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First Report of the Peach Leaf Spot Caused by *Nigrospora sphaerica* in China

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Abstract: Peach (*Prunus persica* L.) is a globally significant fruit valued for its high edible and ornamental qualities. Peach leaf spot disease has become increasingly prevalent in recent years, negatively affecting fruit quality and esthetic appeal. During the summer of 2024, symptoms of leaf spots were observed on peach trees in an orchard in Bazhong City, Sichuan Province, China. Leaf samples displaying typical spot symptoms were collected from peach orchards, and the pathogenic agents were isolated. Based on their morphological characteristics and multi-locus phylogenetic analysis, the isolated and purified fungus SCBZPP6 was identified as *Nigrospora sphaerica*. Furthermore, the pathogenicity of the isolated fungus was verified via Koch's postulates. To our knowledge, this is the first report of *N. sphaerica* causing leaf spot on peach in China.

Keywords: peach; leaf spot; identification; *Nigrospora sphaerica*

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1. Introduction

Peach (*Prunus persica* L.) ranks as the third most economically significant in temperate regions worldwide, valued for its ornamental blooms and nutritious fruits [1]. With a long history of cultivation, peaches are grown globally in countries including China, Spain, Italy, Turkey, Iran, the USA, Egypt, Chile, and India. In China, the peach cultivation area reached 840 thousand hectares, with a production of approximately 15.8 million tons, as reported by the Food and Agriculture Organization of the United Nations (FAO) 2022 Report [2]. Beyond fresh consumption, peaches are employed in various forms, such as dried fruit snacks, fruit juice, and canned fruit. However, the productivity and quality of peach fruits are greatly threatened by various diseases, resulting in a marked reduction in fruit yield and significantly limiting the development of peach production.

Approximately twenty diseases have been reported in peach, including bacterial spot caused by *Xanthomonas arboricola* pv. *pruni* (*Xap*), bacterial canker caused by *Pseudomonas syringae*, brown rot caused by *Monilinia fructicola*, powdery mildew caused by *Podosphaera pannosa*, and peach leaf curl caused by *Taphrina deformans* [3–6]. To date, compared with these peach diseases, less attention has been paid to the peach leaf spots caused by fungi. It has been reported that peach leaf spots occur frequently and affect fruit quality and yield [7–9]. However, until now, the identification of the peach leaf spot diseases caused by fungi has been rarely reported.

Nigrospora species are widely distributed in nature, with 45 species currently recorded in the MycoBank database [10]. This genus has a broad host range and mainly infects plant leaves and stems [11]. Among them, *N. sphaerica* and *N. oryzae* are notable plant pathogens [12]. *N. sphaerica* typically infects plant leaves, causing leaf spot disease. In recent years, new hosts of this pathogen have been continuously discovered, such as pumpkin, blueberry plants, watermelon, *Rhododendron* simsii, cacao, passion fruit, and olive [10,12–17].

Furthermore, *N. sphaerica* can also cause human infections, including onychomycosis and corneal ulcer [11].

In August 2024, leaf spot symptoms were observed on peach in an orchard in Bazhong City, Sichuan Province, China. Initially, the lesions appeared as small, irregular, brown spots with indistinct edges. As the disease progressed, the lesions expanded and often coalesced, occasionally forming perforations in the centers. Over time, the lesion color darkened to black or deep brown. These symptoms were distinct from the water-soaked lesions typically caused by *Xap* [3]. Accurate identification of the causal agent responsible for the peach leaf spot is critical for effective disease control and orchard management. This study aims to identify the pathogen through a combination of morphological and molecular characterization.

2. Materials and Methods

2.1. Sample Collection

Peach leaves with typical lesions were harvested from an orchard in Bazhong City (106°44′35.97″ E, 31°52′12.27″ N), Sichuan Province, China. The collected leaves were wrapped in moistened cotton to retain moisture and placed in a labeled sterile sample bag. The samples were then immediately transported to the laboratory for pathogen isolation. After two weeks, the similar-sized healthy leaves were collected from the orchard for pathogenicity testing.

2.2. Isolation and Purification of Pathogenic Fungi

To isolate pathogens, the boundary tissues of the leaf lesions were cut into 1 cm × 1 cm fragments, disinfected in 75% ethanol for 1 min, rinsed with sterile water for three times, then immersed in a 3% sodium hypochlorite solution for 1 min, followed by rinsing with sterile water three times. The treated fragments were dried on filter paper and incubated on potato dextrose agar (PDA) plates at 25 °C for two days. The isolated strains were purified by picking the hyphal tip from the colonial margin two or three times [18].

2.3. Morphological Identification

For morphological identification, the purified strains were cultured on PDA plates at 25 °C for 5–7 days. The colony morphology was observed when the fungal hyphae reached the plate edges. The characteristics of the mycelium and spores were observed under an optical microscope (Olympus, Chongqing, China).

2.4. Molecular Identification and Phylogenetic Analysis

Approximately 0.5 g of fresh mycelia were harvested from 7-day-old PDA cultures and ground into a fine powder in liquid nitrogen. Total genomic DNA was extracted using CTAB solution (2 M Tris-HCl, 5 M NaCl, 0.5 M EDTA, 2% (*w*/*v*) CTAB, and 0.2% (*v*/*v*) mercaptoethanol) [19]. DNA quantity and quality were assessed using 1.0% (*w*/*v*) agarose gel stained with GelRed nucleic acid stain (Vazyme, Nanjing, China). For molecular identification, primer pairs ITS1/ITS4 [20], EF1-728F/EF1-986R [21], and Bt2a/Bt2b [22] were used to amplify the internal transcribed spacer (ITS), partial translation elongation factor 1-alpha (*TEF1-a*), and *β-tubulin* (*TUB2*) sequences of all isolated fungal strains (Table 1). PCR was carried out with the following conditions: 35 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, and elongation at 72 °C for 1 min, with an initial denaturation step at 95 °C for 5 min. PCR products were purified using Omega Bio-tek's E.Z.N.A.® Gel Extraction Kit and cloned into the pMD18-T vector (Takara, Shiga, Japan) for sequencing. The obtained sequences were compared with reference sequences using the BLAST tool on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on 23 October 2024). The amplified sequences in this study were aligned with the reference sequences using Clustal W2. The phylogenetic tree was constructed using the maximum-likelihood (ML) method via MEGA7.0 software, and the reliability of the tree was assessed using 1000 bootstrap replicates.

Table 1. List of primers used in this study.

Gene	Primer	Sequence (5′–3′)	Size
ITS	ITS1 ITS4	TCCGTAGGTGAACCTGCGG TCCTCCGCTTATTGATATGC	555 bp
TEF1-a	EF1-728F EF1-986R	CATCGAGAAGTTCGAGAAGG TACTTGAAGGAACCCTTACC	279 bp
TUB2	Bt2a Bt2b	GGTAACCAAATCGGTGCTGCTTTC ACCCTCAGTGTAGTGACCCTTGGC	434 bp

2.5. Pathogenicity Test

To assess the pathogenicity of the fungal isolates, healthy peach leaves were surface sterilized by immersion in 75% ethanol for 1 min, followed by being rinsed in sterilized distilled water three times. The leaves were wounded by pin-pricking the surface with a sterilized needle. Subsequently, 5 mm diameter mycelial plugs were obtained from the edges of a fresh colony and inoculated on the wounded leaves. Plugs of PDA were used as controls. The petioles of the inoculated leaves were wrapped with moistened cotton to retain moisture. The inoculated leaves were kept in a plastic box with high humidity at 28 °C in the dark for disease development [23]. To fulfill Koch's postulates, re-isolations were made from the diseased leaves.

3. Results

During the summer investigation, 15% of peach plants exhibited leaf spot symptoms under high humidity conditions. A total of six isolates were obtained from the diseased leaf samples and cultured on PDA plates for 5 days. These isolates exhibited consistent morphological features. Colonies on PDA were white and flocculent, but their reverse side was pale yellow. Under microscopic examination, conidiogenous cells were colorless and subglobose. The conidia were acrogenous, solitary, black, and globose with diameters of 15–19 μm (Figure 1). Based on the morphological characteristics, these isolates were tentatively identified as belonging to the genus *Nigrospora* sp.

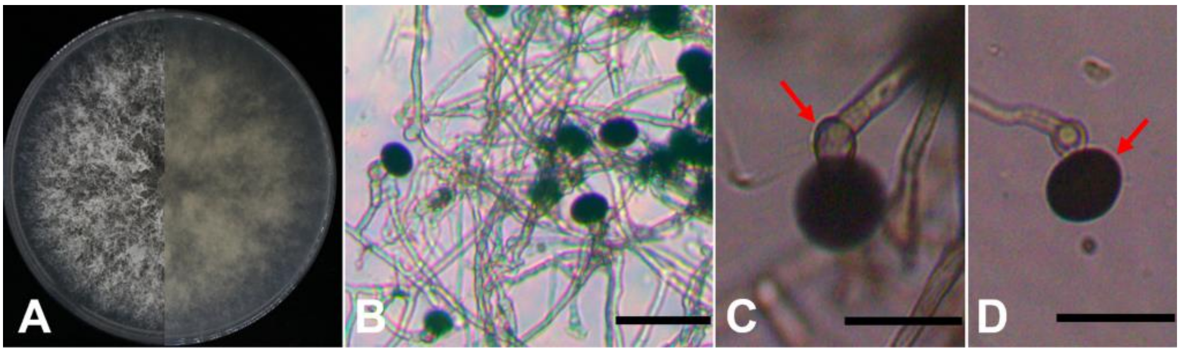

Figure 1. Morphological characteristics of the SCBZPP6 strain. (**A**) Front and reverse view of the colony on PDA after five days; (**B**) Colony on PDA; (**C**,**D**) Conidiogenous cell (red arrow) and conidia. Scale bars: (**B**) = 50 μm; (**C**,**D**) = 20 μm.

For precise molecular identification, we performed PCR using primer pairs ITS1/ITS4, EF1-728F/EF1-986R and Bt2a/Bt2b. The fragment sizes of PCR products were about 555 bp, 279 bp, and 434 bp, respectively. These fragments were cloned into the pMD18-T vector and then sequenced. All six isolates displayed identical nucleotide sequences in the ITS, *TEF1-a* or *TUB2* regions based on multiple sequence alignment analysis. The ITS, *TEF1-a*, and *TUB2* sequences of the representative isolate SCBZPP6 were further analyzed via NCBI Blast. The ITS region sequence of SCBZPP6 showed a 100% match with *N. sphaerica* strains (KU553345.1, KC519729.1, and PQ289163.1). The partial *TEF1-a* sequences of SCBZPP6 showed 99.2–100% identity with *N. sphaerica* (OM826971.1, MN053315.1, KY019393.1, and KY513872.1). Similarly, the *TUB2* region sequence was 99.5–99.7% identical to *N. sphaerica* (MK408565.1, OM826974.1, and MN719407.1).

Then, the ITS, *TEF1-a*, and *TUB2* sequences were submitted to the NCBI and assigned accession numbers PQ496816, PQ505108, and PQ505109, respectively. To analyze evolutionary relationships, a phylogenetic tree was constructed using the ITS, *TEF1-a*, and *TUB2* concatenated sequence. The phylogenetic results showed that SCBZPP6 was most closely related to *N. sphaerica* (Figure 2). Based on these findings, the causal agent of the leaf spot on peach was identified as *N. sphaerica* based on morphological and molecular characteristics.

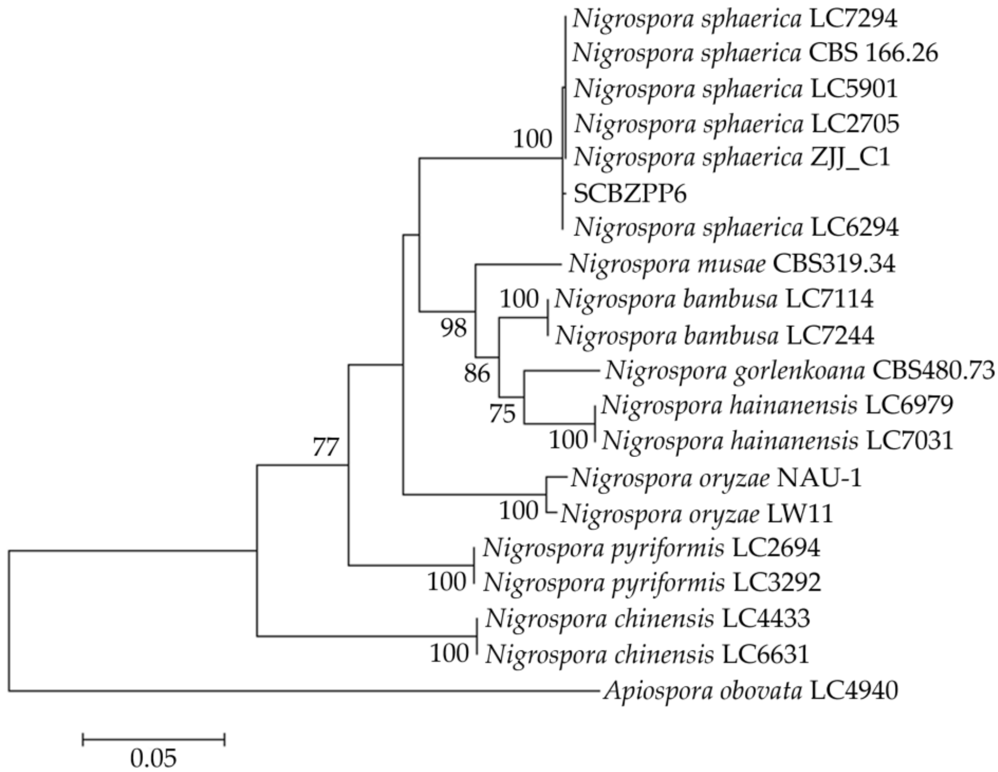

Figure 2. Phylogenetic tree generated from the maximum likelihood analysis based on ITS, *TEF1-a*, and *TUB2* sequences of the isolate SCBZPP6 and 20 strains representing 9 species of the *genus Nigrospora*. Bootstrap values greater than 70% are shown at the nodes. *Apiospora obovata* was chosen as an outgroup.

To assess the pathogenicity of SCBZPP6, an inoculation experiment was conducted under controlled conditions. Leaves inoculated with SCBZPP6 began to exhibit visible brown spots ten days after inoculation. These spots closely resembled the symptoms observed on naturally infected plants in the field, including size, shape, and color (Figure 3). In contrast, no symptoms were observed on the control leaves inoculated with PDA plugs (Figure 3).

To confirm that SCBZPP6 was indeed the causal agent, the pathogen was re-isolated from the diseased leaves and subjected to morphological and molecular characterization. The re-isolated pathogen displayed characteristics identical to the original isolate SCBZPP6, both in colony morphology and genetic sequence analysis, fulfilling Koch's postulates. This demonstrated that SCBZPP6 is capable of causing the observed leaf spot symptoms on peach.

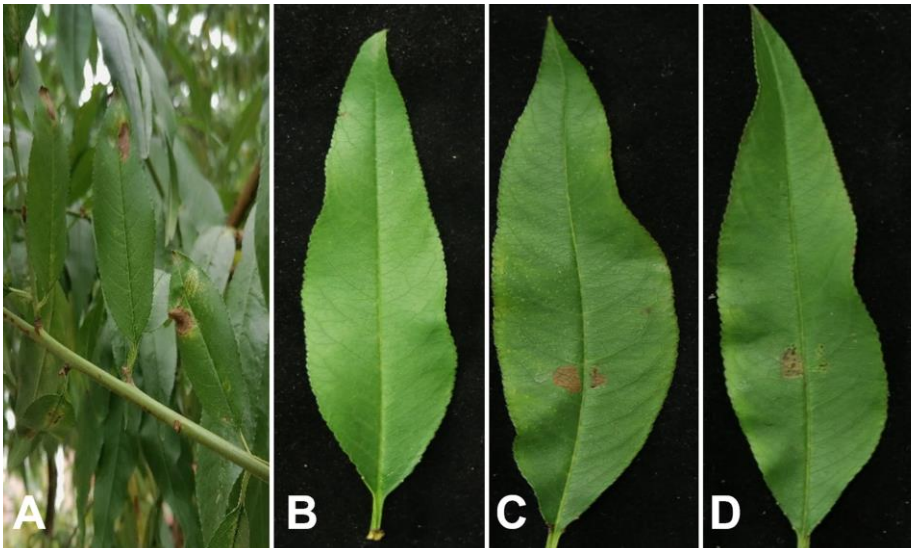

Figure 3. Symptoms of leaf spots on peach (**A**) peach leaf spots symptom in the field; (**B**) control peach leaf inoculated with PDA; (**C**,**D**) peach leaves inoculated with SCBZPP6.

4. Discussion

In recent years, significant climate changes, such as rising temperatures and increased precipitation, are believed to have contributed to a higher incidence of leaf diseases. Among these, leaf spot caused by *Xap* is the most common on peaches [3]. The initial symptoms on leaves typically presented as small, brown, and water-soaked lesions. As the lesions enlarge, the centers become surrounded by yellow halos and eventually fall off, forming irregular perforations. In this study, the symptoms of the peach leaf spot were distinguishable from those caused by bacterial pathogens. Morphological and molecular analyses identified the causal agent as *N. sphaerica*. To our knowledge, this is the first report of peach leaf spot disease caused by *N. sphaerica*.

Nigrospora species are known for their broad host range, with *N. sphaerica* being one of the most reported *Nigrospora* species. So far, *N. sphaerica* has been documented to cause diseases in over 40 plants, including vegetables, fruits, flowers, herbs, and ornamental trees [10,15–17,24–29]. While the fungus primarily causes leaf spot diseases, it can also infect twigs, roots, and fruits. Its broad host range may facilitate its spread to other nearby plants in Bazhong City, emphasizing the need for timely and effective management measures based on accurate pathogen identification.

Historically, morphological characteristics, particularly conidial dimensions, have been the main criteria for identifying *Nigrospora* species. However, these methods often fall short of providing precise identification. In this study, we obtained isolates from peach leaves that displayed morphological characteristics consistent with *N. sphaerica* [23]. Despite this, morphological identification alone is not always reliable, which is why we employed molecular characterization techniques, including ITS, *TEF1-α*, and *TUB* gene sequence analysis, to accurately identify the isolates at the species level.

This study highlights the importance of accurate pathogen identification and the adoption of sustainable management practices to mitigate the impact of *N. sphaerica* on peach production.

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References

1. RawanRawandoozi, Z.J.; Hartmann, T.P.; Carpenedo, S.; Gasic, K.; da Silva Linge, C.; Cai, L.; Van de Weg, E.; Byrne, D.H. Identification and characterization of QTLs for fruit quality traits in peach through a multi-family approach. *BMC Genom.* **2020**, *21*, 522. [CrossRef]
2. Karim, M.M.; Usman, H.M.; Tan, Q.; Hu, J.-J.; Fan, F.; Hussain, R.; Luo, C.-X. Fungicide resistance in *Colletotrichum fructicola* and *Colletotrichum siamense* causing peach anthracnose in China. *Pestic. Biochem. Physiol.* **2024**, *203*, 106006. [CrossRef] [PubMed]
3. Luo, C.-X.; Schnabel, G.; Hu, M.; De Cal, A. Global distribution and management of peach diseases. *Phytopathol. Res.* **2022**, *4*, 30. [CrossRef]
4. Dong, J.; Shi, H.; Wu, Y.; Yang, L.; Zhu, F.; Ji, Z. Identification and pathogenicity analysis of *Fusarium* spp. on peach in China. *BMC Microbiol.* **2023**, *23*, 211. [CrossRef]
5. Zhou, Y.; Manawasinghe, I.S.; He, Z.; Zhang, W.; Liu, M.; Song, J.; Li, S.; Fan, Z.; Yan, J. Microfungi Associated with Peach Branch Diseases in China. *J. Fungi* **2024**, *10*, 217. [CrossRef]
6. Yang, L.; Wang, L.; Cao, J.; Zhu, Y.; Zhang, L.; Jin, W.; Zhu, F.; Ji, Z. Molecular and Biological Characterization of Two New Species Causing Peach Shoot Blight in China. *Plant Dis.* **2022**, *106*, 182–189. [CrossRef]
7. Jin, S.; Wang, N.; Zhou, Z.; Ji, Z.; Zhang, J. Identification of *Bacillus* species causing bacterial leaf spot of peach in China. *J. Phytopathol.* **2022**, *170*, 811–819. [CrossRef]
8. Inoue, K.; Nasu, H. Black Spot of Peach Caused by *Alternaria alternata* (Fr.) Keissler. *J. Gen. Plant Pathol.* **2000**, *66*, 18–22. [CrossRef]
9. Ji, Z.L.; Zhang, S.W.; Zhu, F.; Wan, B.X.; Liang, R.Z. First Report of *Arthrinium arundinis* Causing Leaf Edge Spot of Peach in China. *Plant Dis.* **2020**, *104*, 3077. [CrossRef]
10. Petrović, E.; Vrandečić, K.; Ćosić, J.; Đermić, E.; Godena, S. First Report of *Nigrospora* Species Causing Leaf Spot on Olive (Olea europaea L.). *Horticulturae* **2023**, *9*, 1067. [CrossRef]
11. Wang, M.; Liu, F.-l.; Crous, P.W.; Cai, L.-Z. Phylogenetic reassessment of *Nigrospora*: Ubiquitous endophytes, plant and human pathogens. *Persoonia Mol. Phylogeny Evol. Fungi* **2017**, *39*, 118–142. [CrossRef] [PubMed]
12. Alam, M.W.; Rehman, A.; Ahmad, S.; Sarwar, M.; Nawaz, A.; Khan, S.M.; Ali, S.; Aslam, S.; Mannan, A. First report of *Nigrospora sphaerica* causing leaf spot of date palm in Pakistan. *J. Plant Pathol.* **2020**, *102*, 223. [CrossRef]
13. Ismail, S.I.; Abd Razak, N.F. First Report of *Nigrospora sphaerica* Causing Leaf Spot on Watermelon (Citrullus lanatus) in Malaysia. *Plant Dis.* **2021**, *105*, 488. [CrossRef] [PubMed]
14. Wang, Y.; Cernava, T.; Zhou, X.; Yang, L.; Baccelli, I.; Wang, J.; Gou, Y.; Sang, W.; Chen, X. First Report of Passion Fruit Leaf Blight Caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2022**, *106*, 323. [CrossRef]
15. Wang, Y.; Li, H.; Liu, L.; Javed, K.; Lin, J.; Li, Z.; Ding, H. First Report of *Nigrospora sphaerica* Causing Leaf Spot on *Rhododendron simsii* in China. *Plant Dis.* **2024**, *108*, 1395. [CrossRef]
16. Villanueva, J.D.; Solpot, T.C.; Tangonan, N.G. *Nigrospora sphaerica* causing Leaf Blight Disease of Cacao in the Philippines. *Indian Phytopathol.* **2023**, *76*, 915–922. [CrossRef]
17. Liu, J.; Deng, Y.; Liu, Q.; Zhang, K.; Zhao, Y.; Ma, T.; Chang, W.; Wang, H. First Report of Leaf Blight on Pumpkin Caused by Nigrospora sphaerica in China. *Plant Dis.* **2024**, *108*, 2574. [CrossRef]
18. Hao, Y.; Aluthmuhandiram, J.V.S.; Chethana, K.W.T.; Manawasinghe, I.S.; Li, X.; Liu, M.; Hyde, K.D.; Phillips, A.J.L.; Zhang, W. *Nigrospora* Species Associated with Various Hosts from Shandong Peninsula, China. *Mycobiology* **2020**, *48*, 169–183. [CrossRef]
19. Lee, S.B.; Milgroom, M.G.; Taylor, J.W. A rapid, high yield mini-prep method for isolation of total genomic DNA from fungi. *Fungal Genet. Rep.* **1988**, *35*, 23–24. [CrossRef]
20. White, T.J.; Bruns, T.D.; Lee, S.B.; Taylor, J.W. 38—Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In *PCR—Protocols and Applications—A Laboratory Manual*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press, Inc.: Cambridge, MA, USA, 1990; pp. 315–322.
21. Woudenberg, J.H.C.; Aveskamp, M.M.; Gruyter, J.; Spiers, A.G.; Crous, P. Multiple Didymella teleomorphs are linked to the Phoma clematidina morphotype. *Persoonia* **2009**, *22*, 56–62. [CrossRef]
22. Carbone, I.; Kohn, L.M. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **1999**, *91*, 553–556. [CrossRef]
23. Shi, J.; Li, B.; Wang, S.; Zhang, W.; Shang, M.; Wang, Y.; Liu, B. Occurrence of *Neopestalotiopsis clavispora* Causing Apple Leaf Spot in China. *Agronomy* **2024**, *14*, 1658. [CrossRef]
24. Bansal, N.; Juwantha, R.; Pandey, S. Morphology and ITS-based phylogeny revealed *Nigrospora sphaerica* causing leaf spot and blight disease of *Bambusa polymorpha* in India. *Physiol. Mol. Plant Pathol.* **2024**, *132*, 102315. [CrossRef]
25. Khoo, Y.W.; Khaw, Y.S.; Tan, H.T.; Li, S.-F.; Chong, K.P. First Report of Stem Brown Spot on 'Thai Gold' Selenicereus megalanthus in Malaysia Caused by *Nigrospora sphaerica*. *Plant Dis.* **2023**, *107*, 223. [CrossRef]

26. Sadid, K.N.E.; Hossain, M.A.; Munshi, A.R.; Karim, M.R. First detection, isolation and molecular characterization of banana leaf spot diseases caused by *Nigrospora sphaerica* and *Neocordana musae* in Bangladesh. *Arch. Phytopathol. Plant Prot.* **2023**, *56*, 1112–1125. [CrossRef]
27. Li, J.; Xu, J.; Wang, H.; Wu, C.; Zheng, J.; Zhang, C.; Han, Y. First Report of Fungal Pathogens Causing Leaf Spot on *Sorghum–Sudangrass* Hybrids and Their Interactions with Plants. *Plants* **2023**, *12*, 3091. [CrossRef]
28. Zeng, Y.; Luo, M.-Y.; Yang, M.-f.; Jiang, Y.-L. First Report of Leaf Spot on *Zanthoxylum bungeanum* Caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2024**, *108*, 812. [CrossRef]
29. Tejaswini, G.S.; Mahadevakumar, S.; Joy, J.; Chandranayaka, S.; Niranjan Raj, S.; Devi, L.; Sowjanya, R.; Sowmya, R. First Report of *Nigrospora sphaerica* Associated with Leaf Spot Disease of Crossandra infundibuliformis in India. *Plant Dis.* **2023**, *107*, 2218. [CrossRef]