

## Article

# First Report of *Fusarium vanettenii* Causing *Fusarium* Root Rot in *Fatsia japonica* in China

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**Abstract:** *Fatsia japonica* plays an important role as a commonly used plant in urban landscaping. From 2022 to 2023, a root rot infestation was observed that caused extensive wilting of *Fatsia japonica* without leaf shedding and eventual death of the plant, severely reducing the ornamental qualities of the plant as well as the vigor of its growth. *Fusarium* species were isolated from the roots of the affected plants, exhibiting abundant and dense yellow mycelial colonies that proliferated radially from the center of the Petri dishes. Morphological examinations revealed the presence of falciform macro- and microconidia consistent with *Fusarium*, as well as chlamydospores characterized by their thick walls. For further identification, the amplification and sequencing of the ITS, *TEF1* alpha, and *RPB2* alpha genes were performed. Finally, healthy *Fatsia japonica* plants were inoculated with a spore suspension of the pathogen, to confirm that the disease symptoms were compatible with naturally occurring infection. *Fusarium vanettenii* was identified as the causative agent of *Fatsia japonica* root rot. To the best of our knowledge, this is the first report of *F. vanettenii* causing root rot of *Fatsia japonica* in China.

**Keywords:** first report; *Fusarium vanettenii*; virulence; phylogenetic analysis



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## 1. Introduction

*Fatsia japonica* is an important shade-tolerant, evergreen, clump-forming shrub with palmate, broad leaves that are green in all seasons and yellowish-white flowers in panicle-like, terminal cymes [1]. It typically grows tall and erect, with young branches and leaves that are covered in a dense layer of woolly tomentum, while the stems and large, shiny leaves remain smooth. During the flowering season, the yellowish-white flowers are like small, unfolded umbrellas. The fruit is a drupe, similar to a ball, and starts out light green when young and turns almost black when ripe. *Fatsia japonica* blooms from October to November and bears fruit from May to February. It is named after the shape of its leaves, which are made up of eight golden leaves [2]. The plant is native to Japan and is found in both northern and southern China. It is mainly grown indoors in the north and outdoors in the south. It is slightly shade tolerant, cold tolerant but drought intolerant. The main propagation methods are cutting, sowing, and dividing [3,4]. Due to its strong resistance to sulfur dioxide and other harmful gases, it can be planted in front of gardens, under window sills, or in beds on lawn edges and under woodlands and is a common plant for urban greenery [5]. *Fatsia japonica* is also an important medicinal plant with an overall efficacy rate of more than 90% in the treatment of hepatocellular carcinoma [6], and has obvious effects on prolonging survival in patients with esophageal squamous cell carcinoma [7].

Of the many genera of fungi that are harmful to plants, *Fusarium* is one of the most well-known. Not only plants, but also humans and domestic animals can all become ill as a direct result of this genus [8–10]. *Fusarium* was included in the top 10 most important

genera of plant pathogenic fungi worldwide based on scientific and economic importance [11,12], particularly because of the members of the *F. sambucinum* species complex (FSAMSC) and the *F. oxysporum* species complex (FOSC) [13], which include some of the most destructive agricultural pathogens. Members of the *F. fujikuro* species complex (FFSC) are also important *Fusarium*, *F. verticillioides* (teleomorphic synonym, *Gibberella moniliformis*), *F. fujikuroi* (teleomorphic synonym, *G. fujikuroi*), and *F. proliferatum* (teleomorphic synonym, *G. intermedia*), which are known for their ability to cause devastating diseases such as rice bakanae, corn ear rot, and soybean root rot, leading to significant reductions in crop yields and economic income [14,15].

*Fusarium* was first described by Link (1809) and typified as *Fusarium roseum* (presently *F. sambucinum* nom. cons.) [16]. Since the foundation of phenotypical-based taxonomic treatments categorizing species into sections, morphological varieties, or forms, and later formae speciales based on pathogenicity and host ranges [17,18], the generic and species concepts in *Fusarium* have undergone significant changes. Later, the species were redistributed into species complexes following the introduction of modern molecular tools [19]. Over the past decade, there has been increasing controversy over the general definition of *Fusarium*. Geiser et al. (2013) [20] supported the retention of the genus *Fusarium* and called for the recognition of the genus *Fusarium* as the sole name for a group that includes virtually all *Fusarium* species of importance in plant pathology, mycotoxicology, medicine, and basic research, and the retained genus *Fusarium* includes *F. solani* species complex (FSSC). A challenge to this treatment was later made by Lombard et al. (2015), who distinguished the FSSC as *Neocosmospora* and divided the genus *Fusarium* into seven genera [21]. A study by Crous et al. (2021) recombined 40 species previously identified as *Neocosmospora*. On the basis of phylogenetic analyses utilizing sequence data from eight loci, the authors maintain that *Fusarium* are polyphyletic in Nectriaceae such that a more limited generic concept involving a combination of features is required for the majority of fusarioid species [22]. The Wollenweber concept of *Fusarium* is divided into 20 genera. O'Donnell et al. (2022), based on synapomorphic traits, expressed the opinion that *Fusarium* is still the most useful, nomenclatural, and scientific taxonomic option [23], but there is still much debate on the matter.

The traditional identification of *Fusarium* spp. has historically been based primarily on the morphological characterization of sporocarps, sporocarp stalks, chlamydospores, sexual organs, hyphae, and colonies. It is difficult to use the taxonomic identification of *Fusarium* spp. to identify *Fusarium* parts due to its unstable basis [24,25]. Thus, to obtain more reliable results, *Fusarium* identification must be combined with the use of molecular biology methods. There are many important loci for the systematic studies of *Fusarium*, but currently ribosomal DNA (rDNA) and *EF-1 $\alpha$*  elongation factor are the most commonly used [26,27]; rDNA-ITS sequences have multiple copy repeats, and the complete sequence is generally 500–800 bp long. rDNA-ITS sequences are easily amplified via PCR using universal primers from small and large DNA samples.

From 2022 to 2023, symptomatic *Fatsia japonica* plants appeared in Xuanwu District, Nanjing, and on the campus of Nanjing Forestry University, China. We discovered numerous severely diseased *Fatsia japonica* roots that showed signs of blackened root rot when dug up, low-hanging dead leaves that did not fall off, and complete plant wilting. In addition, impacted plants might show signs of reduced development and a general waning of overall vitality. The main objective of this work was to isolate and characterize the pathogenic agent of *Fatsia japonica* root rot of the pathogen through pathogenicity tests, morphological characterization, and phylogenetic analysis to provide a reference basis for the study of *Fatsia japonica* root rot diseases.

## 2. Materials and Methods

### 2.1. Isolation and Identification of Etiological Agents

In September 2022, dozens of diseased roots of *Fatsia japonica* were dug up near the Xuanwu district of Nanjing and under several dormitory buildings of Nanjing Forestry

University. They were taken to the plant pathology lab in a plastic bag; then the roots were rinsed with water for about 30 min and transferred to an ultra-clean laboratory bench. Washed diseased roots were cut into several small square pieces of approximately 3 mm, the specimen was treated via immersion in 75% bioethanol for 30 s and in 1% sodium hypochlorite for 90 s; then it was rinsed with clean aseptic water three times, and then excess water was removed using sterile filter paper in pre-prepared 90 mm potato dextrose agar (PDA) dishes. Once the specimen was completely dry, it was ready for further analysis or preservation. Each Petri dish was inoculated with 3–5 roots and distributed evenly, wrapped twice with sealing film, and then placed in a dark incubator (Incubator MIR-553 located in Osaka, Japan and manufactured by Sanyo) at 25 °C for 3–5 days. Then, pure cultures were obtained by subculturing them in new PDA Petri dishes.

2.2. Morphological Identification

To facilitate the observation of the morphology of different spores, the colonies in Petri dishes were cut into several dry 1 cm<sup>3</sup> pieces using a scalpel on an ultra-clean bench and placed in conical flasks (100 mL) with sterile PDB (Potato Dextrose Broth) liquid (approximately 20–30 mL), sealed with a sealing film and then placed in a shaker at 200 rpm at 25 °C for 60 h. Once the spore suspension was obtained, approximately 10 µL of the liquid was pipetted onto a clean microscope slide as the next step in the testing process. The morphology of macroconidia, microconidia, and chlamydospores was measured using a Zeiss Axio Imager A2 m microscope (Carl Zeiss, Oberkochen, Germany), and size measurements and morphologic descriptions were performed (*n* = 50).

2.3. DNA Extraction, PCR Amplification, and Sequencing

Isolates were grown on PDA plates for five days and then the mycelium was collected in 2 mL tubes for DNA extraction by using a modified CTAB method [28]. Tubes were put in a shaker at 25 °C for two hours at 200 rpm, and then 500 µL of chloroform and 500 µL of hexadecyltrimethyl ammonium bromide (CTAB) extraction buffer (0.2 M Tris, 1.4 M NaCl, 20 mM EDTA, 0.2 g/L CTAB) were added. Samples were centrifuged at 15,800× *g* for 5 min. The supernatant was then poured into a fresh tube along with 600 µL of pure ethanol, and then the tubes were centrifuged at 15,800× *g* for 5 min. The precipitate was then mixed with 600 µL of 70% ethanol. After 5 min at 15,800× *g* centrifugation, the suspension was separated, and the supernatant was thrown away. After drying, 30 µL of deionized water was used to resuspend the DNA pellet.

With the extracted DNA, a polymerase chain reaction (PCR) amplification was performed. Primers for EF-1α and EF-1H [28,29], ITS1 and ITS4 [30], and RPB2-5F2 and fRPB2-7cR [31] were used to amplify multiple sites, translation elongation factor 1-α(*TEF1*), internal transcribed spacer region (ITS), and RNA polymerase II (*RPB2*). Table 1 below lists the primers used and the PCR conditions.

Table 1. This table lists the primers used to amplify the PCR.

Locus	Primer	Sequence (5'-3')	PCR Conditions	Reference
The internal Transcribed Spacer (ITS) Elongation factor 1-alpha ( <i>TEF1</i> ) RNA polymerase II genes ( <i>RPB2</i> )	ITS1	TCCGTAGGTGAACCTGCGG	94 °C, 3 min; (94 °C, 30 s, 55 °C, 30 s; 72 °C, 30 s) × 35; 72 °C, 10 min	[30]
	ITS4	TCCTCCGCTTATTGATATGC		
	EF-1	ATGGGTAAGGA(A/G)GACAAGAC	94 °C, 3 min; (94 °C, 30 s, 63 °C, 30 s; 72 °C, 45 s) × 35; 72 °C, 10 min	[28,29]
	EF-1H	GTGGGGCATTTACCCCGCC		
	RPB2-5F2	TTGCTATCGACAAAGAGATCC	94 °C, 3 min; (94 °C, 30 s, 57 °C, 30 s; 72 °C, 1 min) × 35; 72 °C, 10 min	[31]
	fRPB2-7cR	ATATAAGACGCGAACCCTTTT		

For PCR amplification, a 50 µL system was used, and the reaction system contained 19 µL of ddH<sub>2</sub>O, 25 µL of Taq DNA polymerase, 2 µL each of upstream and downstream primers (10 µmol/L), and 2 µL of template DNA (100 ng/µL). Using agarose gel electrophoresis, the PCR-amplified product was purified. Subsequently, PCR products were sent to Shanghai Jieli Biotechnology Company Limited (Nanjing, China) for amplicon sequencing.

#### 2.4. Phylogenetic Analysis

To enhance the assay's precision, we conducted additional identification analyses and subjected the isolated sequences to a BLAST search of the NCBI databank to identify highly similar orthologues and submitted them to the NCBI/GenBank, and the results of the search are shown in Table 2. The fungal isolate gene sequence from this investigation has been deposited into GenBank (<http://www.ncbi.nlm.nih.gov>, (accessed on 5 January 2024)). Reference sequences of closely related species and isolates were downloaded from GenBank to perform phylogenetic analysis (Table 3). Gene sequences have been aligned in “bioEdit ver.7.0.9.1” using ClustalW Multiple Alignment [32]. To ensure accuracy, the first bases of each gene sequence pair were eliminated, to avoid potential bias in subsequent analysis. In addition, to ensure that each sequence had the same base, the tail of each sequence was also truncated. The multilocus tandem sequences ITS, *TEF1*, and *RPB2* were used for phylogenetic analysis using ML and BI in PhyloSuite version 1.2.2 [33]. ITS, *TEF1*, and *RPB2* sequences were concatenated using Concatenate Sequence, followed by multigene tandem maximum likelihood phylogenetic analysis using IQ-TREE version 1.6.8 [34]. The nucleotide replacement models were selected using the Akaike Information Criterion (AIC) criteria and ModelFinder [35]. The GTR exchange was selected with the site discriminating factor adjusted to be invgamma. The support for phylogenies was calculated for 1000 replicates using the bootstrap test (BS). Phylogenetic BI analysis of interspecific relationships was performed using MrBayes version 3.2.6 [36]. ModelFinder and Bayesian Information Criterion (BIC) were used for statistical selection of the best replacement model. The operating algorithm was Markov Chain Monte Carlo (MCMC). For over  $2 \times 10^6$  generations, the operation continued. Samples were taken at 1000 generation intervals until the split frequencies' average standard deviation was less than 0 points. We computed each branch's posterior probabilities (PP). Finally, the three files were displayed using FigTree software version 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>, (accessed on 13 January 2024)) and enhanced by editing the dendrograms using drawing tools.

**Table 2.** The BLAST results were obtained using the ITS, *RPB2*, and *TEF1* gene-amplified sequencing from the 3 isolates that were representative of this experimental set.

Isolate	DNA Target	GenBank Accession No.	Blast Match Sequence	
			Reference Accession No.	Sequence Identity (%)
BJ4-1	ITS	PP059592	<i>Fusarium</i> sp. JCM 28442 (LC133858.1)	100% (590/590)
	<i>RPB2</i>	PP066262	<i>F. vanettenii</i> F330 (OR371940.1)	100% (915/915)
	<i>TEF1</i>	PP140664	<i>F. vanettenii</i> F330 (OQ511144.1)	99.43% (700/704)
BJ5-2	ITS	PP060633	<i>Fusarium</i> sp. JCM 28442 (LC133858.1)	100% (590/590)
	<i>RPB2</i>	PP066263	<i>F. vanettenii</i> F274 (OR371921.1)	100% (913/913)
	<i>TEF1</i>	PP145300	<i>F. vanettenii</i> F310 (OQ511138.1)	99.29% (716/737)

Table 2. Cont.

Isolate	DNA Target	GenBank Accession No.	Blast Match Sequence	
			Reference Accession No.	Sequence Identity (%)
BJ5-6	ITS	PP060634	<i>Fusarium</i> sp. JCM 28442 (LC133858.1)	100% (590/590)
	<i>RPB2</i>	PP078612	<i>F. vanettenii</i> F127 (OR371880.1)	99.89% (915/916)
	<i>TEF1</i>	PP145301	<i>F. vanettenii</i> 840047 (AB513842.1)	99.72% (733/935)

Table 3. Sequences from the phylogenetic studies have NCBI accession numbers. The internal transcribed spacer (ITS); Elongation factor 1-alpha (*TEF1*); RNA polymerase II genes (*RPB2*).

Species	Voucher	GenBank Accession Numbers		
		ITS	<i>RPB2</i>	<i>TEF1</i>
<i>Fusarium vanettenii</i>	NRRL 22820	DQ094310	EU329532	AF178355
<i>Fusarium vanettenii</i>	CBS 123669	KM231796	KM232215	KM231925
<i>Fusarium breve</i>	NRRL 28009	DQ094351	EF470135	DQ246869
<i>Fusarium breve</i>	NRRL 32792	DQ094561	EU329621	DQ247101
<i>Fusarium borneense</i>	NRRL 22579	NR169885	EU329515	AF178352
<i>Fusarium bataticola</i>	NRRL 22402	OR519899	MW218100	DQ247681
<i>Fusarium crassum</i>	NRRL 46703	EU329712	EU329661	HM347126
<i>Fusarium cucurbiticola</i>	NRRL 22153	DQ094302	EU329492	DQ236344
<i>Fusarium cicatricum</i>	CBS 125552	MH863560	HQ728145	HM626644
<i>Fusarium ferrugineum</i>	NRRL 32437	DQ094446	EU329581	DQ246979
<i>Fusarium helgardnirenbergiae</i>	NRRL 22387	NR169883	EU329505	AF178339
<i>Fusarium illudens</i>	NRRL 22090	JX171601	EU329488	KZ303538
<i>Fusarium liriodendri</i>	NRRL 22389	DQ094314	EU329506	OP920672
<i>Fusarium mori</i>	NRRL 22230	DQ094305	EU329499	AB674290
<i>Fusarium parceramosum</i>	CBS 115695	OP782205	JX435249	JX435149
<i>Fusarium piperis</i>	NRRL 22570	NR169886	EU329513	AF178360
<i>Fusarium pseudoradicicola</i>	NRRL 25137	JF740899	JF741084	JF740757
<i>Fusarium protoensiforme</i>	NRRL 22178	AF178399	EU329498	DQ094313
<i>Fusarium staphyleae</i>	NRRL 22316	MH582406	EU329502	AF178361
<i>Fusarium solani</i>	NRRL 25388	MH582401	EU329535	DQ246858
<i>Fusarium solani</i>	MRC 2565	MH582400	MH582226	MH582420
<i>Fusarium virguliforme</i>	NRRL 31041	MN310695	JX171643	HM453369
<i>Fusarium venezuelense</i>	NRRL 22395	NR172367	EU329507	AF178341
<i>Fusarium waltegersii</i>	NRRL 32323	DQ094420	EU329576	DQ246951

## 2.5. Pathogenicity Tests

Pathogenicity tests were conducted on the supra-root parts of annual *F. japonica*. One-year-old potted seedlings of *Fatsia japonica* (30 cm tall,  $n = 12$ ) were placed in a greenhouse under the following conditions: 25 °C, 90% relative humidity, and 14 h of daylight. Colonies on PDA were cut into several dry 1 cm<sup>3</sup> pieces with a scalpel on an ultra-clean bench, placed in conical flasks (100 mL) with aseptically treated PDB liquid (approximately 20–30 mL) and placed on a shaker at 200 rpm and 25 °C for approximately 60 h to obtain spore suspensions. One-year-old *F. japonica* roots were dug up to expose the root ball. A small wound was made in the root balls with a sterile needle prior to inoculation. Ten milliliters of spore suspension (10<sup>6</sup> spores/mL) was used to inoculate each plant [37]. The suspension was added to sterile potting soil (~500 g) and mixed. Six seedlings were considered as one replicate, with three replicates per treatment and per control. All pathogenicity experiments were performed in 3 replicates. The treatment inoculated with sterile water served as a mock-inoculated control. The inoculated fungus was re-isolated to prove Koch's postulate and compared with the original one.



### 3. Results

#### 3.1. Natural Symptoms

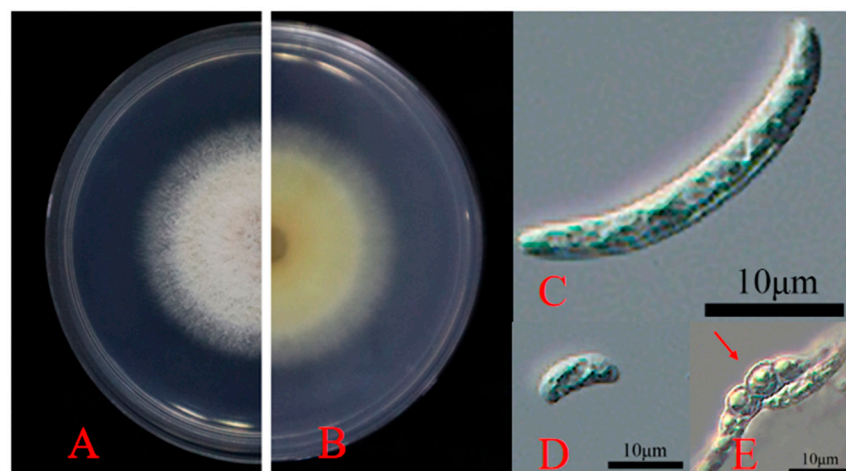
From 2022 to 2023, dozens of *Fatsia japonica* plants with disease symptoms were observed near the Xuanwu district of Nanjing and on the campus of Nanjing Forestry University. In the initial phase of the disease, the plants droop at the base of the stem, and the leaves curl up without falling off, turning green without any visible necrosis (Figure 1A). Wilting of the leaves indicates a more advanced stage of the disease (Figure 1B). Subterranean roots decay and turn black where they are (Figure 1C,D). The disease was also discovered to be highly prevalent around some roads in the Xuanwu District, including Nanjing Forestry University. This occurrence suggests that the disease has the potential to cause substantial damage to the local ecosystem and plant life.



**Figure 1.** Symptoms of root rot of *Fatsia japonica* in the field. (A,B) In the field, infestations of *Fatsia japonica* were observed. (C) Field symptoms of root rot of *Fatsia japonica*. (D) *Fatsia japonica* root rot cross section.

#### 3.2. Morphological Identification of the Isolates

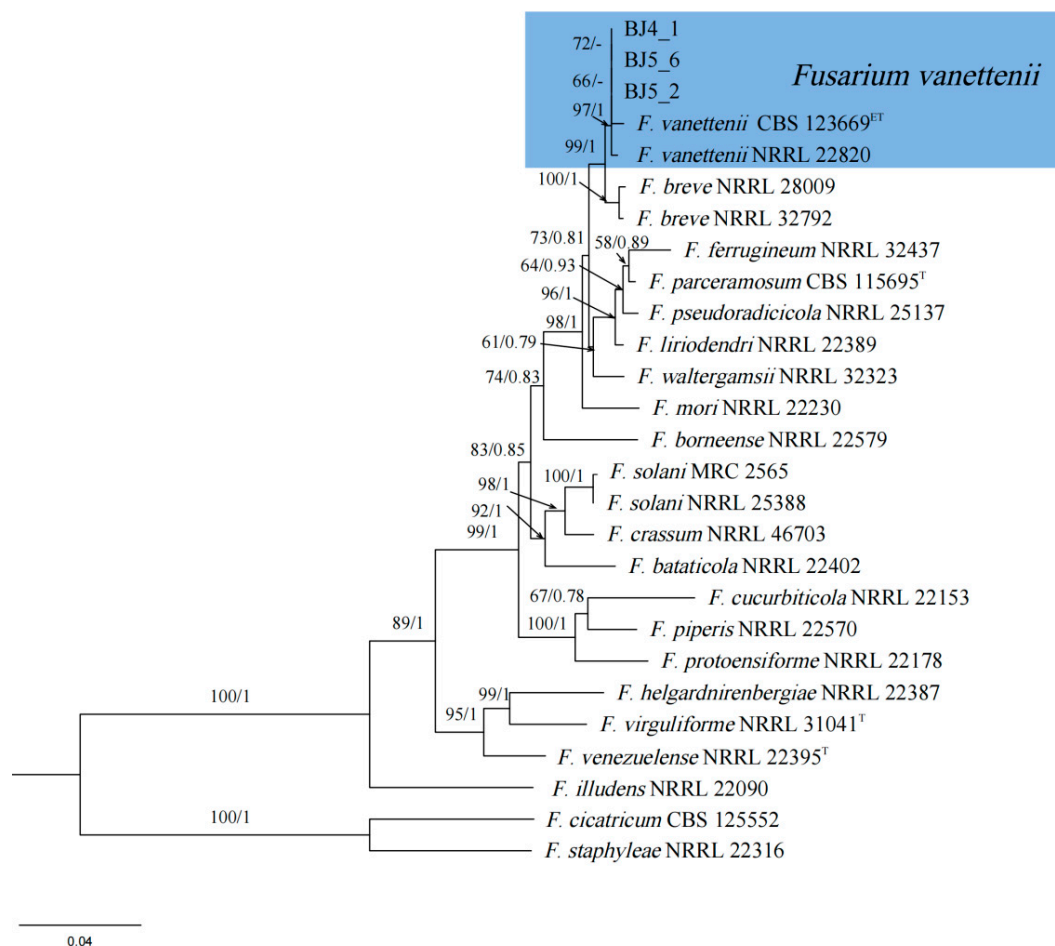
The collected samples showed abundant, dense white mycelial colonies, and cottony growth throughout the margins (Figure 2A,B). The isolated strains were kept in the pathology laboratory of Nanjing Forestry University. The macroconidia have a slightly curved sickle shape (Figure 2C), the microconidia are ovoid (Figure 2D), and the chlamydospores are formed in the mycelium and are round or ovoid, smooth-walled, and raised (Figure 2E).



**Figure 2.** Morphological features of *F. vanettenii* isolates from *Fatsia japonica*. (A,B) The morphological characteristics of BJ4-1 colonies grown on PDA after three days of isolation were analyzed. (C) Macroconidia. (D) Microconidia. (E) Chlamydospore.

### 3.3. Molecular Characterization

The genomic DNA of the strain was amplified using three pairs of primers, and gel electrophoresis yielded bands of the expected sizes (650 bp-*TEF1*, 550 bp-*ITS*, and 990 bp-*RPB2*). Upon receipt of a target fungal DNA sequence, it was further analyzed to determine its identity and potential impact. This analysis includes determining the specific species or strain of fungus by comparing the sequence to existing databases of known fungal DNA sequences. The construction of a multigene phylogenetic space between trees was achieved by using *Fusarium cicatricum* and *Fusarium staphyleae* as outgroups and combining *ITS*, *RPB2*, and *TEF1* sequences. Phylogenetic analyses revealed robust trees with well-supported clades. The Bayesian Inference (BI) and Maximum-likelihood (ML) phylogenetic analyses of the isolates of *F. vanettenii* produced topologically similar trees, and the BI posterior probabilities (PP) were plotted on the ML tree (Figure 3). Phylogenetic analyses showed that the three isolates (*BJ4-1*, *BJ5-2*, and *BJ5-6*) clustered in a distinct clade with *F. vanettenii*, which was distinct from all other known species and closely related to *F. breve* (NRRL 28009 and NRRL 32792) (Figure 3).

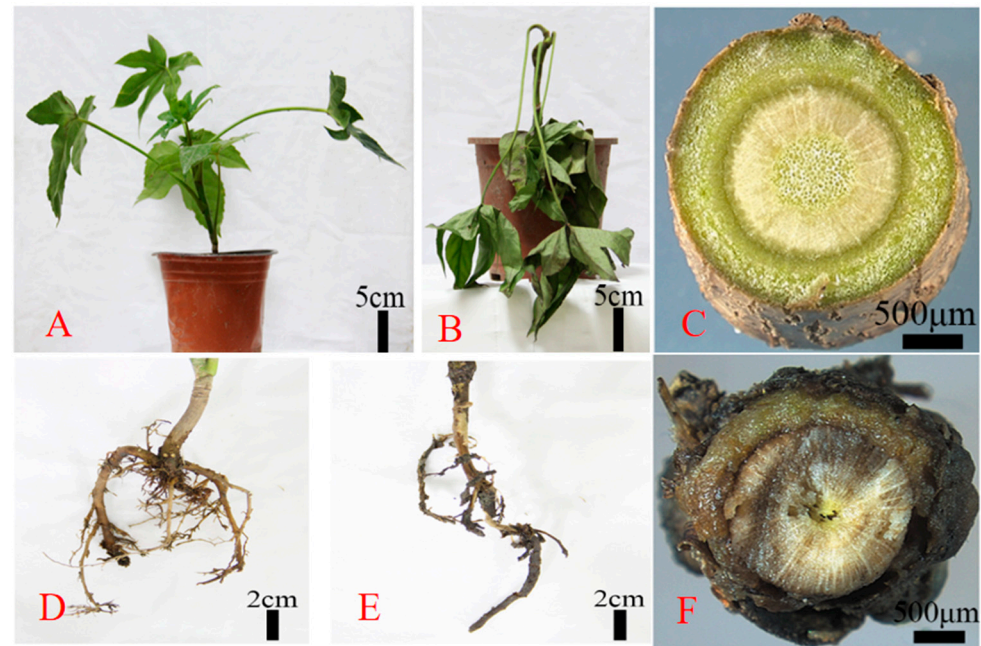


**Figure 3.** The maximum likelihood and Bayes estimation analyses of *Fusarium* cultivars were performed on the concatenated data set (*ITS*, *RPB2*, and *TEF1*). The *Fusarium vanettenii* isolates (*BJ4-1*, *BJ5-2*, and *BJ5-6*) identified in this study represent a unique lineage and have been found to form a distinct lineage with other related strains. The predicted number of substitutions per nucleotide position is indicated by the scale bar. *Fusarium cicatricum* and *Fusarium staphyleae* were used as the outgroup for this analysis. <sup>ET</sup> indicates ex-epitypes. <sup>T</sup> indicates ex-types or ex-epitypes.

### 3.4. Pathogenicity Test

The findings indicate that after 32 days of inoculation, all inoculated seedlings ( $n = 9$ ) exhibited symptoms similar to those of naturally infected plants (see Figure 4B,E,F). In

contrast, control seedlings ( $n = 3$ ) showed no symptoms (Figure 4A,C,D). After re-isolating the pathogen from all inoculated plants, the experiment was repeated three times to ensure the reliability and consistency of the results. This rigorous approach allowed the researchers to validate their findings and confirm the presence of the pathogen in each plant sampled. By conducting multiple repetitions, they were able to minimize the impact of any potential variability and obtain a more accurate representation of the pathogen's behavior and effects on the plants.



**Figure 4.** Symptoms of *Fatsia japonica* 32 days after inoculation of roots with a spore suspension of *F. vanettenii*. (A) Control plant treated with sterile water. (B) Pathogenicity of *F. vanettenii* on artificially spore-inoculated *Fatsia japonica* roots. (C) Cross section through the basal stem of a healthy control plant. (D) Healthy root system of a control plant. (E) Root rot symptoms after the inoculation of the pathogen. (F) Section of the basal stem of a diseased plant that has been inoculated with *F. vanettenii* spore suspension.

#### 4. Discussion

*Fatsia japonica*, also known as Japanese aralia, is a plant with both esthetic and practical value. It is a versatile plant that offers significant benefits. Its popularity as an ornamental plant is due to its large, glossy leaves and attractive growth habit. In addition to its visual appeal, *Fatsia japonica* has been recognized for its medicinal properties and its role in microbiology [38]. But widespread cultivation of *Fatsia japonica* has led to the emergence of several diseases. These have affected the beauty, vigor, and longevity of the plant. Anthracnose has been described from *Fatsia japonica* in China. It is infected by the pathogens, *Colletotrichum fructicola*, *Colletotrichum karstii* as well as *Colletotrichum gloeosporioides* [39,40]. In Iran and China, gray mold has been reported to cause stem ulcers and leaf blight, respectively, in *Fatsia japonica* [41]. In Europe and Korea, there have been reports of foliar diseases caused by *Alternaria panaxae* and *P. cactume* [42]. However, there are very few reports concerning *Fatsia japonica* rooting diseases.

The genus *Fusarium* is a diverse group of filamentous fungi that includes a large number of different species. These fungi have become a major concern for growers and producers worldwide because of their ability to cause disease in a wide variety of horticultural crops [43]. The ability to produce harmful toxins and enzymes that damage plant tissues and disrupt normal physiological processes is responsible for the pathogenicity of *Fusarium* spp. These fungi can cause a variety of symptoms, including wilting, stunting, discoloration, and decay of plant parts, by infecting plants through wounds, natural



openings, and even roots. *Fusarium* spp. are the subject of ongoing research by scientists and plant pathologists to gain a better understanding of their biology and pathogenic mechanisms and to develop effective control measures [44]. In the present study, it was observed that *Fusarium vanettenii* causes root rot on octocarpus plants. This fungus is an established species under the *F. solani* species complex (FSSC) [45], which mainly infects the roots of plants. For example, *F. vanettenii* has been reported to cause root rot of tomato in India [46]. Until now, there have not been many reports on *F. vanettenii*, so there may be many undiscovered natural hosts in nature.

In this study, we found that in some areas of Xuanwu District, Nanjing, *Fatsia japonica* were affected by *Fusarium*-caused root rot. This disease is a serious threat to the cultivation of *Fatsia japonica* and can lead to the death of the entire plant, reducing the ornamental value of *Fatsia japonica* and even affecting the growth of the plant. Sample collection and identification were carried out on the basis of a multi-site survey in Nanjing. In the samples collected, it was found that the disease tends to develop in humid conditions with low light, especially after rain. This affects the ecological and economic value of *Fatsia japonica*. Therefore, it is crucial for authorities and stakeholders to take immediate action to mitigate the spread of the disease and prevent further damage. Implementing control measures, such as regular monitoring, proper sanitation practices, and targeted treatments, can minimize the impact of fungal disease and protect the affected areas from further devastation. Raising the awareness among the local community about the disease and its prevention can also reduce its prevalence and safeguard the district's green spaces.

## 5. Conclusions

To conclude, the etiology of *Fatsia japonica* root disease was elucidated in this investigation. Diseased tissues were taken from symptomatic plants to perform isolations. A phylogenetic tree was constructed using the ITS, *RPB2*, and *TEF1* multigene series to identify the pathogen based on morphology, including macroconidia, microconidia and chlamydospores. Eventually, the disease was determined to be caused by *Fusarium vanettenii*. The results of the study will improve our understanding of this disease in a comprehensive and systematic manner. The detailed description of the new disease may provide plant pathologists and mycologists with new tools to better identify the disease.

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## References

1. Luo, W. Shade-tolerant Foliage Plants—Octocotyledon. *Gardening* **1997**, *5*, 15.
2. Fan, L. High-quality greening ornamental plants. Octagonal gold plate. *Agric. Sci. Technol. Newsl.* **2004**, *8*, 24–25.
3. Liu, J.; Cao, D. Practical application of ground cover plants in landscape greening. *South. Agric. Hortic. Floric.* **2010**, *4*, 33–34.
4. Du, R.; Zhu, L.; Zhou, Y.; Li, F. Application analysis of landscape plant resources in horticulture in Guiyang city. *South. Hortic.* **2013**, *5*, 42–44.
5. Qiu, S.; Chen, J.; Zhang, X.; Qiu, W.; Hu, Z. Uses and propagation technology of anise goldpan. *Mod. Agric. Sci. Technol.* **2014**, *11*, 173.
6. Lu, Z. Clinical experience in the treatment of liver cancer with octagonal gold plate. *J. Zhejiang Coll. Tradit. Chin. Med.* **1995**, *2*, 13.

7. Ma, J. Treatment of 178 cases of esophageal cardia cancer by Compound Anise Jinpan Tang. *Liaoning J. Tradit. Chin. Med.* **1985**, *8*, 23.
8. Rabodonirina, M.; Piens, M.A.; Monier, M.F.; Guého, E.; Fiévre, D.; Mojon, M. *Fusarium* infections in immunocompromised patients: Case reports and literature review. *Eur. J. Clin. Microbiol. Infect. Dis.* **1994**, *13*, 152–161. [\[CrossRef\]](#)
9. Vismer, H.F.; Marasas, W.F.O.; Rheeder, J.P.; Joubert, J.J. *Fusarium dimerum* as a cause of human eye infections. *Med. Mycol.* **2002**, *40*, 399–406. [\[CrossRef\]](#)
10. Dean, R.; Van Kan, J.A.; Pretorius, Z.A. The Top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* **2012**, *13*, 414–430. [\[CrossRef\]](#)
11. O'donnell, K.; Ward, T.J.; Robert, V.A.R.G.; Crous, P.W.; Geiser, D.M.; Kang, S. DNA sequence-based identification of *Fusarium*: Current status and future directions. *Phytoparasitica* **2015**, *43*, 583–595. [\[CrossRef\]](#)
12. Qiu, J.; Lu, Y.; He, D.; Lee, Y.W.; Ji, F.; Xu, J.; Shi, J. *Fusarium fujikuroi* species complex associated with rice, maize, and soybean from Jiangsu province, China: Phylogenetic, pathogenic, and toxigenic analysis. *Plant Dis.* **2020**, *104*, 2193–2201. [\[CrossRef\]](#)
13. Gams, W.; Nirenberg, H.I.; Seifert, K.A.; Brayford, D.; Thrane, U. Proposal to conserve the name *Fusarium sambucinum* (Hyphomycetes). *Taxon* **1997**, *46*, 111–113. [\[CrossRef\]](#)
14. Snyder, W.C.; Hansen, H.N. The species concept in *Fusarium*. *Am. J. Bot.* **1940**, *27*, 64–67. [\[CrossRef\]](#)
15. Gerlach, W.; Nirenberg, H.I. The genus *Fusarium*: A pictorial atlas. Mitteilungen aus der Biologischen Bundesanstalt fuer Land und Forstwirtschaft. *Berl. Dahl.* **1982**, *209*, 1–406. [\[CrossRef\]](#)
16. Link, J.H.F. Observationes in ordines plantarum naturales. Dissertatio Ima. Ges. Naturforschender Freunde Zu Berlin. *Magazin.* **1809**, *3*, 3–42.
17. Kelly, A.; Proctor, R.H.; Belzile, F.; Chulze, S.N.; Clear, R.M.; Cowger, C.; Elmer, W.; Lee, T.; Obanor, F.; Waalwijk, C.; et al. The geographic distribution and complex evolutionary history of the NX-2 trichothecene chemotype from *Fusarium graminearum*. *Fungal Genet. Biol.* **2016**, *95*, 39–48. [\[CrossRef\]](#)
18. Cui, W.L.; Bian, J.Y.; Li, D.W.; Wang, J.W.; Huang, L. First report of leaf blight on Chinese fir (*Cunninghamia lanceolata*) caused by *Bipolaris setariae* in China. *Plant Dis.* **2020**, *104*, 2523. [\[CrossRef\]](#)
19. Chung, P.C.; Wu, H.Y.; Wang, Y.W.; Ariyawansa, H.A.; Hu, H.P.; Hung, T.H.; Tzean, S.S.; Chung, C.L. Diversity and pathogenicity of *Colletotrichum* species causing strawberry anthracnose in Taiwan and description of a new species, *Colletotrichum miaoliense* sp. nov. *Sci. Rep.* **2020**, *10*, 146. [\[CrossRef\]](#)
20. Geiser, D.M.; Aoki, T.; Bacon, C.W. One fungus, one name: Defining the genus *Fusarium* in a scientifically robust way that preserves longstanding use. *Phytopathology* **2013**, *103*, 400–408. [\[CrossRef\]](#)
21. Lombard, L.; van der Merwe, N.A.; Groenewald, J.Z.; Crous, P.W. Generic concepts in Nectriaceae. *Stud. Mycol.* **2015**, *80*, 189–245. [\[CrossRef\]](#)
22. Crous, P.W.; Lombard, L.; Sandoval-Denis, M. *Fusarium*: More than a node or a foot-shaped basal cell. *Stud. Mycol.* **2021**, *98*, 100116. [\[CrossRef\]](#)
23. O'Donnell, K.; Whitaker, B.K.; Laraba, I.; Proctor, R.H.; Brown, D.W.; Broders, K.; Kim, H.S.; McCormick, S.P.; Busman, M.; Aoki, T.; et al. DNA sequence-based identification of *Fusarium*: A work in progress. *Plant Dis.* **2022**, *106*, 1597–1609. [\[CrossRef\]](#)
24. Hong, J.P.; Guo, M.X.; He, Y.C. A newly recorded species of *Fusarium* in China. *J. Fungal Res.* **2007**, *5*, 129–130, 133.
25. Sampietro, D.A.; Marín, P.; Iglesias, J. A molecular based strategy for rapid diagnosis of toxigenic *Fusarium* species associated to cereal grains from Argentina. *Fungal Biol.* **2010**, *114*, 74–81. [\[CrossRef\]](#)
26. Yli-Mattila, T.; Paavanen-Huhtala, S.; Bulat, S.A.; Alekhina, I.A.; Nirenberg, H.I. Molecular, morphological and phylogenetic analysis of the *Fusarium avenaceum*/F. *arthrosporioides*/F. *tricinctum* species complex—A polyphasic approach. *Mycol. Res.* **2002**, *106*, 655–669. [\[CrossRef\]](#)
27. Damm, U.; Mostert, L.; Crous, P.W.; Fourie, P.H. Novel *Phaeoacremonium* species associated with necrotic wood of *Prunus* trees. *Persoonia* **2008**, *20*, 87–102. [\[CrossRef\]](#)
28. O'Donnell, K.; Kistler, H.C.; Cigelnik, E.; Ploetz, R.C. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2044–2049. [\[CrossRef\]](#)
29. O'Donnell, K.; Nirenberg, H.I.; Aoki, T.; Cigelnik, E. A multigene phylogeny of the *Gibberella fujikuroi* species complex: Detection of additional phylogenetically distinct species. *Mycoscience* **2000**, *41*, 61–78. [\[CrossRef\]](#)
30. White, T.J.; Bruns, T.; Lee, S.J.W.T.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., Eds.; Academic Press: Cambridge, MA, USA, 1990; pp. 315–322.
31. Liu, Y.J.; Whelen, S.; Hall, B.D. Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerase II subunit. *Mol. Biol. Evol.* **1999**, *16*, 1799–1808. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Hall, T.A. Bioedit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/ nt. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95–98.
33. Zhang, D.; Gao, F.; Jakovlić, I.; Zou, H.; Zhang, J.; Li, W.X.; Wang, G.T. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Mol. Ecol. Resour.* **2020**, *20*, 348–355. [\[CrossRef\]](#) [\[PubMed\]](#)

34. Nguyen, L.T.; Schmidt, H.A.; Von Haeseler, A.; Minh, B.Q. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [[CrossRef](#)]
35. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.F.; Von Haeseler, A.; Jermini, L.S. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* **2017**, *14*, 587–589. [[CrossRef](#)]
36. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [[CrossRef](#)] [[PubMed](#)]
37. Zhou, J.; Xu, T.; Xu, X.; Dai, T.; Liu, T. The New Report of Root Rot on *Fatsia japonica* Caused by *Phytophthora nicotianae* in China. *Forests* **2023**, *14*, 1459. [[CrossRef](#)]
38. Ho, H.H. The taxonomy and biology of *Phytophthora* and *Pythium*. *J. Bacteriol. Mycol.* **2018**, *6*, 40–45. [[CrossRef](#)]
39. Wang, G.L. Study on Scab-anthrax of *Fatsia japonica*. *J. Zhejiang For. Sci. Technol.* **2007**, *27*, 64–67. Available online: <https://kns.cnki.net/kcms/detail/detail.aspx?FileName=ZJLK200705016&DbName=CJFQ2007> (accessed on 15 May 2023).
40. Shi, N.N.; Du, Y.X.; Chen, F.R.; Ruan, H.C.; Yang, X.J. First report of leaf spot caused by *Colletotrichum fructicola* on Japanese *Fatsia* (*Fatsia japonica*) in Fujian Province in China. *Plant Dis.* **2017**, *101*, 1552. [[CrossRef](#)]
41. Li, Y.L.; Wang, S.B.; Wang, Y.H.; Lin, Q.K.; Zhou, Z. First Report of *Botryosphaeria dothidea* Causing a Leaf Wilt on *Fatsia japonica* in Henan Province, China. *Plant Dis.* **2018**, *102*, 450. [[CrossRef](#)]
42. Mehrabi-Koushki, M.; Artand, S.; Ahmadpour, S.A. *Botryosphaeria dothidea* causes stem canker on *Fatsia japonica* in Iran. *Australas. Plant Dis. Notes* **2021**, *16*, 31. [[CrossRef](#)]
43. Garibaldi, A.; Gilardi, G.; Gullino, M.L. First Report of Alternaria Leaf Blight of *Aralia japonica* Caused by *Alternaria panax* in Europe. *Plant Dis.* **2004**, *88*, 82. [[CrossRef](#)]
44. Deng, J.X.; Paul, N.C.; Park, M.S.; Yu, S.H. Molecular characterization, morphology, and pathogenicity of *Alternaria panax* from araliaceous plants in Korea. *Mycol. Prog.* **2013**, *12*, 383–396. [[CrossRef](#)]
45. Porter, L.; Pasche, J.; Chen, W.; Harveson, R. Isolation, identification, storage, pathogenicity tests, hosts, and geographic range of *Fusarium solani* f. sp. pisi causing *Fusarium root rot* of Pea. *Plant Health Prog.* **2015**, *16*, 136–145. [[CrossRef](#)]
46. Rubin, D.; Deeba, K.; Bishnu, B.M. First report of root rot disease on *Solanum lycopersicum* L. caused by *Fusarium vanettenii* in India. *J. Phytopathol.* **2021**, *169*, 752–756.

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