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Neofusicoccum parvum: A NOVEL PATHOGEN SPECIES CAUSING WILTED LEAF AND DIEBACK PETIOLES ON LOTUS (*Nelumbo nucifera*) IN THUA THIEN HUE, VIETNAM

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Abstract. Lotus (*Nelumbo nucifera*) is an essential species in many countries. In Vietnam, the lotus is a plant with cultural and spiritual significance, representing purity, spiritual growth, and enlightenment. However, petiole dieback and wilted dry leaves are severe diseases that weaken the host and decrease leaf photosynthesis, reducing lotus production. In this study, a new dieback and wilted leaf pathogen were identified via its morphology, phylogeny, and pathogenicity. Four representative Botryosphaeriaceae isolates from lotus fields in Hue, Phong Dien, and Phu Loc were selected for identification and pathogenicity testing. Based on morphological and phylogenetic analyses and by using the ribosomal internal transcribed spacer region (ITS) and β-tubulin (tub-2) gene sequences, we identified four isolates as *Neofusicoccum parvum*. In the pathogenicity test, typical symptoms appear on the inoculated lotus petioles, including dieback, curving, and wilted leaves. These symptoms are consistent with those observed in the field. In addition to identifying the pathogen species responsible for lotus disease, this study provides valuable insights into the taxonomy and phylogenetic relationships of new fungal pathogens that affect lotus fields. These findings can contribute to effective management strategies to control these diseases and improve lotus production. To the best of our knowledge, this is the first report of characterization and phylogenetic analysis of *N. fusicoccum* as the causal agent of wilted leaves and dieback disease in Thua Thien Hue, Vietnam.

Keywords: *Neofusicoccum parvum*, dieback disease, phylogeny, lotus, Vietnam

1 Introduction

The Botryosphaeriaceae are diverse fungi, including 24 genera of ecologically diverse host plants that are either plant pathogens, endophytes, or saprobes [1, 2]. The diseases associated with this family of fungi are often triggered by stressors, such as environmental stressors like drought, extreme temperature changes, heavy metals, nutrient deficiencies, and leaf injuries. These factors

can promote the pathogenicity of Botryosphaeriaceae species [2, 3]. However, they can also be primary pathogens, especially when non-adapted hosts are exposed to an exotic pathogen [4–7]. In recent years, there have been a growing number of reports on diseases caused by *Neofusicoccum* species in numerous countries. For example, *Neofusicoccum parvum* has been identified as a pathogen in different plants, including *Zanthoxylum bungeanum*, *Rhododendron*, *Eucalyptus* spp. in China [8] and nut rot of chestnut (*Castanea sativa*). In the United States, *N. parvum* has been identified as the cause of dieback and canker of hemp (*Cannabis sativa*) in Italy, causing brown spots on gallnuts of *Rhus potaninii* in China, black spots of *Rosa chinensis* in China [9], twig blight and branch dieback of walnut in Turkey [10], stem canker and dieback in blueberries in Chile [11], and wilting and stem rot of *Santalum album* in China [9]. It is worth noting that *N. luteum*, *N. batangarum*, *N. mangiferae*, and some other species in *Neofusicoccum* have also been reported to be related to various plant diseases. It is important to mention that in the past, several species of *Neofusicoccum* were incorrectly classified because of their morphological similarities. However, with the development of phylogenetic analysis, most new species have been discovered, and some known species have been reclassified. Therefore, the identification and management of *Neofusicoccum* species have become increasingly important in the field of plant pathology.

Lotus (*Nelumbo nucifera* Gaertn.) is a perennial aquatic crop that is grown and consumed in Asia for food, ornamental, and medicinal purposes. Currently, despite the importance attributed to the species of this family, knowledge of the taxonomy and diversity of *Neofusicoccum* pathogens associated with the lotus in Vietnam is limited. During the summer of 2022–2023, petioles blight and dieback symptoms were observed in several lotus fields in Thua Thien Hue, Central Vietnam. The present study aims to identify *Neofusicoccum* isolates collected from lotus with symptoms of dieback, blight, and wilted leaves. Species were identified based on their morphology and comparison of the DNA sequence data for ITS rDNA and β-tubulin (tub-2). The taxonomy and phylogenetic relationships of pathogen isolates were investigated, and their pathogenic abilities were determined.

2 Materials and methods

Sampling, isolation, and purification of pathogenic fungi

From 2022 to 2023, nine samples were collected from lotus fields in three regions in Thua Thien Hue, Vietnam (Hue, Phong Dien, and Phu Loc). Before being transferred to the laboratory for further testing, the samples were placed in plastic bags labelled with the date, site, and symptoms. Infected tissues were cut into small pieces (5 × 5 mm), and their surfaces were disinfected with 1% sodium hypochlorite (NaOCl) for 30 seconds and washed three times with sterile distilled water. Tissue samples were dried on sterile absorbent paper and transferred onto WA (water

agar) plates. The inoculated plates were incubated at 30 °C for seven days. To obtain pure culture, we transferred fungal hyphae to fresh potato dextrose agar (PDA) plates for further culture. Obtained isolates were preserved in 50% (v/v) glycerol at −40 °C for future use.

Table 1. List of nine isolates collected from *N. nucifera* in Thua Thien Hue province in this study

Isolate Code	Source – Host plant	Collection date	Geographical Origin
HX.DB.SH.01	*N. nucifera* cv. Cao san pink/ Petiole	June, 2022	Dong Ba, Hue City, Thua Thien Hue province 16°28'40.6"N 107°35'01.7"E
HX.DB.SH.02	*N. nucifera* cv. Cao san pink/ Petiole	July, 2022	Thuan Hoa, Hue City, Thua Thien Hue province 16°28'07.4"N 107°34'19.3"E
HX.DB.SH.03	***N. nucifera* cv. Cao san pink/ Petiole**	March, 2023	Dong Ba, Hue City, Thua Thien Hue province 16°28'40.1"N 107°35'02.4"E
HX.DB.SH.04	***N. nucifera* cv. white/ Leaf**	March, 2023	Dong Ba, Hue City, Thua Thien Hue province 16°28'38.4"N 107°35'03.3"E
HX.DB.SH.05	*N. nucifera* cv. white/ Petiole	May, 2023	Dong Ba, Hue City, Thua Thien Hue province 16°28'40.4"N 107°35'02.0"E
KN.SH.PD.1004	***N. nucifera* cv. Cao san pink/ Leaf**	April, 2022	Phong Hien, Phong Dien District, Thua Thien Hue province 16°33'26.1"N 107°27'17.6"E
KN.PH.PD.1004	*N. nucifera* cv. Cao san pink/ Leaf	May, 2023	Phong Hien, Phong Dien District, Thua Thien Hue province 16°33'21.9"N 107°27'22.8"E
TN.TL.SH.02	*N. nucifera* cv. white/leaf	April, 2023	Phu Loc District, Thua Thien Hue province 16°16'26.3"N 107°52'09.7"E
TN.TL.SH.03	***N. nucifera* cv. white/Petiole**	April, 2023	Phu Loc District, Thua Thien Hue province 16°16'21.3"N 107°52'28.5"E

Morphological analysis

The isolates were cultured on PDA to observe colony and conidia morphology. Mycelial discs of 5 × 5 mm diameters were inoculated at the centre of PDA plates and incubated in the dark at ambient temperature (30 ± 2 °C). Colony diameters were measured after seven days, and cultural features were examined and photographed. The characteristics of conidia were observed under an Olympus BX51 photographic microscope.

DNA extraction, PCR amplification

Pathogenic fungi were inoculated and grown for seven days on PDA; then, the mycelia were collected and harvested by using Whatman filter paper. Fungal DNA genomic was extracted by using the Genomic DNA Extraction Kit, following the manufacturer's instructions (ABT, Vietnam). Polymerase chain reaction (PCR) amplification of the ribosomal DNA internal transcribed spacer (ITS) region, β-tubulin (tub-2) region, was performed using the primers pairs ITS1/ITS4, and βt2a/Bt-2b [12, 13]. ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′)/ITS4 (5′-TCCTCCGCTTATTGATATGC-3′); βt2a (5′-GGTAACCAAATCGGTGCTGCTTTC-3′)/ βt2b (5′ ACCCTCAGTGTAGTGACCCTTGGC-3′). Polymerase chain reaction amplification was carried out in a SimpliAmp™ Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific Inc., USA) in a 50 μL reaction mixture containing 25 μL of Go Taq Green 2X Master Mix (Promega, USA), 5 μL (50 ng) of the DNA template, 5 μL of each primer (10 pmol), and 15 μL of ddH_2O.

The PCR conditions were as follows: initial denaturation at 95 °C for five minutes, followed by 35 cycles: 95 °C for 30 seconds, annealing at a suitable temperature for 30 seconds, and 72 °C for 60 seconds. The final extension was performed at 72 °C for seven minutes. The annealing temperature for each gene was 52 °C for ITS and 55 °C for tub-2.

Sequencing and phylogenetic analysis

Polymerase chain reaction products were sequenced at the First BASE sequencing service in Malaysia. The sequences obtained were read and compared by using The Basic Local Alignment Search Tool (BLASTn) in GenBank at National Center for Biotechnology Information NCBI ((http://www.ncbi.nlm.nih.gov, accessed on 15 July 2023) (Table 2).

The DNA sequences obtained from both strands were assembled to obtain consensus sequences for each isolate by using BioEdit v7.0.5 [14]. Multiple sequence alignment was manually performed with the closely related reference sequences available in the NCBI database by using MUSCLE in MEGA 11 software under default parameters. Aligned gene regions were adjusted manually where necessary. Concatenated alignment was performed by using MEGA 11 software [15]. To establish the identity of fungal isolates, we conducted phylogenetic analysis by

using a concatenated session formed by two loci (ITS, tub-2). The phylogenetic tree was reconstructed according to the maximum likelihood method by using IQ-TREE v. 2.1.3 [16]. The ModelFinder was used to determine the best-fit model [17]. Branch support was determined by using 10,000 ultra-bootstraps, a Bayesian posterior probabilities support, and 10,000 SH-aLRT bootstrap replicates. The resulting trees were plotted by using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree), Interactive Tree of Life (iTOL) v.6 [18], and edited by using PowerPoint (Microsoft, CA, USA) and Adobe Illustrator CC 2021 (Adobe Systems, CA, USA).

Pathogenicity tests

The pathogenicity and virulence of four representative isolates of *N. parvum* were examined via Koch's postulates. Lotus plants (*N. nucifera* cv. Cao san – high yield pink, and cv. white) at the two- to three-leaf stage were used for all inoculation tests in this study. The inoculation points of petioles were surface-disinfected with 1% sodium hypochlorite solution for 1 min, followed by 70% ethanol for 2 min with filter paper. Mycelium plugs (6 × 6 mm) of ½ PDA were cut from an actively growing colony, placed on inoculation points, and wrapped in paraffin. The plants were kept under high humidity conditions (>85% relative humidity) in a covered plastic container for 1–3 days at 28 °C. Plants were then transferred to a net house maintained at ambient temperature (30 ± 2 °C under a 12 h/12 h photoperiod). The area of the lesion developed on the inoculated petioles was measured with a grid table, converted with ImageJ v1.5.2 (National Institute of Health, USA). For each trial, we used three plant replicates (three inoculation points/plant) for fungal inoculation. In the control plants, the sterile PDA plug was used for inoculation (control). Differences in lesion area were evaluated by using the one-way method ANOVA, and means were compared with Tukey's post-hoc test ($p < 0.05$) by using IBM SPSS Statistics v.20 software. To confirm Koch's postulates, the fungal pathogens from symptomatic inoculated leaves and petioles were reisolated and reidentified by using morphological characteristics.

3 Results

Fungal isolation and morphological identification

The symptomatic lotus plants in the field exhibit different symptoms, characterized with severely infected petiole browning and leaf browning and wilting (Fig. 1b, 1c, and 1d). The tissues with symptoms were collected for fungal pathogen isolation. Nine isolates of pathogens were successfully recovered from the diseased samples collected from the lotus fields in the study location. These isolates were fast-growing on PDA and formed abundant aerial mycelia that were initially white, turning grey to dark grey over time (after 4–5 days). The reverse of the colonies

was white, which also became greyish to greenish-grey with ageing (14–15 days). These morphological characteristics are similar to those of other members of the parvum-ribis species complex. The colonies formed dark pycnidia in pine needles (Figure 2e). The conidial sizes range from 17.5–21.3 (avg. 19.8) μm × 9.7–12.2 (10.5) μm.

Fig. 1. Disease symptoms observed on *N. nucifera* from lotus fields in Dong Ba, Hue City, Thua Thien Hue

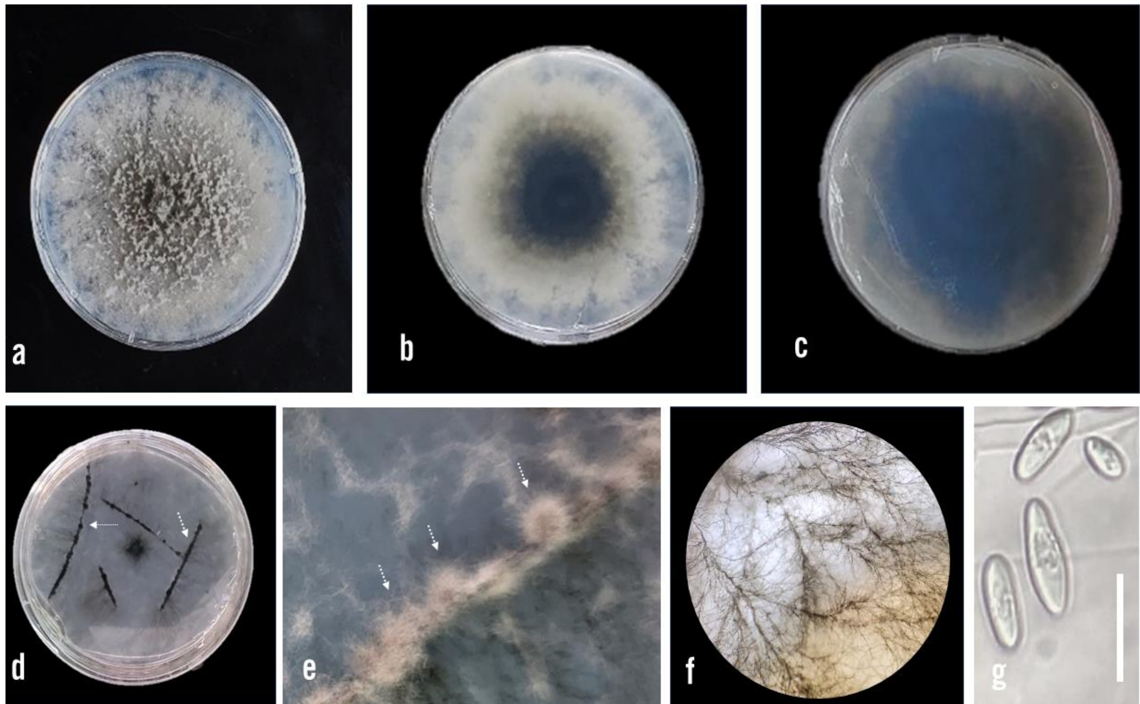

Fig. 2. *Neofusicoccum parvum* (HX.DB.SH.03). a: Upper side of colony grown on PDA (five days); b: Reverse side; c: Aged colony on PDA (14 days); d: Conidiomata on WA 2% + pine needles; e: Pycnidia and conidia induced to form after 15 days in WA 2% + pine needles; f: Hyphae form in WA 2% + pine needles; g: Conidia. Scale bar = 20 μm.

Molecular identification and phylogenetic analysis

The representative isolates: HX.DB.SH.03 (isolated from cultivar Cao san/petiole), HX.DB.SH.04 (cv. white/leaf), KN.SH.PD.1004 (cv. Cao san/leaf), and TN.TL.SH.03 (cv. white/petiole) were selected for molecular identification and phylogenetic analysis. The amplicon size of these isolates is 500–650 bp for ITS and 300–400 bp for tub-2. The BLASTn analysis homology of the sequences indicates that the sequences from the present study match well with the reliable reference sequences of *N. parvum* and show 100% sequence similarity in ITS with BRIP66334, CMW28386, 99.78% in tub-2 with L12, KARE1198.

Table 2. Taxa of isolates and their GenBank accession numbers used in the phylogenetic analysis

Species	Accession Number	ITS	TUB-2	Host	Origin	
N. arbuti	CBS116131	AY819720	KF531793	*Arbutus menziesii*	USA	**Type**
N. australe	CMW6837 = CBS139662	AY339262	AY339254	*Acacia sp.*	Australia	
N. batangarum	**CBS124924 = CMW28363**	FJ900607	FJ900634	*Terminalia catappa*	Cameroon	**Type**
N. batangarum	CBS124923 = CMW28320	FJ900608	FJ900635	*Terminalia catappa*	Cameroon	
N. brasiliense	**CMM1338**	JX513630	KC794031	*Mangifera indica*	Brazil	**Type**
N. brasiliense	CMM1285	JX513628	KC794030	*Mangifera indica*	Brazil	
N. cordaticola	**CBS123634**	EU821898	EU821838	*Syzygium cordatum*	South Africa	**Type**
N. cordaticola	CBS123635	EU821903	EU821843	*Syzygium cordatum*	South Africa	
N. cryptoaustrale	**CBS 122813 = CMW23785**	FJ752742	FJ752756	*Eucalyptus sp.*	South Africa	**Type**
N. eucalypticola	**CBS115679 = CMW6539**	AY615141	AY615125	*Eucalyptus grandis*	Australia	**Type**
N. eucalypticola	CBS115766 = CMW6217	AY615143	AY615127	*Eucalyptus rossi*	Australia	
N. eucalyptorum	**CBS115791 = CMW10125**	AF283686	AY236920	*Eucalyptus grandis*	South Africa	**Type**
N. hellenicum	**CERC1947**	KP217053	KP217069	*Pistacia vera*	Greece	**Type**
N. hellenicum	CERC1948	KP217054	KP217070	*Pistacia vera*	Greece	
N. hongkongense	CERC2973	KX278052	KX278261			
N. hongkongense	CERC2967	KX278050	KX278259			
N. kwambonambiense	CBS123641 = CMW14140	EU821919	EU821859	*Syzygium cordatum*	South Africa	
N. kwambonambiense	**CBS 123639 = CMW14023**	MH863317	EU821840	*Syzygium cordatum*	South Africa	**Type**
N. luteum	**CBS110299**	AY259091	DQ458848	*Vitis vinifera*	Portugal	**Type**
N. luteum	CBS110497	EU673311	EU673092	*Vitis vinifera*	Portugal	
N. lumnitzerae	**CMW 41469**	KP860881	KP860801	*Luminitzera racemosa*	South Africa	**Type**

Species	Accession Number	ITS	TUB-2	Host	Origin	
N. macroclavatum	**CBS118223**	DQ093196	DQ093206	*Eucalyptus globulus*	Australia	**Type**
N. mediterraneum	PD82	GU251192	GU251852	*almond*	USA	
N. mediterraneum	**CBS121718**	GU251176	GU251836	*Eucalyptus sp.*	Greece	**Type**
N. nonquaesitum	**CBS126655 = PD484**	GU251163	GU251823	*Umbellularia californica*	USA	**Type**
N. occulatum	**CBS 128008 = MUCC227**	EU301030	EU339472	*Eucalyptus grandis*	Australia	**Type**
N. occulatum	MUCC 286	EU736947	EU339474	*Eucalyptus pellita*		
N. parvum	CPC31234	MN860181	MN905746	*Persea americana*	Greece	
N. parvum	CERC 3503	KX278059	KX278268	*E. urophylla × E. grandis*	China	
N. parvum	MAN55	KY052933	KY000117			
N. parvum	MFLUCC 11-0184	JX646795	JX646843			
N. parvum	**CBS 138823 = ATCC58191 = CMW9081**	AY236943	AY236917	*Populus nigra*	New Zealand	**Type**
N. parvum	LC013	OM392021	OM453641	*Macadamia*	China	
N. pennatisporum	**MUCC510**	EF591925	EF591959	*Allocasuarin a fraseriana*	Australia	**Type**
N. ribis	**CBS 115475**	AY236935	AY236906			**Type**
N. stellenboschiana	**CBS110864 = CPC4598**	AY343407	KX465047	*Vitis vinifera*	South Africa	**Type**
N. stellenboschiana	CPC 31232	MN860179	MN905744	*Persea americana*	Greece	
N. umdonicola	**CBS 123645 = CMW14058**	EU821904	EU821844	*Syzygium cordatum*	South Africa	**Type**
N. umdonicola	CBS 123646 = CMW14060	EU821905	EU821845	*Syzygium cordatum*	South Africa	
N. vitifusiforme	**CBS110887 = STE-U 5050**	AY343382	KX465061	*Vitis vinifera*	South Africa	**Type**
Botryosphaeria dothidea	**CBS 115476**	AY236949	AY236927			**Type**

Strains bolded are ex-type or ex-epitype. ATCC: *American Type Culture Collection, Virginia, USA*; CBS: *Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands*; CMM: *Culture Collection of*

Phytopathogenic Fungi Prof. Maria Menezes, Federal Rural University of Pernambuco, Brazil; CMW: *Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa*; CPC: *Culture collection of Pedro Crous, housed at CBS*; CERC: *China Eucalypt Research Center, Chinese*; MFLUCC: *Mae Fah Luang University Culture Collection, Thailand*; ICMP: *International Collection of Micro-organisms from Plants, Landcare Research, New Zealand*.

To further identify these isolates, we performed the phylogenetic analysis with ITS and tub-2 sequence alignment comprising 45 isolates with *Boytryosphaeria dothidea* CBS115476 as the outgroup for individual loci sequenced as well as on the combined dataset (Fig. 3). The best-fit models of nucleotide substitution used in the analysis of individual genes were as follows: TNe + I + G4 (ITS) and HKY + F + G4 (tub-2). The tree topologies resulting from ML analysis of independent alignments of ITS, a portion of the tub-2 gene, and a concatenated data set containing both loci were congruent, with strongly supported species-level clades for *N. parvum*. The tree constructed with optimal log-likelihood of ML analysis is –2681.773. The HKY+F+I+G4 best-fit model was chosen according to the Bayesian information criterion (BIC). The estimated base frequencies were recorded as follows: $A = 0.209$; $C = 0.321$; $G = 0.249$; $T = 0.20$; while substitution rates were established as $AC = 1$; $AG = 8.83977$; $AT = 1$; $CG = 1$; $CT = 8.83977$; $GT = 1$; the gamma distribution shape parameter (α) is 0.611. The results show that the four isolates: HX.DB.SH.03, HX.DB.SH.04, KN.SH.PD.1004, and TN.TL.SH.03 cluster with strong statistical support in the clade contain the type strain *N. parvum* CMW9081 (SH-aLRT/PP/MLBS: 97.4/1/98-ITS, 92.1/1/94-tub2, 96.7/1/99-concatenated ITS+tub), where *N. batagarum*, *N. umdonicola*, and *N. ribis* are associated (PP/MLBS = 1/93) as the closest sister species of these pathogenic fungi.

The phylogenetic relationships of the *Neofusicoccum* species fully match the topology of the phylogeny tree reported by Bezerra et al., Hilário et al., and Lopes et al. [19–21]. The combined sequence data of these isolates group with the high bootstrap value of the reference *N. parvum* CMW9081 (Fig. 3). Therefore, these isolates were confirmed as *N. parvum* based on molecular identification.

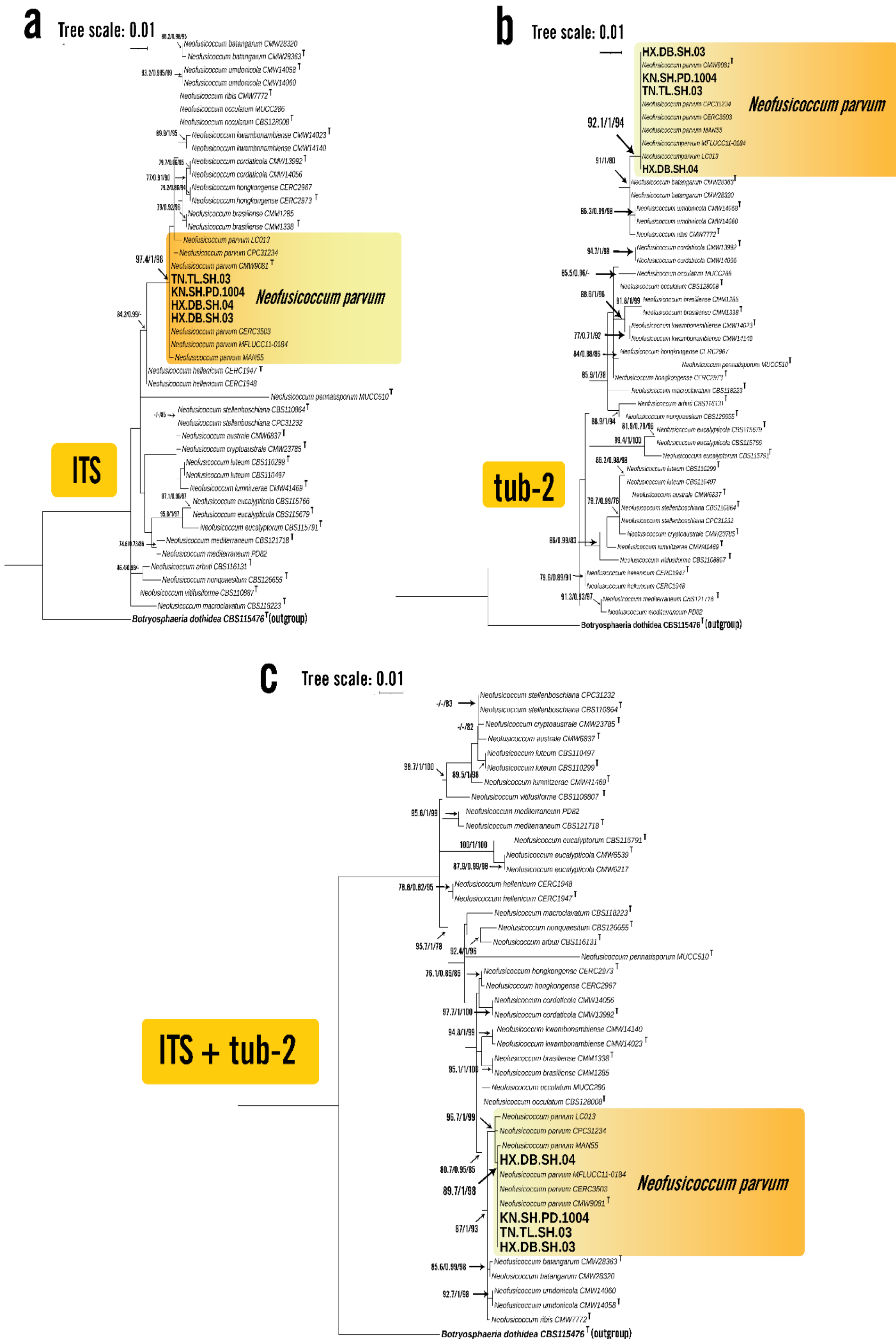
a
Tree scale: 0.01
ITS
TN.TL.SH.03
KN.SH.PD.1004
HX.DB.SH.04
HX.DB.SH.03
Neofusicoccum parvum
Botryosphaeria dothidea CBS115476T (outgroup)
b
Tree scale: 0.01
tub-2
HX.DB.SH.03
KN.SH.PD.1004
TN.TL.SH.03
HX.DB.SH.04
Neofusicoccum parvum
92.1/1/94
Botryosphaeria dothidea CBS115476T (outgroup)
c
Tree scale: 0.01
ITS + tub-2
HX.DB.SH.04
KN.SH.PD.1004
TN.TL.SH.03
HX.DB.SH.03
Neofusicoccum parvum
89.7/1/98
Botryosphaeria dothidea CBS115476T (outgroup)

Fig. 3. Phylogenetic trees based on maximum likelihood (ML) analysis for species in *Neofusicoccum* sp. a. ITS region; b. tub-2 gene region; c. combination of ITS + tub regions. Isolates sequenced in this study are in bold. Bootstrap support values are shown at each node (SH-aLRT/BPP/MLBS). Isolates representing type sequences are marked with 'T'. *Botryosphaeria dothidea* CBS 115476 was used as the outgroup taxon. The bar indicates the substitutions number per position.

N. parvum has previously been reported to cause canker and dieback symptoms in blueberries in Chile, avocado orchards and *Eucalyptus* in Spain [22], walnut in Turkey [23], grapevine trunk in Algeria [24], *Cannabis sativa* in Italy, and Canada [25, 26], *Olea europaea* in Italy [27], sequoias (*Sequoiadendron giganteum*) in Switzerland [4], and *Osmanthus fragans* in China [28]. It was reported causing stem end rot in mango, leaf spot disease of *Machilus thunbergii* – Japan, *Geodorum eulophioides*, *Cinnamomum cassia* in China [29, 30], and leaf blight of Indian hawthorn (*Rhaphiolepsis indica*) in Italy. To our best knowledge, this is the first report of *N. parvum* as a new agent associated with leaf spot and petioles dieback in *Nelumbo nucifera* in Vietnam. Furthermore, the lotus plants facing environmental stress or mechanical injuries due to natural disasters, such as strong winds, floods, and droughts, create favourable conditions, highly vulnerable to this pathogen. In addition, several studies have shown that fungi belonging to the Botryosphaeriaceae family can infiltrate plants via endophytic colonization, wounds, contaminated soil, and insect attacks [30, 31].

Pathogenicity tests

Morphological observations on microscopes show that all pathogen isolates are identical, and four isolates were selected for the pathogenicity tests. Fulfilling Koch's postulates shows that *Neofusicoccum* spp. are pathogens causing wilted leaves and dieback petioles on the lotus, the symptoms similar to those observed in the field.

The petioles inoculated with these isolates were inspected for the appearance of canker symptoms at the early stage (7–10 days post-inoculation (dpi)). All isolates cause lesions on the petioles, with larger lesion sizes observed with the wounded method. When the petioles were inoculated with the unwounded method, the disease area was smaller. As the disease progresses, the lesions expand longitudinally from the inoculation sites and spread to leaves, causing the leaves to turn brown, gradually curling and wilting. After 35–40 days, the plant-inoculated pathogen isolates completely dried and died. A cross-section of the fungal inoculated plant shows the rooting area of the internal xylem of petioles, and the diseased area has a dark brown colour. In contrast, the plants treated with sterile PDA (control) do not exhibit any signs of dieback (Fig. 4g).

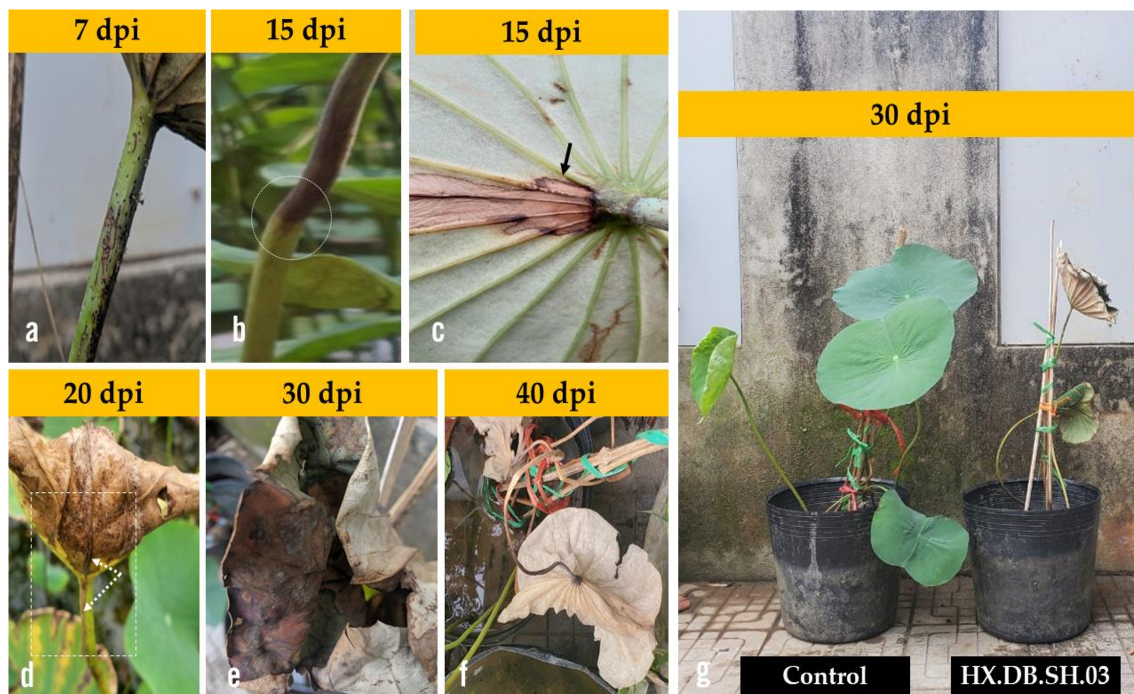

Fig. 4. Pathogenicity test results. Disease symptoms in different parts of lotus plant induced by *N. parvum* – isolate HX.DB.SH.03. a. Early stage (7dpi) with browning of necrotic areas; b, c. Lesion spread on petioles and leaves; d: e, f: leaves gradually curving, dry, and dead; g: The lotus plant-inoculated isolate HX.DB.SH.03 exhibited disease, while the control remained symptomless.

Since *Neofusicoccum* is known to have a wide host range, we investigated whether the isolates in this study could cross-infect a range of lotus cultivars (cv. white and cv. Cao san). Although all isolates cause dieback in both lotus cultivars, the area and speed of dieback development differ significantly, suggesting that virulence varies among isolates and inoculation methods. In the wounded method, there are significant differences in lesion areas produced on the *N. nucifera* inoculated petioles that range from 67.38 to 82.6 mm^2 for cv. Cao san lotus and 26.86–46.60 mm^2 for cv. white lotus. These findings suggest that *N. nucifera* cv. cao san is more susceptible to *N. parvum* infection than *N. nucifera* cv. white, with greater disease severity ($p <$ 0.05).

Table 3. Area (mm^2) of lesion caused by four isolates *N. parvum* on petioles of various cultivar of lotus at 15 dpi

Inoculation method	Cultivar	HX.DB.SH.03	HX.DB.SH.04	KN.SH.PD.1004	TN.TL.SH.03
Agar plugs on wounded	cv. Cao san lotus	82.60 ± 5.32^a	80.94 ± 5.11^a	67.38 ± 2.31^b	72.36 ± 4.54^b
	cv.white lotus	45.06 ± 3.56^a	46.60 ± 1.21^a	26.86 ± 2.48^b	30.18 ± 1.91^b
	Control	0	0	0	0
Agar plugs on unwounded	cv. Cao san lotus	36.74 ± 1.06^a	35.76 ± 2.25^a	30.38 ± 1.99^b	31.94 ± 2.24^b
	cv.white lotus	25.92 ± 2.04^a	26.74 ± 1.98^a	20.66 ± 2.83^b	21.02 ± 2.00^b
	Control	0	0	0	0

Note: Average lesion area (mm^2) ± SD (standard deviation). The letters indicate that the treatments are significantly different ($p < 0.05$, Turkey HSD test). Data were recorded at 15 days post-inoculation (dpi).

4 Conclusion

In this study, we first reported *Neofusicoccum parvum* as a causal pathogen of petioles dieback, leaf blight, and curving lotus leaves in Thua Thien Hue, Vietnam. The pathogen was identified by using morphological features, multigene DNA sequencing, phylogenetic analysis, and pathogenicity testing. This information is useful in establishing quarantine measures and developing targeted disease management strategies.

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