

Cyptotrama glabra and *Hymenopellis raphanipes* newly recorded in Taiwan

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ABSTRACT

Two wood-inhabiting agarics, *Cyptotrama glabra* and *Hymenopellis raphanipes*, are newly recorded in Taiwan. Their detailed descriptions and illustrations are provided. Phylogenetic analyses using sequences of the internal transcribed spacer (ITS rDNA) were carried out to confirm our morphology-based determinations.

Key words: Agaricales, *Oudemansiella radicata*, phylogeny, Physalacriaceae, taxonomy

Introduction

The agaric genera *Cyptotrama* Singer and *Hymenopellis* R.H. Petersen are classified in the family Physalacriaceae Corner, belonging to the order Agaricales Underw. *Cyptotrama* is characterized by its lignicolous habitat, non-viscid and smooth-floccose pilei surface, simple- or nodose-septate generative hyphae, and smooth basidiospores (Qin & Yang 2016). Within the genus, *C. asprata* (Berk.) Redhead & Ginns is the only species reported in Taiwan (Tzean et al. 2015). *Hymenopellis* is characterized by its slightly convex to plane pilei with a slippery and viscid-glutinous surface when moist, presence of pseudorhizae, smooth and large basidiospores, and association with buried wood (Petersen & Hughes 2010). Species of *Hymenopellis* has never been reported in Taiwan. In this study,

Cyptotrama glabra and *Hymenopellis raphanipes* are reported in Taiwan for the first time based on morphological and molecular characterization.

Materials and Methods

The studied specimens are deposited at the herbarium of the National Museum of Natural Science, Taichung, Taiwan (TNM). Freehand thin sections of basidiocarps were prepared with the sharp scalpel and mounted in (i) 5% KOH with 1% phloxine stain for observation and measurement and (ii) Melzer's solution to examine amyloidity and dextrinoidity. The abbreviations are defined as follows: L = the average length of all basidiospores; W = the average width of all basidiospores; Q = length/width ratio of spore in side view; and Q_m = the average Q of all basidiospores \pm sample standard deviation.

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Table 1. Species and sequences used in the phylogenetic analyses. Newly generated sequences are shown in bold.

Taxon	Strain/Specimens	ITS	Locality
<i>Cyptotrama angustispora</i>	Yang5681	KR607194	Laos
<i>C. asprata</i>	Ge2627	KJ024098	China
<i>C. asprata</i>	Hao781	KR607190	China
<i>C. asprata</i>	LIP CL/ Mart03156	KM588682	France
<i>C. asprata</i>	PDD78164	KR607177	New Zealand
<i>C. chrysopepla</i>	Ge2258	KR607197	USA
<i>C. fagiphila</i>	BRNM 751955	KM588683	Spain
<i>C. fagiphila</i>	SEST 09081503	KM588684	Spain
<i>C. glabra</i>	Li820	KR607208	China
<i>C. glabra</i>	QT1084201	LC512056	Taiwan
<i>C. glabra</i>	Yang4687	KR607207	China
<i>C. glabra</i>	Yang5658	KR607205	Laos
<i>C. shenmongjia</i>	Cai805	KR607195	China
<i>C. shenmongjia</i>	Qin558	KR607196	China
<i>C. songolarum</i>	LIP Y AnkeiA3	KM588685	Congo, Brazzaville
<i>Dactylosporina glutinosa</i>	MCA1775	HM005074	Guyana
<i>D. glutinosa</i>	MCA1860	HM005075	Guyana
<i>D. macracantha</i>	CLO3711	HM005077	Panama
<i>D. macracantha</i>	TFB10789	HM005076	Argentina
<i>D. steffanii</i>	TFB10698c2	HM005071	Costa Rica
<i>D. steffanii</i>	TFB10698c3	HM005073	Costa Rica
<i>Hymenopellis colensoi</i>	ZT12902	HM005139	New Zealand
<i>H. colensoi</i>	ZT12902h2	HM005140	New Zealand
<i>H. furfuracea</i>	TFB12535h1	GQ913366	USA
<i>H. furfuracea</i>	TFB13415	GQ913364	USA
<i>H. furfuracea</i>	TFB4078	GQ913365	USA
<i>H. gigaspora</i>	TENN50050	GQ913359	Australia
<i>H. gigaspora</i>	TENN50056	GQ913358	Australia
<i>H. incognita</i>	TENN58768	GQ913424	USA
<i>H. incognita</i>	TENN60228	GQ913419	USA
<i>H. japonica</i>	HKAS61674	KX688225	China
<i>H. japonica</i>	HKAS83175	KX688226	China
<i>H. limonispora</i>	TENN50261	GQ913402	USA
<i>H. limonispora</i>	TENN59438	GQ913406	USA
<i>H. limonispora</i>	TENN61379	GQ913403	USA
<i>H. megalospora</i>	TENN51257	GQ913411	USA
<i>H. orientalis</i>	TM I2IX2002	GQ913396	Japan
<i>H. orientalis</i>	TM I2IX2002	GQ913397	Japan
<i>H. orientalis</i>	TM I2IX2002	GQ913398	Japan
<i>H. radicata</i>	TENN59329	GQ913380	Austria
<i>H. radicata</i>	TENN62837	GQ913375	Sweden
<i>H. raphanipes</i>	CWN04019	LC512055	Taiwan
<i>H. raphanipes</i>	HKAS42503	GU980130	China
<i>H. raphanipes</i>	HKAS42555	GU980129	China
<i>H. raphanipes</i>	JBZ 2122002	KX688229	China
<i>H. raphanipes</i>	YLH0074	LC512057	Taiwan
<i>H. raphanipes</i>	YLH0185	LC512059	Taiwan
<i>H. rubrobrunnescens</i>	TENN51262	GQ913373	USA
<i>H. rubrobrunnescens</i>	TENN52479	GQ913371	USA
<i>H. rubrobrunnescens</i>	TENN52654	GQ913372	USA
<i>H. rugosoceps</i>	TENN57307	GQ913395	USA
<i>H. rugosoceps</i>	TENN60604	GQ913394	USA
<i>H. sinapicolor</i>	TENN56566	GQ913350	USA
<i>H. sinapicolor</i>	TENN56566	GQ913351	USA
<i>H. sinapicolor</i>	TENN56566	GQ913352	USA
<i>H. superbiens</i>	MEL2291946	GQ913360	Australia
<i>H. superbiens</i>	MEL2291946	GQ913361	Australia
<i>H. trichofera</i>	MEL2293664	GQ913354	Australia
<i>H. vinocontusa</i>	TMI7669	GQ913370	Japan
<i>Mucidula brunneomarginata</i>	TENN53020	GQ844243	Russia
<i>M. brunneomarginata</i>	TENN52362	GQ844244	Russia
<i>M. mucida</i> var. <i>asiatica</i>	LFZ265	GQ844242	China
<i>M. mucida</i> var. <i>asiatica</i>	TENN48240	GQ844231	Japan
<i>M. mucida</i> var. <i>mucida</i>	TENN50662	GQ844233	UK, Scotland
<i>M. mucida</i> var. <i>mucida</i>	TENN59324	GQ844235	Australia
<i>M. mucida</i> var. <i>mucida</i>	TENN62246	EU598203	Germany
<i>M. venosola-mellata</i>	SU20040912	AB499077	Japan
<i>M. venosola-mellata</i>	SU20080524	AB688122	Japan
<i>M. venosola-mellata</i>	YLH0076	LC512058	Taiwan
<i>Oudemansiella apalosarca</i>	RV95/297	AF321472	Australia
<i>O. apalosarca</i>	RV95/416	AF321473	Australia
<i>O. apalosarca</i>	RV95/852	AF321475	Papua New Guinea
<i>O. canarii</i>	RV96/35	AF321477	Costa Rica
<i>O. canarii</i>	RVPR100	AF321479	Puerto Rico

<i>O. canarii</i>	TENN62802	GQ892793	USA
<i>Paraxerula americana</i>	CLO4744	HM005141	USA
<i>P. americana</i>	CLO4746	HM005142	USA
<i>P. americana</i>	DBG21746	HM005143	USA
<i>P. hongoi</i>	C60612	HM005144	Russia
<i>Ponticulomyces kedrovayae</i>	TENN60767	HM005145	Russia
<i>P. kedrovayae</i>	TENN60767	HM005146	Russia
<i>Protixerula flavo-olivacea</i>	NY REH8781	HM005147	Australia
<i>P. flavo-olivacea</i>	NY REH8781	HM005148	Australia
<i>P. flavo-olivacea</i>	NY REH8781	HM005149	Australia
<i>Strobilurus occidentalis</i>	TENN52255	GQ892806	USA
<i>S. occidentalis</i>	TENN52275	GQ892805	USA
<i>Xerula pudens</i>	TENN59208	HM005154	Australia
<i>X. pudens</i>	C63308	HM005155	Spain

DNA were extracted from dried specimens using the Plant Genomic DNA Extraction Miniprep System (Viogene). The commercial primer pairs ITS1/ITS4 were used for the internal transcribed spacer (ITS) rDNA region. The PCR products were purified and sequenced by the MB Mission Biotech Company, Taiwan. Obtained sequences were submitted to the DNA Data Bank of Japan (DDBJ). Two separate ITS-based phylogenies inferred from the Maximum Likelihood (ML) algorithm were carried out to confirm morphological determinations of *Cyptotrama glabra* and *Hymenopellis raphanipes*. The newly generated sequences as well as other representative sequences retrieved from GenBank (NCBI) (Table 1) were aligned using MAFFT version 7 and then manually adjusted using BioEdit version 7.0.5. The ML analyses were performed using RAxML BlackBox (Stamatakis et al. 2014) with parameter settings following Wu et al. (2018).

Taxonomy

Cyptotrama glabra Zhu L. Yang & J. Qin, Fungal Biology 120:519. 2016.

Figs. 1A–C & 2

Pileus hemispherical when young, becoming convex to plane when mature, 3–6 cm in diam, center often slightly depressed, ochraceous; other parts of pileus orange-yellow; margin pinkish to olivaceous grey with faintly translucent striations. Lamellae adnexed to nearly free, distant, with lamellulae, white to cream. Stipe subcylindrical, 3–5 cm in length, 3–7 mm in diam, hollow, slightly enlarged at base, cream to white, sometimes slightly colorless.

Hyphal system monomitic; hyphae simple-septate. Basidiospores hyaline, oblong to cylindrical, $(10\text{--})10.2\text{--}11.6\text{--}(12) \times (5\text{--})5.4\text{--}6.8\text{--}(7) \mu\text{m}$ ($L = 10.9 \mu\text{m}$, $W = 6.1 \mu\text{m}$, $Q = 1.43\text{--}2.20$, $Q_m = 1.8 \pm 0.2$), thin-walled, smooth, inamyloid, non-dextrinoid. Basidia clavate to narrowly clavate, $37\text{--}42 \times 7\text{--}8 \mu\text{m}$, 4-sterigmate. Cheilocystidia hyaline, clavate to broadly clavate, subfusiform, $18\text{--}25 \times 6\text{--}11 \mu\text{m}$, crowded, thin-walled. Pleurocystidia hyaline, subfusiform, narrow to acute at apex, $92\text{--}97 \times 15\text{--}18 \mu\text{m}$, scattered, slightly thick-walled. Pileipellis an epithelium composed of subglobose, clavate, ovoid to obovoid cells. Caulocystidia hyaline, clavate, $17\text{--}24 \times 10\text{--}12 \mu\text{m}$, scattered, sometimes narrowly clavate to subfusiform, $35\text{--}60 \times 6\text{--}15 \mu\text{m}$, thin-walled.

Specimens examined. TAIWAN. Nantou County: Aowanda National Forest Recreation Area, $23^{\circ}57'N$, $121^{\circ}10'E$, 1200 m, on angiosperm wood, 20 Apr 2019, *G.T. Wu*, *QT1084201* (TNM F33101); Lienhuachih, on rotten wood, 16 Mar 2000, *W.N. Chou*, *CWN 04558* (TNM F14212).



Fig. 1. Basidiocarps of *Cyptotrama glabra* (A–C: QT1084201) and *Hymenopellis raphanipes* (D: YLH0074, E–F: YLH0185). Bars: A–C = 1 cm; D–F = 2 cm.

Ecology and distribution. On rotten angiosperm wood in tropical to subtropical forests, during spring and summer at 300–2600 m in SW China, Laos (Qin & Yang 2016) and Taiwan (this study).

Notes. Morphological features of our specimens correspond well with the original description of this species (Qin & Yang 2016) except that cheilocystidia are slightly narrower and subfusiform, pleurocystidia possess slightly thicken walls, and

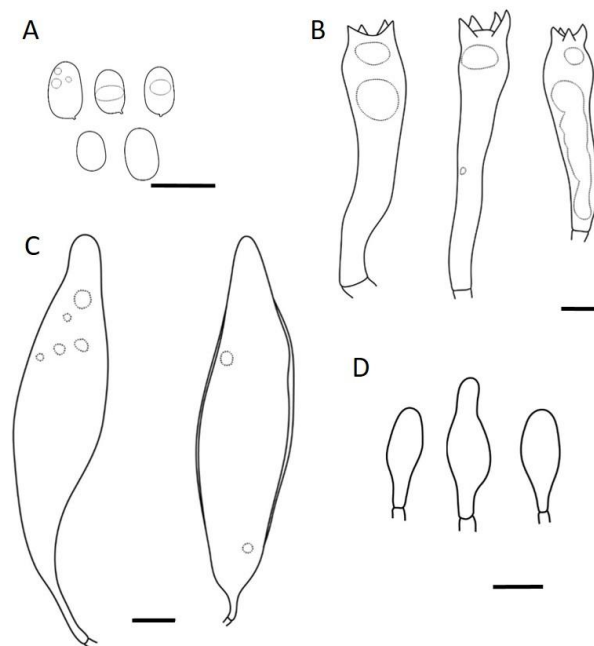


Fig. 2. Micromorphological features of *Cyptotrama glabra* (drawn from QT1084201). A. Basidiospores. B. Basidia. C. Pleurocystidia. D. Cheilocystidia. Bars: Bars: A–D = 10 μ m.

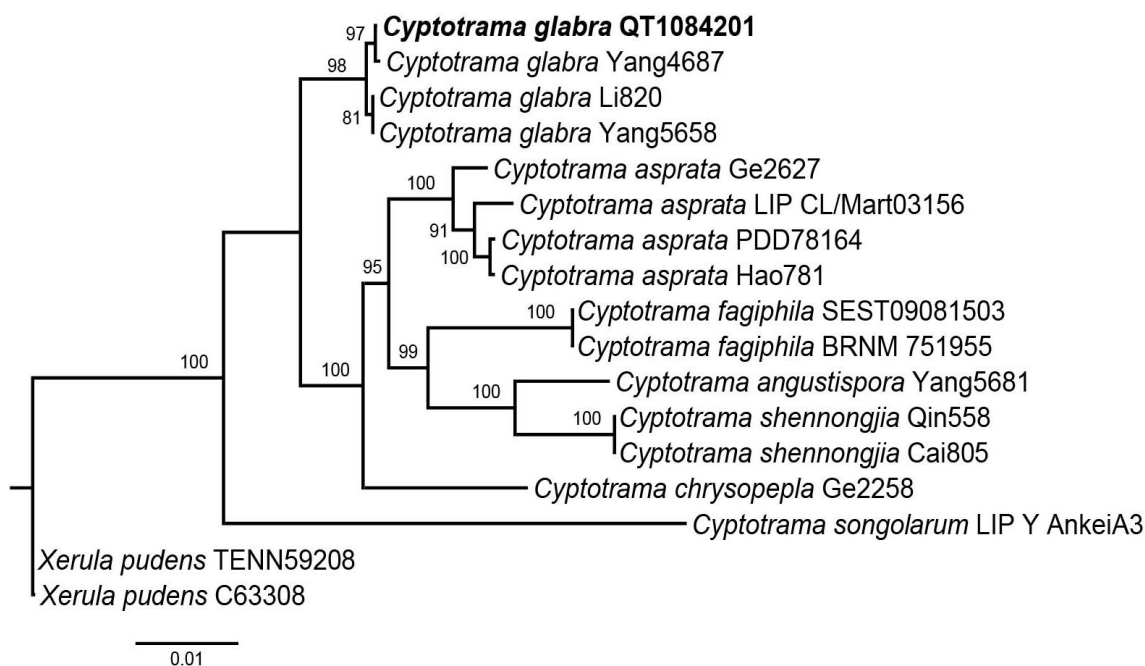


Fig. 3. The phylogram inferred from ML analysis of the ITS dataset verifying the identity of *Cyptotrama glabra* of a specimen (*QT1084201*). ML bootstrap proportional values $\geq 70\%$ were labeled on the branches. The newly obtained sequence was in bold. Bar = substitutions per site.

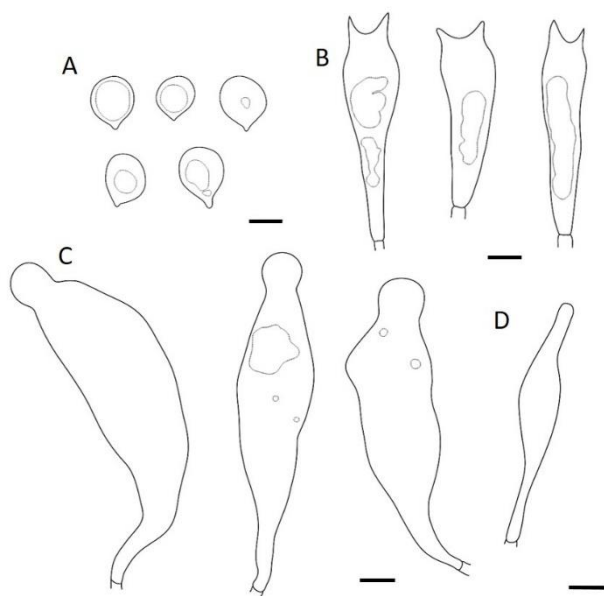


Fig. 4. Micromorphological features of 2-sterigmate *Hymenopellis raphanipes* (drawn from *YLH0074*). A. Basidiospores. B. Basidia. C. Pleurocystidia. D. Cheilocystidia. Bars: Bars: A–C = 10 μm ; D = 25 μm .

caulocystidia are slightly smaller. Phylogenetically, the sequence of *QT1084201* clustered with sequences of *C. glabra* from China and Laos with a high support (ML bootstrap proportional values = 98 %, Fig. 3).

Hymenopellis raphanipes (Berk.) R.H. Petersen, Beihefte zur Nova Hedwigia 137:213. 2010.

Figs. 1D–F & 4

Pileus convex to plane, center slightly umbo to slightly concave, 5–10 cm in diam; surface glutinous, light brown, brown to blackish brown, translucent when moist, with black veins radiating from center towards the margin; context white, becoming yellowish when dry. Lamellae adnate to sinuate or subdistant, with lamellulae, white to cream, thick, becoming yellow when dry. Stipe radicating, subcylindrical, enlarged at

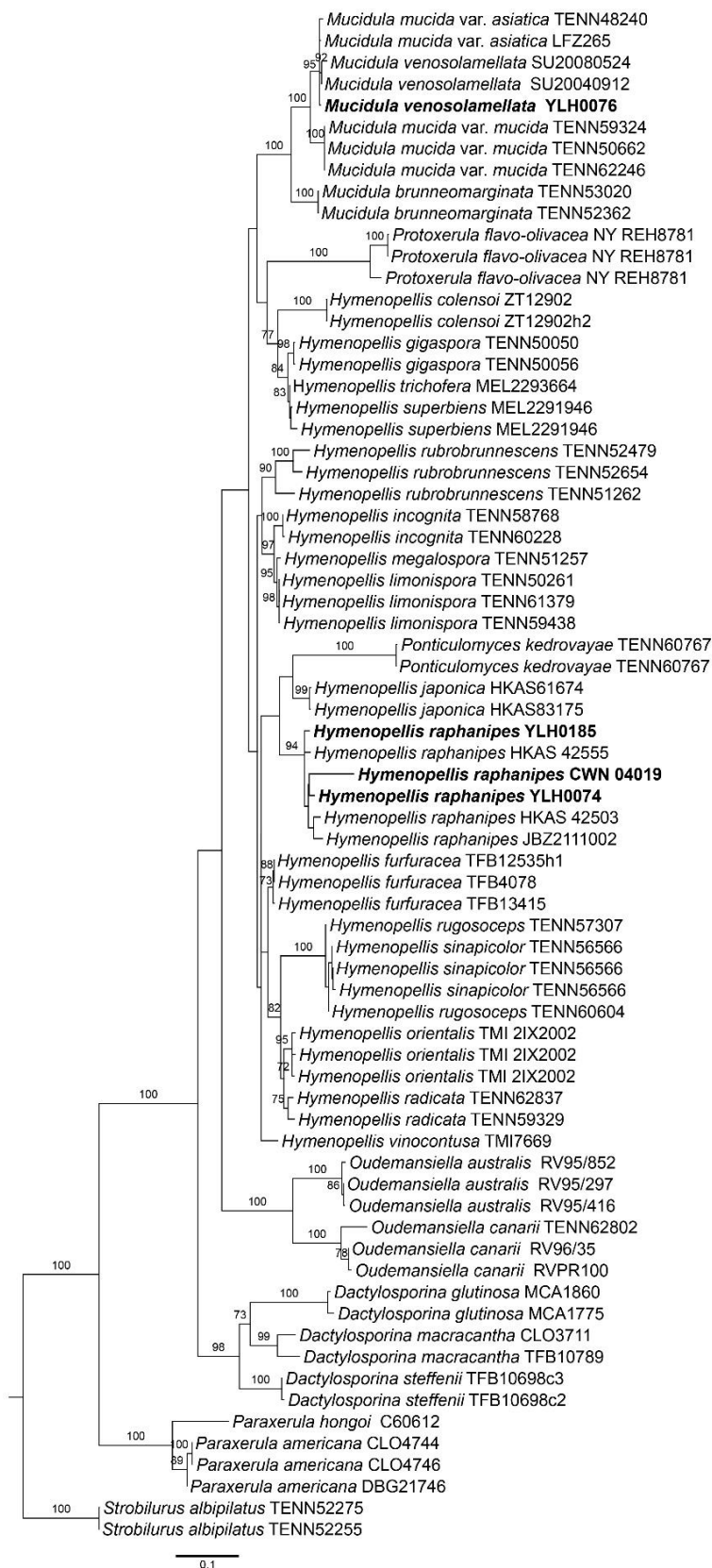


Fig. 5. The phylogram inferred from ML analysis of the ITS dataset confirming the identity of *Hymenopellis raphanipes* of three specimens (*CWN 04019*, *YLH0074* and *YLH0185*). ML bootstrap proportional values $\geq 70\%$ were labeled on the branches. The newly obtained sequences were in bold. Bar = substitutions per site.

base, 3–30 cm in length, 2–20 mm in diam, white, covered with felted squamules, brownish at base, white at apex; pseudorhizae brownish to white.

Hyphal system monomitic; all hyphae simple-septate. Basidiospores hyaline, subglobose, broadly ellipsoid, to sublimoniform, (13–)14.5–17.9(–20) × (11–)11.5–13.7(–15) μm (L = 16.2 μm, W = 12.6 μm, Q = 1.08–1.64, Q_m = 1.29 ± 0.12), thin-walled, smooth, inamyloid and non-dextrinoid. Basidia hyaline, clavate, 42–56 × 12–16 μm, thin-walled, 2 or 4-sterigmate. Cheilocystidia hyaline, fusiform, narrowly clavate, subcylindrical, 53–56 × 4–10 μm, numerous, thin-walled. Pleurocystidia hyaline, fusiform, capitate at apex, 86–93 × 24–27 μm, scattered, thin-walled. Pileipellis an ixohymeniderm composed of clavate to broadly clavate, sphaeropedunculate, brownish cells embedded in gelatinous matrix. Caulocystidia fasciculate, clavate, narrowly clavate to subcylindrical, 77–133 × 11–18 μm, numerous, crowded, thin to slightly thick-walled.

Specimens examined. TAIWAN. Hsinchu county: Kuanwu, on rotten wood, 15 Jun 2004, *W.N. Chou*, *CWN 06655* (TNM F25804). Nantou County: Jenai Township, Aowanda National Forest Recreation Area, 23°57'N, 121°10'E, 1200 m, on the ground, 12 Aug 2015, *C.C. Chen*, *GC 1508-24* (TNM F29047); 1231 m, on rotten wood, 28 Aug 2017, *Y.L. Huang & C.L. Wei*, *YLH0074* (TNM F31772); Huisun Forestry Station, on soil, 29 Apr 2004, *C.H. Yen*, (TNM F16537); Lienhuachien, on soil, 27 Apr 2006, *S.Z. Chen*, *Chen 1471* (TNM F20023); Tsuifeng, Juiyenhsi Nature Reserve Alt., 2300 m, on soil, 01 May 2002, *W.N. Chou*, *CWN 05383* (TNM F24168); Yuanfeng, on soil, 19 Jun 2002, *W.N.*

Chou, *CWN 05490* (TNM F24205). Taichung City: Heping District, Wuling, on rotten wood, 19 May 1999, *W.N. Chou*, *CWN 04019* (TNM F12955). Taipei City: Peitou District, Mt. Shamao Trail, 25°09'N, 121°32'E, 339 m, on soil, 9 Oct 2018, *Y.L. Huang*, *YLH0185* (TNM F33102).

Ecology and distribution. On rotten wood buried in soil during spring and fall at 300–2400 m. Widely distributed in eastern and southern Asia (Hao et al. 2016; this study). Originally described from Darjeeling, Sikkim, India (Berkeley 1850).

Notes. Morphological features of the studied specimens correspond well with other descriptions of *H. raphanipes* (Petersen & Hughes 2010; Hao et al. 2016) except that cheilocystidia are slightly smaller. Phylogenetically, sequences of *CWN 04019*, *YLH0074* and *YLH0185* clustered together with those of three strains of *H. raphanipes* from China in the same lineage with a high support (ML bootstrap proportional values = 94%, Fig. 5).

Hymenopellis raphanipes was regarded as *Oudemansiella radicata* (Relhan) Singer [≡ *H. radicata* (Relhan) R.H. Petersen] in Taiwan (Chen 1975; Wang & Chou 2001; Tschen et al. 2004). However, *H. radicata* is distinguished from *H. raphanipes* by having glabrous stipes, having utriform to tenpin-shaped pleurocystidia, lacking pileocystidia and caulocystidia, and occurring in Europe (Petersen & Nagasawa 2006; Petersen & Hughes 2010). The present phylogenetic analyses showed that *H. radicata* and *H. raphanipes* are distinct from each other (Fig. 5), which agrees with Hao et al. (2016).

Hymenopellis raphanipes is edible (Wu et al. 2019) and widely cultivated in China in recent years (Hao et al. 2016).

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臺灣新記錄種：無毛鱗蓋傘(*Cyptotrama glabra*)及長根菇 (*Hymenopellis raphanipes*)

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摘 要

本文報導臺灣兩種新記錄木棲傘菌：無毛鱗蓋傘 (*Cyptotrama glabra*) 及長根菇 (*Hymenopellis raphanipes*)。本文亦提供此兩種詳細的特徵描述及繪圖，並使用核糖體核酸 (rDNA) 內轉錄間隔區 (internal transcribed spacers, ITS) 序列進行親緣關係分析以確定形態鑑定結果。

關鍵詞：傘菌目、長根小奧德蘑、親緣關係、膨瑚菌科、分類學