Leaf spot of *Hosta ventricosa* caused by *Fusarium oxysporum* in China

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Leaf spot of *Hosta ventricosa* is a new disease in China. This disease seriously affects the ornamental value and greening function of *H. ventricosa*. Identification of the causal agent can prevent and control leaf spot in *H. ventricosa* and promote the healthy development of the *H. ventricosa* industry. Known incidents of leaf spot of *H. ventricosa* occurred in three places, and samples were collected. After the fungus were isolated, its pathogenicity was tested according to Koch's postulates. Isolates ZE-1b and ZE-2b were identified as *Fusarium oxysporum* based on morphological features and multigene phylogenetic analyses of calmodulin (CMDA), RNA polymerase II subunit A(RPB1), RNA polymerase II second largest subunit (RPB2) and translation elongation factor 1-alpha (TEF1). These results provide a theoretical basis for the control of this disease of *H. ventricosa*.

[1]Leaf spot of *Hosta ventricosa* caused by *Fusarium oxysporum* in China

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Abstract

Leaf spot of *Hosta ventricosa* is a new disease in China. This disease seriously affects the ornamental value and greening function of *H. ventricosa*. Identification of the causal agent can prevent and control leaf spot in *H. ventricosa* and promote the healthy development of the *H. ventricosa* industry. Known incidents of leaf spot of *H. ventricosa* occurred in three places, and samples were collected. After the fungi were isolated, their pathogenicity was tested according to Koch's postulates. Isolates ZE-1b and ZE-2b were identified as *Fusarium oxysporum* based on morphological features and multi gene phylogenetic analyses of calmodulin (CMDA), RNA polymerase II subunit A(RPB1), RNA polymerase II second largest subunit (RPB2) and translation elongation factor 1-alpha (TEF1). These results provide a theoretical basis for the control of this disease of *H. ventricosa*.

Introduction

H. ventricosa, is a perennial herbaceous plant of the *Hosta* genus in Liliaceae. It originated in China, South Korea and Japan (*Yu et al.2015*) and is widely distributed in China, including

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21 Jiangsu, Anhui, Hebei and other places (*Liu et al., 2008*). In addition to its bright leaves and

22 graceful habit, this species has strong adaptability to the environment and is suitable for planting

23 under trees, in the shade of buildings or other exposed shaded places. It is an excellent ground

24 cover with ornamental value and greening functions (*Zhao, Chen & Lv, 2009*). In addition, the

25 whole plant, flowers, leaves or roots can be used as a traditional Chinese medicinal material with

26 the ability to dissipate blood stasis and relieve pain (*Zeng, Zhao & Li, 2020*).

27 However, *H. ventricosa* is impacted by several major pathogens, such as *Sclerotium rolfsii Sacc*.

28 This disease manifests because the *H. ventricosa* leaves are especially thick, and during the rainy

29 season, the *H. ventricos*a rhizome is in contact with water for a prolonged time (*Zhao, Chen &*

30 *Lv, 2009*).Thus, the epidermis of the affected area becomes brown and necrotic, and finally a

31 white mycelial layer is formed, which leads to cortex decay (*Li, Yang & Zhu, 2013*). In addition,

32 excessive humidity and poor drainage in the rainy season can also favor diseases caused by

33 *Colletotrichum*, which mainly damages the leaves, petioles and pedicels of *H. ventricosa*. The

34 plants present round or nearly round discolloid spots that are gray or grayish brown (*Zhao, Chen*

35 *& Lv, 2009*). Leaf spot of *H. ventricosa* caused by *F. oxysporum* is a very serious fungal disease

36 (*Fisher et al., 2012*). *F. oxysporum* is one of the top ten most important plant pathogenic fungi in

37 the world, with high virulence and a wide distribution area. The pathogen can cause plant drying

38 and wilting. *F. oxysporum* can be a saprophytic or parasitic fungus. It is widely found in nature,

39 animals and plants, and has been isolated in cold Arctic areas and arid deserts. This strain can

40 cause wilt and decay of roots, stems, leaves, flowers and fruits in over one hundred plant hosts

41 (*Maryani et al., 2019*).

42 **Materials and methods**

43 **Experimental materials**

44 Leaves of infected *H. ventricosa* were collected in Nanjing from 2020 to 2021. Materials used in

45 this study included PDA plates, tissue separation tools, 2% CTAB, and chloroform.

46 **Sampling and isolation**

47 To isolate the fungus, *H. ventricose* leaves showing leaf spots were collected from three places in

48 Nanjing, China (32°4′47″N, 118°48′31″E; 32°4′45″N, 118°48′31″E; 32°4′44″N, 118°48′31″E.),

49 in September 2020. The collected leaves were rinsed under tap water for 15-30 min. After the

50 leaves were dried, both healthy and affected tissues were cut into small pieces, each of which

51 was 2 × 2 mm in size. The pieces were disinfected in 75% ethanol for 30 s and in 2 % NaClO for

52 90 s, then rinsed with sterile water 3 times for 20 s each time (*Si et al., 2021*), dried on sterile

53 filter paper and inoculated onto PDA. After the appearance of fungal colonies, blocks of tissue

54 were removed from the edges of the colonies for purification. The morphological characteristics,

55 color, size and shape of the purified colonies were observed and described (*Chang et al., 2020*).

56 Two single-spore cultures were used for further study and were also deposited in the China

57 Forestry Culture Collection Center (CFCC).

58 **Pathogenicity test**

59 The experiments were replicated three times, and a total of 30 seedings were used. Healthy *H.*

60 *ventricosa* leaves were collected and rinsed with clean water. The leaves surface were disinfected

61 and dried on an aseptic bench. Pathogenic isolates were inoculated on PDA plates and cultured in

62 an incubator at 25°C for 5 days. To test the pathogenicity of the isolates, *H. ventricosa* leaves

63 were wounded with a sterile needle and then inoculated with 5 mm plugs cut out from the

64 growing edges of 5-day-old cultures (*Feng et al., 2019*). Three replicates were used (*Yang et al.,*

65 *2021*). At the same time, isolates were inoculated onto plants in the natural environment in the

66 wild. Leaves mock inoculated without isolates were used as controls, and the incidence of leaf

67 spot was observed after three days (*Yang et al., 2021*).

68 **Morphological analysis**

69 Pathogenic isolates were inoculated on PDA plates and incubated in an incubator at 25°C for one

70 week to observe and record the morphology, color, surface characteristics and growth status at

71 the edges of the colonies (*Zhang, 2014*). The morphology, size and presence of spore septations

72 were recorded under a microscope (*Murugan et al., 2020*).

73 **DNA extraction, amplification, sequencing and phylogenetic analyses**

74 Before DNA extraction, a small portion of mycelia taken from a 7-day-old culture of the

75 pathogen grown on PDA plates at 25°C was collected and transferred to 2-ml Eppendorf tubes.

76 Genomic DNA was extracted by the CTAB method (*Freeman et al., 1996*). After passing the test,

77 the mycelia were stored at -18°C (*Guo, Hyde & Liew, 2000*; *Sahai-Maroof et al., 1984*).

78 The extracted DNA was subjected to polymerase chain reaction (PCR) amplification of partial

79 regions of five genes/regions, namely, calmodulin (CMDA), RNA polymerase II subunit A

80 (RPB1), RNA polymerase II second largest subunit (RPB2) and translation elongation factor 1-

81 alpha (TEF1), which were amplified with primers CL1/CL2A, FA/G2R, 5F2/7CR, and EF1/EF2,

82 respectively (Table 1).

83 The total volume of the PCR mixture was 50μL (*Lombard, Van & Crous, 2019*), containing 19

84 μL double-distilled water, 2 μL genomic DNA, 2 μL of each primer, and 25 μL Taq DNA

85 polymerase mix. After PCR, the products were sent to Shanghai Jieli Biotechnology Co., Ltd. for

86 DNA sequencing. All sequences with primers CL1/CL2A, FA/G2R, 5F2/7CR, and EF1/EF2 of

87 ZE-1b was deposited in GenBank under accession numbers MW890756, MZ146450,

88 MW890757, MZ088053, and ZE-2b was deposited in GenBank under accession numbers

89 MW885175, MZ127817, MZ126726 and MW885176, respectively.

90 **The CAMD, RPB1, RPB2, and TEF sequences were compared to sequences in GenBank**

91 **using BLAST. The sequences were obtained from GenBank for phylogenetic analyses**

92 **(Table 2).** We downloaded sequences for which the comparison results showed higher than 99%

93 similarity. Using *Fusarium aywerte* as the outgroup. The arrangement of each gene/region was

94 compared with MAFFTver.7.313 (*Katoh & Standley, 2013*) and manually adjusted with

95 BioEditver.7.0 (*Hall, 1999*). It was a combination of these five genes/regions. The ModelFinder

96 was used to select the best-fit model (*Kalyaanamoorthy et al., 2017*). In IQTree ver.1.6.8, the

97 alternative model of GTR + F + I + G4 was adopted, 1000 iteration guidance methods were used,

98   and the maximum - likelihood ground method (ML) analysis was used to estimate the system

99   relationship (*Nguyen et al., 2015*). In the GTR + I + G + F model (2 parallel runs, 2 million

100   generations), MRBayesver.3.2.6 was used for Bayesian analysis. Using burn-in, 25% of sampled

101   data were discard (*Ronquist et al., 2012*). The phylogenetic trees were drawn with FigTree ver.

102   1.4.4 (http://tree.bio.ed.ac.uk/soft- ware/figtree/)

103   **Results**

104   **Incidence of disease and symptoms**

105   The incidence of leaf spot of *H. ventricosa* in three areas of Nanjing City was investigated, and

106   the results showed that the incidence of leaf spot of *H. ventricosa* in the field was 40%. When the

107   *H. ventricosa* leaves were infected, the edge of leaves will turn green and yellow, and be dull.

108   With the development of disease, the leaf spots extended and gradually turned yellowish brown.

109   **Pathogenicity of fungal isolates**

110   Base on the colony morphology, fifty fungal samples were divided into seven types. More than

111   50% are classified as ZE-1b/ZE-2b types. According to the colony morphology, fungi were

112   divided into 7 kinds namely ZE-a - ZE-g. According to the ITS sequence ZE-a - ZE-g were

113   identified as *Fusarium oxysporum* (50%), *Fusarium ipomoeae* (20%), *Fusarium equsiti* (10%),

114   *Colletotrichum spaethianum* (9%), *Nigrospora spherica* (5%), *Colletotrichum gloeosporioide*

115   (4%), *Colletotrichum siamense* (2%). All of the seven kinds of isolates were inoculated seedings,

116   replicated three times.

117   Inoculated *H. ventricosa* showed leaf spot disease consistent with that observed previously. Two

118   isolates (ZE-1b and ZE-2b) were proven pathogenic to H. ventricosa leaves. Lesions appeared on

119   detached leaves 3 days after inoculation using mycelial plugs (Fig. 1G-H). In live plants, one

120   week after inoculation, the leaves began to show obvious symptoms of infection, turning yellow

121   and withering (Fig. 1D-E). In addition, no lesions were observed on leaves   from the control

122   plants (*Cong et al., 2017*) (Fig. 1C, F). The symptoms on detached leaves and live plants after

123   inoculation were the same as those in the field (Fig. 1A-B). The reisolated pathogens from

124   inoculated diseased leaves were consistent with those obtained in the first isolation. Therefore, it

125   was determined that ZE-1b and ZE-2b were the main pathogens causing *H. ventricosa* leaf spot.

126   **Morphological characteristics of fungal isolates**

127   Morphological observations of the pathogenic fungi were carried out. Colonies were inoculated

128   on PDA plates and cultured at 25°C for 4 days, and the colony diameter was 7 cm (Fig. 2I, N).

129   The hyphae grew radially, luxuriously and densely, and the aerial hyphae were velvety, white or

130   pink-white (*Liu et al., 2020*).

131   *Fusarium* has three types of conidia for reproduction and survival under adverse environments:

132   microconidia, macroconidia, and chlamydospores. Microconidia were numerous, oval or kidney-

133   shaped, and scattered, with the size of 4.7 - 8.6 μm × 2.5 - 4.7μm (Fig. 2J, O). Macroconidia

134   were sickle-shaped, generally symmetrical, slightly curved, and tapering toward the ends, with

135   the size of 23 - 50.6 μm× 3 - 5 μm (Fig. 2K, P). Chlamydospores were readily produced, with

136   smooth and spherical surfaces (Fig. 2L, Q). They were solitary, paired or clustered between

137   hyphae (*Du, 2017*).

138   **Phylogenetic analyses**

139   Sequences of the genes/regions CAMD, RPB1, RPB2, and TEF1 from the two isolates (ZE-1b

140   and ZE-2b) were deposited in GenBank, and the accession numbers are shown in Table 2. The

141   sequences from ZE-1b and ZE-2b showed 100% similarity with *F. oxysporum*. These results

142   further indicate that isolation, purification, morphological identification and molecular biology

143   can be used in combination for accurate and reliable results (*Cong et al., 2017*).

144   In the ML phylogenetic tree, two isolates (ZE-1b and ZE-2b) were in the same cluster as *F.*

145   *oxysporum* with 100% RAxML bootstrap support values (Fig. 3). The phylogenetic tree obtained

146   by Bayesian analysis was consistent with the ML tree. Bayesian analyses showed that the

147   isolates clustered with *F. oxysporum* with a high Bayesian posterior probability. Two isolates

148 (ZE-1b and ZE-2b) were identified as *F. oxysporum* based on multigene phylogeny and
149 morphology.

Discussion

151 In this study, a novel leaf spot disease was studied through pathogenicity determination,
152 morphological identification, and molecular biological identification, and the results showed that
153 the pathogen was *F. oxysporum*. Herein, wilt of *H. ventricosa* leaves caused by *F. oxysporum*
154 was reported for the first time in China.

155 *F. oxysporum* is a facultative parasitic fungus that can both infect plants and live in soil (*Yang et*
156 *al., 2021*; *Cong et al., 2017*). The transmission of the isolate is either vertical transmission
157 through the mother line to the next generation of seeds or horizontal transmission when the fungi
158 in soil or crops infects the host through wounds. The main means of horizontal transmission are
159 as follows: fungal isolates infect and destroy the vascular bundle from the roots (*Foley, 1962*)
160 and stems of the plant and spread to various parts of the plant. Due to the exposure of stomata
161 and other external tissues of crops as well as plant wounds, spore and mycelial infection via the
162 air can also occur (*Headrick, 1991*). Most *Fusarium* enter through natural openings in plants or
163 seeds, such as stomata *(Lin et al., 2014)*.

164 *F. oxysporum* is highly destructive and can destroy many plant organs and cause very severe
165 diseases, such as leaf spot, root rot, stem rot, flower rot and grain wilt (*Liu et al., 2020*). Globally,
166 *F. oxysporum* has been identified as a wilt pathogen in many host plants, such as bananas
167 (*Maryani et al., 2019*; *Forsyth, Smith & Aitken, 2006*), cotton (*Xie et al., 2020*; *Davis et al.,*
168 *2006*; *Zhu et al., 2020*), cucumbers (*Jaber et al., 2020*), sesame (*Khalifa, 1997*), grapes (*El-*
169 *Sayed et al., 2011*), basil (*Chen, Lin & Chung, 2017*; *Mamta et al., 2013*; *Salim, Salman & Jasim,*
170 *2017*; *Basco et al., 2017*), lettuce (*Guerrero et al., 2020*) and pecan (*Rolom et al., 2020*). Leaves
171 wilt and eventually drop to the ground, leading to a large area of growth decline; at worst, the
172 whole plant winters and dies, which eventually leads to reductions in yield and quality, causing
173 huge economic losses (*Yang et al., 2021*; *Li et al., 2020*; *Cong et al., 2017*).

174   Originally by scientists abroad, *Fusarium* was considered a crescent-shaped fungus born on the

175   seed coat. Because many other fungi also produce such sickle-like spores and fungal culture

176   techniques have limitations, the classification of *Fusarium* has long been in a state of confusion.

177   Later, German scientists introduced the first systematic classification of *Fusarium* and proposed

178   a relatively complete classification system based on the biological characteristics of these fungi,

179   combined with their morphological structures, which laid the foundation for classification

180   research on *Fusarium* (*Du, 2017*). *Fusarium* was initially divided into 44 strains, with 35 strains

181   in China, laying a foundation for the study of *Fusarium* here (*Yu, 1977*). Currently, more than

182   3000 strains of *Fusarium* have been studied, 40 physiological strains have been identified and

183   collected, and 1 new strain was found. Twenty-eight strains of *Fusarium* zhejiangensis were

184   identified in Zhejiang, and *Fusarium* zhejiangensis was first recorded in the literature. "*Fusarium*

185   disease in Taiwan" was published in Plant Pathology, Chung Hsing University, Taiwan (*Du,*

186   *2017*).

187   In recent years, research on *Fusarium* taxonomy in China has developed rapidly based on both

188   morphology and molecular biology. This experiment provides a basis for field prevention and

189   treatment of *H. ventricosa* leaf spot caused by *Fusarium* and provides a reference for further

190   genetic analysis and cultivation of disease resistant varieties of *H. ventricosa* (*Zhi, 2020*).

191   ## Conclusion

192   This is the first report of *H. ventricosa* leaf spot in China and Chinese *H. ventricosa* is a new host

193   of *F. oxysporum*. We should take reasonable preventive measures against diseases. This study

194   provided theoretical guidance for the control of Chinese *H. ventricosa* leaf spot.

195   ## Additional Information and Declarations

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199 **Reference**

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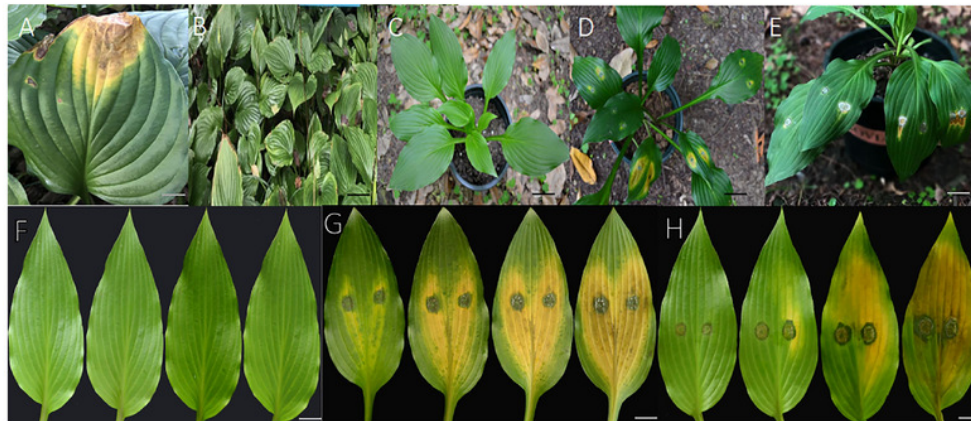
Figure 1

Pathogenicity in detached leaves and in live plants.

3   (A and B) Diseased leaves naturally infected. (C) No symptoms were observed on

4   leaves from control plants 7 days after inoculation with sterile water; (D) Symptoms on

5   live leaves 7 days after

6   inoculation with mycelial plugs of ZE-1b; (E) Symptoms on live leaves 7 days after

7   inoculation with mycelial plugs of ZE-2b; (F) No symptoms were observed on detached

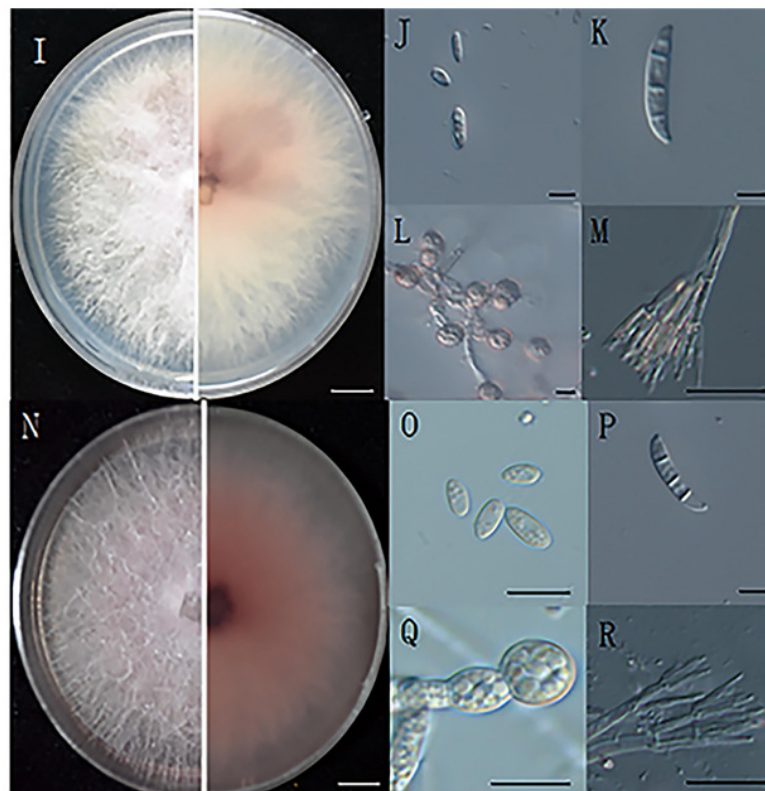
8   leaves from control plants 3, 5, 7 or 10 days after inoculation with sterile water; (G)

9   Symptoms on detached leaves 3, 5, 7 and 10 days after inoculation with mycelial plugs

10 of ZE-1b; (H) Symptoms on detached leaves 3, 5, 7 and 10 days after inoculation with

11 mycelial plugs of ZE-2b; Bars A=2 cm; B=5 cm; C-E=3 cm; F-H=1 cm.

Figure 2

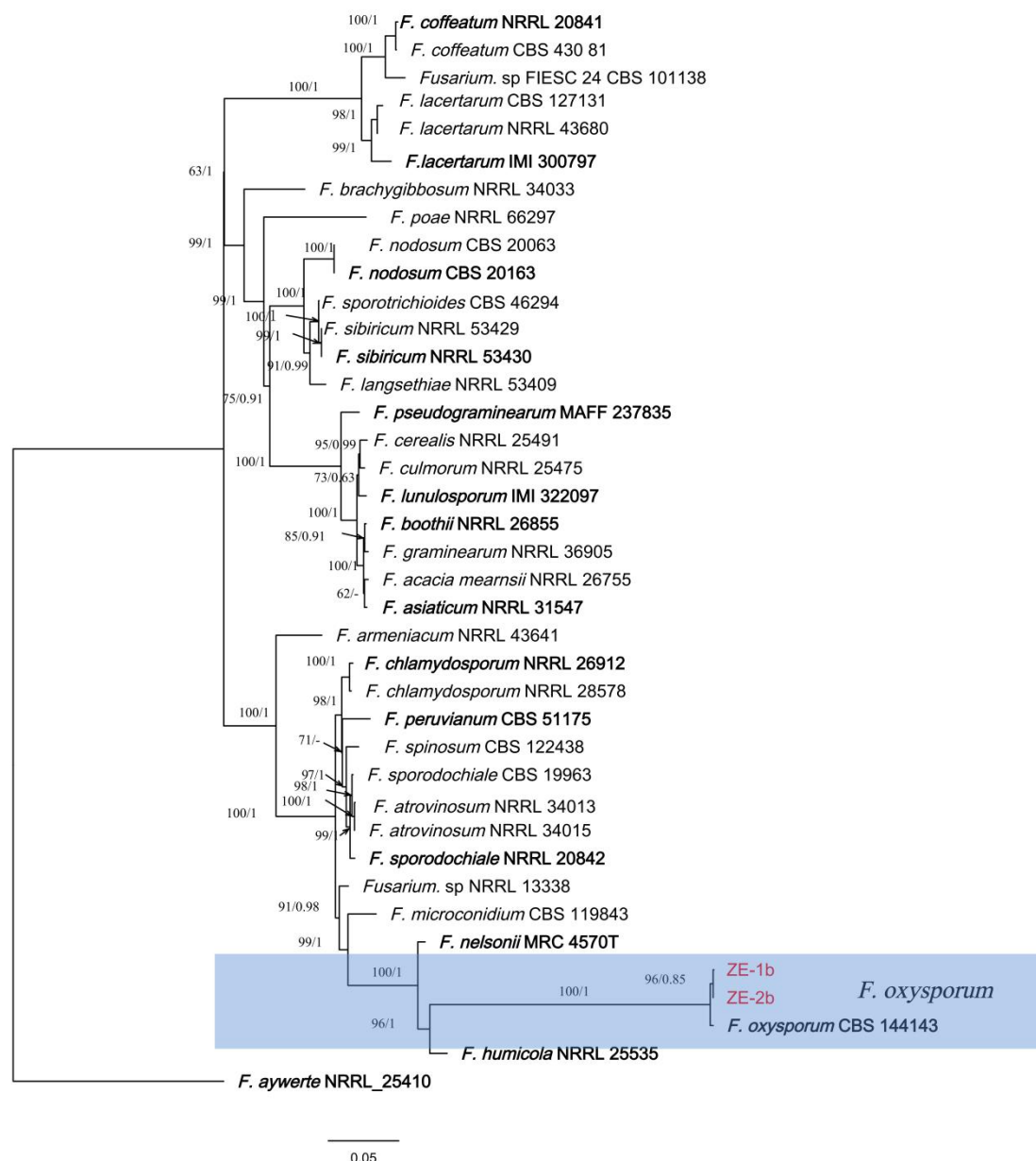
The morphology of hyphae and conidia.

3 (I) The front and reverse colony morphology of ZE-1b; (J) microconidia of ZE-1b; (K)

4 macroconidia of ZE-1b; (L) chlamydospore formation of ZE-1b; (M) conidiophores of ZE-1b; (N)

5 front and reverse colony morphology of ZE-2b; (O) microconidia of ZE-2b; (P) macroconidia of

6 ZE-2b; (Q) chlamydospore formation of ZE-2b; (R) conidiophores of ZE-2b; Bars I, N=1 cm; J-M,

7 O-R=10μm.

A maximum parsimony phylogeny for *Fusarium oxysporum*.

Phylogenetic relationship of ZE-1b and ZE-2b with related taxa derived from maxmum-likelihood (ML) analysis using combined CAMD, RPB1, RPB2, and TEF sequence alignment of *Fusarium* spp., With *Fusarium aywerte* (NRRL 25410) as the outgroup. RAxML bootstrap support values (ML ≥ 50) and Bayesian posterior probability (PP ≥ 0.80) are shown at the nodes (ML/PP). Ex-type strains are marked in bold. Isolates from *H. ventricosa* marked in red.

Primers for PCR and DNA sequencing.

1 **Table 1 Primers for PCR and DNA sequencing.**

Locus	Primer Designation	Sequence (5'- 3') *	PCR amplification procedures	Reference
TEF1	EF1	ATGGGTAAGGARGACAAGAC	94 ° C to 90 s; Cycles of 94 °C 45 s, 55 °C 45 s, 72 °C 1 min; 72 ° C for 10 min; Soak 10 ° C	O 'donnell et al., 1998
	EF2	GGARGTACCAGTSATCATG		O 'donnell et al., 1998
CAMD	CL1	GARTWCAAGGAGGCCTTCTC	94 ° C to 90 s; Cycles of 94 °C 45 s, 55 °C 45 s, 72 °C 1 min; 72 ° C for 10 min; Soak 10 ° C	Lombard et al., 2019
	CL2A	TTTTTGCATCATGAGTTGGAC		Lombard et al., 2019
RPB1	Fa	CAYAARGARTCYATGATGGGWC	94 ° C to 90 s; Cycles of 94 °C 45 s, 58 °C 45 s, 72 °C 2 min; Cycles of 94 °C 45 s, 57 °C 45 s, 72 °C 2 min; Cycles of 94 °C 45s, 56 °C 45s, 72 °C 2 min; 72 ° C for 10 min; Soak 10 ° C	O'donnell et al., 2010
	G2R	GTCATYTGDGTDGCDGGYTCDCC		O 'donnell et al., 2010
RPB2	5F2	GGGGWGAYCAGAAGAAGGC	94 ° C to 90 s; Cycles of 94 °C 45 s, 58 °C 45 s, 72 °C 2 min; Cycles of 94 °C 45 s, 57 °C 45 s, 72 °C 2 min; Cycles of 94 °C 45 s, 56 °C 45 s, 72 °C 2 min; 72 ° C for 10 min; Soak 10 ° C	O 'donnell et al., 2010
	7CR	CCCATRGCTTGYTTRCCCAT		O 'donnell et al., 2010

2   * R = A or G; S = C or G; W = A or T; Y = C or T.

Isolates and sequences used in this study.

1 **Table 2 Isolates and sequences used in this study.**

| | | | | GenBank accession | | | |
Species	Culture accession	Host/substrate	Origin	CAMD	RPB1	RPB2	TEF1
F. acacia-mearnsii	NRRL 26755 = CBS 110255 = MRC 5122	*Acacia mearnsii*	South Africa	-	KM361640	KM361658	AF212449
F. armeniacum	NRRL 43641	*Horse eye*	USA	GQ505398	HM347192	GQ505494	GQ505430
F. asiaticum	**NRRL 13818 = CBS110257 = FRC R-5469 = MRC 1963 = NRRL 31547[T]**	**Hordeum vulgare**	**Japan**	**-**	**JX171459**	**JX171573**	**AF212451**
F. atrovinosum	NRRL 34013	*Human toenail*	USA	GQ505378	-	GQ505472	GQ505408
	NRRL 34015	*Human eye*	USA	GQ505380	-	GQ505474	GQ505410
F. aywerte	**NRRL 25410[T]**	**Soil**	**Australia**	**KU171417**	**JX171513**	**JX171626**	**KU171717**
F. boothii	**NRRL 26916 = ATCC 24373 = CBS 316.73 = NRRL 26855[T]**	**Zea mays**	**South Africa**	**-**	**KM361641**	**KM361659**	**AF212444**
F. brachygibbosum	NRRL 34033	*Human foot*	USA	GQ505388	HM347172	GQ505482	GQ505418
F. cerealis	NRRL 25491 = CBS 589.93	*Iris hollandica*	Netherlands	-	MG282371	MG282400	AF212465
F. chlamydosporum	**CBS 145.25 =NRRL 26912[NT]**	**Musa sapientum**	**Honduras**	**MN120695**	**MN120715**	**MN120735**	**MN120754**
	CBS 615.87 = NRRL 28578	*Colocasia esculenta*	Cuba	GQ505375	JX171526	GQ505469	GQ505405
F. coffeatum	**CBS 635.76 = BBA 62053 = NRRL 20841[T]**	**Cynodon lemfuensis**	**South Africa**	**MN120696**	**MN120717**	**MN120736**	**MN120755**
	CBS 430.81 = NRRL 28577	*Grave stone*	Romania	MN120697	-	MN120737	MN120756
F. culmorum	NRRL 25475 = CBS 417.86 = FRC R-8504 = IMI 309344	*Hordeum vulgare*	Denmark	-	JX171515	JX171628	AF212463

GenBank accession

Species	Culture accession	Host/substrate	Origin	CAMD	RPB1	RPB2	TEF1
F. graminearum	NRRL 36905	*Triticum aestivum*	USA	-	KM361646	KM361664	DQ459742
F. humicola	**CBS 124.73= NRRL 25535ᵀ**	**Soil**	**Pakistan**	**MN120698**	**MN120718**	**MN120738**	**MN120757**
F. lacertarum	**NRRL 20423 = ATCC 42771 = CBS 130185 = IMI 300797ᵀ**	**Lizard skin**	**India**	**GQ505505**	**JX171467**	**JX171581**	**GQ505593**
	CBS 127131	*Soil*	USA	MN120699	MN120720	MN120739	MN120758
	NRRL 43680	*Contact lens fluid*	USA	-	-	EF470046	EF453007
F. langsethiae	NRRL 53409	*Hordeum vulgare*	Finland	-	-	HQ154455	HM744667
F. lunulosporum	**NRRL 13393 = BBA 62459 = CBS 636.76 = FRC R-5822 = IMI 322097ᵀ**	**Citrus paradisi**	**South Africa**	**-**	**KM361637**	**KM361655**	**AF212467**
F. microconidium	CBS 119843 = MRC 839	*Unknown*	Unknown	MN120700	MN120721	-	MN120759
F. nelsonii	**CBS 119876 = FRC R 8670 = MRC 4570ᵀ**	**Plant debris**	**South Africa**	**MN120701**	**MN120722**	**MN120740**	**MN120760**
F. nodosum	CBS 200.63	*Arachis hypogaea*	Portugal	MN120703	MN120724	MN120742	MN120762
	CBS 201.63 ᵀ	**Arachis hypogaea**	**Portugal**	**MN120704**	**MN120725**	**MN120743**	**MN120763**
F. oxysporum	**CBS 144143ᵀ**	**Solanum tuberosum**	**Germany**	**MH484771**	**-**	**MH484953**	**MH485044**
	CFCC 55679 = ZE-1b	**Hosta ventricosa**	**China**	**MW890756**	**MZ146450**	**MW890757**	**MZ088053**
	CFCC 55680= ZE-2b	**Hosta ventricosa**	**China**	**MW885175**	**MZ127817**	**MZ126726**	**MW885176**
F. peruvianum	**CBS 511.75ᵀ**	**Gossypium sp.**	**Peru**	**MN120707**	**MN120728**	**MN120746**	**MN120767**
F. poae	NRRL 66297		-	-	MG282363	MG282392	-
F. pseudograminearum	**NRRL 28062 = CBS 109956= FRCR 5291= MAFF 237835ᵀ**	**Hordeum vulgare**	**Australia**	**-**	**JX171524**	**JX171637**	**AF212468**
F. sibiricum	NRRL 53429	*Avena sativa*	Russia	-	-	HQ154471	HM744683
	NRRL 53430ᵀ	**Avena sativa**	**Russia**	**-**	**-**	**HQ154472**	**HM744684**

GenBank accession

Species	Culture accession	Host/substrate	Origin	CAMD	RPB1	RPB2	TEF1
F. sporodochiale	CBS 199.63= MUCL 6771	*Termitary*	Unknow	MN120709	MN120730	MN120748	MN120769
	CBS 220.61 = ATCC 14167 = NRRL 20842ᵀ	***Soil***	**South Africa**	**MN120710**	**MN120731**	**MN120749**	**MN120770**
F. sporotrichioides	CBS 462.94	*Glycosmis citrifolia*	Austria	MN120711	MN120732	MN120750	MN120771
FIESC 24	CBS 101138 = BBA 70869	*Phaseolus vulgaris*	Turkey	MN120712	MN120733	MN120751	MN120772
Fusarium sp.	NRRL 13338	*Soil*	Australia	GQ505372	JX171447	JX171561	GQ505402

2   *Isolates in this study. Ex-type cultures are shown in bold.