

蓮霧黑腐病菌之鑑定與致病力比較

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摘要

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蓮霧黑腐病為蓮霧上常見的病害，發病初期於果實上造成水浸狀病徵，而後水浸狀擴大至整顆果實，並在果實表面產生大量黑色柄子殼，使果實呈現黑色外觀，之後逐漸失水呈木乃伊狀。本研究收集了來自高雄、嘉義、南投及屏東等產地的罹病果實，並分離菌株，經人工接種後確認收集到的所有菌株皆有病原性，病原菌經形態鑑定為*Lasiodiplodia*屬及*Neofusicoccum*屬，並進一步利用核糖體核糖核酸內轉錄區間隔 (rRNA-ITS)、 β -微管蛋白 (*TUB2*)、轉錄延伸因子1 α (*TEF1 α*) 以及RNA聚合酶II第二大次單元 (*RPB2*) 之序列，進行多基因序列鑑定所收集的菌株，分別有*L. theobromae*、*L. pseudotheobromae*及*N. parvum*等三種。以此三種菌之代表菌株進行比較，探討不同溫度下三種菌對於蓮霧果實的感染力。結果顯示32 ~ 37°C環境下*L. theobromae*與*L. pseudotheobromae*的感染較*N. parvum*快，且37°C下明顯不利於*N. parvum*的感染；三種菌在24°C下的感染能力相當，但在16°C下的感染能力皆顯著受到抑制，在8°C環境下則完全無法感染。其中*L. pseudotheobromae*及*N. parvum*引起蓮霧果實病害為世界首次紀錄。

關鍵詞：蓮霧、親緣分析、*Lasiodiplodia theobromae*、*Lasiodiplodia pseudotheobromae*、*Neofusicoccum parvum*、溫度

緒言

蓮霧 (*Syzygium samarangense*) 為臺灣重要經濟作物，屬

於桃金娘科蒲桃屬常綠熱帶果樹，原產於馬來半島及安達曼群島，現今主要栽培國家包括印尼、馬來西亞、泰國及臺灣等⁽¹²⁾。蓮霧於17世紀由荷蘭人自印尼爪哇引進臺灣種植，初期僅為零星栽培，由於臺灣夏季高溫多雨、多病蟲害等原因，造成果實易裂，品質不佳，直到民國七十年代前後，由於藥劑防治、品種改良、催花方法及產期調節技術之成熟，栽培面積及產量才逐漸提升^(11, 38)。目前市場上常見的品種包含南洋種(粉紅種)、泰國種、大果品系、印尼大果種，以及各品系的改良種等，例如臺農三號(黑糖芭比)⁽¹¹⁾。根據農業統計年報顯示，我國2020年的蓮霧栽種面積為3,167公頃，產量為54,501公噸，其中高屏地區為最重要產區，占全國總產量八成以上 (<https://agrstat.coa.gov.tw/sdweb/public/book/Book.aspx>)。

根據前人研究，臺灣主要為害蓮霧果實的病害包括果腐病 (*Pestalotiopsis euginae*)、炭疽病 (*Colletotrichum gloeosporioides*)、黑腐病 (*Lasiodiplodia theobromae*)、果實疫病 (*Phytophthora palmivora*)、黃腐病 (*Calonectria sulawesiensis*)等^(35, 36)。其中，蓮霧黑腐病在臺灣的紀錄主要由*Lasiodiplodia theobromae*引起，發生於10月至隔年3月，其中又以11-12月及3月為主，可感染蓮霧的果實及葉片，感染果實初期果皮會呈現水浸狀、果肉轉為黃褐色，之後病斑逐漸擴大，並伴隨表面出現密集的黑色柄子殼，最終覆蓋全果，使整顆果實轉為灰黑色至黑色的皺縮狀乾果，為臺灣蓮霧生產的主要病害之一^(1, 15, 16)。*L. theobromae*屬葡萄座腔菌科 (Botryosphaeriaceae)，其最常被使用的同物異名為*Botryodiplodia theobromae*，臺灣病害名彙紀錄的主要作物病害尚有柑橘黑色蒂腐病、枇杷果腐病、椪果蒂腐病、酪梨蒂腐病、波羅蜜果腐病及番石榴莖潰瘍病等病害，也曾在與蓮霧同屬的*Syzygium longana*上被報導造成果實炭化病⁽³⁶⁾。

葡萄座腔菌科包含許多屬的植物病原菌，在植物上引起

相似的病徵，並常同時感染同一種寄主植物。例如分別造成椪果 (*Mangifera indica*) 及酪梨 (*Persea americana*) 蒂腐病徵的病原菌包含 *L. theobromae*、*Neofusicoccum parvum*、*N. mangiferae*、*Fusicoccum aesculi* 等四種葡萄座腔科的病原菌^(18, 19)；可引起無花果 (*Ficus carica*) 葉枯及莖腐的病原菌有 *L. theobromae*、*Lasiodiplodia brasiliensis* 及 *Neoscytalidium dimidiatum*^(20, 29)。由上述研究，推測蓮霧黑腐病實際上很可能是由多種同科不同種的病原菌所造成病徵相似的危害，因此本研究從蓮霧黑腐病之病果上收集菌株，並利用多基因序列進行分子鑑定，以確認造成蓮霧黑腐病的病原菌種類。此外，由於蓮霧黑腐病亦為蓮霧的採後病害之一，因此進一步探討，在不同的溫度下不同的病原菌是否會對蓮霧果實的感染及致病力有差異性。

材料與方法

供試菌株分離與保存

本研究從南投縣信義鄉、嘉義縣中埔鄉、屏東縣林邊鄉與東港鎮以及高雄市鳳山區等地各擇一果園，蒐集具有典型黑腐病徵的蓮霧果實 (表一)，若果實表面有柄子殼，則挑取分生孢子進行單孢分離菌株，並繼代培養於馬鈴薯葡萄糖瓊脂 (Potato Dextrose Agar, PDA) 培養基；若未有產孢，則採用組織分離方法，以0.5%的次氯酸鈉 (NaClO) 水溶液消毒罹病組織30秒後，再過兩次無菌水潤洗，之後移至水瓊脂 (Water Agar, WA) 培養基上，待長出菌絲後繼代至PDA培養基上。另外再將各菌株繼代到置有滅菌濾紙片的PDA培養基上，於28°C恆溫培養，待菌絲長滿濾紙片後即可將濾紙片取下風乾，再於-20°C

低溫保存。

菌株病原性測試

使用臺農三號蓮霧果實進行病原性測試，健康果實直接購自嘉義縣梅山鄉之果園，由農友採收成熟度一致之果實，於採收當天送達，先以自來水清洗表面後，將果實浸泡於0.1%次氯酸鈉水溶液中消毒1分鐘，再以自來水沖洗，拭乾備用。蓮霧黑腐病菌菌株繼代於PDA培養基上，於28°C、12小時光照及黑暗週期輪替的生長箱中培養2天後，以直徑6 mm打孔器從菌落邊緣處取下菌絲塊作為接種源，接種在果實果頂至果底的曲線中略為內凹之處 (約為果實縱軸線一半高度之果實表面)，每個菌株各接種兩顆果實，其一做無傷口處理，另一則是傷口接種，以消毒之針頭刺入果實內3 mm的深度，每顆果實接種2處，20株菌共接種40顆果實。接種時將菌絲塊長有菌絲的一面平貼在果實表面，之後將果實放入保鮮盒，以潤濕紙巾保濕，置於恆溫26°C、12小時光照及黑暗週期輪替的生長箱內，之後於第四天將蓋子打開通風，使盒內濕度降低，並以此狀態繼續觀察進一步的侵染過程。

多基因分子親緣鑑定

為了進一步確認蓮霧黑腐病菌之種類，本研究將菌株之新鮮菌絲以phenol:chloroform方法⁽³¹⁾抽取DNA後，再以聚合酶鏈鎖反應 (polymerase chain reaction, PCR) 增幅多個基因片段，包括internal transcribed spacer (*ITS*)、 β -tubulin 2 (*TUB2*)、translation elongation factor 1-alpha (*TEF1* α) 以及RNA polymerase subunit II (*RPB2*) 等基因，PCR引子分別以ITS1及ITS4增幅ITS⁽³⁹⁾，Bt2a及Bt2b增幅*TUB2*⁽⁹⁾，EF1-668F及EF2增幅*TEF1* α ^(2, 21)，rpb2-LasF及rpb2-LasR增幅*RPB2*⁽⁶⁾。增幅後之產物送交源資國際生物科技股份有限公司進行定序，得到之

表一、本研究所使用的蓮霧黑腐病菌的來源分布。

TABLE 1. Distribution of pathogens causing fruit black rot of wax apple.

Fungal species	Frequency of occurrence (%) ¹	Cultivars of wax apple	Locations	Number of isolates	Pathogenicity with / without wound
<i>Lasiodiplodia theobromae</i>	65	Tainung No.3	Donggang Township, Pingtung County;	9	+ / +
		Pink	Fengshan Dist., Kaohsiung City	4	+ / +
<i>Lasiodiplodia pseudotheobromae</i>	5	Pink	Linbian Township, Pingtung County	1	+ / +
<i>Neofusicoccum parvum</i>	30	Tainung No.3	Zhongpu Township, Chiayi County;	5	+ / +
		Tainung No.3	Xinyi Township, Nantou County	1	+ / +

¹ The frequency = (isolate number of a species / total isolate number of the three species in this study) * 100%

基因序列先以BioEdit v7.2.5 軟體⁽¹⁰⁾整理後，於美國生物訊息資料庫 (National Center for Biotechnology Information Genebank, NCBI) 網站使用BLAST功能進行比對，獲得相近種後，再從

NCBI 上下載相近種參考菌株之基因序列 (表二、表三)，與本研究中的菌株序列整理後以MAFFT version 7 (<https://mafft.cbrc.jp/alignment/server/>)⁽¹⁴⁾進行排列 (alignment)，而後進一步

表二、本研究用來建構*Lasiodiplodia*多基因親緣分析之菌株資訊。

TABLE 2. Information of strains used in a phylogenetic analysis of *Lasiodiplodia* species in this study.

Species	Isolate ¹	Origin	Source	ITS ²	<i>TEF1</i> α	<i>TUB2</i>	<i>RPB2</i>
<i>Diplodia scrobiculata</i>	CBS 118110 = CMW 189 = BOT 1195	USA	<i>Pinus banksiana</i>	AY253292	AY624253	AY624258	KX463959
<i>Lasiodiplodia acaciae</i>	CBS 136434 = CPC 20820	Indonesia	<i>Acacia</i> sp.	MT587421	MT592133	MT592613	MT592307
<i>L. avicenniae</i>	CBS 139670 = CMW 41467	South Africa	<i>Avicennia marina</i>	KP860835	KP860680	KP860758	KU587878
	LAS 199	South Africa	<i>Avicennia marina</i>	KU587957	KU587947	KU587868	KU587880
<i>L. brasiliensis</i>	CBS 120395 = GJS 06-247	Cameroon	<i>Theobroma cacao</i>	MT587423	MT592135	MT592615	MT592309
	CBS 129756 = H8	Venezuela	<i>Ficus insipida</i>	MH865372	JX545118	JX545138	MT592310
<i>L. bruguiera</i>	CBS 139669 = CMW 41470	South Africa	<i>Bruguiera gymnorrhiza</i>	KP860832	KP860677	KP860755	KU587876
	CBS 139638 = CMW 41614	South Africa	<i>Bruguiera gymnorrhiza</i>	KP860833	KP860678	KP860756	KU587877
<i>L. cinnamomi</i>	CFCC 51997	China	<i>Cinnamomum camphora</i>	MG866028	MH236799	MH236797	MH236801
	CFCC 51998	China	<i>Cinnamomum camphora</i>	MG866029	MH236800	MH236798	MH236802
<i>L. citricola</i>	CBS 124707 = IRAN 1552C	Iran	<i>Citrus</i> sp.	GU945354	GU945340	KU887505	KU696351
	CBS 124706 = IRAN 1521C	Iran	<i>Citrus</i> sp.	GU945353	GU945339	KU887504	KU696350
<i>L. crassispora</i>	CBS 118741 = WAC 12533	Australia	<i>Santalum</i> sp.	DQ103550	DQ103557	KU887506	KU696353
	CBS 125626 = STE-U 5799	South Africa	<i>Vitis vinifera</i> cv. <i>Ruby Cabernet</i>	MT587424	GU233658	MT592617	MT592312
<i>L. euphorbiaceicola</i>	CMW 33268	Senegal	<i>Adansonia</i> sp.	KU887131	KU887008	KU887430	KU887367
	CMW 36231	Zimbabwe	<i>Adansonia</i> sp.	KU887187	KU887063	KU887494	KU696347
<i>L. gilanensis</i>	CBS 124704 = IRAN 1523C	Iran	<i>Citrus</i> sp.	GU945351	GU945342	KU887511	KU696357
	CBS 124705 = IRAN 1501C	Iran	<i>Citrus</i> sp.	GU945352	GU945341	KU887510	KU696356
<i>L. gonubiensis</i>	CBS 115812 = CMW 14077	South Africa	<i>Syzygium cordatum</i>	AY639595	DQ103566	DQ458860	KU696359
	CBS 138654 = CPC 22781	Thailand	<i>Phyllanthus emblica</i>	KM006443	KM006474	MT592619	MT592314
<i>L. hormozganensis</i>	CBS 124709 = IRAN 1500C	Iran	<i>Olea</i> sp.	GU945355	GU945343	KU887515	KU696361
	CBS 168.28	Indonesia	<i>Cocos nucifera</i>	MT587427	MT592138	MT592621	MT592316
<i>L. iraniensis</i>	CBS 124710 = IRAN 1520C	Iran	<i>Salvadora persica</i>	GU945348	GU945336	KU887516	KU696363
	CMW 35881	ND	<i>Adansonia</i> sp.	KU887092	KU886970	KU887464	KU887388
<i>L. laeliocattleyae</i>	CBS 167.28	Italy	<i>Laelio cattleya</i>	MT587425	MT592136	MT592618	MT592313
<i>L. laeliocattleyae</i> (syn. <i>L. egyptiaca</i>)	CBS 130992 = BOT 10	Egypt	<i>Mangifera indica</i>	JN814397	JN814424	KU887508	KU696354
<i>L. lignicola</i>	CBS 134112 = MFLUCC 11-0435	Thailand	Dead wood	JX646797	KU887003	JX646845	KU696364
<i>L. lignicola</i> (syn. <i>L. chinensis</i>)	CGMCC 3.18061	China	Woody branch	KX499889	KX499927	KX500002	KX499965
<i>L. lignicola</i> (syn. <i>L. sterculiae</i>)	CBS 342.78	Germany	<i>Sterculia oblonga</i>	KX464140	KX464634	KX464908	KX463989
<i>L. mahajangana</i>	CBS 124925 = CMW 27801	Madagascar	<i>Terminalia catappa</i>	FJ900595	FJ900641	FJ900630	KU696365
	CBS 124926 = CMW 27818	Madagascar	<i>Terminalia catappa</i>	FJ900596	FJ900642	FJ900631	KU696366
<i>L. margaritacea</i>	CBS 122519 = CMW 26162 = MOZ 11A	Australia	<i>Adansonia gibbosa</i>	EU144050	EU144065	KU887520	KU696367

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Species	Isolate ¹	Origin	Source	ITS ²	<i>TEF1</i> α	<i>TUB2</i>	<i>RPB2</i>	
<i>L. mediterranea</i>	CBS 138290 = CMW 40957	Zambia	<i>Combretum collinum</i>	KP872321	KP872350	KP872380	KP872430	
	CBS 137783 = BL 1	Italy	<i>Quercus ilex</i>	KJ638312	KJ638331	KU887521	KU696368	
<i>L. microconidia</i>	CBS 137784 = BL 101	Italy	<i>Vitis vinifera</i>	KJ638311	KJ638330	KU887522	KU696369	
	BE35	China	<i>Citrus reticulata</i> cv. Succosa	MW880669	MW884172	MW884201	MW884146	
<i>L. parva</i>	BE88	China	<i>Citrus maxima</i>	MW880667	MW884170	MW884199	MW884144	
	CBS 456.78	Colombia	Cassava-field soil	EF622083	EF622063	KU887523	KU696372	
<i>L. plurivora</i>	CBS 494.78	Colombia	Cassava-field soil	EF622084	EF622064	EU673114	KU696373	
	STE-U 5803	South Africa	<i>Prunus salicina</i>	EF445362	EF445395	KP872421	KP872479	
	CBS 121103 = STE-U 4583 = CPC 4583	South Africa	<i>Vitis vinifera</i>	AY343482	EF445396	KU887525	KU696375	
<i>L. pseudotheobromae</i>	CBS 116459 = KAS 2	Costa Rica	<i>Gmelina arborea</i>	EF622077	EF622057	EU673111	KU696376	
	CBS 116460 = KAS 17	Costa Rica	<i>Acacia mangium</i>	EF622078	EF622058	KU198428	MT592322	
	CBS 130991 = BOT-8	Egypt	<i>Mangifera indica</i>	MT587433	MT592145	MT592629	MT592325	
	CBS 121772 = CMW 25417 = CAMS 1172	Namibia	<i>Acacia mellifera</i>	EU101310	EU101355	MT592627	MT592323	
<i>L. rubropurpurea</i>	BLB10	Taiwan	<i>Syzygium samarangense</i>	OP584321	OP594266	OP594290	OP620022	
	CBS 118740 = WAC 12535	Australia	<i>Eucalyptus grandis</i>	DQ103553	DQ103571	EU673136	KU696380	
<i>L. thailandica</i>	WAC 12536 = CMW 15207	Australia	<i>Eucalyptus grandis</i>	DQ103554	DQ103572	KU887530	KU696381	
	BJFU DZP160119-9	China	ND	KY676788	KY676797	KY676794	KY676791	
<i>L. thailandica</i> (syn. <i>L. hyalina</i>)	BJFU DZP160123-13	China	ND	KY676789	KY676798	KY676795	KY676792	
	CGMCC 3.18383 = B 6180	China	Cankered branches	KY767661	KY751302	KY751299	KY751296	
<i>L. theobromae</i>	CBS 164.96	Papua	Fruit along coral reef coast	AY640255	AY640258	KU887532	KU696383	
	CPC 28505	Indonesia	<i>Dillenia suffruticosa</i>	MT587442	MT592154	MT592640	MT592336	
<i>L. viticola</i>	CBS 111530 = CPC 2095 = JT 695	USA	<i>Leucospermum</i> sp.	EF622074	EF622054	KU887531	KU696382	
	CBS 130989 = BOT 4	Egypt	<i>Mangifera indica</i>	MT587437	MT592149	MT592634	MT592330	
	WL1-1	Taiwan	<i>Syzygium samarangense</i>	OP584308	OP594257	OP594277	OP620009	
	WL1-5	Taiwan	<i>Syzygium samarangense</i>	OP584309	OP594267	OP594278	OP620010	
	WL1-71	Taiwan	<i>Syzygium samarangense</i>	OP584310	OP594268	OP594279	OP620011	
	BDG01	Taiwan	<i>Syzygium samarangense</i>	OP584311	OP594258	OP594280	OP620021	
	BDG02	Taiwan	<i>Syzygium samarangense</i>	OP584312	OP594269	OP594281	OP620020	
	BDG03	Taiwan	<i>Syzygium samarangense</i>	OP584313	OP594259	OP594282	OP620012	
	BDG04	Taiwan	<i>Syzygium samarangense</i>	OP584314	OP594260	OP594283	OP620013	
	BDG05	Taiwan	<i>Syzygium samarangense</i>	OP584315	OP594270	OP594284	OP620014	
	BDG06	Taiwan	<i>Syzygium samarangense</i>	OP584316	OP594261	OP594285	OP620015	
	BDG07	Taiwan	<i>Syzygium samarangense</i>	OP584317	OP594262	OP594286	OP620016	
	BDG08	Taiwan	<i>Syzygium samarangense</i>	OP584318	OP594263	OP594287	OP620017	
	BDG09	Taiwan	<i>Syzygium samarangense</i>	OP584319	OP594264	OP594288	OP620018	
	BFS11	Taiwan	<i>Syzygium samarangense</i>	OP584320	OP594265	OP594289	OP620019	
	<i>L. tropica</i>	CGMCC 3.18477	Laos	<i>Aquilaria crassna</i>	KY783454	KY848616	KY848540	KY848574
	<i>L. venezuelensis</i>	CBS 118739 = WAC 12539	Venezuela	<i>Acacia mangium</i>	DQ103547	DQ103568	KU887533	KU696384
		CMW 13512 = WAC 12540	Venezuela	<i>Acacia mangium</i>	DQ103548	DQ103569	KU887534	KP872491
	<i>L. viticola</i>	CBS 128313 = UCD 2553AR	USA	<i>Vitis vinifera</i>	HQ288227	HQ288269	HQ288306	KU696385

Species	Isolate ¹	Origin	Source	ITS ²	<i>TEF1</i> α	<i>TUB2</i>	<i>RPB2</i>
<i>L. vitis</i>	CBS 128314 = UCD 2604MO	USA	<i>Vitis vinifera</i>	HQ288228	HQ288270	HQ288307	KU696386
	CBS 124060 = PvPa	Italy	<i>Vitis vinifera</i>	KX464148	MN938928	KX464917	KX463994

¹ CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CFCC: China Forestry Culture Collection Center, Beijing, China; CGMCC: China General Microbiological Culture Collection Center; CMW: Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; CPC: Working collection of P.W. Crous, housed at CBS; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; JT: Personal number of J.E. Taylor; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; STE-U: Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa; UCD: University of California, Davis, Plant Pathology Department Culture Collection; WAC: Department of Agriculture, Western Australia Plant Pathogen Collection, South Perth, Western Australia. Isolates in bold are ex-type or holotype strain.

² ITS: internal transcribed spacer; *TEF-1* α : translation elongation factor 1-alpha; *TUB2*: beta-tubulin; *RPB2*: DNA-directed RNA polymerase II second largest subunit.

表三、本研究用來建構*Neofusicoccum*多基因親緣分析之菌株資訊。

TABLE 3. Information of strains used in a phylogenetic analysis of *Neofusicoccum* species in this study.

Species	Isolate ¹	Origin	Source	ITS ²	<i>TEF1</i> α	<i>TUB2</i>
<i>Dothiorella sarmentorum</i>	IMI 63581b	United Kingdom	<i>Ulmus</i> sp.	AY573212	AY573235	EU673102
<i>Neofusicoccum arbuti</i>	CBS 116131	USA	<i>Arbutus menziesii</i>	AY819720	KF531792	KF531793
	CBS 117090	USA	<i>Arbutus menziesii</i>	AY819724	KF531791	KF531794
<i>N. arbuti</i> (syn. <i>N. andinum</i>)	CBS 117453	Venezuela	<i>Eucalyptus</i> sp.	GU251155	GU251287	GU251815
<i>N. australe</i>	CBS 117921	Venezuela	<i>Eucalyptus</i> sp.	KX464152	KX464647	KX464924
	CMW 6837	Australia	<i>Acacia</i> sp.	AY339262	AY339270	AY339254
	CMW 6853	Australia	<i>Sequoiadendron giganteum</i>	AY339263	AY339271	AY339255
	CAA 327	Portugal	<i>Pyracantha coccinea</i>	KX871854	KX871810	KX871721
<i>N. brasiliense</i>	CBS 110851	South Africa	<i>Vitis vinifera</i>	MT587453	MT592162	MT592653
	CMM 1338	Brazil	<i>Mangifera indica</i>	JX513630	JX513610	KC794031
	CMM 1285	Brazil	<i>Mangifera indica</i>	JX513628	JX513608	KC794030
<i>N. buxi</i>	CBS 113714	Sweden	<i>Buxus sempervirens</i>	KX464164	KX464677	KX464954
<i>N. cordaticola</i>	CBS 123634	South Africa	<i>Syzygium cordatum</i>	EU821898	EU821868	EU821838
	CBS 123635	South Africa	<i>Syzygium cordatum</i>	EU821903	EU821873	EU821843
<i>N. cryptoaustrale</i>	CMW 23785	South Africa	<i>Eucalyptus</i> sp.	FJ752742	FJ752713	FJ752756
	CMW 20738	South Africa	<i>Eucalyptus citriodora</i>	FJ752740	FJ752710	FJ752754
<i>N. eucalypticola</i>	CBS 115679	Australia	<i>Eucalyptus grandis</i>	AY615141	AY615133	AY615125
	CBS 115766	Australia	<i>Eucalyptus rossi</i>	AY615143	AY615135	AY615127
<i>N. eucalyptorum</i>	CBS 115791	South Africa	<i>Eucalyptus grandis</i>	AF283686	AY236891	AY236920
	CAA 369	Portugal	<i>Eucalyptus globulus</i>	KT440922	KT440982	KX871773
<i>N. hellenicum</i>	CERC 1947	Greece	<i>Pistacia vera</i>	KP217053	KP217061	KP217069
	CERC 1948	Greece	<i>Pistacia vera</i>	KP217054	KP217062	KP217070
<i>N. hongkongensis</i>	CERC 2973	China: HongKong	<i>Araucaria cunninghamii</i>	KX278052	KX278157	KX278261
	CERC 2968	China: HongKong	<i>Araucaria cunninghamii</i>	KX278051	KX278156	KX278260
<i>N. illicii</i>	CGMCC 3.18311 (BJFU 2038)	China	<i>Illicium verum</i>	KY350150	KY817756	KY350156
	CGMCC 3.18312 (BJFU 2039)	China	<i>Illicium verum</i>	KY350151	KY817757	KY350157
<i>N. kwambonambiense</i>	CBS 123639	South Africa	<i>Syzygium cordatum</i>	EU821900	EU821870	EU821840
	CBS 123641	South Africa	<i>Syzygium cordatum</i>	EU821919	EU821889	EU821859

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Species	Isolate ¹	Origin	Source	ITS ²	<i>TEF1</i> α	<i>TUB2</i>
	CAA 755	Portugal	<i>Eucalyptus globulus</i>	KT440946	KT441006	KX505917
<i>N. lumnitzeriae</i>	CMW 41469	South Africa	<i>Lumnitzera racemosa</i>	KP860881	KP860724	KP860801
	CBS 139676	South Africa	<i>Lumnitzera racemosa</i>	MT587481	MT592194	MT592686
<i>N. luteum</i>	CBS 110299	Portugal	<i>Vitis vinifera</i>	AY259091	AY573217	DQ458848
	CBS 110497	Portugal	<i>Vitis vinifera</i>	EU673311	EU673277	EU673092
	CAA 203	Portugal	<i>Melia azedarach</i>	KX871870	KX871826	KX871755
<i>N. macroclavatum</i>	CBS 118223	Australia	<i>Eucalyptus globulus</i>	DQ093196	DQ093217	DQ093206
	WAC 12446	Australia	<i>Eucalyptus globulus</i>	DQ093197	DQ093218	DQ093207
<i>N. mangiferae</i>	CBS 118532 (CMW 7797)	Australia	<i>Mangifera indica</i>	AY615186	DQ093220	AY615173
	CBS 118531 (CMW 7024)	Australia	<i>Mangifera indica</i>	AY615185	DQ093221	AY615172
<i>N. mediterraneum</i>	CBS 121718	Greece	<i>Eucalyptus</i> sp.	GU251176	GU251308	GU251836
	CBS 121558	USA	<i>Vitis vinifera</i>	GU799463	GU799462	GU799461
<i>N. microconidium</i>	CERC 3497	China	<i>Eucalyptus urophylla</i> x <i>E. grandis</i>	KX278053	KX278158	KX278262
	CBS 118821	South Africa	<i>Syzygium cordatum</i>	MT587497	MT592212	MT592704
<i>N. nonquaesitum</i>	CBS 126655	USA	<i>Umbellularia californica</i>	GU251163	GU251295	GU251823
	PD301	Chile	<i>Vaccinium corymbosum</i>	GU251164	GU251296	GU251824
<i>N. oculatum</i>	CBS 128008	Australia	<i>Eucalyptus grandis</i> hybrid	EU301030	EU339509	EU339472
	MUCC 286	Australia	<i>Eucalyptus pellita</i>	EU736947	EU339511	EU339474
<i>N. parvum</i>	CMW 9081	New Zealand	<i>Populus nigra</i>	AY236943	AY236888	AY236917
	CBS 110301	Portugal	<i>Vitis vinifera</i>	AY259098	AY573221	EU673095
	CAA 189	Portugal	<i>Ferula communis</i>	KX871879	KX871835	KX871766
	WN5-161	Taiwan	<i>Syzygium samarangense</i>	OP584341	OP594271	OP594291
	WN5-172	Taiwan	<i>Syzygium samarangense</i>	OP584342	OP594272	OP594292
	WN5-188	Taiwan	<i>Syzygium samarangense</i>	OP584343	OP594273	OP594293
	WN5-190	Taiwan	<i>Syzygium samarangense</i>	OP584344	OP594274	OP594294
	WN5-191	Taiwan	<i>Syzygium samarangense</i>	OP584345	OP594275	OP594295
	WNNT01	Taiwan	<i>Syzygium samarangense</i>	OP584346	OP594276	OP594296
<i>N. parvum</i> (syn. <i>N. algeriense</i>)	CBS 137504	Algeria	<i>Vitis vinifera</i>	KJ657702	KX505893	KX505915
	CAA 322	Portugal	<i>Malus domestica</i>	KX505906	KX505894	KX505916
	CAA 366	Portugal	<i>Eucalyptus globulus</i>	KT440951	KT441011	KX871764
<i>N. parvum</i> (syn. <i>N. italicum</i>)	CBS 140889	France	<i>Vitis vinifera</i> cv. <i>Alfrocheiro</i>	MT587479	MT592192	MT592684
<i>N. parvum</i> (syn. <i>N. pandanicola</i>)	CBS 118832	South Africa	<i>Syzygium cordatum</i>	MT587501	MT592216	MT592708
<i>N. pennatisporum</i>	MUCC 510	Australia	<i>Allocasuarina fraseriana</i>	EF591925	EF591976	EF591959
<i>N. pistaciae</i>	CBS 595.76	Greece	<i>Pistacia vera</i>	KX464163	KX464676	KX464953
<i>N. podocarpi</i>	CBS 131677	South Africa	<i>Podocarpus henkelii</i>	MT587508	MT592223	MT592715
	CBS 131678	South Africa	<i>Podocarpus henkelii</i>	MT587509	MT592224	MT592716
<i>N. protearum</i>	CBS 114176	South Africa	<i>Leucadendron salignum</i> x <i>L. laureolum</i>	AF452539	KX464720	KX465006
	MUCC 497	Australia	<i>Santalum acuminatum</i>	EF591912	EF591965	EF591948
	CBS 115177	South Africa	<i>Protea magnifica</i>	FJ150703	MT592239	MT592731
<i>N. rapanae</i>	CBS 145973	South Africa	<i>Myrsine melanophloeos</i>	MT587511	MT592226	MT592718
	CPC 32578	South Africa	<i>Rapanea</i> sp.	MT587512	MT592227	MT592719

Species	Isolate ¹	Origin	Source	ITS ²	<i>TEF1</i> α	<i>TUB2</i>
<i>N. ribis</i>	CBS 115475	USA	<i>Ribes</i> sp.	AY236935	AY236877	AY236906
	M1-105	Canada	<i>Prunus armeniaca</i>	OK287034	OK346636	OK346633
<i>N. ribis</i> (syn. <i>N. batangarum</i>)	CBS 124924	Cameroon	<i>Terminalia catappa</i>	FJ900607	FJ900653	FJ900634
<i>N. ribis</i> (syn. <i>N. umdonicola</i>)	CBS 123645	South Africa	<i>Syzygium cordatum</i>	EU821904	EU821874	EU821844
<i>N. sinense</i>	CGMCC 3.18315	China	unknown woody plant	KY350148	KY817755	KY350154
<i>N. sinoeucalypti</i>	CERC 2005	China	<i>Eucalyptus urophylla</i> x <i>E. grandis</i>	KX278061	KX278166	KX278270
	CERC 3415	China	<i>Eucalyptus urophylla</i> x <i>E. grandis</i>	KX278063	KX278168	KX278272
<i>N. stellenboschiana</i>	CBS 133326	USA	<i>Persea americana</i>	MT587519	MT592244	MT592736
	CBS 139666	South Africa	<i>Rizophora mucronata</i>	MT587520	MT592245	MT592737
<i>N. terminaliae</i>	CBS 125263 = CMW 26679	South Africa	<i>Terminalia sericea</i>	GQ471802	GQ471780	KX465052
	CBS 125264 = CMW 26683	South Africa	<i>Terminalia sericea</i>	GQ471804	GQ471782	KX465053
<i>N. ursorum</i>	CBS 122811 (CMW 24480)	South Africa	<i>Eucalyptus</i> sp.	MH863237	FJ752709	KX465056
	CBS 131680	South Africa	<i>Podocarpus henkelii</i>	MT587527	MT592253	MT592745
<i>N. variabile</i>	CMW 37742	South Africa	<i>Mimusops caffra</i>	MH558609	MH576585	MH569154
	CMW 37745	South Africa	<i>Mimusops caffra</i>	MH558610	MH576586	MH569155
<i>N. viticlavatum</i>	CBS 112878 (STE-U 5044)	South Africa	<i>Vitis vinifera</i>	AY343381	AY343342	KX465058
	CBS 112977 (STE-U 5041)	South Africa	<i>Vitis vinifera</i>	AY343380	AY343341	KX465059
<i>N. vitifusiforme</i>	CBS 110887 (STE-U 5252)	South Africa	<i>Vitis vinifera</i>	AY343383	AY343343	KX465061
	5H022	California	<i>Juglans regia</i>	KF778869	KF779059	KF778964
	B8	Italy	<i>Vitis vinifera</i>	KC469638	KX505897	KX505922

¹ CAA: Personal culture collection Artur Alves, Universidade de Aveiro, Portugal; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CERC: Culture collection of China Eucalypt Research Centre, Chinese Academy of Forestry, ZhanJiang, GuangDong, China; CGMCC: China General Microbiological Culture Collection Center; CMM: Culture Collection of Phytopathogenic Fungi “Prof. Maria Menezes”, Universidade Federal Rural de Pernambuco, Recife, Brazil; CMW: Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; IMI: International Mycological Institute, CBI-Bioscience, Egham, Boreham Lane, UK; MUCC (Australia): Murdoch University Culture Collection, Murdoch, Australia; STE-U: Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa; WAC: Department of Agriculture, Western Australia Plant Pathogen Collection, South Perth, Western Australia. Isolates in bold are ex-type or holotype strain.

² ITS: internal transcribed spacer; *TEF1* α : translation elongation factor 1-alpha; *TUB2*: beta-tubulin.

用Gblocks Server v0.91b ⁽³⁾去除帶有gap之片段，合併每個基因的序列檔，用於建構多基因親緣樹。本研究使用MEGA 6.0軟體⁽³³⁾建構親緣關係樹，首先使用軟體中Find Best DNA/Protein Models功能，分析最佳的運算模型 (model) 後，再以最大似然法 (Maximum likelihood method, ML) 建構親緣樹，序列分析的模型為T92 model，Bootstrap重複數為1,000次，於親緣樹各節點 (node) 僅呈現達到60%以上的Bootstrap值。同時也將多基因序列進行貝氏分析 (Bayesian inference (BI) analyses)，使用軟體為MrBayes v. 3.2.7⁽³⁰⁾，以馬可夫鏈蒙地卡羅方法 (Markov chain Monte Carlo, MCMC)運算1,000,000代，每1,000代進行一次取樣，運算結束後捨棄前25%之取樣樹，剩餘750棵樹則合併為一致性樹 (consensus tree)，將親緣樹在FigTree v. 1.4.4開啟並檢視各節點之後驗概率 (posterior probabilities, PP)，數值大於0.9

者則標註至最大似然法構架之親緣樹對應節點。

分生孢子及菌落形態觀察

將黑腐病菌菌株以上述病原性測試之條件接種在蓮霧果實上，成功感染後，繼續將果實放置於接種環境中，待產孢構造形成及分生孢子成熟，從中挑取觀察並紀錄產孢構造及分生孢子形態。另外將菌株繼代於PDA培養基上，於28°C、12小時光照及黑暗週期輪替的生長箱中培養3天後，拍照紀錄菌落型態。

溫度對黑腐病菌感染蓮霧果實之影響

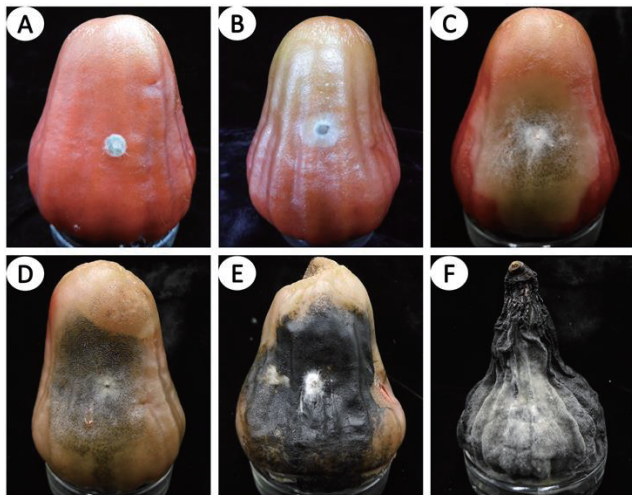
挑選已經由多基因鑑定得知分別為*L. theobromae*的WL1-

5、*L. pseudotheobromae*的BLB10以及*N. parvum*的WNNT01三株菌株進行試驗。本試驗之接種條件與病原性測試之條件一致，唯接種之果實皆為無傷口處理，且放置果實之保鮮盒都以鋁箔紙完全包覆，形成全黑暗之試驗條件，而不同溫度之處理組分別放置於8°C、16°C、24°C、32°C、37°C的生長箱中，每菌株於每種溫度中分別接種三顆果實，之後於接種後第四天將盒蓋打開通風，使盒內濕度降低。為了比較在各種溫度下蓮霧黑腐病菌的病徵發展差異，於接種後第1、3、5、9及13天紀錄罹病等級，罹病等級之定義則是由黑腐病菌感染過程中各階段的病徵外觀進行區分，根據果實上的褪色病斑及黑色柄子殼佔總表面積大小分成0~5級的罹病級數。0級代表果實上無病斑；1級為水浸狀褪色病斑出現，病斑佔全顆果實表面積1~25%；2級為水浸狀褪色病斑佔全顆果實表面積達26%~75%；3級為、水浸狀病褪色斑擴散至整顆果實，此時可見到25%以下果實表面長出一些黑色的柄子殼；4級時柄子殼逐漸擴散，使整顆果實表面約26%~75%呈現黑色；5級則是整顆果實皆為黑色，表面分佈柄子殼，有時可以在表面看見白色菌絲體或是擠壓出的分生孢子(圖一)。

結果

供試菌株分離與病原性測試

本試驗從罹患蓮霧黑腐病的蓮霧果實中共分離出20株菌，其中9株來自屏東縣東港鎮，5株來自嘉義縣中埔鄉，4株來自



圖一、蓮霧黑腐病接種病徵及罹病級數。(A)0級，無病徵；(B)1級，1~25%面積水浸狀；(C)2級，26~75%面積水浸狀；(D)3級，>75%面積水浸狀，且<25%面積分佈黑色柄子殼；(E)4級，26~75%面積分佈黑色柄子殼；(F)5級，全果分佈黑色柄子殼。

Fig. 1. Symptoms and disease indexes of inoculated wax apples. (A) 0, symptomless; (B) 1, 1~25% area of water soaking; (C) 2, 26~75% area of water soaking; (D) 3, >75% area of water soaking and <25% area of black pycnidia; (E) 4, 26~75% area of black pycnidia; (F) 5, entire fruit with black pycnidia.

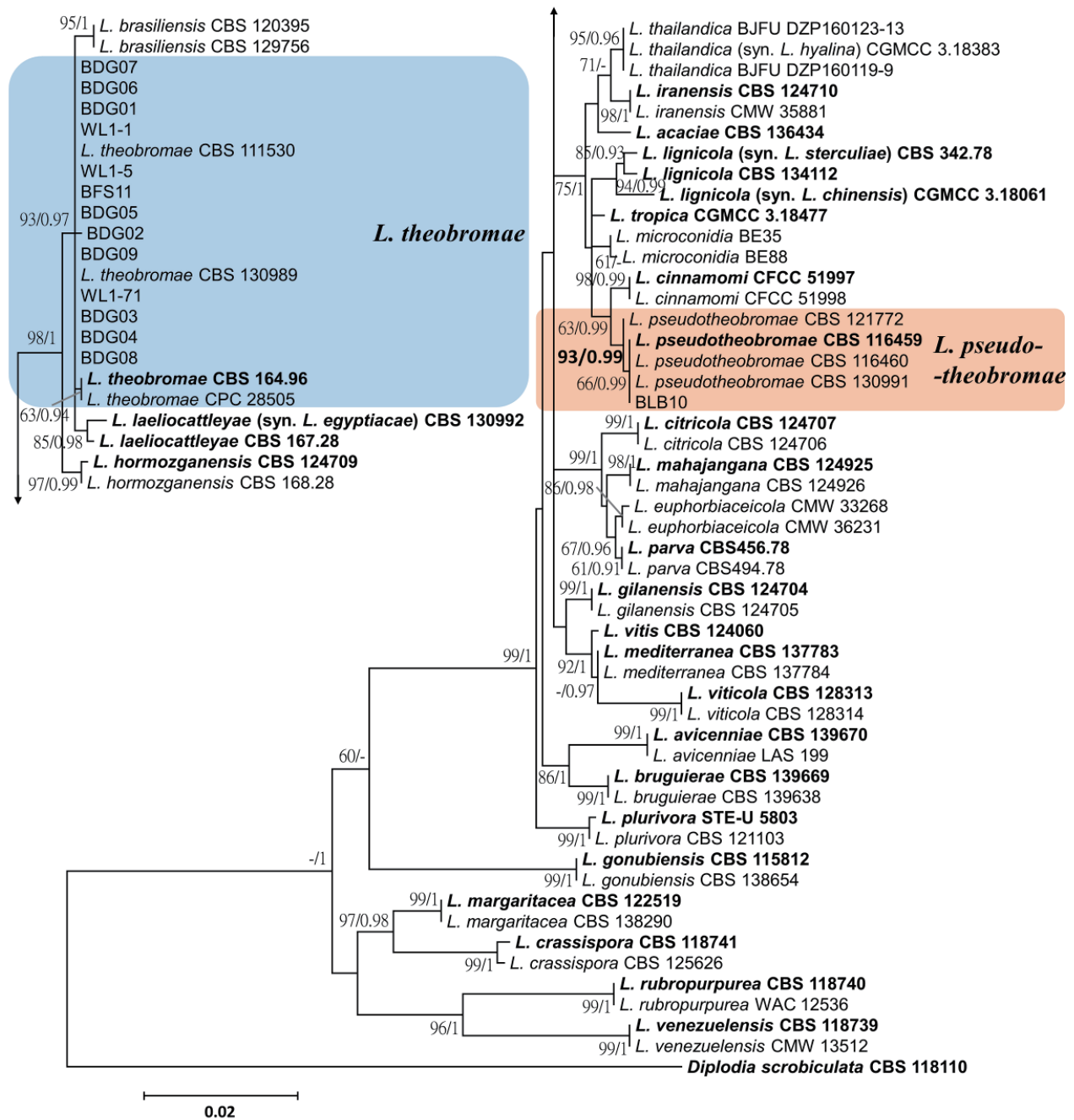
高雄市鳳山區，南投縣信義鄉與屏東縣林邊鄉各1株；其中來自屏東縣的10株菌株及高雄市鳳山區的1株菌，寄主皆為粉紅種蓮霧，其餘9株的寄主為臺農三號蓮霧(表一)。為進一步測試所分離菌株的病原性，將各菌株的菌絲塊接種於臺農三號蓮霧果實上，於26°C的溫度條件下，無傷口處理組在接種3天後皆可在果實表面觀察到水浸狀褪色病斑，在第4-5天病斑即會迅速擴展，7-9天病斑表面開始出現黑色的柄子殼，果實內部逐漸軟化，隨後柄子殼逐漸遍布整顆果實，有時會在表面擠出分生孢子堆。約在接種14天後，果實已整顆轉為黑色，且逐漸脫水、皺縮及變硬。而傷口處理組的發病情形與前者描述相似，相異處在於每個發展階段會更提前1-2天。經接種結果得知，本試驗所有的供試菌株皆具有病原性，且不論以無傷口或製造傷口之條件下接種，皆可在蓮霧果實上成功感染造成病徵。

多基因分子親緣鑑定

在*Lasiodiplodia*的親緣樹中，來自屏東縣東港鎮的9株菌及高雄市鳳山區的4株菌皆與*L. theobromae*的模式菌株(CBS 164.96)及參考菌株歸屬在一個複合的支序群(clade)中，此複合支序群之節點有93%的bootstrap值以及0.97的PP值，而此支序群除了以*L. theobromae*為主體，同時還包含*L. brasiliensis*次支序群(95%的bootstrap值以及1.0的PP值)及*L. laeliocattleyae*次支序群(85%的bootstrap值以及0.98的PP值)，本研究菌株及*L. theobromae*的參考菌株則集中分佈於上述兩次支序群之外，此複合支序群的結構與Zhang (2021)⁽⁴¹⁾等人的研究結果相似。而來自屏東縣林邊鄉的1株菌則是與*L. pseudotheobromae*的模式菌株(CBS 116459)及3株參考菌株形成一個獨立的支序群，該節點有93%的bootstrap值以及0.99的PP值(圖二)。在*Neofusicoccum*的親緣樹中，來自嘉義縣中埔鄉的5株菌及南投縣信義鄉的1株菌皆與*N. parvum*的模式菌株(CMW 9081)及參考菌株集中在一個支序群(clade)中，該節點有74%的bootstrap值以及1.0的PP值(圖三)。

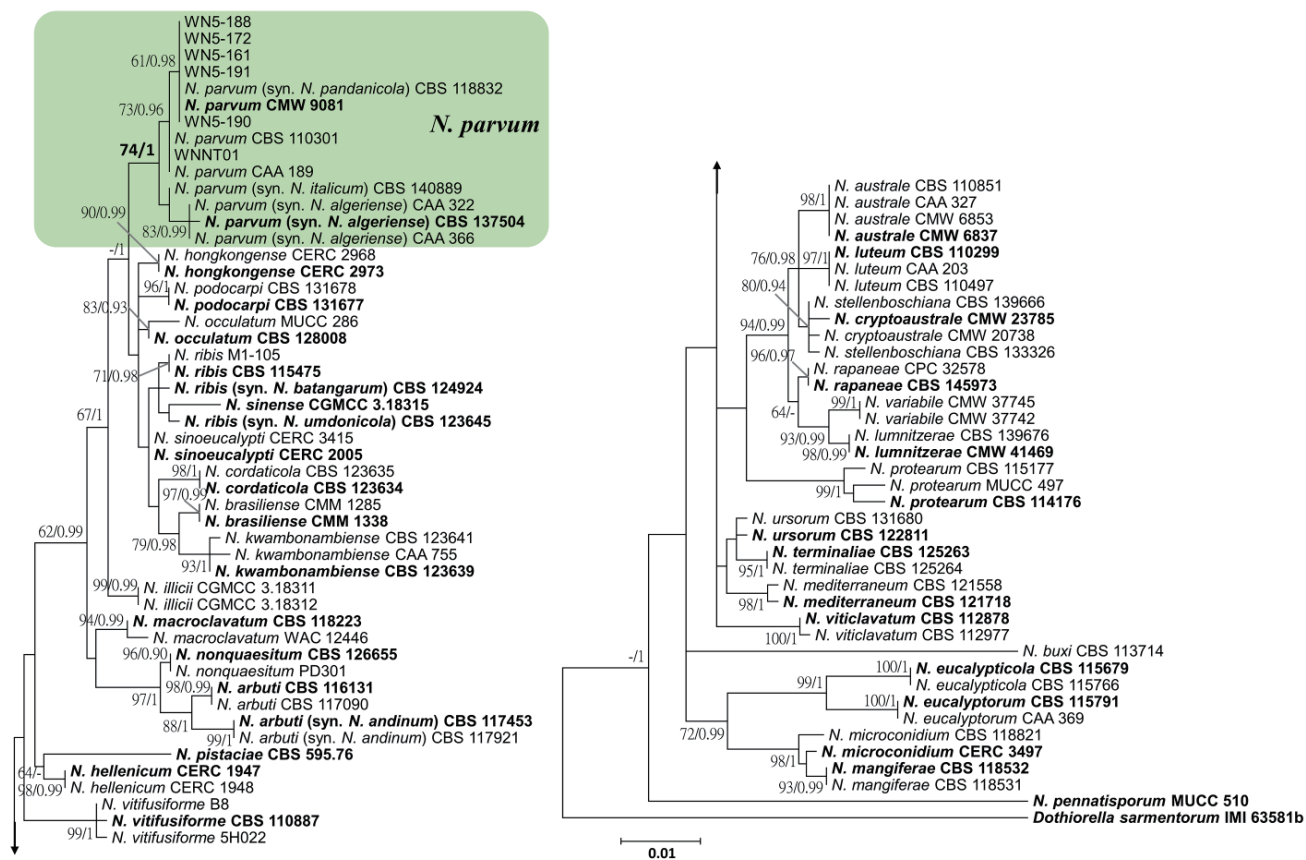
菌落及分生孢子形態觀察

造成蓮霧黑腐病的病原菌中主要有兩類，一類為*Lasiodiplodia*屬，菌落形成初期為白色的氣生菌絲，之後菌絲成熟後會轉變為灰黑至墨綠色，產孢梗與側絲皆為透明無色，分生孢子外形呈卵形至橢圓形，未成熟時為透明無色的無隔單細胞孢子，成熟後轉為黑褐色並有一層隔膜的孢子，其表面上具縱向的紋路，其中*L. theobromae*之分生孢子長度約為23~31 μm，寬度約為12~16 μm(圖四)，*L. pseudotheobromae*之分生孢子長度約為25~33 μm，寬度約為11~17 μm(圖五)；另一類為*Neofusicoccum*屬，菌落則是灰白色，部分菌絲成熟也會轉為灰綠色，但需較長時間，主要的特徵為成熟分生孢子呈透明無色，且形狀大多為梭形或紡錘形，有的則為一端鈍圓一端尖



圖二、*Lasiodiplodia*屬菌株之多基因親緣分析。親緣分析合併ITS、*TEF1* α 、*TUB2*及*RPB2*四個基因序列進行，使用最大似然法(Maximum likelihood method)及T92 model建構親緣樹，Bootstrap重複數為1000次；同時也將多基因序列進行貝氏分析(Bayesian inference analyses)。每個節點(node)只顯示大於60%的Bootstrap值(斜線左側)，以及大於0.9的後驗概率(posterior probabilities, PP)(斜線右側)，小於此兩標準之數值以負號呈現。圖中以粗體字呈現之菌株皆為該菌種之模式菌株(type strain)，縮寫syn.則為同物異名(synonyms)。

Fig. 2. A phylogenetic analysis of *Lasiodiplodia* strains. The phylogenetic tree was analyzed by the Maximum likelihood method with T92 model and the Bayesian inference analyses based on the concatenated sequences of ITS, *TEF1* α , *TUB2*, and *RPB2* genes. A thousand times of bootstrap and posterior probabilities (pp) were carried out. Each node of the phylogenetic tree displayed bootstrap values more than 60% at the right side of a slash and pp values more than 0.9 at the left side of a slash. The type strains were in bold. Synonyms were abbreviated as syn.



圖三、*Neofusicoccum*屬菌株之多基因親緣分析。親緣分析合併ITS、TEF1及TUB2三個基因序列進行，使用最大似然法(Maximum likelihood method)及T92 model建構親緣樹，Bootstrap重複數為1000次；同時也將多基因序列進行貝氏分析(Bayesian inference analyses)，每個節點(node)只顯示大於60%的Bootstrap值(斜線左側)，以及大於0.9的後驗概率(posterior probabilities, PP)(斜線右側)，小於此兩標準之數值以負號呈現。圖中以粗體字呈現之菌株皆為該菌種之模式菌株(type strain)，縮寫syn.則為同物異名(synonyms)。

Fig. 3. A phylogenetic analysis of *Neofusicoccum* strains. The phylogenetic tree was analyzed by the Maximum likelihood method with T92 model and the Bayesian inference analyses based on the concatenated sequences of ITS, *TEF1* α , *TUB2*, and *RPB2* genes. A thousand times of bootstrap and posterior probabilities (pp) were carried out. Each node of the phylogenetic tree displayed bootstrap values more than 60% at the right side of a slash and pp values more than 0.9 at the left side of a slash. The type strains were in bold. Synonyms were abbreviated as syn.

細，尺寸較*Lasiodiplodia*的孢子小，*N. parvum*之分生孢子長度約為15~17 μm ，寬度約為5~8 μm ，產孢梗是透明無色，且不具有側絲(圖六)。

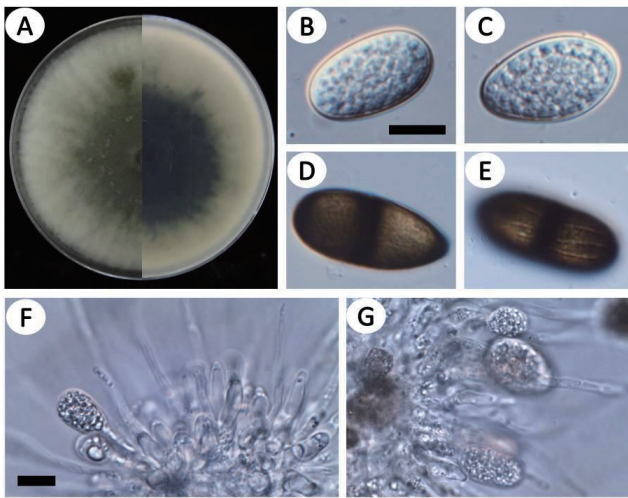
不同溫度對黑腐病菌感染蓮霧果實之致病力

經形態及分子親緣鑑定後，得知蓮霧黑腐病菌有三個種，因此各種挑選一株作為代表菌株，進行後續試驗。*L. theobromae*的代表菌株為WL1-5，*L. pseudotheobromae*的代表菌株為BLB10，*N. parvum*的代表菌株為WNNT01。將三種黑腐病菌之代表菌株以菌絲塊無傷口接種於蓮霧果實上，以0級到5級六個罹病等級比較菌株在不同溫度下的致病力。結果顯示，*L. theobromae* WL1-5、*L. pseudotheobromae* BLB10及*N. parvum* WNNT01 在24°C的發病趨勢相近，第13天的平均罹病等級為3-4；在32°C時*L. theobromae* WL1-5及*L. pseudotheobromae* BLB10

於第13天的罹病等級皆可達到5，發病速度明顯較*N. parvum* WNNT01快。溫度上升到37°C時，*L. theobromae* WL1-5及*L. pseudotheobromae* BLB10 在9-13天可達到4以上的罹病等級，但*N. parvum* WNNT01的病徵發展明顯受阻，第13天罹病度仍少於1；三株菌在16°C的病徵發展明顯較24°C時受到抑制，三者接種後第13天仍然只有1以下的罹病等級；於8°C時，接種13天後仍沒有發病跡象(圖七)。

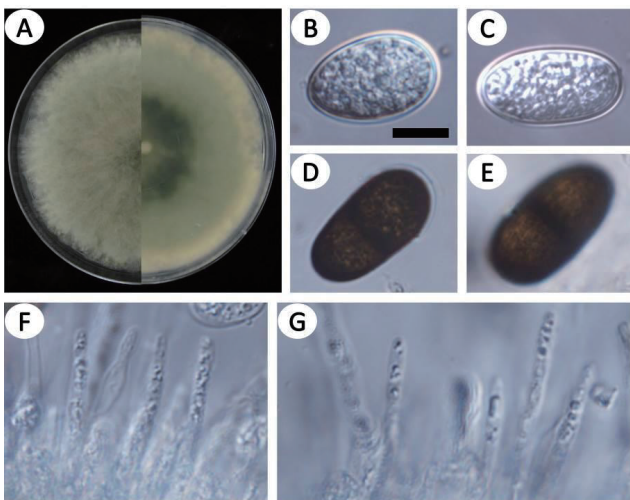
討 論

在本研究初期的調查中，根據分生孢子之形態，即可判斷出臺灣的蓮霧黑腐病菌族群是由一種以上的物種所構成。為了釐清實際造成危害的菌種，在本研究中，主要參考前人發



圖四、*Lasiodiplodia theobromae* WL1-5之菌落及孢子形態。(A)菌落正面(左)及反面(右)。(B、C)未成熟分生孢子。(D、E)成熟分生孢子。(F、G)產孢梗及側絲。比例尺為10 μm ，(B - E)及(F - G)分別使用相同比例尺。

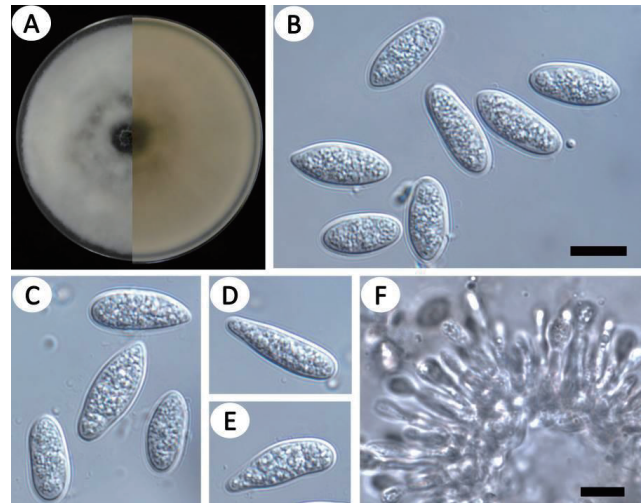
Fig. 4. Colony and conidial morphology of *Lasiodiplodia theobromae* WL1-5. (A) The upper side (left) and the lower side (right) of a colony. (B & C) An immature conidium. (D & E) An mature conidium. (F & G) Conidiophores and paraphyses. Scale bars are 10 μm .



圖五、*Lasiodiplodia pseudotheobromae* BLB10之菌落及孢子形態。(A)菌落正面(左)及反面(右)。(B、C)未成熟分生孢子。(D、E)成熟分生孢子。(F、G)產孢梗及附屬絲。(B - G)參考(B)之比例尺為10 μm 。

Fig. 5. Colony and conidial morphology of *Lasiodiplodia pseudotheobromae* BLB10. (A) The upper side (left) and the lower side (right) of a colony. (B & C) An immature conidium. (D & E) An mature conidium. (F & G) Conidiophores and paraphyses. The scale bar is 10 μm .

表的分類研究及參考菌株⁽⁴¹⁾，利用多基因序列進行分析，建構出親緣關係樹。根據親緣樹及病原性測試的結果，證實造成蓮霧黑腐病的病原菌皆屬於葡萄座腔菌科下的*Lasiodiplodia*及

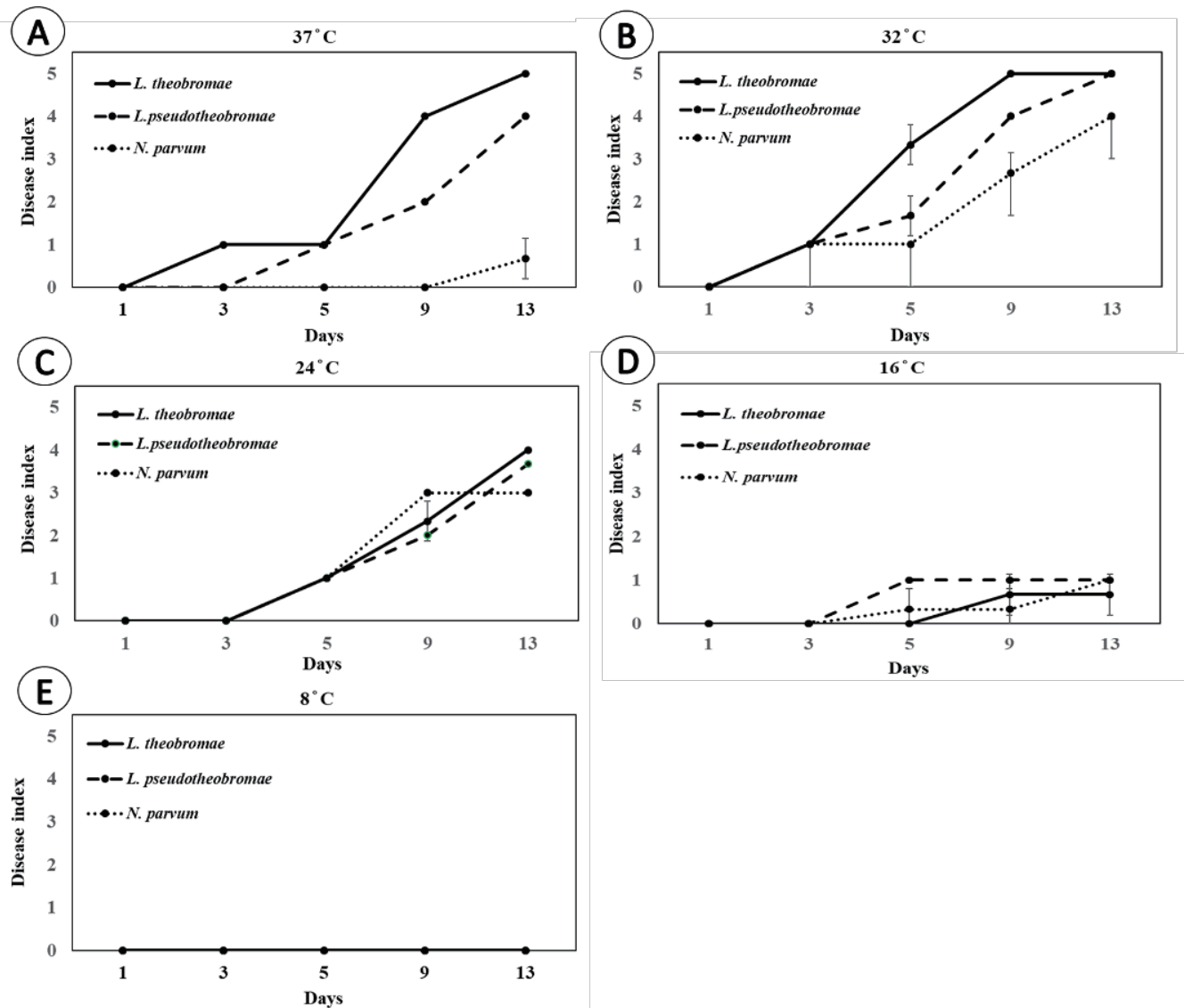


圖六、*Neofusicoccum parvum* WNNT01之菌落及孢子形態。(A)菌落正面(左)及反面(右)。(B - E)分生孢子。(F)產孢梗。(B - E)參考(B)之比例尺為10 μm ，(F)之比例尺為10 μm 。

Fig. 6. Colony and conidial morphology of *Neofusicoccum parvum* WNNT01. (A) The upper side (left) and the lower side (right) of a colony. (B - E) Conidia. (F) Conidiophores. Scale bars are 10 μm .

*Neofusicoccum*屬，包括*L. theobromae*、*L. pseudotheobromae*以及*N. parvum*等三種。目前*Lasiodiplodia*及*Neofusicoccum*屬皆有30個以上的物種，*Lasiodiplodia*屬可為害裸子及被子植物，造成許多重要的農作物及森林植物的莖幹潰瘍及枝枯病害⁽⁴¹⁾，其中以*L. theobromae*為主要代表，可造成許多種植物及作物果實病害⁽²⁸⁾，也是本研究收集到的菌株中數量最多的種類；*L. pseudotheobromae*除可引起莖幹及枝條的病害，近年在不同國家的報導中，亦可造成果腐病害，包含臺灣的酪梨果腐⁽¹⁹⁾、中國的柑橘果腐⁽⁴⁾、巴西的柿子果腐⁽¹³⁾、泰國的龍眼果腐⁽²⁴⁾、波多黎各可可莢腐病⁽³²⁾等。*Neofusicoccum*屬亦可感染許多物種引起莖幹及枝條的病害，經常出現於枯死的枝條上，其分生孢子構造簡單，成熟後皆為透明無色，表面光滑⁽²³⁾。此外*N. parvum*亦在下列案例中引起果實病害，如在中國引起核桃、奇異果及枇杷果腐病^(5, 40, 42)、烏拉圭引起蘋果果腐病⁽⁷⁾、美國引起可可莢腐病及草莓果腐病^(22, 27)、墨西哥引起酪梨果斑病⁽¹⁷⁾及臺灣引起椪果蒂腐病及酪梨果斑病^(18, 19)。本研究結果為首次在蓮霧上報導*L. pseudotheobromae*及*N. parvum*引起的果腐病紀錄，在泰國*N. parvum*雖曾紀錄於蓮霧枯枝上，卻無法分辨是否為病原菌⁽³⁴⁾，因此本報導也是*L. pseudotheobromae*及*N. parvum*首次引起蓮霧病害的記錄⁽⁸⁾。

在本次研究中測試溫度對於病害發展的影響，藉此探討不同物種的生理特性差異。由臺農三號蓮霧的接種結果可得知，*L. theobromae*的致病力最強，其次為*L. pseudotheobromae*，而*N. parvum*則是致病力最弱。在所測試的溫度區間內，*L. theobromae*和*L. pseudotheobromae*兩種菌在24~37°C間皆可快速侵染蓮霧，特別是在32°C的感染速率最快；*N. parvum*的適合



圖七、三種蓮霧黑腐病菌接種於果實上在不同溫度下的病徵發展情形。

Fig. 7. Effect of temperatures on symptom development of the three pathogens causing the fruit black rot of wax apple. Error bars are standard deviations.

感染溫度則是24°C~32°C，若達到37°C時，感染程度就會明顯低於*Lasiodiplodia*屬的兩菌種，顯示*N. parvum*較不耐高溫環境。三種菌在溫度16°C以下時，生長皆會受到抑制。至於在8°C的環境，三株菌皆不能造成病害，也無法正常生長。上述溫度接種試驗的結果說明*Lasiodiplodia*屬的兩種病原菌的適合感染溫度較*N. parvum*高，此結果亦與前人在培養基上測量的病原菌生長速度的趨勢相似^(26, 37)。由於蓮霧黑腐病也是一種蓮霧的採收後病害，可於貯運期間發生，而根據試驗結果也可得知，在16°C環境下蓮霧黑腐病明顯發展受到抑制，8°C下更可完全抑制病害發生，但需要注意過低溫度及貯藏時間過久易導致的寒害問題。此外，由於本研究的供試菌株在培養基中不易收集到大量且成熟度一致的分生孢子，故以菌絲塊進行試驗。推測病

原菌於田間應以分生孢子進行傳播與感染，溫度對於分生孢子的產生、發芽及入侵可能不同於菌絲，也可能影響各種菌的致病力，未來若能建立穩定的產孢方法，以分生孢子接種將更能反應田間狀況。

在菌相分佈中，值得注意的是，*L. theobromae*與*L. pseudotheobromae*的寄主皆來自屏東縣的東港鎮與林邊鄉及高雄的鳳山區，屬沿海及平原地區；而*N. parvum*的寄主來源則是嘉義縣的中埔鄉及南投縣的信義鄉，屬山坡地，顯示此兩個病原菌屬在分佈上具地理差異性；另外菌株數量最多的*L. theobromae*在粉紅種以及臺農三號兩個品種上皆可分離到，說明該菌相差異與寄主品種較無相關，而可能與地理及環境因子較相關。上述溫度試驗的結果進一步呼應菌株的分布，使臺

灣各地的氣溫條件可能會是影響不同病原菌分布多寡的因素之一，推測高雄及屏東的採集地為沿海地區普遍氣溫較高，蓮霧黑腐病可能以*Lasiodiplodia*屬佔大部分，而嘉義及南投等山地區域氣溫相對較低，則*N. parvum*所佔比例可能增加。此外澳洲葡萄莖潰瘍病的研究顯示，*L. theobromae*及*N. parvum*皆為病原菌，*L. theobromae*的分離率明顯較*N. parvum*低，且僅出現於較乾熱的地區⁽²⁵⁾；美國葡萄莖潰瘍病的研究亦有相似的趨勢，*L. theobromae*主要發生在加州南部Coachella Valley的沙漠地區，而*N. parvum*則分布在加州北部⁽³⁷⁾。這些例子顯示引起病害的病原菌會隨著氣候環境的差異而有所不同，其中溫度可能扮演重要角色，因此病原菌的分布及比例在不同地區可能因此有所不同。惟本研究所得之菌株數量僅20株，且各地區的菌株取樣不平均，菌株種類是否受地理環境因子影響需進一步擴大菌株收集後，再進一步驗證。

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ABSTRACT

Wei-Lun Lin, Chong-Wei Yeh, Pu-Jui Ma, Chi-Cho Huang, Chih-Li Wang. 2023. Identification and virulence comparison of pathogens causing fruit black rot of wax apple. *J. Plant Med.* 65(2): 69-84.

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Fruit black rot of wax apple is a common disease. The initial symptom is a water soaking lesion. Later, the lesion expands over the entire fruit. As a huge amount of black pycnidia is produced on the surface of infected fruits, the fruit become black. The diseased fruit gradually lose water and eventually become mummified. This study isolated pathogens of diseased wax apples collected from Kaoshung, Chiayi, Natou and Pintung. Artificial inoculation confirmed that all isolates were pathogenic. All pathogens were morphologically identified as members of *Lasiodiplodia* and *Neofusicoccum*. Further identification was carried out by multigene sequence analysis based on sequences of internal transcribed spacer (ITS), β -tubulin (*TUB2*), translation elongation factor 1-alpha (*TEF1 α*), and RNA polymerase subunit II (*RPB2*). Three species were revealed: *L. theobromae*, *L. pseudotheobromae*, and *N. parvum*. Virulence of the representative isolates of each specie was compared at different temperatures. *L. theobromae* and *L. pseudotheobromae* colonized fruit faster than *N. parvum* at 32 - 37°C. Colonization of *N. parvum* was significantly impeded at 37°C. The three species displayed comparable virulence at 24°C, decreased colonization at 16°C, and complete loss of infection ability at 8°C. This is the first report globally that *L. pseudotheobromae* and *N. parvum* cause fruit rot of wax apple.

Keywords: *Syzygium samarangense*, phylogenetic analysis, *Lasiodiplodia theobromae*, *Lasiodiplodia pseudotheobromae*, *Neofusicoccum parvum*, temperature

