

## 研究報告

## 雲南常見貿易黑塊菌的親緣及分群

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## 摘 要

以產自雲南省之黑塊菌為研究標的，將每顆塊菌子實體作形態鑑定及ITS親緣分析，結果顯示雲南黑塊菌可分為3個分群，分別為擬外孔塊菌分群(Pseudoexcavatum cluster)、印度塊菌分群(Indicum cluster)及臺灣塊菌分群(Formosanum cluster)。2017年的294條黑塊菌ITS序列當中，有76%序列屬於印度塊菌分群，23%序列屬於臺灣塊菌分群，1%序列屬於擬外孔塊菌分群；2016年10條黑塊菌序列當中，80%序列屬於印度塊菌分群，20%條序列屬於臺灣塊菌分群。當中擬外孔塊菌分群可以根據形態及顯微特徵之明顯差異而分辨出來，可是成體很少。另外印度塊菌及臺灣塊菌兩分群，比例接近4:1，成對比較分析亦得出兩分群間ITS序列相似度有一定的差異(90.56~93.78%)，但比較兩基因分群之子實體，並沒有明顯外觀或顯微特徵可作分辨。高黎貢山產區之黑塊菌表皮包覆的土壤有特別的金屬光澤，亦帶有干貝般的特殊香氣，主要以臺灣塊菌分群的塊菌佔大多數(75%)。

關鍵詞：黑松露、ITS親緣分析、基因分群、成對比較分析、形態鑑定。

黃勁暉、傅春旭、林介龍、張詠怡、李鎧彤。2020。雲南常見貿易黑塊菌的親緣及分群。台灣林業科學35(1):61-80。

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2018年12月送審 2020年3月通過 Received December 2019, Accepted March 2020.

Research paper

## Phylogenetic Clusters of Commercial Blackish *Tuber* Species from Yunnan, China

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### 【 Summary 】

Morphological and phylogenetic analyses were individually conducted for each commercial blackish *Tuber* from Yunnan Province, China. An internal transcribed spacer (ITS) phylogenetic analysis of *Tuber* ascocarps suggests that 3 phylogenetic clusters exist in Yunnan blackish truffles, which are the Indicum cluster, Formosanum cluster, and Pseudoexcavatum cluster. In total, 294 ITS sequences were obtained in 2017, among which 76% belonged to the Indicum cluster, 23% belonged to the Formosanum cluster, and 1% belonged to the Pseudoexcavatum cluster. Among the 10 ITS sequences obtained in 2016, 80% belonged to the Indicum cluster, and 20% belonged to the Formosanum cluster. The ratio of Indicum clusters to Formosanum clusters was approximately 4:1. *Tuber* ascocarps of the Pseudoexcavatum cluster were from Huize County; its members had a distinct morphology and could readily be distinguished from the others. *Tuber* ascocarps of Indicum and Formosanum clusters had no significant morphological differences, but a pairwise comparison suggested that their ITS sequences only shared 90.56~93.78% similarities. Blackish *Tuber* specimens from Gaoligong Mountain had a good aroma and were covered with soil containing metallic fragments; 75% of *Tuber* specimens from Gaoligong Mountain belonged to the Formosanum cluster.

**Key words:** black truffles, ITS phylogenetic analysis, morphology, pairwise comparison, phylogenetic clusters.

**Wong KF, Fu CH, Lin CL, Cheung WY, Li HT. 2010.** Phylogenetic Clusters of Commercial Blackish *Tuber* Species from Yunnan, China. *Taiwan J For Sci* 35(1):61-80.

### 前言

中國雲南省出產之黑塊菌以印度塊菌 (*Tuber indicum* Cooke & Masee) 為主要貿易種類，另外也常混入少量的擬外孔塊菌 (*T. pseudoexcavatum* Y. Wang, G. Moreno, Rioussset, Manjón & G. Rioussset)。印度塊菌於1892年首先發現於印度的喜瑪拉雅山脈 (Cooke and Masee 1892)，於1992年在中國境內也被發現並且產量豐富，現今主要產於中國西部的四川省及雲南省一帶 (Zhang et al. 2005, Wang et al. 2006,

Wang and Liu 2009)。印度塊菌在外觀形態上與法國黑孢塊菌 (*T. melanosporum* Vittad.) 有極高之相似度，基因分析上屬同一基因分群，有密切的親緣關係，唯法國黑孢塊菌之氣味比印度塊菌濃郁，以及子囊果外皮、孢子囊內含的孢子數量及孢子紋飾等微細特徵上有些差異 (Bonito et al. 2010, 2011, García-Montero et al. 2010, Zambonelli et al. 2016)，要分辨此兩種塊菌並不容易，得靠有經驗的專家以顯微特徵或甚至需

要分子生物技術輔助，才能鑑定及辨別，有不法商人混入印度塊菌出口，充當高價的法國黑袍塊菌販賣，亦有歐洲的法國黑袍塊菌園受到印度塊菌入侵及污染(Bonito et al. 2011)。

臺灣目前有兩個黑塊菌的發表記錄，皆由退休臺大教授胡弘道所發表，分別為1992年的臺灣塊菌(*T. formosanum* Hu)及2009年發表的屑塊菌(*T. furfuraceum* Hu & Wang) (Hu 1992, Huang et al. 2009)，胡弘道於1996年發表以人工接種方式成功產出臺灣塊菌子實體，說明臺灣本土之氣候環境及殼斗科之青剛櫟適合培育塊菌，然而產量非常少(Hu et al. 2005)。國外研究指出印度塊菌寄主廣泛、不少樹種都能與之共生並形成菌根，臺灣的相關研究也指出印度塊菌能與青剛櫟(*Cyclobalanopsis glauca* (Thunb.) Oerst.)小苗共生並形成菌根，而且菌根形成率較臺灣塊菌佳(Lin 2005)，因此本研究團隊選擇印度塊菌作為接種試驗，初步研究印度塊菌人工栽培的可行性。

本研究團隊早期利用雲南省出產之印度塊菌製作成孢子懸浮液接種青剛櫟小苗，成功形成菌根形態，後來嘗試利用Internal transcribed spacer (ITS) 分子技術鑑定菌根之菌種，結果卻發現小苗所形成之菌根為臺灣塊菌菌根，卻沒有印度塊菌的菌根被發現，同時形成菌根化之菌根大約佔整體接種苗木的22%，因此開始懷疑雲南省的黑塊菌在基因上可能非單一族群。中國雲南省販買之黑塊菌，自多個產區收集混售，寄主植物不確定，而且販買時都是帶土的狀態，難以準確觀察到外皮疣突特徵，亦缺乏鏡檢觀察顯微特徵，主要依據塊菌盤商及市場銷售商的民間經驗而「瞎子摸象」的鑑定，因此極有可能有混合其他塊菌種類。本實驗以大批的雲南黑塊菌作研究，以每顆塊菌為研究單位個別取樣、進行形態及分子鑑定，詳細調查當中之親緣關係，以了解雲南常見貿易黑塊菌的分類，並留下資料後續追蹤接種小苗的確實塊菌種類。

## 材料與方法

### 一、塊菌材料

實驗之塊菌子實體於2015~2017年購自中

國雲南省，產季為9-10月，子實體大部分產自會澤縣，產區海拔2300~2400 m，以華山松為主要寄主植物，根據商家鑑定為印度塊菌及擬外孔塊菌；另外亦有購自昆明市木水花野生菌交易市場者，根據商家鑑定及外觀初判有印度塊菌，「中國的夏塊菌」及來自高黎貢山的、香氣特殊及表覆金屬光澤的「夏塊菌」。

### 二、形態鑑定方法

每顆塊菌子實體以火滅菌過之手術刀切開一角，製作切片以觀察其孢子成熟程度，初步篩選出孢子成熟度大於一半之子實體才進行仔細鑑定，每顆塊菌獨立量度其子實體的直徑長寬、重量、顏色、蟲洞孔洞、軟硬及成熟程度等基本資料後，配以編號，並於攝影箱中拍攝其不同角度之表皮和疣突特徵；接著把子實體剖半，記錄果肉的顏色、內部腔洞的形態及曝露於空氣後氧化的顏色變化等。其後切片加入乳酚溶液製成玻片封存，於Leica DLMB光學顯微鏡下觀察子實體的果皮、子囊及子囊孢子的形態、顏色及紋飾等顯微特徵，拍照記錄後配合Pixitretre 5.8軟體測量子囊孢子大小( $n \geq 100$ )，作為鑑定依據。

### 三、分子生物鑑定方法

#### (一)核酸萃取(DNA extraction)

切一小塊塊菌子實體的菌肉移置離心管內，加lysis buffer後使用研磨棒徹底磨碎，磨碎後按照產品廠商(台灣圓點奈米技術股份有限公司)的說明書指引，利用TANBead® Nucleic Acid Extraction Kit Fungal DNA Auto Plate/Auto Tube，配合萃取機器Smart LabAssist-32 (64)的L-BNA-PK-AUTO Program萃取DNA，萃取完成之DNA樣本即可繼續PCR程序，或保存於-30°C冰箱。

#### (二)聚合酶鏈鎖反應(polymerase chain reaction; PCR)及電泳分析

本實驗以ITS4及5通用性引子對(5'-TCCTCCGCTTATTGATATGC-3'及5'-GGAAGTAAAAGTCGTAACAAGG-3') (White

et al. 1990)進行PCR，混合2.5  $\mu$ L DNA萃取樣本、12.5  $\mu$ L 2xTaq Master Mix (基龍米克斯生物科技股份有限公司)、各0.5  $\mu$ L 10  $\mu$ M引子ITS4/5及8  $\mu$ L ddH<sub>2</sub>O，以配製總容量為24  $\mu$ L之PCR反應溶液。PCR是用Multigene Thermal Cycler (Labnet International)進行，起始變性(denaturation)溫度為94°C 3 min；變性溫度為94°C 30s，黏合(annealing)溫度為56°C 30s，增幅溫度72°C 30s，子實體樣本循環30週期、菌根樣本循環40週期；最後延展(extension)溫度72°C 5 min。經過PCR增幅的DNA樣本，以含1.4% agarose、0.5 M Tris-acetate-EDTA (TAE)溶液之洋菜膠及5  $\mu$ L/100 mL Healthview™ nucleic acid stain，於100 V電壓下進行電泳分析約25 min。電泳完成後，以Multimage™ light cabinet (Alphamager 2200)照相，比較DNA ladder與所得之DNA樣本，以估計各樣本之長度(bp)，然後把產物送至昕穎生醫技術股份有限公司進行定序。

### (三)與基因資料庫之比對佐證及親緣樹分析

取得定序結果後，與美國生物技術資訊中心網站(NCBI)之資料庫內登錄的基因序列，以核酸基因庫序列群組分析程式(BLAST)作序列相似度比對，綜合比對相似度後得出最相似及具參考價值的佐證結果。由於對NCBI資料庫內印度塊菌序列的確定性存疑，所以使用2015~2016年經形態記錄之塊菌子實體、純培養菌絲及菌根之序列作基因親緣樹分析，另外再從結果分群(clusters)中各取代表，與2015年在臺灣南投縣東埔山區找到的臺灣塊菌子實體及分離純培養得到的菌絲的序列，以及GenBank中其他序列作基因親緣樹分析，各ITS序列以網路程式MAFFT 7 (Katoh and Standley 2013)對齊後，再以網路程式Gblocks 0.91b (Castresana 2000)檢查及去掉排列不佳的序列片段，然後以MEGA 6.0 (Tamura et al. 2013)的最大似然分析(ML)作基因親緣分析，Bootstrap值設定為1000，最後以FigTree 1.4.3 (Rambaut 2014)整合出基因親緣關係圖。

### (四)序列成對比較分析(pairwise comparisons)

從基因親緣樹分析的結果分群中各取代表，以網路程式MAFFT 7對齊後，再以網路程

式Gblocks 0.91b檢查及去掉排列不佳的序列片段，然後以PHYLP 3.695 (Felsenstein 2005)的DNADIST作成對比較分析，以計算各序列之相似度(similarities)。

## 結果

### 一、分子鑑定結果

本研究中得到294條雲南黑塊菌的ITS序列(accession no.: LC415613~LC415904, LC419993, LC419994) (Table 1)利用MEGABLAST與GenBank比對，發現21條序列(7%)與日本發表近似臺灣塊菌及喜馬拉雅塊菌之序列(Kinoshita et al. 2001, 2018)，相似度達97~100%，亦與臺灣塊菌之模式標本序列JN655530 (Qiao et al. 2013)相似度達99%；另外271條序列(92%)與印度塊菌之序列相似度達96~99%；最後有2條序列(1%)與擬外孔塊菌之序列相似度達97%，與形態鑑定的兩顆擬外孔塊菌(編號YNB17102及YNB17107)結果吻合。

294條ITS序列加入2015年雲南黑塊菌中抽取4顆雲南黑塊菌的序列(2015YNB1~4)、2016年10顆塊菌的序列(2016YNB161~YNB1610)、從臺灣塊菌子實體中分離純培養得到的2個菌絲樣本的序列(*T. formosanum\_culture* S1/S2)及從接種塊菌的青剛櫟小苗中得到的臺灣塊菌菌根之序列(*T. formosanum\_mycorrhizae*)，共311條序列作ITS親緣關係分析，311條序列排列後序列長947 bp，以Gblocks 0.91b檢查及去掉排列不佳的序列片段後，序列長466 bp。親緣分析結果顯示雲南黑塊菌有3個主要分群，配合形態鑑定及GenBank比對佐證，2個擬外孔塊菌序列以高支持度(BS = 1.00)歸到擬外孔塊菌分群；其餘序列分為兩分群，與臺灣塊菌純培養絲樣本及臺灣塊菌菌根之序列歸到同一分群的為臺灣塊菌分群(BS = 0.62)，另外為印度塊菌分群(BS = 0.79)。2016及2017兩年得到的印度塊菌分群及臺灣塊菌分群比例相約，接近4:1；2017年的294條黑塊菌序列當中，有223條序列(76%)屬於印度塊菌分群，69條序列(23%)屬於臺灣塊菌分群，2條序列(1%)屬於擬外孔塊菌

分群；2016年黑塊菌序列當中，8條序列(80%)  
屬於印度塊菌分群，2條序列(20%)屬於臺灣塊

菌分群；2015年4條黑塊菌序列全屬於印度塊  
菌分群。

**Table 1. List of internal transcribed spacer sequences deposited into GenBank in this study**

Taxon	Origin	Isolated from	Voucher no.	GenBank accession no.
<i>Tuber formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17001	LC415613
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17002	LC415614
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17004	LC415615
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17006	LC415616
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17008	LC415617
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17010	LC415618
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17011	LC415619
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17012	LC415620
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17013	LC415621
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17014	LC415622
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17015	LC415623
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17016	LC415624
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17018	LC415625
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17019	LC415626
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17033	LC415627
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17039	LC415628
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17042	LC415629
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17135	LC415630
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17141	LC415631
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17145	LC415632
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17154	LC415633
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17155	LC415634
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17157	LC415635
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17162	LC415636
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17163	LC415637
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17170	LC415638
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17181	LC415639
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17183	LC415640
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17185	LC415641
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17186	LC415642
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17192	LC415643
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17193	LC415644
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17202	LC415645
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17203	LC415646
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17205	LC415647
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17206	LC415648
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17207	LC415649
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17208	LC415650
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17209	LC415651

Table 1 (continue)

<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17210	LC415652
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17211	LC415653
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17213	LC415654
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17215	LC415655
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17218	LC415656
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17219	LC415657
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17221	LC415658
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17222	LC415659
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17224	LC415660
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17225	LC415661
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17226	LC415662
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17227	LC415663
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17228	LC415664
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17230	LC415665
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17231	LC415666
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17232	LC415667
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17233	LC415668
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17234	LC415669
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17235	LC415670
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17236	LC415671
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17237	LC415672
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17239	LC415673
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17240	LC415674
<i>T. formosanum</i>	Gaoligong Mt., China	Ascocarp	YNB17243	LC415675
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17281	LC415676
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17287	LC415677
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17290	LC415678
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17303	LC415679
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17310	LC415680
<i>T. formosanum</i>	Huize, Yunnan, China	Ascocarp	YNB17316	LC415681
<i>T. formosanum</i>	Dung-Pu, Nantou, Taiwan	Pure culture	S1	LC437961
<i>T. formosanum</i>	Dung-Pu, Nantou, Taiwan	Pure culture	S2	LC437962
<i>T. formosanum</i>	Dung-Pu, Nantou, Taiwan	Ascocarp	TF1	LC437963
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17003	LC415682
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17005	LC415683
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17007	LC415684
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17009	LC415685
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17017	LC415686
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17020	LC415687
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17021	LC415688
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17022	LC415689
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17023	LC415690
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17024	LC415691
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17025	LC415692

Table 1 (continue)

<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17026	LC415693
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17027	LC415694
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17028	LC415695
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17029	LC415696
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17030	LC415697
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17031	LC415698
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17032	LC415699
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17034	LC415700
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17035	LC415701
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17036	LC415702
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17037	LC415703
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17038	LC415704
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17040	LC415705
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17041	LC415706
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17044	LC415707
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17048	LC415708
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17049	LC415709
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17050	LC415710
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17051	LC415711
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17052	LC415712
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17053	LC415713
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17056	LC415714
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17061	LC415715
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17062	LC415716
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17063	LC415717
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17064	LC415718
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17065	LC415719
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17067	LC415720
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17068	LC415721
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17070	LC415722
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17072	LC415723
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17075	LC415724
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17076	LC415725
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17077	LC415726
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17078	LC415727
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17079	LC415728
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17080	LC415729
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17081	LC415730
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17082	LC415731
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17083	LC415732
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17084	LC415733
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17086	LC415734
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17087	LC415735
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17088	LC415736

Table 1 (continue)

<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17089	LC415737
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17090	LC415738
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17091	LC415739
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17092	LC415740
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17093	LC415741
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17094	LC415742
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17095	LC415743
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17096	LC415744
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17097	LC415745
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17098	LC415746
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17099	LC415747
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17100	LC415748
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17101	LC415749
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17103	LC415750
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17104	LC415751
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17105	LC415752
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17106	LC415753
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17108	LC415754
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17109	LC415755
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17110	LC415756
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17111	LC415757
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17112	LC415758
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17113	LC415759
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17114	LC415760
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17115	LC415761
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17116	LC415762
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17117	LC415763
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17118	LC415764
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17119	LC415765
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17120	LC415766
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17121	LC415767
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17122	LC415768
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17123	LC415769
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17124	LC415770
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17125	LC415771
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17126	LC415772
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17127	LC415773
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17128	LC415774
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17129	LC415775
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17130	LC415776
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17131	LC415777
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17132	LC415778
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17133	LC415779
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17134	LC415780



Table 1 (continue)

<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17136	LC415781
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17137	LC415782
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17138	LC415783
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17139	LC415784
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17140	LC415785
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17142	LC415786
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17143	LC415787
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17144	LC415788
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17146	LC415789
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17147	LC415790
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17148	LC415791
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17149	LC415792
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17150	LC415793
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17151	LC415794
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17152	LC415795
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17153	LC415796
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17156	LC415797
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17158	LC415798
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17159	LC415799
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17160	LC415800
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17161	LC415801
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17164	LC415802
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17165	LC415803
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17166	LC415804
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17167	LC415805
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17168	LC415806
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17169	LC415807
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17171	LC415808
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17172	LC415809
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17173	LC415810
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17175	LC415811
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17176	LC415812
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17177	LC415813
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17178	LC415814
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17179	LC415815
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17180	LC415816
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17182	LC415817
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17184	LC415818
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17187	LC415819
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17188	LC415820
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17189	LC415821
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17190	LC415822
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17191	LC415823
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17194	LC415824

Table 1 (continue)

<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17195	LC415825
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17196	LC415826
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17197	LC415827
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17198	LC415828
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17199	LC415829
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17200	LC415830
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17201	LC415831
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17212	LC415832
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17214	LC415833
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17216	LC415834
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17217	LC415835
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17220	LC415836
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17223	LC415837
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17238	LC415838
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17241	LC415839
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17242	LC415840
<i>T. indicum</i>	Gaoligong Mt., China	Ascocarp	YNB17244	LC415841
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17245	LC415842s
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17246	LC415843
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17247	LC415844
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17248	LC415845
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17249	LC415846
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17250	LC415847
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17251	LC415848
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17252	LC415849
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17253	LC415850
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17254	LC415851
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17255	LC415852
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17256	LC415853
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17257	LC415854
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17258	LC415855
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17259	LC415856
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17260	LC415857
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17261	LC415858
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17262	LC415859
<i>T. indicum</i>	Yunnan market, China	Ascocarp	YNB17263	LC415860
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17264	LC415861
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17265	LC415862
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17266	LC415863
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17267	LC415864
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17268	LC415865
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17269	LC415866
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17270	LC415867
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17271	LC415868

Table 1 (continue)

<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17272	LC415869
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17273	LC415870
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17275	LC415871
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17276	LC415872
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17277	LC415873
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17278	LC415874
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17279	LC415875
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17280	LC415876
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17282	LC415877
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17283	LC415878
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17284	LC415879
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17285	LC415880
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17286	LC415881
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17288	LC415882
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17289	LC415883
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17291	LC415884
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17292	LC415885
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17293	LC415886
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17294	LC415887
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17295	LC415888
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17296	LC415889
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17297	LC415890
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17298	LC415891
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17299	LC415892
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17300	LC415893
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17301	LC415894
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17302	LC415895
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17304	LC415896
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17308	LC415897
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17309	LC415898
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17313	LC415899
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17314	LC415900
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17318	LC415901
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17320	LC415902
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17323	LC415903
<i>T. indicum</i>	Huize, Yunnan, China	Ascocarp	YNB17326	LC415904
<i>T. pseudoexcavatum</i>	Huize, Yunnan, China	Ascocarp	YNB17102	LC419993
<i>T. pseudoexcavatum</i>	Huize, Yunnan, China	Ascocarp	YNB17107	LC419994

從以上結果的3個分群中隨機各取20個代表序列，與2015年在臺灣南投縣東埔山區找到的臺灣塊菌子實體及其分離純培養得到的菌絲序列(accession no.: LC437961~LC437963)，以及

GenBank中其他序列(Table 2)作基因親緣樹分析圖 (Fig. 1)，62條序列排列後序列長959 bp，以Gblocks 0.91b檢查及去掉排列不佳的序列片段後，序列長505 bp。結果顯示雲南黑塊菌的

3個分群狀況相同，擬外孔塊菌分群的2條序列與GenBank的擬外孔塊菌序列以高支持度(BS = 0.98)分為一群，其他雲南黑塊菌與法國黑孢塊菌為姐妹群(BS = 1.00)，但亦以高支持度(BS = 0.85/0.84)分為兩分群，印度塊菌分群包括了雲

南黑塊菌的20個印度塊菌分群代表，以及4條GenBank的印度塊菌序列；其他20個臺灣塊菌分群代表、臺灣塊菌子實體及分離純培養得到的菌絲的序列及GenBank的序列分到臺灣塊菌分群。

**Table 2. Details of internal transcribed spacer sequences used in the phylogenetic study in Fig. 1**

Taxon	Origin	Voucher no.	GenBank accession no.	Reference
<i>Tuber formosanum</i>	Gaoligong Mt., China	YNB 17001	LC415613	This study
<i>T. formosanum</i>	Gaoligong Mt., China	YNB 17004	LC415615	This study
<i>T. formosanum</i>	Gaoligong Mt., China	YNB 17011	LC415619	This study
<i>T. formosanum</i>	Gaoligong Mt., China	YNB 17015	LC415623	This study
<i>T. formosanum</i>	Gaoligong Mt., China	YNB 17019	LC415626	This study
<i>T. formosanum</i>	Huize, Yunnan, China	YNB 17033	LC415627	This study
<i>T. formosanum</i>	Huize, Yunnan, China	YNB 17042	LC415629	This study
<i>T. formosanum</i>	Huize, Yunnan, China	YNB 17135	LC415630	This study
<i>T. formosanum</i>	Huize, Yunnan, China	YNB 17157	LC415635	This study
<i>T. formosanum</i>	Huize, Yunnan, China	YNB 17170	LC415638	This study
<i>T. formosanum</i>	Huize, Yunnan, China	YNB 17183	LC415640	This study
<i>T. formosanum</i>	Gaoligong Mt., China	YNB 17202	LC415645	This study
<i>T. formosanum</i>	Gaoligong Mt., China	YNB 17222	LC415659	This study
<i>T. formosanum</i>	Gaoligong Mt., China	YNB 17228	LC415664	This study
<i>T. formosanum</i>	Gaoligong Mt., China	YNB 17230	LC415665	This study
<i>T. formosanum</i>	Gaoligong Mt., China	YNB 17243	LC415675	This study
<i>T. formosanum</i>	Huize, Yunnan, China	YNB 17281	LC415676	This study
<i>T. formosanum</i>	Huize, Yunnan, China	YNB 17287	LC415677	This study
<i>T. formosanum</i>	Huize, Yunnan, China	YNB 17290	LC415678	This study
<i>T. formosanum</i>	Huize, Yunnan, China	YNB 17303	LC415679	This study
<i>T. formosanum</i>	Dung-Pu, Nantou, Taiwan	S1	LC437961	This study
<i>T. formosanum</i>	Dung-Pu, Nantou, Taiwan	S2	LC437962	This study
<i>T. formosanum</i>	Dung-Pu, Nantou, Taiwan	TF1	LC437963	This study
<i>T. formosanum</i>	Ho-She, Nantou, Taiwan	HKAS62628(holotype)	JN655530	Qiao et al. 2013
<i>T. formosanum</i>	Ho-She, Nantou, Taiwan	T12_HKAS48268	GU979048	Qiao et al. 2013
<i>T. formosanum</i>	Dung-Pu, Nantou, Taiwan	HU070105C_DungPu03	FJ176919	Huang et al. 2009
<i>T. formosanum</i>	Dung-Pu, Nantou, Taiwan	HU070105A_DungPu01	FJ176914	Huang et al. 2009
<i>T. himalayense</i>	Japan	K464	AB553394	Kinoshita et al. 2018
<i>T. himalayense</i>	Japan	N123	AB553402	Kinoshita et al. 2011
<i>T. himalayense</i>	Huize, Yunnan, China	HKAS25689	AY773356	Wang et al. 2006
<i>T. indicum</i>	Gaoligong Mt., China	YNB 17003	LC415682	This study
<i>T. indicum</i>	Gaoligong Mt., China	YNB 17005	LC415683	This study
<i>T. indicum</i>	Gaoligong Mt., China	YNB 17007	LC415684	This study
<i>T. indicum</i>	Gaoligong Mt., China	YNB 17009	LC415685	This study
<i>T. indicum</i>	Gaoligong Mt., China	YNB 17017	LC415686	This study

Table 2 (continue)

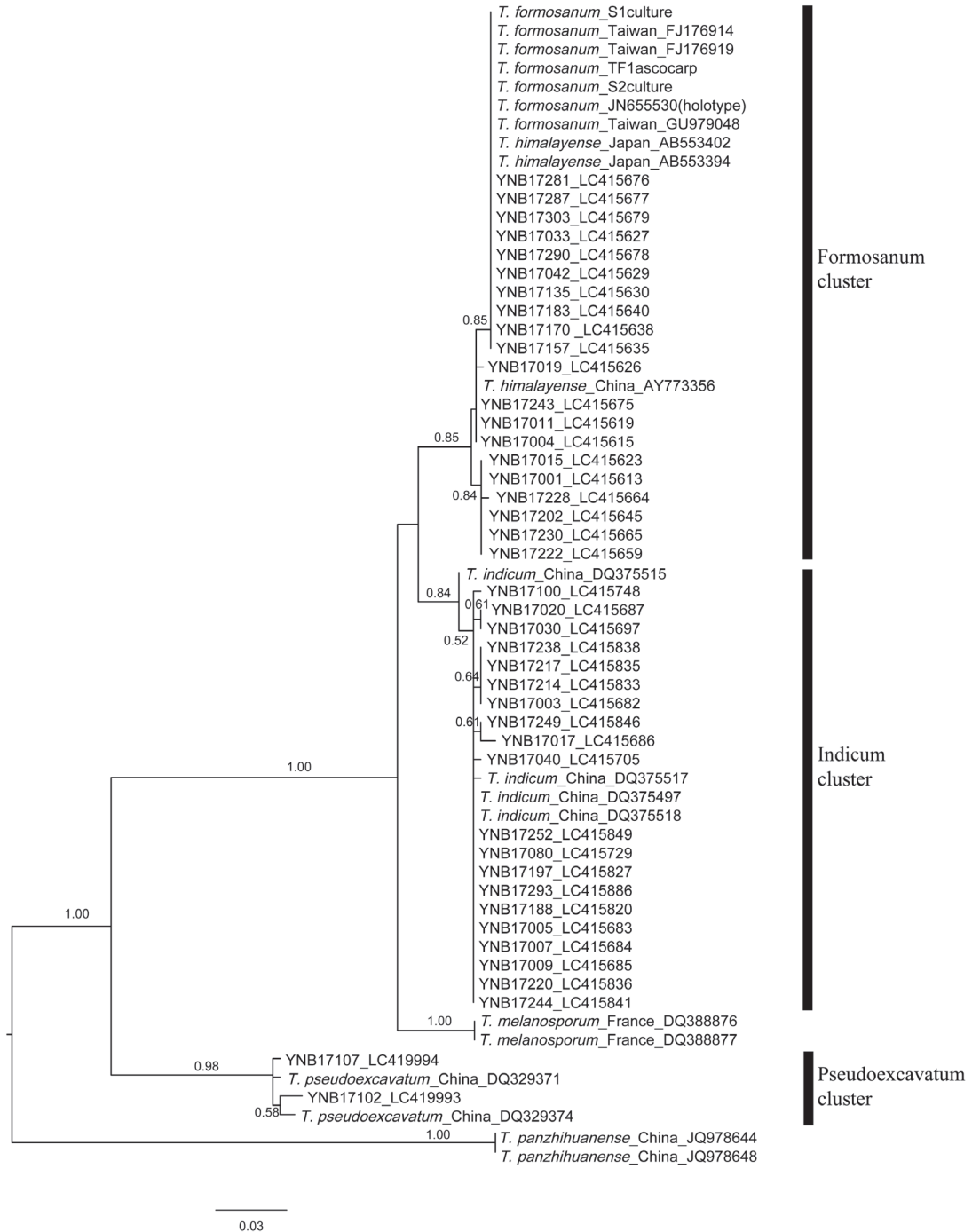
<i>T. indicum</i>	Huize, Yunnan, China	YNB 17020	LC415687	This study
<i>T. indicum</i>	Huize, Yunnan, China	YNB 17030	LC415697	This study
<i>T. indicum</i>	Huize, Yunnan, China	YNB 17040	LC415705	This study
<i>T. indicum</i>	Yunnan market, China	YNB 17080	LC415729	This study
<i>T. indicum</i>	Yunnan market, China	YNB 17100	LC415748	This study
<i>T. indicum</i>	Huize, Yunnan, China	YNB 17188	LC415820	This study
<i>T. indicum</i>	Huize, Yunnan, China	YNB 17197	LC415827	This study
<i>T. indicum</i>	Gaoligong Mt., China	YNB 17214	LC415833	This study
<i>T. indicum</i>	Gaoligong Mt., China	YNB 17217	LC415835	This study
<i>T. indicum</i>	Gaoligong Mt., China	YNB 17220	LC415836	This study
<i>T. indicum</i>	Gaoligong Mt., China	YNB 17238	LC415838	This study
<i>T. indicum</i>	Gaoligong Mt., China	YNB 17244	LC415841	This study
<i>T. indicum</i>	Yunnan market, China	YNB 17249	LC415846	This study
<i>T. indicum</i>	Yunnan market, China	YNB 17252	LC415849	This study
<i>T. indicum</i>	Huize, Yunnan, China	YNB 17293	LC415886	This study
<i>T. indicum</i>	Yunnan, China	Tind-yn20	DQ375515	Wang et al. 2006
<i>T. indicum</i>	Yunnan, China	Tind-yn23	DQ375518	Wang et al. 2006
<i>T. indicum</i>	Yunnan, China	Tind-yn22	DQ375517	Wang et al. 2006
<i>T. indicum</i>	Hulli, Sichuan, China	Tind-hl04	DQ375497	Wang et al. 2006
<i>T. melanosporum</i>	France	Tmel-eu05	DQ388876	Wang et al. 2006
<i>T. melanosporum</i>	France	Tmel-eu08	DQ388877	Wang et al. 2006
<i>T. panzhihuanense</i>	Panzhihua, Sichuan, China	DXJ267 (holotype)	JQ978644	Deng et al. 2012
<i>T. panzhihuanense</i>	Kunming, Yunnan, China	DXJ260	JQ978648	Deng et al. 2012
<i>T. pseudoexcavatum</i>	China	Tpse-pzh02	DQ329371	Huang et al. 2009
<i>T. pseudoexcavatum</i>	China	Tpse-yn05	DQ329374	Huang et al. 2009
<i>T. pseudoexcavatum</i>	Yunnan, China	YNB 17102	LC419993	This study
<i>T. pseudoexcavatum</i>	Yunnan, China	YNB 17107	LC419994	This study

各序列經成對比較之相似度列於Table 3，臺灣塊菌分群內序列相似度為96.73~100%，印度塊菌分群內序列相似度為97.53~100%，比較臺灣塊菌及印度塊菌分群之序列相似度為90.56~93.78%，而比較擬外孔塊菌分群與臺灣塊菌及印度塊菌分群之序列相似度為68.23~74.84%。

## 二、形態鑑定結果

塊菌子實體材料中，以顯微鏡觀察孢子成熟程度，篩選出25%為不成熟，無法用作後續接種材料，所以沒有作形態及分子鑑定，未成熟的塊菌子實體外皮紅棕色，切開菌肉淡橘色，白色菌脈不明顯。成熟的塊菌子實體

大小範圍為17~73×16~59 mm，平均大小為36.3×30.4 mm，平均重量17.5 g，從外型及表皮特徵看，大部分塊菌子實體外觀相似，未有任何明顯特徵可分辨種類，外皮皆為深褐或黑色，外皮有4至6邊錐型疣突，1 cm有5~6個疣突，外觀合乎印度塊菌或臺灣塊菌之描述；當中只有編號YNB17102及YNB17107明顯不同，兩顆外皮褐色，疣狀突出比印度塊菌細而淺，1 cm有16個疣突，以顯微鏡觀察子囊內以5~7個孢子為主，與印度塊菌及臺灣塊菌中每子囊主要2~4個孢子可以明顯區別(Fig. 2)，以形態鑑定為擬外孔塊菌。氣味方面，只有產自高黎貢山產區之塊菌有干貝般的特殊香氣，其他塊菌都不具明顯香味。



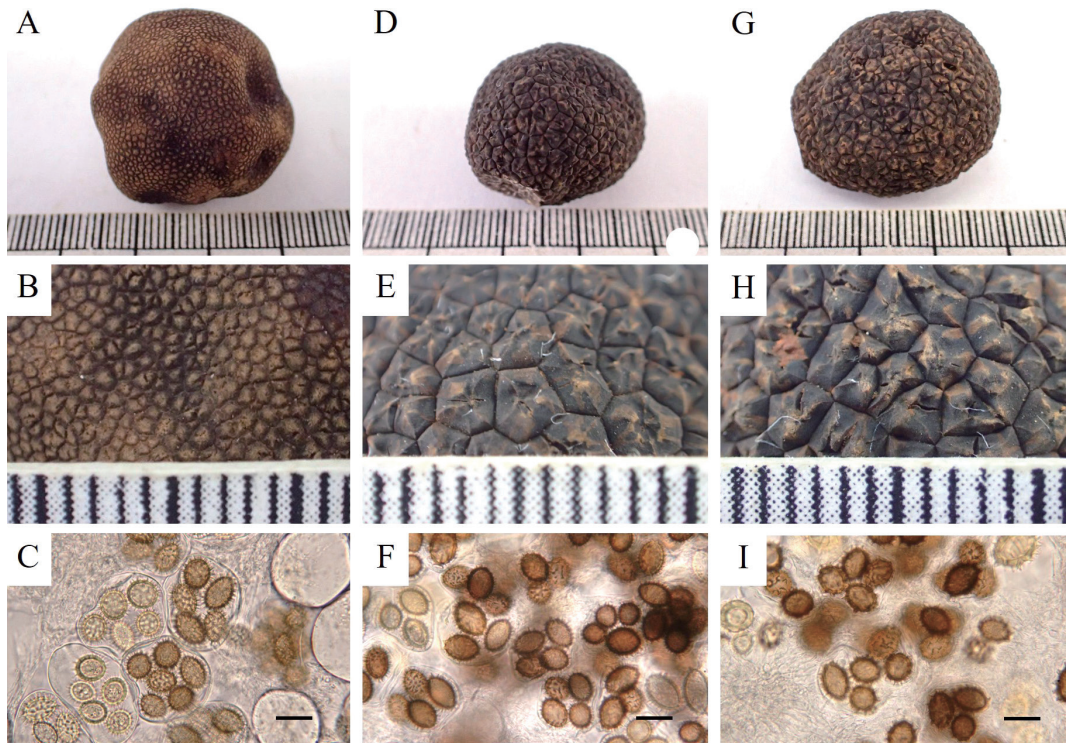
**Fig. 1.** Phylogenetic tree of Yuannan blackish *Tuber* and related *Tuber* species of the Melanosporum Clade based on the ITS-rDNA sequences. *Tuber panzhihuanense* was used as the outgroup taxa. Numbers near branches indicated the bootstrap values (BS) obtained from maximum likelihood (ML) analysis. Values below 50% are not indicated.

Table 3. Pairwise comparisons of sequences of *Indicum*, *Formosanum*, and *Pseudoxacatum* clusters of Yunnan blackish *Thber* species

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
1 LC415613																							
2 LC415615	99.44																						
3 LC415619	99.59	99.79																					
4 LC415626	99.44	99.63	99.79																				
5 LC415623	100.0	99.44	99.59	99.44																			
6 LC415627	99.06	99.25	99.38	99.25	99.06																		
7 LC415629	99.06	99.25	99.38	99.25	99.06	100.0																	
8 LC415630	99.06	99.25	99.38	99.25	99.06	100.0	100.0																
9 LC415635	96.73	96.92	99.38	96.92	96.73	97.71	97.71	97.71															
10 LC415638	96.73	96.93	99.38	96.93	96.73	97.71	97.71	97.71	99.81														
11 LC415682	92.81	92.81	93.31	92.81	92.81	93.21	93.21	93.21	91.89	91.69													
12 LC415683	93.16	93.16	93.55	93.16	93.16	93.55	93.55	93.55	91.41	91.22	99.04												
13 LC415687	93.57	93.57	93.78	93.57	93.57	93.55	93.55	93.55	91.42	91.23	98.07	98.30											
14 LC415697	93.57	93.57	93.78	93.57	93.57	93.55	93.55	93.55	91.42	91.23	98.07	98.30	100.0										
15 LC415729	93.17	93.17	93.34	93.17	93.17	93.15	93.15	93.15	91.00	90.81	98.07	98.30	99.63	99.63									
16 LC415705	92.95	92.95	93.11	92.95	92.95	92.94	92.94	90.78	90.59	97.87	98.11	99.44	99.44	99.81									
17 LC415685	92.12	92.12	93.11	92.12	92.12	92.51	92.51	90.75	90.56	98.65	99.06	97.72	97.72	97.72	97.53								
18 LC415748	93.04	93.04	93.12	93.04	93.04	93.44	93.44	91.92	91.72	97.88	98.46	98.66	98.66	98.66	98.46	98.07							
19 LC415686	93.38	93.38	93.56	93.38	93.38	93.15	93.15	92.70	92.50	98.05	98.05	99.03	99.03	99.03	99.03	97.85	98.06						
20 LC415684	92.12	92.12	93.11	92.12	92.12	92.51	92.51	90.75	90.56	98.65	99.06	97.72	97.72	97.72	97.53	100.0	98.07	97.85					
21 LC419993	68.23	68.51	74.22	69.07	68.23	68.79	68.79	68.79	69.32	69.12	71.08	70.18	69.02	69.02	68.46	68.79	69.63	69.96	70.70	69.63			
22 LC419994	68.51	68.79	74.84	69.35	68.51	69.07	69.07	69.07	69.42	69.50	71.63	70.46	69.30	69.30	68.74	69.07	69.90	70.24	71.59	69.90	97.48		

Note: <sup>1)</sup> Similarities are shown in percentages; (1~10) represent *Formosanum* clusters, (11~20) represent *Indicum* clusters, and (21 and 22) represent *Pseudoxacavatum* clusters.

<sup>2)</sup> The gray area highlights similarities between *Formosanum* and *Indicum* clusters.



**Fig. 2.** Morphological characteristics of Yuannan blackish *Tuber*. **A**, ascocarp of *Tuber* of *Pseudoexcavatum* cluster; **B**, warts on peridium surface of *Tuber* of *Pseudoexcavatum* cluster; **C**, asci and ascospores of *Tuber* of *Pseudoexcavatum* cluster; **D**, ascocarp of *Tuber* of *Formosanum* cluster; **E**, warts on peridium surface of *Tuber* of *Formosanum* cluster; **F**, asci and ascospores of *Tuber* of *Formosanum* cluster; **G**, ascocarp of *Tuber* of *Indicum* cluster; **H**, warts on peridium surface of *Tuber* of *Indicum* cluster; **I**, asci and ascospores of *Tuber* of *Indicum* cluster. Scale bars: 30  $\mu\text{m}$ .

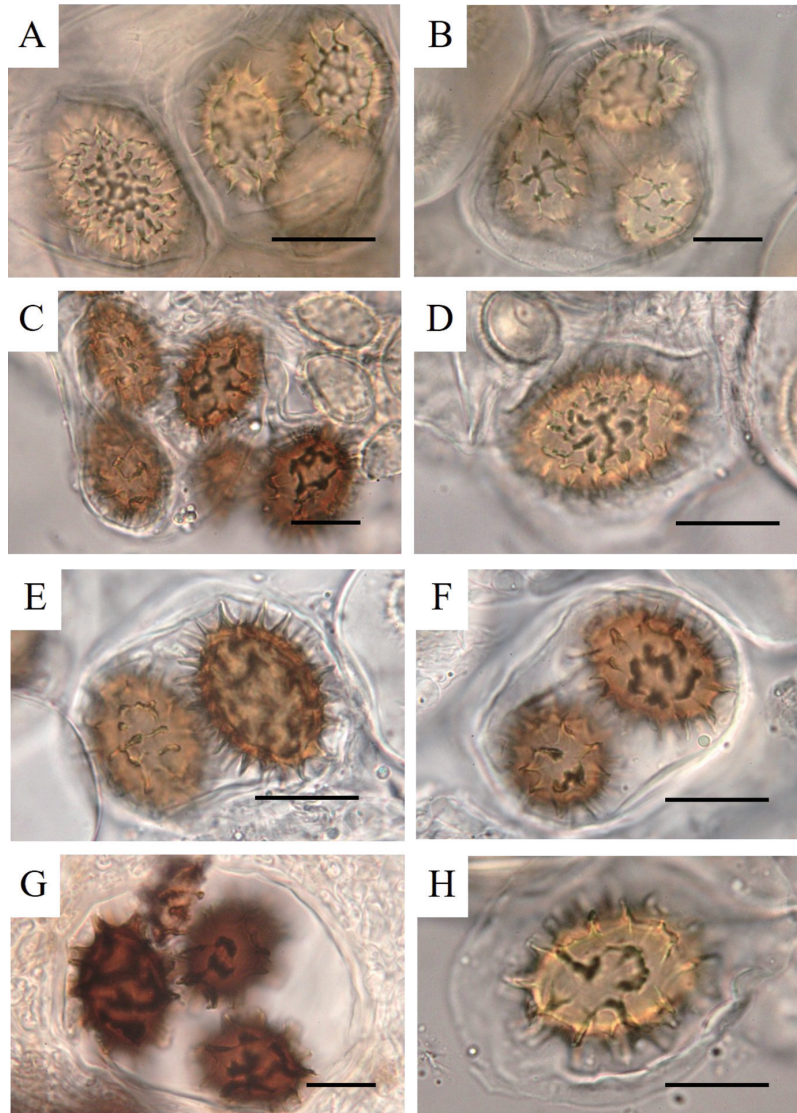
根據ITS親緣關係分析結果，隨機挑選印度塊菌分群及臺灣塊菌分群內各14顆塊菌子實體作詳細孢子大小量測，各子實體測量孢子數超過100個，兩分群各測量孢子約1800個，得到印度塊菌分群之孢子大小範圍為(18~) 24~44 (~53) × (17~) 20~35 (~38)  $\mu\text{m}$ ，平均大小32 × 25  $\mu\text{m}$ ，臺灣塊菌分群之孢子大小範圍為(17~) 22~36 (~45) × (14~) 17~26 (~33)  $\mu\text{m}$ ，平均大小28 × 22  $\mu\text{m}$ ，兩分群的平均孢子長寬比同樣為1.3。測量結果顯示臺灣塊菌分群之孢子較小，但是兩分群之孢子大小範圍大部分重疊，未能作明顯之分辨依據。另外在同一顆臺灣或印度塊菌分群的塊菌子實體內皆可以觀

察到針刺及刺網格狀之兩種孢子紋飾/皆可以觀察到針刺、刺網格或兩者混合的三種孢子紋飾 (Fig. 3)，觀察是因為孢子針刺紋飾的底座寬度不一，有寬有細，寬底座可能連在一起，以致顯微鏡下部分孢子帶有網格狀紋飾。

## 討論

產自雲南的黑塊菌當中，只有擬外孔塊菌外型有明顯差異而可以分辨出來，其外皮褐色及疣突細而淺，再配合子囊內主要5-7個孢子的特徵，可以作確實鑑定，不過雲南商家販買之塊菌都是帶土的狀態，難以準確觀察到外皮疣突特





**Fig. 3.** Ascospore ornamentation of Yuannan blackish *Tuber* of *Fornosamum* and *Indicum* clusters. A, B, C, D, ascospore ornamentation of *Tuber* of *Fornosamum* cluster; E, F, G, H, ascospore ornamentation of *Tuber* of *Indicum* cluster. Scale bars: 20  $\mu$ m.

徵，加上缺乏鏡檢孢子，以致商家鑑定的5 kg擬外孔塊菌中，只有2顆為正確的，其餘皆為外皮顏色類似之未成熟印度塊菌。本研究收集到的擬外孔塊菌皆來自會澤產區，而且產量稀少、只有

在約10 kg黑塊菌中才混入兩小顆。另外本研究收集到的黑塊菌材料中，只有產自高黎貢山的黑塊菌帶有干貝般的特殊香氣，表皮包覆的土壤亦有特別的金屬光澤，雖然於清除表土後子實體在

外型及顯微構造上沒有明顯差別，唯該產區塊菌的成熟比例比較高，在ITS親緣關係中亦顯示高比例(75%)屬於臺灣塊菌分群，相對會澤塊菌盤商的黑塊菌中只有12%屬於臺灣塊菌分群之差別甚大，或許可以進一步分析含金屬光澤的表土成份，以更深入了解該產區塊菌的特性。

除擬外孔塊菌之外，產自雲南的黑塊菌在外型或孢子特徵上差異不明顯，難以分辨，因此希望配合ITS親緣分析協助鑑定，以後繼續追蹤接種小苗的確實塊菌種類。回顧中國黑塊菌之前人研究，皆指出中國黑塊菌的外觀及形態非常相似，以致形態鑑定困難，親緣關係分析普遍認同分為兩大基因群：印度塊菌A群及B群(Bonito et al. 2010, 2016, Chen et al. 2011, Kinoshita et al. 2011)，A群為印度塊菌分群，B群因為包括臺灣塊菌及喜馬拉雅塊菌比較有爭議性，有學者因為臺灣塊菌的寄主植物及地理差異認同為獨立種(Huang et al. 2009, Chen et al. 2011, Qiao et al. 2013)，但亦有學者因為臺灣塊菌及喜馬拉雅塊菌在形態及基因上都沒有明顯差異而認為屬同種異名(Kinoshita et al. 2018)。印度塊菌的A及B群亦有學者認為為因地理分隔而成之生態型(Wang et al. 2006, Feng et al. 2016)，或是形態一致但不同種的隱存種(cryptic species) (Chen et al. 2011)。形態鑑定方面，前人研究皆指出中國黑塊菌的外觀非常相似，比較多是利用孢子囊內孢子數量及孢子紋飾作辨認特徵，孢子紋飾可以分為針刺(spiny)、刺網格(spiny-reticulate)或兩者混合(partial reticulate)。Zhang and Minter (1988)從印度塊菌的模式標本當中，以不同的網格孢子紋飾把喜馬拉雅塊菌分辨出來，經多個學者證實此特徵在模式標本上非常明顯，但是沒有再看到另一標本有如此鮮明的差異(García-Montero et al. 2010)，但現時喜馬拉雅塊菌仍然以獨特的網格孢子紋飾普遍接受為獨立種。Kinoshita et al. (2018)觀察臺灣塊菌與喜馬拉雅塊菌的孢子皆以刺網格狀紋飾為主，不過三種紋飾皆有機會觀察到，加上基因上沒有明顯差異，認為兩者屬同種異名。Qiao et al. (2013)以三大差異點分辨臺灣塊菌與印度塊菌：(1)臺灣塊菌之子囊有短柄而印度

塊菌沒有，(2)臺灣塊菌之子囊孢子紋飾為刺網格狀而印度塊菌之為針刺狀，(3)臺灣塊菌之寄主為青剛櫟而印度塊菌之寄主以松屬為主。

本研究的結果與前人研究作比較，ITS親緣樹分析結果與前人研究相似，將雲南商業市場常見之印度塊菌分為兩族群，分別為印度塊菌分群(A群)及臺灣塊菌分群(B群)，經成對比較分析，印度塊菌及臺灣塊菌分群之間ITS序列相似度為90.56~93.78%，而兩分群內的相似度為96.73~100%，顯示兩分群基因上有一定的差異。詳細測量印度塊菌及臺灣塊菌分群之孢子大小( $n > 1,800$ )，顯示臺灣塊菌分群之孢子較小，但是兩分群之孢子大小範圍大部分重疊，孢子大小未能作明顯分辨依據。另外使用光學顯微鏡仔細觀察未能看到臺灣塊菌分群塊菌子實體之子囊小短柄，此結果與Qiao et al. (2013)形態上的結論並不相符，而在同一顆臺灣或印度塊菌內皆可以觀察到針刺、刺網格或兩者混合的三種孢子紋飾，兩分群的形態差異並不明顯。

## 結論

本研究為了追蹤各棵小苗接種塊菌種類，及後續觀察菌根、甚至子實體之形成，而把每顆塊菌子實體作個別紀錄及分析。整合實驗結果，確定無法以外表形態簡單分辨雲南商業市場常見之黑塊菌，得靠顯微特徵、甚至需要分子生物技術輔助才能鑑定及辨別。ITS親緣分析顯示雲南黑塊菌可分為有3個分群，分別為擬外孔塊菌分群、印度塊菌分群及臺灣塊菌分群，當中擬外孔塊菌分群之親緣比較疏遠，亦可以根據形態及顯微特徵之明顯差異而分辨出來。另外雖然可以利用ITS親緣關係分析分辨出印度塊菌及臺灣塊菌兩分群，成對比較分析亦得出兩分群間ITS序列相似度有一定的差異(90.56~93.78%)，但是比較兩基因分群的子實體形態，外觀非常接近，孢子大小範圍大部分重疊，孢子紋飾亦沒有明顯差異；顯示暫時只能以親緣關係分辨雲南黑塊菌分群。兩年實驗結果得到的印度塊菌分群及臺灣塊菌分群比例接近4:1，臺灣塊菌分群只有在會澤產區及高黎貢山產區發現，高黎貢山產區有高比例(75%)的臺灣塊菌

分群，而且該產區之塊菌表皮包覆的土壤有特別的金屬光澤，亦帶有干貝般的特殊香氣，或可進一步分析表土成份以了解該產區塊菌的特性。

## 謝誌

本實驗承蒙行政院農委會林業試驗所計畫(108農科-10.1.1-森-G2)補助經費，謹此致謝。

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