

Two Newly Recorded Species of *Chlorophyllum* (Agaricaceae, Basidiomycota) in Taiwan

Yu-Ling Huang*, Jun-Xiang Xiao, Chia-Ling Wei, and Shu-Wen Chou

Department of Biology, National Museum of Natural Science, Taichung 404, Taiwan.

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Abstract. Mushrooms recently collected from the Botanical Garden and the yard of the National Museum of Natural Science (NMNS) in Taichung, Taiwan were identified as *Chlorophyllum globosum* and *C. hortense* through phylogenetic analyses and morphological characteristics, making new records of these fungi in Taiwan. Examining specimens of *Chlorophyllum* from Taiwan in the herbarium of NMNS (TNM) revealed that some *C. globosum* had previously been identified as *C. molybdites* in the collection. *Chlorophyllum molybdites* is widely distributed across Taiwan, and *C. globosum* is predominantly found in central Taiwan, where it is co-distributed with *C. molybdites* from late spring to fall. Additionally, a specimen which was previously recorded as *C. alborubescens* is actually *C. hortense* based on the phylogenetic analyses.

Key words: Agaricales, *Chlorophyllum alborubescens*, *Chlorophyllum globosum*, *Chlorophyllum hortense*, poisonous mushroom.

INTRODUCTION

The genus *Chlorophyllum* Massee (Agaricaceae, Agaricales) is recognized by the large basidiocarp with a hymenidermal pileus and a distinct annulus. Members of the genus usually have dextrinoid basidiospores with or without a germ pore, and basidiospores vary in color, including green, white, yellow, and brown (Loizides *et al.*, 2020). Green basidiospores were used as an easily recognized character for the genus, but molecular phylogenetic analyses combined some white-spore taxa from the genus *Macrolepiota* into *Chlorophyllum* (Vellinga, 2002). The genus also contains some sequestrate species which were previously placed in the genera *Endoptychum* Czern. and *Secotium* Kunze (Loizides *et al.*, 2020). Based on molecular phylogeny, the genus is divided into six sections (Ge *et al.*, 2018). Currently, 27 accepted species

of *Chlorophyllum* are listed in Mycobank (<https://www.mycobank.org/>, accessed on Feb. 23, 2024).

Members in the genus *Chlorophyllum* are saprotrophic and distributed worldwide, and they are often found in urban areas. *Chlorophyllum molybdites* (G. Mey.) Massee and *C. alborubescens* (Hongo) Vellinga are the only two species that have been recorded in Taiwan. The record of *C. molybdites* in Taiwan was based on specimens collected in Changhua, Taichung, and Tainan in 1981 (Liou, 1985). The large fruiting bodies of *C. molybdites* usually form a fairy ring in lawns after a rain, and it is one of the poisonous mushrooms which is most accidentally eaten by people (Lehmann and Khazan, 1992). *Chlorophyllum alborubescens* was first recorded as *Lepiota alborubescens* Hongo based on a specimen collected in Changhua in 1980 (Liou, 1985), and it was also reported as *Macrolepiota alborubescens* (Hongo) Hongo in Taiwan based

*Corresponding author. E-mail: ylhuang@nmns.edu.tw

on a specimen collected in Taichung (Chou, 2010). After examining *Chlorophyllum* specimens deposited in the mycological herbarium (TNM) of the National Museum of Natural Science (NMNS; Taichung, Taiwan) using phylogenetic analyses and morphological characters, we report two newly recorded species of *Chlorophyllum* in Taiwan: *C. globosum* (Mossebo) Vellinga and *C. hortense* (Murrill) Vellinga.

MATERIALS AND METHODS

Specimen Collection and Morphological Features

Five fresh specimens were recently collected from the Botanical Garden and the yard of NMNS, Taichung, Taiwan. Macroscopic features were recorded and photographed at the sample site, and then the specimens were freeze-dried or oven-dried for long-term storage. Additionally, TNM specimens originally identified as *Chlorophyllum* species and related species, including 20 *C. molybdites* and one *Macrolepiota alborubescens*, were selected for phylogenetic analyses to confirm their identity (Table 1).

Morphological features were examined by a Leica DM2500 light microscope. Microscopic samples were mounted in 1% phloxine in a 5% KOH solution for observation and measurement. Melzer's reagent was used to detect amyloid or dextrinoid reactions of basidiospores. Microscopic features were photographed under a 1000× lens using a Canon EOS60 camera and measured using ImageJ software. Specimens collected in this study were deposited in the TNM after examination.

Molecular Phylogenetic Study

Genomic DNA was extracted from fungal mycelia using the Viogene Genomic DNA Extraction Miniprep System (Viogene BioTek, New Taipei City, Taiwan) following the manufacturer's instructions. Genomic DNA was stored at -20 °C until polymerase chain reaction (PCR) amplification. ITS1/ITS4 primers were mainly used to amplify the nuclear ribosomal DNA fragment containing the ITS1-5.8S-ITS2 sequence. ITS1F/ITS4 or ITS1/LR3 primers were used when ITS1/ITS4 failed to amplify a fragment. Each PCR included 1 µL of the DNA template, 0.8 µL of each primer,

10 µL 2× SuperRed MasterMix with loading dye (BIOTOOLS, New Taipei City, Taiwan), and 7.4 µL distilled water (dH₂O). The PCR protocol began at 95 °C for 1 min, followed by 30 cycles of 95 °C for 30 s, 54 °C for 30 s, 72 °C for 1 min 30 s, and then 72 °C for 7 min before ending at 15 °C. All PCRs were conducted using a SimpliAmp™ thermal cycler (Applied Biosystems, MA, USA). To confirm PCR success, PCR products were loaded in a 1% agarose gel in 1× TAE buffer with 5% HealthView nucleic acid stain (Genomics, Taipei, Taiwan) and run for 25 min at 100 V. Gels were visualized using a G:Box Mini imaging system with GeneSys software (SYNGENE, MD, USA). For each sample, the PCR product and 1 µL of 10 µM primer were mixed and sent to Genomics BioSci & Technology Co. Ltd. (New Taipei City, Taiwan) for Sanger sequencing on an ABI 3730 xl DNA Analyzer (ThermoFisher Scientific Inc, MA, USA). Resulting sequences were manually edited using Chromaseq Package vers. 1.53 (Maddison and Maddison, 2021). ITS sequences of other *Chlorophyllum* species were acquired from GenBank or previous publications (Ge *et al.*, 2018; Maubet *et al.*, 2022). Totally, 65 ITS sequences were used for the phylogenetic analyses, and 26 of them were newly generated from this study using TNM specimens (Table 2). All sequences were aligned using MAFFT (Kuraku *et al.*, 2013; Katoh *et al.*, 2019). A phylogenetic tree was inferred by the maximum likelihood method with 1000 bootstrap replicates using RAxML (Stamatakis, 2014) on the CIPRES Science Gateway (Miller *et al.*, 2010).

RESULTS

Phylogenetic Analyses

The phylogenetic tree of the ITS locus using 19 *Chlorophyllum* species and *Clarkeinda trachodes* (Berk.) Singer as the outgroup is shown in Fig. 1. *Chlorophyllum molybdites* and *C. globosum* were clustered into two clades sister to each other with strong supports, respectively. The four specimens (F0037741~F0037744; Fig. 2) collected from the Botanical Garden in this study clustered with *C. globosum*, and the microscopic morphology also confirmed that they were *C. globosum*. Five TNM specimens which were identified as *C. molybdites* also clustered with *C. globosum* in the phylogenetic

Table 1. Collection data of TNM specimens used in this study

TNM no.	Collector's no.	Taxon	Site	Collector	Date
F0003840*	CWN 01230	<i>Chlorophyllum globosum</i>	Shuili, Nantou	W.-N. Chou	1995/8/24
F0008371*	CWN 02346	<i>Chlorophyllum globosum</i>	Tunghai University, Taichung	M.-M. Su	1997/5/28
F0016151*	CWN 05557	<i>Chlorophyllum globosum</i>	on the way to Hsiehshankeng, Tungshi, Taichung	W.-N. Chou	2002/7/16
F0025667*	CWN 06516	<i>Chlorophyllum globosum</i>	National Museum of Natural Science, Taichung	W.-N. Chou	2004/4/9
F0034522*	Chen 3982	<i>Chlorophyllum globosum</i>	Botanical Garden, National Museum of Natural Science, Taichung	S.-Z. Chen	2018/6/25
F0037741*	YLH0428	<i>Chlorophyllum globosum</i>	Botanical Garden, National Museum of Natural Science, Taichung	Y.-L. Huang	2022/3/21
F0037742*	YLH0432	<i>Chlorophyllum globosum</i>	Botanical Garden, National Museum of Natural Science, Taichung	Y.-L. Huang	2022/4/8
F0037743*	YLH0450	<i>Chlorophyllum globosum</i>	Botanical Garden, National Museum of Natural Science, Taichung	Y.-L. Huang	2022/5/23
F0037744*	YLH0454	<i>Chlorophyllum globosum</i>	Botanical Garden, National Museum of Natural Science, Taichung	Y.-L. Huang	2023/4/7
F0016369**	CWN 05896	<i>Chlorophyllum hortense</i>	National Museum of Natural Science, Taichung	W.-N. Chou	2002/10/8
F0036445*	WEI 20-001	<i>Chlorophyllum hortense</i>	National Museum of Natural Science, Taichung	C.-L. Wei	2020/1/6
F0000641	93050607	<i>Chlorophyllum molybdites</i>	National Museum of Natural Science, Taichung	S.-H. Wu	1992/5/4
F0002651	CWN 00677	<i>Chlorophyllum molybdites</i>	National Museum of Natural Science, Taichung	W.-N. Chou	1994/8/9
F0003276	CWN 00826	<i>Chlorophyllum molybdites</i>	National Museum of Natural Science, Taichung	W.-N. Chou	1995/5/23
F0003391	CWN 00949	<i>Chlorophyllum molybdites</i>	Tungshan Park, Ilan	W.-N. Chou	1995/6/15
F0004361	CWN 01275	<i>Chlorophyllum molybdites</i>	Tunghsiao, Miaoli	S.-H. Chang	1995/9/28
F0004362	CWN 01276	<i>Chlorophyllum molybdites</i>	Lintsoliao, Yunlin	W.-N. Chou	1995/9/29
F0008374	CWN 02349	<i>Chlorophyllum molybdites</i>	Chungming Primary School, Taichung	S.-Y. Chuang	1997/5/28
F0008394	CWN 02371	<i>Chlorophyllum molybdites</i>	Lienhuaohih, Nantou	W.-N. Chou	1997/6/6
F0015918	CWN 05141	<i>Chlorophyllum molybdites</i>	Wenchang Primary School, Taichung	S.-H. Liu	2001/7/18
F0016084	CWN 05457	<i>Chlorophyllum molybdites</i>	Taichung Veterans General Hospital, Taichung	T.-L. Li	2002/6/7
F0023193	CWN-TF843	<i>Chlorophyllum molybdites</i>	Peimen, Taipei	W.-N. Chou	1991/9/29
F0025893	CWN 06752	<i>Chlorophyllum molybdites</i>	Tunghsiao, Miaoli	W.-N. Chou	2004/9/13
F0026122	CWN 06339	<i>Chlorophyllum molybdites</i>	National Museum of Natural Science, Taichung	W.-N. Chou	2003/8/18
F0026167	–	<i>Chlorophyllum molybdites</i>	National Kinmen Senior High School, Kinmen	C.-C. Tung	2012/6/25
F0036369	CWN 07899	<i>Chlorophyllum molybdites</i>	National Museum of Natural Science, Taichung	W.-N. Chou	2008/5/26

Notes: * Specimen was originally identified as *C. molybdites*. ** Specimen was originally identified as *Macrolepiota alborubescens*.[†] Specimen was collected in this study.

Specimens examined in this study are shown in **bold**. TNM, herbarium of the National Museum of Natural Science, Taichung, Taiwan.

Table 2. List of ITS sequences for the phylogenetic analysis

Taxon	Specimen no.	GenBank no.
<i>Chlorophyllum africanum</i>	PREM 62140	MG741961
<i>Chlorophyllum africanum</i>	PREM 62141	MG741963
<i>Chlorophyllum agaricoides</i>	HKAS 101312	MG742003
<i>Chlorophyllum agaricoides</i>	HMAS 71678	MG742004
<i>Chlorophyllum arizonicum</i>	AH31724	KR233490
<i>Chlorophyllum arizonicum</i>	Trappe 11481	HQ020416
<i>Chlorophyllum brunneum</i>	H. Manson 8	AY083206
<i>Chlorophyllum demangei</i>	Z. W. Ge 3112	MG741965
<i>Chlorophyllum globosum</i>	CWN 01230 (F0003840)	PP727215
<i>Chlorophyllum globosum</i>	CWN 02346 (F0008371)	PP727218
<i>Chlorophyllum globosum</i>	CWN 05557 (F0016151)	PP727223
<i>Chlorophyllum globosum</i>	CWN 06516 (F0025667)	PP727226
<i>Chlorophyllum globosum</i>	Chen 3982 (F0034522)	PP727230
<i>Chlorophyllum globosum</i>	YLH0428 (F0037741)	PP727233
<i>Chlorophyllum globosum</i>	YLH0432 (F0037742)	PP727234
<i>Chlorophyllum globosum</i>	YLH0450 (F0037743)	PP727235
<i>Chlorophyllum globosum</i>	YLH0454 (F0037744)	PP727236
<i>Chlorophyllum globosum</i>	PREM 62147	MG742002
<i>Chlorophyllum globosum</i>	Z. W. Ge 2006-1	MG741995
<i>Chlorophyllum hortense</i>	CWN 05896 (F0016369)	PP727224
<i>Chlorophyllum hortense</i>	WEI 20-001 (F0036445)	PP727232
<i>Chlorophyllum hortense</i>	HKAS 101317	MG741967
<i>Chlorophyllum hortense</i>	HKAS 90470	MG741971
<i>Chlorophyllum hortense</i>	Z. W. Ge 3115	MG741968
<i>Chlorophyllum levantinum</i>	ACAM 2012-320	NR_169974
<i>Chlorophyllum lusitanicum</i>	AH43927	KR233483
<i>Chlorophyllum lusitanicum</i>	AH45540	KR233482
<i>Chlorophyllum molybdites</i>	93050607 (F0000641)	PP727211
<i>Chlorophyllum molybdites</i>	CWN 00677 (F0002651)	PP727212
<i>Chlorophyllum molybdites</i>	CWN 00826 (F0003276)	PP727213
<i>Chlorophyllum molybdites</i>	CWN 00949 (F0003391)	PP727214
<i>Chlorophyllum molybdites</i>	CWN 01275 (F0004361)	PP727216
<i>Chlorophyllum molybdites</i>	CWN 01276 (F0004362)	PP727217
<i>Chlorophyllum molybdites</i>	CWN 02349 (F0008374)	PP727219
<i>Chlorophyllum molybdites</i>	CWN 02371 (F0008394)	PP727220
<i>Chlorophyllum molybdites</i>	CWN 05141 (F0015918)	PP727221
<i>Chlorophyllum molybdites</i>	CWN 05457 (F0016084)	PP727222
<i>Chlorophyllum molybdites</i>	CWN-TF843 (F0023193)	PP727225
<i>Chlorophyllum molybdites</i>	CWN 06752 (F0025893)	PP727227
<i>Chlorophyllum molybdites</i>	CWN 06339 (F0026122)	PP727228

Table 2. (continued)

Taxon	Specimen no.	GenBank no.
<i>Chlorophyllum molybdites</i>	F0026167	PP727229
<i>Chlorophyllum molybdites</i>	CWN 07899 (F0036369)	PP727231
<i>Chlorophyllum molybdites</i>	HKAS 101322	MG741988
<i>Chlorophyllum molybdites</i>	HKAS 45051	MG741985
<i>Chlorophyllum molybdites</i>	Z. W. Ge 3146	MG741987
<i>Chlorophyllum molybdites</i>	Z. W. Ge 3377	MG741992
<i>Chlorophyllum molybdites</i>	Z. W. Ge 3381	MG741993
<i>Chlorophyllum neomastoideum</i>	HKAS 83208	MG741976
<i>Chlorophyllum nothorachodes</i>	H. Lepp 1142	AF482855
<i>Chlorophyllum olivieri</i>	HKAS 31587	MG742016
<i>Chlorophyllum olivieri</i>	HKAS 53466	MG742017
<i>Chlorophyllum palaeotropicum</i>	HKAS 93747	MG741983
<i>Chlorophyllum palaeotropicum</i>	PREM 62142	MG741978
<i>Chlorophyllum palaeotropicum</i>	PREM 62145	MG741982
<i>Chlorophyllum pseudoglobosum</i>	AM155	KP642506
<i>Chlorophyllum rhacodes</i>	E.C. Vellinga 2106	AF482849
<i>Chlorophyllum rhacodes</i>	VPI-OKM19588	U85312
<i>Chlorophyllum sphaerosporum</i>	HMAS 66153	MG742011
<i>Chlorophyllum sphaerosporum</i>	HMAS 71683	MG742012
<i>Chlorophyllum subrhacodes</i>	Z. W. Ge 3232	MG741973
<i>Chlorophyllum subrhacodes</i>	Z. W. Ge 3242	MG741974
<i>Chlorophyllum subrhacodes</i>	Z. W. Ge 3385	MG741972
<i>Chlorophyllum subrhacodes</i>	Z. W. Ge 3411	MG741975
<i>Chlorophyllum venenatum</i>	IZS20117147/12A-149A	MZ005539
<i>Clarkeinda trachodes</i>	E.C. Vellinga 3550 (UC)	HM488751

Note: Newly generated sequences are shown in bold. ITS, internal transcribed spacer.

tree. Another specimen (F0036445; Fig. 3) collected from the yard of the NMNS in this study was morphologically identified as *C. hortense* and clustered with other *C. hortense*. The TNM specimen (F0016369) which was identified as *Macrolepiota alborubescens* clustered with *C. hortense* as well.

Taxonomy

Chlorophyllum globosum (Mossebo) Vellinga, Mycotaxon 83: 416, 2002. Fig. 2.

≡ *Macrolepiota globose* Mossebo, Mycotaxon 76: 268, 2000.

Pileus 9–18 cm broad, subglobose when young,

convex to flat when mature; surface dry, off-white with large light-brownish patches at the center and small scales scattered to the margin; margin sulcate to plicate and sometimes wavy. Lamellae free, crowded, and smooth; off-white when young and becoming grayish-green when mature. Stipe 14–26 cm, off-white to light reddish-brown, fibrous, swollen at base. Annulus double, persistent. Basidiospores 10–13.4 × 6.4–9.1 μm, Q = 1.34–1.75, colorless or pale green, amygdaliform, thick-walled, with apical germ pore, dextrinoid. Basidia 29–51 × 11.5–16 μm, clavate, 4-spored. Cheilocystidia 28.5–75.5 × 14.5–40 μm, broadly clavate to pyriform,

occasionally with long stalk. Clamp connections absent.

Specimens examined: See Table 1.

Habitat: Fruiting in groups, on soils, grassland.

Distribution: Taiwan (this study), Africa, China, India, Laos, and Thailand (Sysouphanthong *et al.*, 2021).

Notes: This is the first record of *C. globosum* in Taiwan. Specimens of *C. globosum* were previously identified as *C. molybdites* in Taiwan. The two *Chlorophyllum* species are similar in their basidiocarps and other characteristics, but their basidiospores differ in size and shape. *Chlorophyllum globosum* has broader

basidiospores than *C. molybdites*, and the hilar appendix end of the basidiospore protrudes more in *C. globosum* than in *C. molybdites*. In addition, *C. globosum* has longer cheilocystidia with a distinct stalk (Sysouphanthong *et al.*, 2021). Distinguishing the two species can be difficult without molecular data. Both species have been found in the same grassland area in Taiwan, with *C. globosum* usually found from late spring to summer in central Taiwan, and *C. molybdites* found from summer to fall throughout the main island of Taiwan, Kinmen (Liou, 1985), and Lanyu (Orchid Is.; Yeh and Chen, 1991).

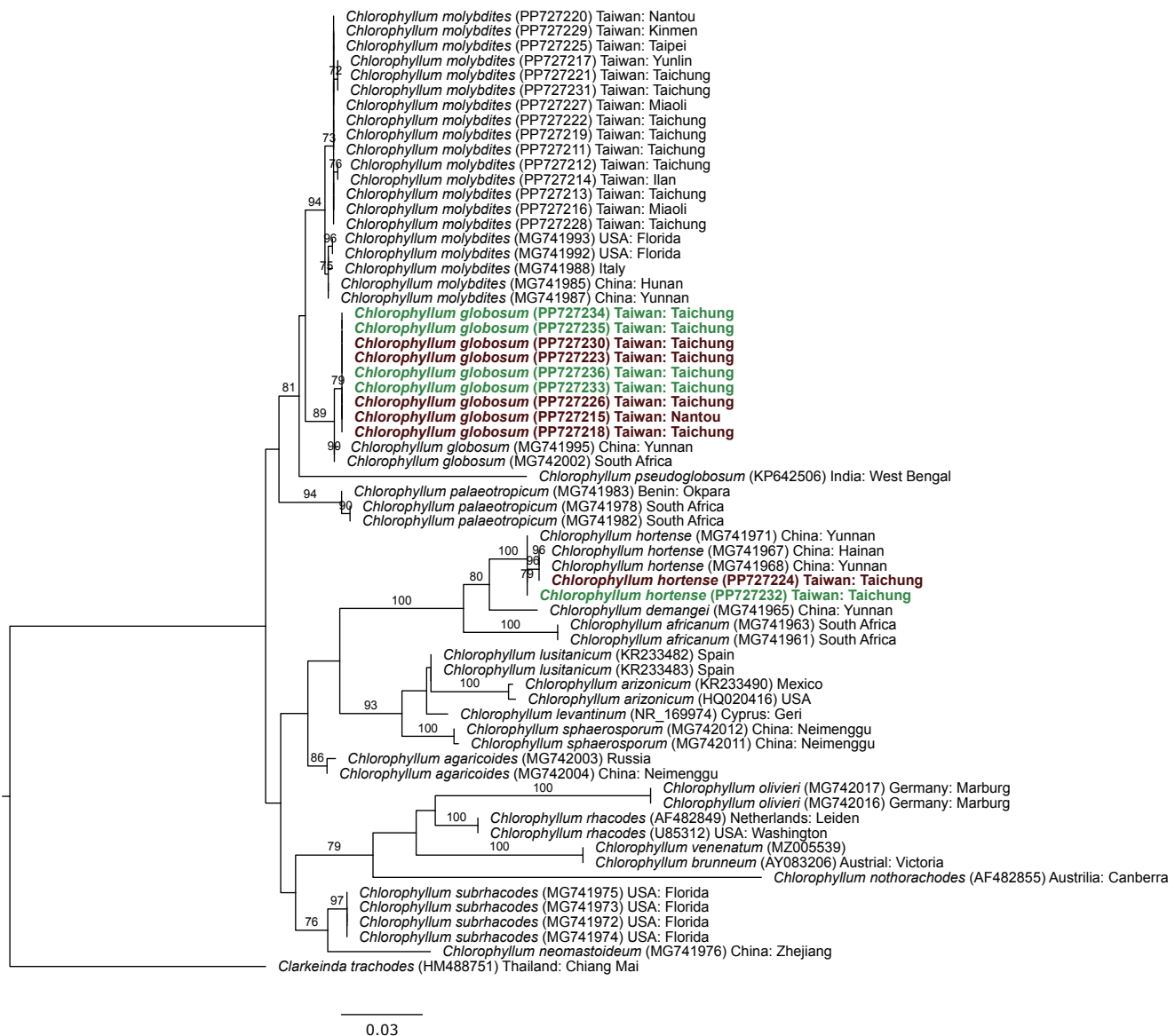


Fig. 1. Maximum likelihood phylogenetic tree generated from the ITS locus. GenBank accession numbers are shown in parentheses after species names with sample country and location after them. Specimens collected in this study are in green. TNM specimens which were identified as other species are in brown.

Chlorophyllum hortense (Murrill) Vellinga, Mycotaxon 83: 416, 2002. Fig. 3.

≡ *Lepiota hortensis* Murrill, North American Flora 10(1): 59, 1917.

≡ *Leucoagaricus hortensis* (Murrill) Pegler, Kew Bulletin Additional Series 9: 414, 1983.

Pileus 3.8–5.2 cm broad, conic to convex, umbonate when young, flat when mature; surface dry, white to off-white, squamulose with a light orange-brown patch in center, squamules scattered to margin; margin slightly sulcate. Lamellae free, crowded, off-white. Stipe 3.5 × 0.8 cm, reddish-brown. Annulus double, persistent. Basidiospores 8–12.7 × 5.9–8.1 μm, Q = 1.33–1.84, ellipsoid to oblong, thick-walled, dextrinoid. Basidia 23–37.5 × 6.5–10 μm, clavate, 2-spored. Cheilocystidia 30–45.5 × 6–7.5 μm, cylindro-clavate or strangulated. Clamp connections absent.

Habitat: Grows in groups, on soils, grassland.

Specimens examined: See Table 1.

Distribution: Taiwan (this study), China, Pakistan (Jabeen *et al.*, 2021), Thailand, (Sysouphanthong *et al.*, 2021), the USA, South America (Maubet *et al.*, 2022), and Australia (Vellinga, 2003).

Notes: This is the first record of *C. hortense* in Taiwan. The specimen CWN 05896 (TNM F0016369) was previously identified as *Macrolepiota alborubescens*. *Chlorophyllum hortense* is the type species for *Chlorophyllum* sect. *Ellipsoidospororum* Z.W. Ge (Ge *et al.*, 2018). *Chlorophyllum alborubescens* was considered a synonym of *C. hortense* in some studies (Ge *et al.*, 2018; Sysouphanthong *et al.*, 2021), but no official combination of this treatment has been published. Both MycoBank and Index Fungorum treat *C. hortense* and *C. alborubescens* as different species. Further confirmation of their status is necessary. *Chlorophyllum alborubescens* has been recorded



Fig. 2. *Chlorophyllum globosum*. A, B. F0037741. C, D. F0037742. E, F. F0037743. G, H. F0037744. I. Basidiospores. J. Dextrinoid basidiospores. K. Basidium. L, M. Cheilocystidia. Scale bars: A–H = 2 cm, I–M = 10 μm.

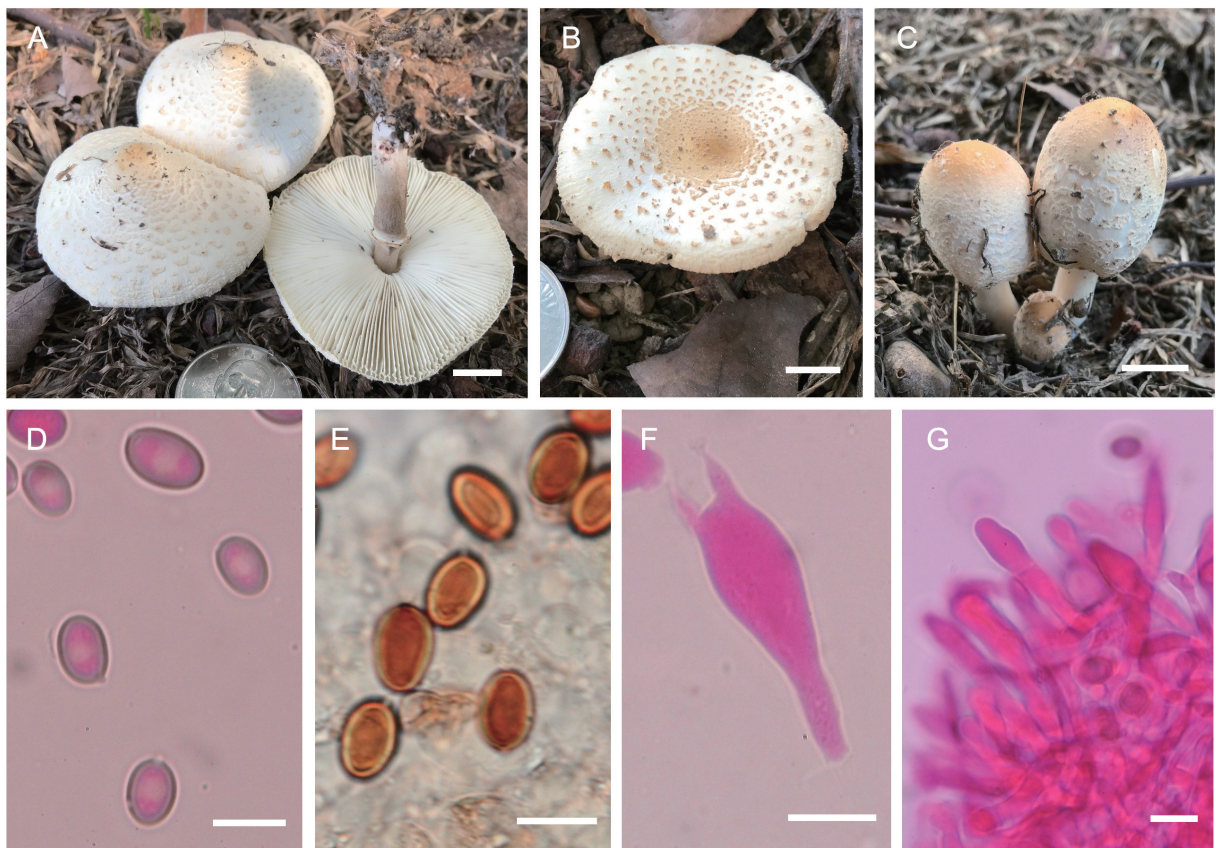


Fig. 3. *Chlorophyllum hortense*. A–C. F0036445. D. Basidiospores. E. Dextrinoid basidiospores. F. Basidium. G. Cheilocystidia. Scale bars: A–C = 1 cm, D–G = 10 μ m.

as *Lepiota alborubescens* (Liou, 1985) and *Macrolepiota alborubescens* (Chou, 2010) in Taiwan. The latter record was based on specimen CWN 05896 (TNM F0016369) which was identified as *C. hortense* by phylogenetic analyses and morphological examination in this study. Since we did not have access to specimens examined by Liou (1985), we could not confirm if the earlier record of *C. alborubescens* is also *C. hortense*. Here, we suggest retaining *C. alborubescens* on the list of fungal flora in Taiwan, with *C. hortense* as a newly recorded species. Currently, four *Chlorophyllum* species are recorded in Taiwan.

Key to the 4 species of *Chlorophyllum* in Taiwan

- 1. Basidia 2-spored.....2
Basidia 4-spored.....3
- 2. Pileus up to 10 cm; cheilocystidia clavate.....
.....*C. alborubescens*
Pileus up to 6 cm; cheilocystidia cylindro-clavate or strangulated.....*C. hortense*
- 3. Spore print green; cheilocystidia clavate.....
.....*C. molybdites*

Spore print pale yellow; cheilocystidia clavate, occasionally with long stalk.....*C. globosum*

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台灣兩種綠褶菇屬新紀錄種

黃俞菱、蕭均祥、魏佳玲、周舒雯

國立自然科學博物館生物學組

透過系統支序學分析和形態特徵鑑定於國立自然科學博物館轄內與植物園採集到的真菌標本，確認為球蓋綠褶菇(*Chlorophyllum globosum*)與庭院綠褶菇(*Chlorophyllum hortense*)，兩者皆為台灣之新紀錄種。另檢視蒐藏於標本館之台灣各地綠褶菇(*Chlorophyllum molybdites*)標本時，發現其中部分標本實為球蓋綠褶菇。綠褶菇廣泛分佈在台灣各地，而球蓋綠褶菇則主要分布在台灣中部，兩者在中部的分佈地區相近且皆於春末到秋季的時期出現。此外，曾在台灣發表過之記錄種淡紅環柄菇(*Chlorophyllum alborubescens*)之引證標本，經分子支序學分析後，應為庭院綠褶菇。

關鍵詞：傘菌目、淡紅環柄菇、球蓋綠褶菇、庭院綠褶菇、毒菇