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ORIGINAL ARTICLE

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Canker on culm of *Bambusa multiplex* (Lour.) Raeusch. ex Schult. caused by *Fusarium incarnatum* (Roberge) Sacc.

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Abstract

Bambusa multiplex has been broadly cultivated in China and has significant economical, ecological and ornamental importance. A canker on the culm of *B. multiplex* was first time discovered in 2015 in Shanghai, China. In this study, the fungal isolate XSZ-1 isolated from the infected tissues was determined to be a pathogen of canker on the culm of *B. multiplex* by fulfilling Koch's postulates. The fungal pathogen was identified as *Fusarium incarnatum* based on the morphological characteristics and phylogenetic analyses with the sequences of ITS, *TEF-1α* and *RPB2*. To our knowledge, this is the first report of a canker on the culm of *B. multiplex* caused by *F. incarnatum* worldwide.

KEYWORDS
FIESC, fungal pathogen, hedge bamboo, new disease

1 | INTRODUCTION

Bambusa multiplex (Lour.) Raeusch. ex Schult. & Schult.f. (Poaceae) (Hedge Bamboo) is a bamboo species native to China and southeastern Asia: Bhutan, India (Assam), Nepal, Sri Lanka, Vietnam and northern Indochina and it had been naturalized in Iraq, Madagascar, Mauritius, Seychelles, the Indian subcontinent, parts of South America, the West Indies and the southeastern United States (Geng & Wang, 1996; Govaerts, Dransfield, Zona, Hodel, & Henderson, 2009; Kress, Defilipps, Farr, & Kyi, 2003; Newman et al., 2007; Noltie, 2000; Press, Shrestha, & Sutton, 2000; Yuan et al., 2009). *B. multiplex* is a fast-growing bamboo, which will provide a dense living hedge capable of reaching up to 8 m in height (Zhang, 1995). The fibres of *B. multiplex* are long and strong and can be used for papermaking and weaving (Zhang, 1995). These fibres may also prove useful in the production of cellulose nanofibers (Kawasaki et al., 2017; Zhang, 1995). *B. multiplex* has also been planted to control soil erosion because of its strong network of rhizomes and roots (Zhou, Fu, Xie, Yang, & Li, 2005). In addition to its significant economic and ecological importance, *B. multiplex* is also widely planted as an evergreen ornamental and hedge (Yuan et al., 2009).

A number of diseases have been reported on *B. multiplex*: leaf and sheath rust (*Dasturella divina* (Syd.) Mundk. et Khesw.), leaf spot (*Linochora howardii* Syd.), rounded leaf spot (*Myriangium haraeanum* Tai et Wei), tar spot (*Phyllachora gracilis* Speg., *Phyllachora orbicular* Rehm, *Phyllachora lelebae* Saw.), leaf brown spot (*Prosthemiella bambusina* Syd.), rust (*Uredo inflexa* S. Ito) and *Balladyna lelebae* Yamam (Xu et al., 2006).

A canker of *B. multiplex* was found in 2015 in Shanghai, China. A causal agent of canker on *B. multiplex* has not been reported previously worldwide. Thus, this study was conducted to isolate and identify the causal agent of the observed canker on *B. multiplex* using morphological and molecular techniques and pathogenicity tests.

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2 | MATERIALS AND METHODS

2.1 | Specimens of canker on *B. multiplex* and isolation of the pathogen

Five specimens of canker on *B. multiplex* (Lour.) Raeusch. ex Schult. & Schult.f. (Herbarium number: CSH0076238) were collected from Baiyang park (E121°14′7″, N32°50′43″), Jinshan District, Shanghai on 20 July 2015 and stored in the Forest Pathology Laboratory at Nanjing Forestry University.

Infected areas of the diseased stems were surface sterilized with 75% ethanol, and the epidermal layer of infected areas was cut off with a sterile surgical scalpel. The plant tissues were cut into 2–3 × 2–3 mm pieces from the transitional area between infected and healthy tissues. The tissue pieces were placed in dishes (four pieces per dish) containing PDA medium. The dishes were incubated at 25°C for 3 days. Fungal isolates growing from infected plant tissues were purified using the monosporic isolation method described by Huang, Li, Zhang, Li, and Ye (2016).

2.2 | Morphological analysis

Fungal isolates were inoculated at the centre of PDA Petri dishes and incubated in the dark at 25°C. The diameter, colour and texture of the colonies were observed and measured daily for a week. For observation of morphological characters of conidiophores, conidiogenous cells and conidia, mycelial blocks of the isolates were inoculated to steamed rice medium according to the method by Chen (2007). Morphological characters of the fungal structures were observed and measured, and microphotographs were taken with a ZEISS Axio Imager A2m microscope (ZEISS, Germany). Measurements of the fungal structures were statistically analysed using Descriptive Statistics with Microsoft Office Excel 2013 with 95% confidence interval of means. The results were presented as ranges calculated based on mean ± standard deviation; *n*, the number of fungal structures measured. The extreme ends of the measurements of each fungal structure were presented in parentheses. The experiments have three replicates in each of three trials. For the morphological study, "The *Fusarium* Laboratory Manual" by Leslie and Summerell (2006) was consulted.

2.3 | Pathogenicity determination by Koch's postulates test

2.3.1 | Inoculation under field conditions

Carboxymethylcellulose (CMC) medium broth was used to induce sporulation, and conidial suspensions of isolate XSZ-1 were prepared following the method of Huang Yang Li Zhang and Ye (2017). The concentration of the resultant conidial suspension was adjusted to 1×10^6 conidia/ml with sterile ddH_2O for use as inoculum.

Healthy plants of *B. multiplex* without diseased symptom in nature on the campus of Nanjing Forestry University were used for the pathogenicity test. Culm surfaces were sterilized with 75% ethanol, and the mid-sections of bamboo culms were drilled through with a 0.5 cm sterilized drill bit. The drill bit was sterilized with 75% ethanol after each use. Each hole was inoculated with 10 μl of conidial suspension of the isolate XSZ-1 using a pipette. The inoculated holes were sealed with parafilm. The parafilm was removed after 1 week to allow symptoms to develop naturally. Control plants were treated with the sterile distilled water following the same procedures. Disease occurrence and its symptoms on the culms were observed and measured 6 months after inoculation, and developing cankers were collected for re-isolation of the fungus for pathogen verification and completion of Koch's postulates. The experiment was conducted twice on 15 August 2015 and 23 July 2016, respectively, and each treatment had at least three replicates.

2.3.2 | Inoculation under laboratory conditions

Culms of healthy *B. multiplex* were vertically split in half. Culms were sterilized on exterior and interior surfaces by spraying with 75% ethanol. Half of the split-culms were wounded with a red-hot needle on the exterior surface, while the others were wounded on the interior surface. The wounded area of each split culm was inoculated with 10 μl of conidial suspension of the isolate XSZ-1. Control culms were treated with sterile distilled water. The culms were placed in moisture chambers containing wet paper towel to retain the moisture and kept in the dark at 25°C in an incubator. After 24 hr, the moisture chambers were placed under light conditions of 12 hr light/dark and kept at room temperature of ca. 25°C. At the 7th day postinoculation, fungi were isolated from developing cankers at the transitional area between healthy and diseased tissues. The experiment was conducted three times and each treatment had three replicates.

2.4 | Molecular identification and phylogenetic analyses

Genomic DNA from the fungal isolate XSZ-1 was extracted following the method of Damm, Mostert, Crous, and Fourie (2008). The ITS was amplified and sequenced using the primer set ITS-1F/ITS-4 (Huang et al., 2016). The *TEF-1α* and *RPB2* genes were amplified and sequenced using the method of O'Donnell et al. (2010). The PCR reactions were performed in an Eppendorf Nexus Thermal Cycler (Eppendorf, Germany) in a total volume of 20 μl. The ITS, *TEF-1α* and *RPB2* PCR mixture contained 20 ng genomic DNA, 0.2 μM of each primer and 10 μl of 2X PCR Taq MasterMix (Applied Biological Materials Inc., Canada). PCR reactions were performed following the method by Huang et al. (2018). Amplicons were sequenced by Sangni Biotechnology Company (Shanghai, China). DNA sequences were aligned and edited using MEGA 7.0 software (Kumar et al., 2016).

The isolate XSZ-1 was initially identified to the species complex or species level by using ITS, *TEF-1α* and *RPB2* as a BLAST query, respectively, against the FUSARIUM-ID database (http://isolate.fusariumdb.org) (Wang et al., 2011). The phylogenetic analyses were performed with *TEF-1α*, as well as the concatenated sequence



FIGURE 1 Symptoms of *Bambusa multiplex* that developed naturally in the field. White arrows indicate the canker on culms of *B. multiplex* [Colour figure can be viewed at wileyonlinelibrary.com]

alignment of ITS, *TEF-1α* and *RPB2* using MEGA 7.0 software (Kumar et al., 2016; O'Donnell et al., 2012). ITS, *TEF-1α* and *RPB2* sequences of *Fusarium* isolates/taxa were obtained from Fusarium-ID database and GenBank, respectively. The ITS, *TEF-1α* and *RPB2* sequences of *Cochliobolus heterostrophus* were used as out-groups. Phylogenetic trees were inferred by using a maximum likelihood method based on the Tamura 3-parameter model using all site option, and trees were drawn with branch lengths measured in the number of substitutions per site. The bootstrap method test using 1,000 bootstrap replications was performed to assess the relative stability of the branches.

3 | RESULTS

3.1 | Symptom development of canker on *B. multiplex* in the field

The canker symptoms of *B. multiplex* developed on the culms. The symptoms started as light yellow–brown spots, circular in shape which gradually expanded vertically into elongate, fusiform or irregular shapes. Large cankers measured 22–25 cm in length and became whitish grey with a dark brown edge. Infection sites became necrotic and black (Figure 1). The spots were dry without conspicuous water-soaked lesions and developed typical symptoms of dry-rot canker. The pathogen penetrated the culm wall and reached the interior cavity. The infected area in the bamboo cavity turned dark brown to black and became necrotic. The culm canker severely reduced the growth and ornamental value of *B. multiplex*.

3.2 | Morphological characteristics of fungal isolates

Seven fungal isolates were isolated from infected culms of *B. multiplex*. Since these isolates shared similar colony characteristics and sporulating structures, isolate XSZ-1 was randomly chosen for colony morphological analysis and pathogenicity determination. Colonies of XSZ-1 on PDA dishes were white, floccose, circular, orange on the reverse side due to pigment secretion. Mycelia were greyish white with long, dense aerial hyphae, which formed tufts (Figure 2a). Growth rate of the colonies was 1.3 cm/day.

On steamed rice, conidiophores of isolate XSZ-1 were solitary or in groups, unbranched or branched, smooth, hyaline, (27.5–)39–84.8 (–129.0) × (2.0–)2.9–4.7(–5.5) μm, (mean ± *SD* = 61.9 ± 22.9 × 3.8 ± 0.9 μm, *n* = 35) (Figure 2b i–iii). Conidiogenous cells were phialidic to polyphialidic, lageniform, smooth, hyaline, (12.8–)17.5–28.7(–35.2) μm (23.1 ± 5.6 μm) in length, (1.4–)1.8–2.8(–3.8) μm (2.3 ± 0.5 μm) in width at the apex, (1.5–)2.4–4.0(–4.6) μm (3.2 ± 0.8 μm) in width at the base,

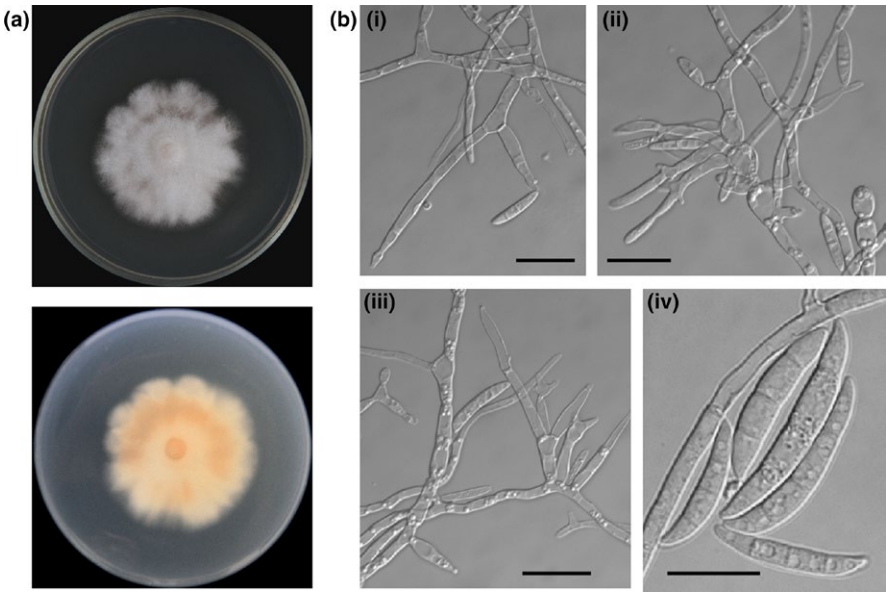


FIGURE 2 Morphological characters of XSZ-1. (a) Morphology of colonies of XSZ-1. (b) Conidiophores, conidiogenous cells (i–iii) and conidia (iv). Scale bars = 10 μm [Colour figure can be viewed at wileyonlinelibrary.com]

(2.1–)3.1–4.7(–6.0) μm, (3.9 ± 0.8 μm) in width at the middle (n = 34) (Figure 2b i–iii). Conidia developed singly or gregariously. Macroconidia were falcate or fusiform, smooth, colourless, multiseptated, 3–7 celled, majority 4–6 celled, (12.4–)17.8–28.4(–35.1) × (1.9–)3.1–4.5(–6.4) μm, (23.1 ± 5.3 × 3.8 ± 0.7 μm, n = 75) (Figure 2b iv). Meso- and micro-conidia and chlamydospores were absent.

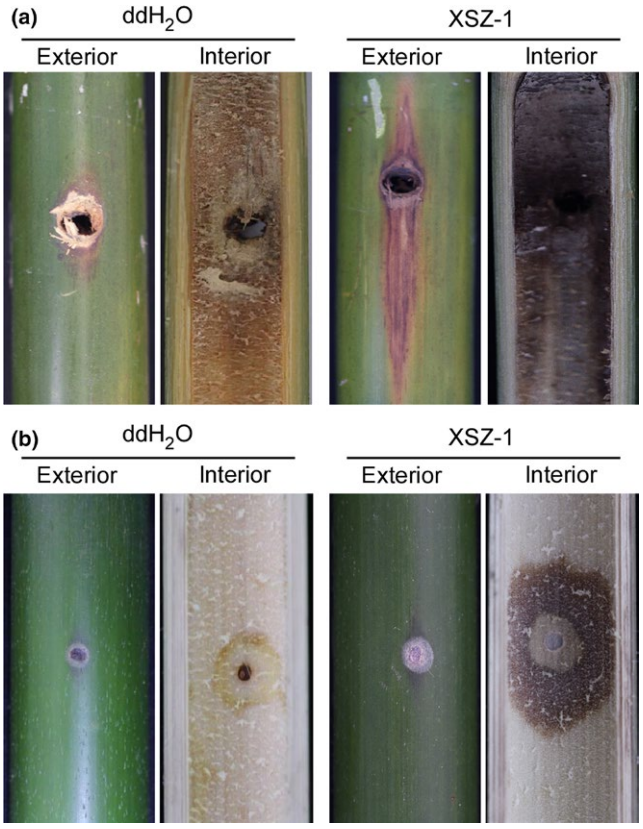


FIGURE 3 Symptoms on culms of *B. multiplex* that developed from inoculation with XSZ-1 in the field (a) and laboratory (b) [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Pathogenicity determination in the field and laboratory

Necrotic spots developed around the inoculation sites in the culms at 6 months after inoculation in the field. The spots were ca. 6.2 × 0.8 cm in size, dry and fusiform. The infected area in bamboo cavities turned black. No symptoms developed on culms of the control treatment (Figure 3a). The fungus was re-isolated from the transitional area between diseased and healthy tissues. The morphological characters of colonies, conidia and conidiophores showed that it was the same as the fungus used for inoculation (Figure 2).

In the laboratory, circular necrotic spots developed around the inoculation sites within 7 days following inoculation. The spots were pale grey on the exterior of the culms and a dark brown spot with a grey centre developed in the cavity. Infected areas in the culm cavity became black and necrotic (Figure 3c). No symptoms developed on the culms of the control treatment. The same fungus was re-isolated from the transitional area between healthy and diseased tissues. The living culture of the XSZ-1 has been deposited in the China Center for Type Culture Collection (CCTCC AF 2017009) at Wuhan University, Wuhan City, Hubei Province, China.

3.4 | Molecular characterizations and phylogenetic analysis of the isolate XSZ-1

To identify the isolate XSZ-1, the ITS, *TEF1-α* and *RPB2* of isolate XSZ-1 were cloned and sequenced. The sequences were deposited

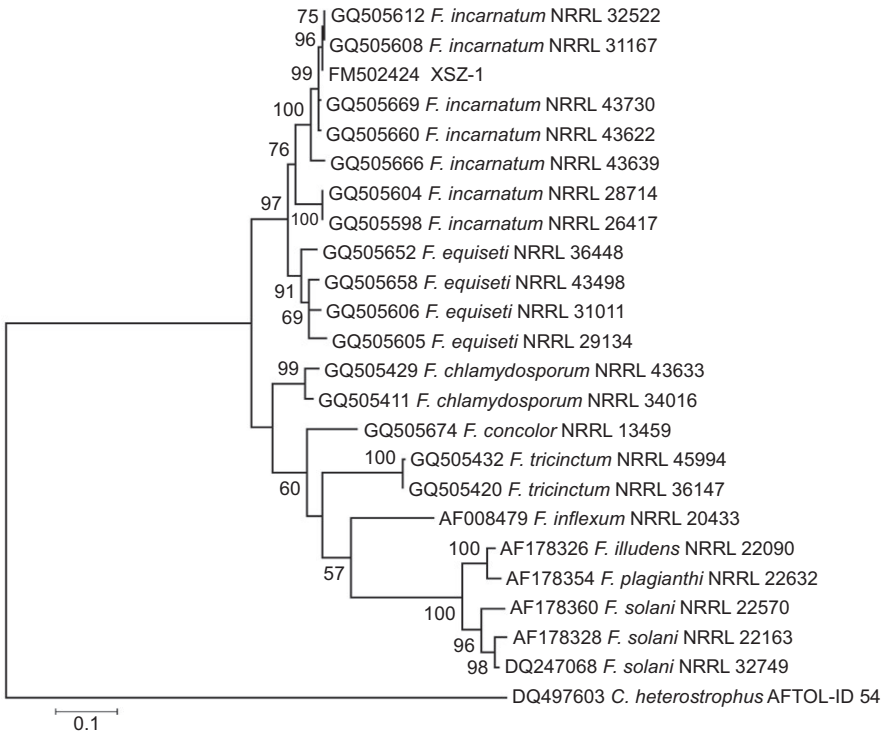


FIGURE 4 Phylogenetic tree of the putative *Fusarium incarnatum* isolate XSZ-1 with allied taxa calculated from *TEF1-α* gene using maximum likelihood method. Bootstrap values >50% (1,000 replications) are provided at the nodes. *Cochliobolus heterostrophus* was used as an out-group. Bar = 0.1 substitutions per nucleotide position

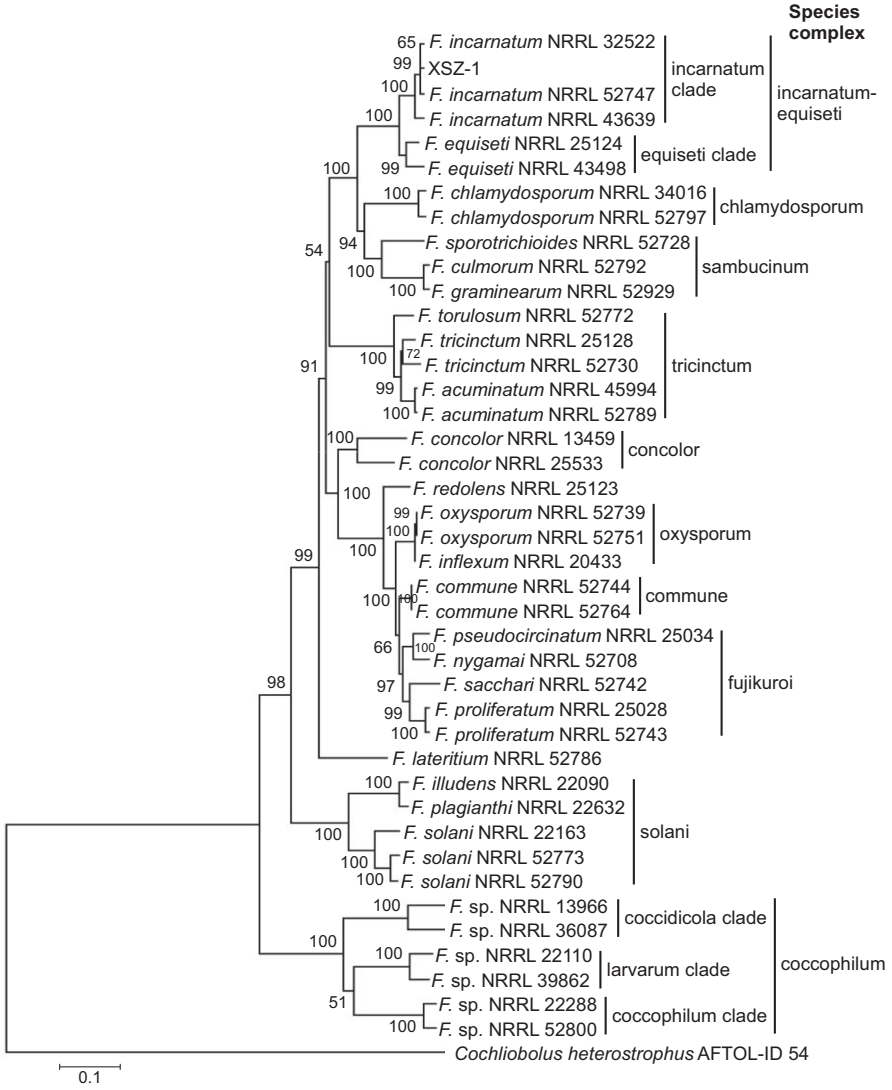


FIGURE 5 Phylogenetic tree of the putative *Fusarium incarnatum* isolate XSZ-1 with allied taxa analysed using concatenated sequences of ITS, *TEF-1α* and *RPB2* with maximum likelihood method. Bootstrap values >50% (1,000 replications) are given at the nodes. *Cochliobolus heterostrophus* was used as an out-group. Different species complexes were indicated. Bar = 0.1 substitutions per nucleotide position

in the GenBank database with the accession numbers: MF480650, MF502424 and MF502425, respectively. The ITS sequence of XSZ-1 showed 100% similarity with *Fusarium incarnatum-equiseti* species complex (FIESC) strains such as NRRL 43730 (GQ505758) and NRRL 43639 (GQ505755). The *TEF1-α* sequence showed 99.7% similarity with *F. incarnatum-equiseti* species complex strain NRRL 32522 (GQ505612) and NRRL 31167 (GQ505608). The *RPB2* sequence showed 99.0% similarity with *F. incarnatum-equiseti* species complex strain NRRL 26417 (GQ505776).

The phylogenetic tree calculated from *TEF1-α* sequences showed that the XSZ-1 is most closely related to *F. incarnatum* and grouped together with *F. incarnatum* isolates with 96% bootstrap support (Figure 4). The phylogenetic tree calculated from concatenated sequences of ITS, *TEF1-α* and *RPB2* also showed that the XSZ-1 was clustered with *F. incarnatum* strains with a high bootstrap support (Figure 5). The FIESC including *Fusarium incarnatum s.l.* and *F. equiseti s.l.* (O'Donnell et al., 2009). The phylogenetic analyses based on the multiloci of the current study showed that the isolate XSZ-1 was monophyletic with *Fusarium incarnatum s.l.* within the FIESC. These results, in combination with its morphological characters, showed that the isolate XSZ-1 belongs to *F. incarnatum s.l.*

4 | DISCUSSION

Fusarium contains ca. 150 described species (Seifert, Morgan-Jones, Gams, & Kendrick, 2011) and many taxa remain undescribed (O'Donnell et al., 2009, 2010). *Fusarium* includes at least 20 genealogically exclusive lineages: *F. albidum*, *F. babinda*, *F. buharicum*, *F. buxicola*, *F. chlamydosporum*, *F. concolor*, *F. decemcellulare*, *F. dimerum*, *F. fujikuroi*, *F. heterosporum*, *F. incarnatum-equiseti* (FIESC), *F. lateritium*, *F. nisikadoi*, *F. oxysporum*, *F. redolens*, *F. sambucinum*, *F. solani*, *F. staphyleae*, *F. tricinctum* and *F. ventricosum* species complexes/clades (Geiser et al., 2013).

The identification of *Fusarium* species traditionally relies on morphological and physiological characters. However, these are limited in their ability to describe species boundaries. This is especially evident among very closely related *Fusarium* species, such as members

of *F. incarnatum-equiseti* species complex (FIESC), due to the high level of cryptic speciation and extreme morphological homoplasy. The ITS region has been used to characterize eukaryotic microbes (Johannesson & Stenlid, 1999). But, for some large fungal genera: *Fusarium*, *Colletotrichum* and *Aspergillus*, these species cannot be totally separated solely using sequences from the ITS region (Balajee et al., 2009; Huang et al., 2016). Other genes such as *TEF1-α* and *RPB2* were also employed for species identification of *Fusarium*. A multilocus sequence typing scheme analysis has been applied to members of FIESC and revealed that it comprises 30 phylogenetically distinct species, evenly divided among two clades designated Incarnatum and Equiseti (O'Donnell et al., 2012).

Fusarium incarnatum (Roberge) Sacc. has been reported to cause various plant disease in the world (Ramdial, Hosein, & Rampersad, 2016; Seta, Gonzalez, & Lori, 2004). In China, *F. incarnatum* causes black spot disease of Chinese Jujube (Guo, Sun, Zhao, & He, 2016), root rot of *Morus alba* (Chen et al., 2017), and was found on corn (Gai et al., 2016). Here, we identified for the first time, the *F. incarnatum* as the pathogen of canker on *B. multiplex* worldwide. The distribution of this disease in other areas in China and potential impact on growth and production of culm on *B. multiplex* need to be investigated in the future.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

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REFERENCES

Balajee, S. A., Borman, A. M., Brandt, M. E., Cano, J., Cuenca-Estrella, M., Dannaoui, E., … Wickes, B. L. (2009). Sequence-based identification of *Aspergillus*, *Fusarium*, and Mucorales species in the clinical mycology laboratory: Where are we and where should we go from here? *Journal of Clinical Microbiology*, *47*, 877–884. https://doi.org/10.1128/JCM.01685-08

Chen, J-S. (2007). *Identification of* Fusarium *spp. collected from Hainan Island*. M.Sc. Thesis, Southern China University of Tropical Agriculture, Dan Zhou, Hainan.

Chen, S. C., Zhao, H. J., Wang, M. M., Ren, J. J., Wang, Z. H., Li, J. D., … Ahammed, G. J. (2017). First report of root rot caused by *Fusarium incarnatum* on *Morus alba* in China. *Plant Disease*, *101*, 1320. https://doi.org/10.1094/PDIS-02-17-0161-PDN

Damm, U., Mostert, L., Crous, P., & Fourie, P. (2008). Novel *Phaeoacremonium* species associated with necrotic wood of *Prunus* trees. *Persoonia*, *20*, 87–102. https://doi.org/10.3767/003158508X324227

Gai, X. T., Yang, R. X., Pan, X. J., Yuan, Y., Wang, S. N., Liang, B. B., & Gao, Z. G. (2016). First report of *Fusarium incarnatum* causing stalk rot on maize in China. *Plant Disease*, *100*, 1010. https://doi.org/10.1094/PDIS-07-15-0766-PDN

Geiser, D. M., Aoki, T., Bacon, C. W., Baker, S. E., Bhattacharyya, M. K., Brandt, M. E., … Zhang, N. (2013). One fungus, one name: defining the genus *Fusarium* in a scientifically robust way that preserves longstanding use. *Phytopathology*, *103*, 400–408. https://doi.org/10.1094/PHYTO-07-12-0150-LE

Geng, B. P., & Wang, Z. P. (1996). *Flora of China*. Beijing, China: Science Press.

Govaerts, R., Dransfield, J., Zona, S., Hodel, D., & Henderson, A. (2009). World checklist of selected plant families. The Board of Trustees of the Royal Botanic Gardens, Kew Published on internet. Retrieved from http://apps.kew.org/wcsp

Guo, K., Sun, J., Zhao, S., & He, L. (2016). Black spot disease of Chinese Jujube (*Ziziphus jujuba*) caused by *Fusarium incarnatum* in China. *Plant Disease*, *100*, 529. https://doi.org/10.1094/PDIS-06-15-0694-PDN

Huang, L., Li, Q., Zhang, Y., Li, D., & Ye, J. (2016). *Colletotrichum gloeosporioides* s.s. is a pathogen of leaf anthracnose on evergreen spindle tree (*Euonymus japonicus*). *Plant Disease*, *100*, 672–678. https://doi.org/10.1094/PDIS-07-15-0740-RE

Huang, L., Yang, J., Li, Q., Zhang, Y., & Ye, J. (2017). Identification of the pathogen causing stem canker on *Sophora japonica* Linn. var. *japonica* f. *pendula* Hort. *Journal of Nanjing Forestry University* (Natural Sciences Edition), *41*, 175–179. https://doi.org/10.3969/j.issn.1000-2006.201701004

Huang, L., Zhu, Y., Yang, J., Li, D., Li, Y., Bian, L., & Ye, J. (2018). Shoot blight on Chinese fir (*Cunninghamia lanceolata*) is caused by *Bipolaris oryzae*. *Plant Disease*, *102*, 500–506. https://doi.org/10.1094/PDIS-07-17-1032-RE

Johannesson, H., & Stenlid, J. (1999). Molecular identification of wood-inhabiting fungi in an unmanaged *Picea abies* forest in Sweden. *Forest Ecology and Management*, *115*, 203–211. https://doi.org/10.1016/S0378-1127(98)00399-5

Kawasaki, M., Yordsri, V., Thanachayanont, C., Junin, C., Asahina, S., Oikawa, T., … Shiojiri, M. (2017). Structures of green culms and charcoal of *Bambusa multiplex*. *Microscopy and Microanalysis*, *23*(Suppl 1), 1294–1295. https://doi.org/10.1017/S1431927617007139

Kress, W. J., Defilipps, R. A., Farr, E., & Kyi, D. Y. Y. (2003). A checklist of the trees, shrubs, herbs, and climbers of Myanmar (revised from the original works by JH Lace, R. Rodger, HG Hundley and U Chit Ko Ko on the" List of trees, shrubs, herbs and principal climbers etc. recorded from Burma"). Washington D.C.: 590p: Department of Systematic Biology - Botany, National Museum of Natural History.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*, 1870–1874. https://doi.org/10.1093/molbev/msw054

Leslie, J. F., & Summerell, B. A. (2006). *The* Fusarium *laboratory manual*. Victoria, Australia: Blackwell Publishing.

Newman, M., Ketphanh, S., Svengsuksa, B., Thomas, P., Sengdala, K., Lamxay, V., & Armstrong, K. (2007). *A checklist of the vascular plants of Lao PDR*. Edinburgh, UK: Royal Botanic Garden Edinburgh.

Noltie, H. (2000). *Flora of Bhutan*. Edinburgh, UK: Royal Botanic Garden.

O'Donnell, K., Sutton, D. A., Rinaldi, M. G., Gueidan, C., Crous, P. W., & Geiser, D. M. (2009). Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum-F. equiseti* and *F. chlamydosporum* species complexes within the United States. *Journal of Clinical Microbiology*, *47*, 3851–3861. https://doi.org/10.1128/JCM.01616-09

O'Donnell, K., Humber, R. A., Geiser, D. M., Kang, S., Park, B., Robert, V. A., … Rehner, S. A. (2012). Phylogenetic diversity of insecticolous fusaria inferred from multilocus DNA sequence data and their molecular identification via FUSARIUM-ID and *Fusarium* MLST. *Mycologia*, *104*, 427–445. https://doi.org/10.3852/11-179

O'Donnell, K., Sutton, D. A., Rinaldi, M. G., Sarver, B. A. J., Balajee, S. A., Schroer, H. J., … Geiser, D. M. (2010). Internet-accessible DNA sequence database for identifying fusaria from human and animal infections. *Journal of Clinical Microbiology*, *48*, 3708–3718. https://doi.org/10.1128/JCM.00989-10

Press, J. R., Shrestha, K. K., & Sutton, D. A. (2000). *Annotated checklist of the flowering plants of Nepal*. London, UK: Natural History Museum Publications.

Ramdial, H., Hosein, F., & Rampersad, S. N. (2016). First report of *Fusarium incarnatum* associated with fruit disease of bell peppers in Trinidad. *Plant Disease*, *100*, 526. https://doi.org/10.1094/PDIS-05-15-0550-PDN

Seifert, K. A., Morgan-Jones, G., Gams, W., & Kendrick, B. (2011). *The genera of Hyphomycetes*. Utrecht, The Netherlands: CBS-KNAW Fungal Biodiversity Centre Utrecht.

Seta, S., Gonzalez, M., & Lori, G. (2004). First report of walnut canker caused by *Fusarium incarnatum* in Argentina. *Plant Pathology*, *53*, 248. https://doi.org/10.1046/j.1365-3059.2003.00968.x

Wang, H., Xiao, M., Kong, F., Chen, S., Dou, H. T., Sorrell, T., … Xu, Y. C. (2011). Accurate and practical identification of 20 *Fusarium* species by seven-locus sequence analysis and reverse line blot hybridization, and an in vitro antifungal susceptibility study. *Journal of Clinical Microbiology*, *49*, 1890–1898. https://doi.org/10.1128/JCM.02415-10

Xu, M. Q., Dai, Y. C., Fan, S. H., Jin, L. X., Lu, Q., Tian, G. Z., & Wang, L. F. (2006). Records of bamboo diseases and the taxonomy of their pathogens in China (I). *Forest Research*, *19*, 692–699.

Yuan, J., Gu, X., Li, L., Yue, J., Yao, N., & Guo, G. (2009). Callus induction and plantlet regeneration of *Bambusa multiplex*. *Scientia Silvae Sinicae*, *45*, 35–39.

Zhang, X. (1995). Study on fiber and paper-making properties of main bamboo species in Guizhou. *Journal of Bamboo Research*, *14*, 14–30.

Zhou, B., Fu, M., Xie, J., Yang, X., & Li, Z. (2005). Ecological functions of bamboo forest: Research and application. *Journal of Forest Research*, *16*, 143–147. https://doi.org/10.1007/BF02857909

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