2007;17(6):475–486.
[17] Xu JT, Guo SX. Fungus associated with nutrition of seed germination of *Gastrodia elata—Mycena osmudicola* Lange. Mycosystema. 1989;8(3):221–226.
[18] Guo SX, Xu JT. Studies on the cell ultrastructure in the course of *Gastrodia elata* digesting *Mycena osmundicola* lange and *Armillaria mellea* FR. Acta Mycol Sin. 1990;9:218–225.
[19] Guo SX, Fan L, Cao WQ, et al. *Mycena anoectochila* sp. nov. Isolated from mycorrhizal roots of *Anoectochilus roxburghii* from Xishuangbanna, China. Mycologia. 1997;89(6):952–954.
[20] Guo SX, Li F, Cao WQ, et al. *Mycena dendrobii*, a new mycorrhizal fungus. Mycosystema. 1999;18(2):141–144.
[21] Hong IP, Kim HK, Park JS, et al. Physiological characteristics of symbiotic fungi associated with the seed germination of *Gastrodia elata*. Mycobiology. 2002;30(1):22–26.

[22] Park EJ, Lee WY. In vitro symbiotic germination of myco-heterotrophic *Gastrodia elata* by *Mycena* species. Plant Biotechnol Rep. 2013;7(2):185–191.
[23] Lee HR, Han M, Choi MN, et al. Enhancement of the germination efficiency of *Gastrodia elata* seeds using a new *Mycena* species. J Plant Biotechnol. 2017;44(1):56–60.
[24] Fan L, Guo SX, Cao WQ, et al. Isolation, culture, identification and biological activity of *Mycena orchidicola* sp. nov in *Cymbidium sinense* (Orchidaceae). Mycosystema. 1996;15:251–255.
[25] Kornerup A, Wanscher JH. Methuen handbook of colour. London, England: Eyre Methuen; 1978. p. 1–252.
[26] Horak E, Hausknecht A. Röhrlinge und blätterpilze in europa: Bestimmu-ngsschlüssel für polyporales (pp), boletales, agaricales, russulales. 6th ed. Munich, Germany: Elsevier, Spektrum A-kad. Verlag; 2005. p. 1–555.
[27] White TJ, Bruns T, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York (NY): Academic Press; 1990. p. 315–322.
[28] Liu LN, Bau T. Two new *Xeromphalina* (Mycenaceae, Agaricales) species with eccentric stipes from subtropical China. Phytotaxa. 2018; 379(4):277–286.
[29] Zhang LC, Chen J, Lv YL, et al. *Mycena* sp. a mycorrhizal fungus of the orchid *Dendrobium officinale*. Mycol Prog. 2012;11(2):395–401.
[30] Higaki K, Rammitsu K, Yamashita Y, et al. A method for facilitating the seed germination of a mycoheterotrophic orchid, *Gastrodia pubilabiata*, using decomposed leaf litter harboring a basidiomycete fungus, *Mycena* sp. Bot Stud. 2017;58(1):59.
[31] Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32(5):1792–1797.
[32] Nylander JM. 2.3. Computer program and documentation distributed by the author. Uppsala, Sweden: Evolutionary Biology Centre, Uppsala University; 2004.
[33] Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 2003;19(12):1572–1574.
[34] Stamatakis A, Ludwig T, Meier H. RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatics. 2005;21(4):456–463.
[35] Rambaut A. FigTree v1. 4. Molecular evolution, phylogenetics and epidemiology. Edinburgh: University of Edinburgh, Institute of Evolutionary Biology; 2012.
[36] Maas Geesteranus RA. Conspectus of the Mycenas of the Northern Hemisphere-9. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Ser. C, Biological and medical sciences. 1988;91:43–83.
[37] Robich G. A revised key to the species of *Mycena* section *Fragilipedes* of the Northern hemisphere. Persoonia. 2006;19(1):1–43.
[38] Aravindakshan DM, Kumar TKA, Manimohan P. A new bioluminescent species of *Mycena* sect. *Exornatae* from Kerala state, India. Mycosphere. 2012;3(5):556–561.