**Nigrospora** Species Associated with Various Hosts from Shandong Peninsula, China

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**ABSTRACT**

*Nigrospora* is a monophyletic genus belonging to Apiosporaceae. Species in this genus are phytopathogenic, endophytic, and saprobic on different hosts. In this study, leaf specimens with disease symptoms were collected from host plants from the Shandong Peninsula, China. The fungal taxa associated with these leaf spots were studied using morphology and phylogeny based on ITS, TEF1, and TUB2 gene regions. In this article, we report on the genus *Nigrospora* with *N. gorlenkoana*, *N. oryzae*, *N. osmanthi*, *N. rubi*, and *N. sphaerica* identified with 13 novel host associations including crops with economic importance such as bamboo and Chinese rose.

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1. Introduction

The genus *Nigrospora* Zimm. (Apiosporaceae, Xylariales, and Sordariomycetes) was established to accommodate *N. panici* Zimm. [1,2]. *Nigrospora* species are cosmopolitan, filamentous, dematiaceous taxa, with a diverse host range including crops with economic importance [2,3]. Species of this genus are pathogens, endophytes, and saprobes of various hosts [4,5]; Table 1 presents a list of reported *N.* occurrences including disease incidents. These studies emphasize *N. oryzae* and *N. sphaerica* as the most frequently reported pathogens of *Nigrospora*.

Species of *Nigrospora* harbor a great potential in bioactive secondary metabolite production. *N. sphaerica* is a rich source of secondary metabolites such as bioactive compounds with antileukemic (tested on HL60 and K562 cell lines), antileishmanial, and antifungal activities [6]. An endophytic *Nigrospora* species isolated from *Moringa oleifera* root produced a few important bioactive secondary metabolites under in vitro conditions, including griseofulvin, dechlorogriseofulvin, and mellein with antifungal activity [7]. A new hydroanthraquinone derivative and new azaphilones produced by *Nigrospora* sp. YE3033 was reported to be successful in inhibiting influenza viral strain of A/Puerto Rico/8/34 (H1N1) [8].

Species delimitation in *Nigrospora* was previously based on morphological characters [9], but it was found that some key morphological characters such as conidial dimensions overlap between phylogenetically distinct species [3]. To address this issue, a polyphasic approach, combining both morphology and molecular phylogeny, is necessary. A recent study reassessing *Nigrospora* species by Wang et al. [3] sequenced previously introduced *Nigrospora* species from their herbarium materials. Further, they affirmed the placement of the genus in Apiosporaceae (Xylariales) based on multi-locus molecular phylogeny (internal transcribed spacer (ITS), translation elongation factor 1-α (TEF1) and β-tubulin (TUB2) gene regions) [3]. In their study, the new species *N. aurantiaca* Mei Wang & L. Cai, *N. bambusae* Mei Wang & L. Cai, *N. camelliasinensis* Mei Wang & L. Cai, *N. chinensis* Mei Wang & L. Cai, *N. guilinensis* Mei Wang & L. Cai,

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Table 1. Occurrences of *Nigrospora* species on different hosts and their nutritional relationship.

| Causative agent | Nutritional relationship | Disease | Host | Region | References |
|-----------------|--------------------------|---------|------|--------|------------|
| *N. lacticonia* | Pathogenic | Reddish brown spots | *Hyllocerus polyrhizus* | Malaysia | Kee et al. [32] |
| *N. musae*     | Endophytic | NA | *Musa acuminate* | Australia | Palmeate et al. [34] |
| *N. oryzae*    | Pathogenic | Lint rot | *Gossypium hirsutum* | Alabama | Sharma et al. [35] |
| *N. oryzae*    | Pathogenic | Stem blight | *Brassica juncea* | India | Zhai et al. [36] |
| *N. oryzae*    | Pathogenic | Leaf spot | *Aloe vera* | China | Wu et al. [37] |
| *N. oryzae*    | Pathogenic | Leaf spot | *Dendrobiun candidum* | China | Li et al. [38] |
| *N. oryzae*    | Pathogenic | Brown/black spot | *Actinidia delicosa* | NA | |
| *N. oryzae*    | Pathogenic | Leaf spots | *Gossypium hirsutum* | China | Zhang et al. [28] |
| *N. oryzae*    | Pathogenic | Leaf spots | *Poa pratensis* | Canada | Zheng et al. [39] |
| *N. oryzae*    | Pathogenic | Foliar and cane rot | *Arundo donax* | France, Crete, Cyprus, Italy, Morocco, and Spain | Widmer et al. [27] |
| *N. osmanthi*  | Pathogenic | Leaf blight | *Pearl millet* | Iran | Kalati et al. [40] |
| *N. osmanthi*  | Pathogenic | Leaf blight | *Phoenix dactylifera* | Iraq | Abass [41] |
| *N. oryzae*    | Endophytic | NA | *Emblica officinalis* | India | Rathod et al. [42] |
| *N. oryzae*    | Endophytic | NA | *Artemisia sp.* | China and Canary Islands | Cosoveanu [43] |
| *N. oryzae*    | Saprobiic | NA | *Musa acuminate* | Hong Kong and Australia | Brown et al. [33] |
| *N. osmanthi*  | Pathogenic | Leaf blight | *Stenotaphrum secundatum* | Tropics and sub-tropics and China | Mei et al. [44] |
| *N. sacchari*  | Pathogenic | Leaf blight | *Ficus pandurata* | China | Liu et al. [45] |
| *N. sphaeric*a | Endophytic | NA | *Bauhinia phoenicea* | India | Raviraja et al. [46] |
| *N. sphaeric*a | Pathogenic | Leaf blight | *Sesamum indicum* | China | Zhao et al. [47] |
| *N. sphaeric*a | Pathogenic | Leaf blight | *Saccharum* | China | Cui et al. [48] |
| *N. sphaeric*a | Pathogenic | Leaf blight | *Camellia sinensis* | India | Dutta et al. [49] |
| *N. sphaeric*a | Pathogenic | Leaf blight | *Morus alba* | India and China | Chen et al. [50], Arunakumar et al. [51] |
| *N. sphaeric*a | Pathogenic | Leaf spots, twigs, and shoot blight | *Vaccinium corymbosum* | Buenos Aires, Entre Ríos | Wright et al. [31] |
| *N. sphaeric*a | Pathogenic | Leaf and stem black spot | *Phoenix dactylifera* | Iraq | Abass [41], Abass et al. [52] |
| *N. sphaeric*a | Pathogenic | Reddish brown spots | *Hyllocerus polyrhizus* | Malaysia | Kee et al. [32] |
| *N. sphaeric*a | Pathogenic | Black end and squirter disease | *Musa sp.* | Australia | Allen [53], Simmonds [54] |
| *N. sphaeric*a | Pathogenic | Leaf spots | *Actinidia sp.* | China | Chen et al. [55] |
| *N. sphaeric*a | Pathogenic | Postharvest rot | *Actinidia sp.* | China | Li et al. [56] |
| *N. sphaeric*a | Pathogenic | Leaf spots | *Lagenaria siceraria* | Georgia | Li et al. [57] |
| *N. sphaeric*a | Pathogenic | Leaf blight | *Camellia sinensis* | China | Liu et al. [58] |
| *N. sphaeric*a | Pathogenic | Leaf blight | *Cunninghamia lanceolata* | China | Xue et al. [59] |
| *N. sphaeric*a | Pathogenic | Leaf spots | *Kinnon Mandarin* | Pakistan | Alam et al. [60] |
| *N. sphaeric*a | Pathogenic | Leaf spots | *Phoenix dactylifera* | Pakistan | Alam et al. [61] |
| *N. sphaeric*a | Pathogenic | Leaf spots | *Mangifera indica* | India | Pandey et al. [62] |
| *N. sphaeric*a | Endophytic | NA | *Artemisia sp.* | China | Cosoveanu [43] |
| *Nigrospora* sp. | Endophytic | NA | *Azadirachta indica* | Southwest China | Wu et al. [63] |

NA: not applicable.

*N. hainanensis* Mei Wang & L. Cai, *N. lacticonlia* Mei Wang & L. Cai, *N. osmanthi* Mei Wang & L. Cai, *N. pyriformis* Mei Wang & L. Cai, *N. rubi* Mei Wang & L. Cai, *N. venezularis* Mei Wang & L. Cai and *N. zimmermanii* Crous. were introduced. *N. vietnamensis* Hol.-Jech. was transferred to *Arthrinium* and synonymized under *Arthrinium vietnamensis* (Hol.-Jech.) Mei Wang & L. Cai. based on the multigene phylogenetic analyses [3].

Shandong Peninsula, the target site of this study, is bordered by the Bohai Sea to the North and Yellow Sea to the Southeast. The fungal ecology in this region would be an interesting aspect to study. This study focuses on *Nigrospora* species associated with leaf spots on forest plants. It also aims to provide molecular data for the genus to support molecular phylogeny based species identification. Furthermore, novel host associations of *Nigrospora* are identified and potential threats on forest plant species and crops with economic importance are predicted.

2. Materials and methods

2.1. Sample collection, isolation, and herbarium specimens

Leaf specimens from various plants with leaf spot symptoms were collected from Shandong Peninsula, China and brought to the laboratory in paper bags. Symptomatic leaves with leaf spots were selected and cut into approximately 2 × 2 mm pieces composed of both the diseased and healthy leaf tissue areas. The leaf pieces were surface sterilized by washing with 1% sodium hypochlorite for 30 s, 70% ethanol for 30 s, and finally, three times in sterilized water prior to culturing on potato dextrose agar (PDA) (1/4 PDA) and incubated at 25°C. Hyphal tips of growing mycelia from leaf tissues on PDA
were carefully picked up with a sterile toothpick and transferred onto fresh PDA plates to obtain pure cultures.

Morphological characters were observed and photographed using an Axio Imager Z2 photographic microscope (Carl Zeiss Microscopy, Oberkochen, Germany) and measurements were made with ZEN PRO 2012 software (Carl Zeiss Microscopy). Fifty conidial measurements were taken per isolate and cultures were allowed to grow until they completely covered a 90 mm petri dish to measure growth rate. The growth rate was calculated as the mean of two perpendicular measurements.

Voucher specimens were deposited in the herbarium collection of Beijing Academy of Agricultural and Forestry Sciences (JZBH) and all the cultures were deposited at the culture collections of Beijing Academy of Agricultural and Forestry Sciences (JZB), China and Kunming Institute of Botany (KUMCC), China. Following Jayasiri et al. [10], Faces of Fungi (FOF) numbers were acquired.

### 2.2. DNA extraction, PCR amplification, and sequencing

Fungal mycelia grown on PDA for 4–7 d were scraped off and collected. Genomic DNA was extracted using a modified CTAB protocol described in Guo et al. [11]. The following loci are amplified for 30 s at 95°C, and 30 s of annealing and 1 min elongation at 72°C, and a final extension for 10 min at 72°C. The annealing temperatures were as follows: 58°C for both ITS and TUB2, and 52°C for TEF1. The PCR reaction mixture was composed of 0.3 μL of TaKaRa Ex-Taq DNA polymerase (TaKaRa, Beijing, China), 2.5 μL of 10x Ex-Taq buffer (TaKaRa), 3.0 μL of dNTPs (TaKaRa), 1 μL of genomic DNA, 1 μL of each primer, and 16.2 μL of double-distilled H2O. The PCR products were visualized on 1% agarose gel followed by ethidium bromide staining, under UV light using a GelDoc XR Molecular Imager (Bio-Rad, Hercules, CA, USA). Sequencing of PCR products was done by Beijing Biomed Gene Technology Co., Ltd, Beijing, China.

### 2.3. Sequence alignment and phylogenetic analyses

Sequence chromatograms were checked with Chromas version 2.6.6 (Technelysium Pty Ltd., South Brisbane, Australia) and low-quality regions were trimmed prior to sequence alignments. Consensus sequences were generated for the TUB2 gene region using DNAStar version 5.1 (DNASTAR, Inc. Madison, WI, USA). All the sequences generated in this study were analyzed using the BLASTn searches in the GenBank. Reference sequences were obtained from GenBank referring to recently published relevant phylogenies and are listed in Table 3 [3]. Individual data sets of ITS, TEF1, and TUB2 were aligned using the default settings of the MAFFT version 7 webserver [13]. The alignments were manually edited further discarding leading or trailing gaps and concatenated in the following order, ITS, TEF1, and TUB2 using BioEdit version 7.0.5.2 (Department of Microbiology, North Carolina State University, NC, USA) [14]. Phylogenetic analyses of the aligned data were based on maximum likelihood (ML), Maximum parsimony (MP), and Bayesian posterior probabilities (BYP) analyses.

ML analysis was performed using RAxML-HPC2 on XSEDE version 8.2.8 (San Diego Supercomputer Center, CA, USA) [15,16] in the CIPRES Science Gateway platform [17] using GTR + CAT model of evolution. MP analysis was performed in PAUP version 4.0b10 (Sinauer Associates, Sunderland, MA, USA) [18], with the heuristic search option. Ambiguous regions in the alignment were excluded from the analyses, and gaps were treated as missing data. The stability of generated trees was evaluated by 1000 random bootstrap replicates. Maxtrees was set to 1000 and branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony (tree length [TL], consistency index [CI], retention index [RI], relative consistency index [RCI], and homoplasy index [HI]) were calculated. Differences between the trees inferred under different optimality criteria were evaluated with Kishino–Hasegawa tests (KHT) [19].

| Table 2. Primers used in the study, with sequences and references. |
|------------------|------------------|------------------|------------------|------------------|------------------|
| Gene abbreviation | Primer | Sequence (5’-3’) | References |
| ITS-5-ITS2 | Internal transcribed spacer | ITS 4 | TCCTCCGCTATGATGATGC | White et al. [12] |
| ITS 5 | | ITS 5 | GGAAGTAAAAACGCGAAAGG |
| TEF1 | Partial translation elongation factor 1-α | TEF1-728F | CATCGAGAAGTCGAGAAGG |
| | | EF-2 | GGA(G/A)GTACCAGTG(CA)TCAGATTGTT |
| TUB2 | β-Tubulin | BT-2F | AACATCCTGAGATTGTTAAGT |
| | | BT-4R | TAGTGACCCTGGGGCAGTAGT |

*References*

[1] White et al. [12]
[2] Carbone et al. [64]
[3] O’Donnell et al. [65]
[4] O’Donnell et al. [66]
Table 3. Strains of the *Nigrospora* species and related GenBank accession numbers of taxa included in this study.

| Taxa                                      | Culture collection Numbera,b | Hostc                        | GenBank Accession numbersd |
|-------------------------------------------|-----------------------------|------------------------------|----------------------------|
| *N. aurantiaca*                           | CGMCC 3.18130 = LC 7302     | *Nelumbo sp.* (leaf)         | KX986064 KY019465 KY019295 |
| *N. aurantiaca*                           | LC 7034                     | *Musa paradisiaca*           | KX986093 KY019598 KY019394 |
| *N. bambusae*                             | CGMCC 3.18327 = LC 7114     | *Bamboo* (leaf)              | KX853307 KY853319 KY853313 |
| *N. bambusae*                             | LC 7244                     | *Bamboo* (leaf)              | KX853306 KY853320 KY853314 |
| *N. bambusae*                             | LC 7245                     | *Bamboo* (leaf)              | KX853305 KY853321 KY853315 |
| *N. camelliae-sinensis*                   | LC 2710                     | *Camastanosp.*               | KX985957 KY019484 KY019310 |
| *N. camelliae-sinensis*                   | LC 3287                     | *Camellia sinensis*          | KX985957 KY019502 KY019323 |
| *N. camelliae-sinensis*                   | LC 3496                     | *Camellia sinensis*          | KX985958 KY019510 KY019327 |
| *N. camelliae-sinensis*                   | CGMCC 3.18125 = LC 3500     | *Camellia sinensis*          | KX985965 KY019481 KY019343 |
| *N. camelliae-sinensis*                   | LC 6684                     | *Nigrospora sp.*             | KX860646 KY019570 KY019449 |
| *N. chinensis*                            | LC 2696                     | *Lindera aggregata*          | KX859547 KY019474 KY019424 |
| *N. chinensis*                            | LC 3493                     | *Camellia sinensis*          | KX859854 KY019509 KY019434 |
| *N. chinensis*                            | LC 4935                     | *Camastanosp.*               | KX860613 KY019536 KY019446 |
| *N. chinensis*                            | LC 4598                     | *Orzya sativa* host plant    | KX860628 KY019543 KY019441 |
| *N. chinensis*                            | LC 6523                     | *Vitis vinifera*             | KX860623 KY019462 KY019422 |
| *N. chinensis*                            | LC 4660                     | *Quercus sp.*                | KX860626 KY019548 KY019445 |
| *N. chinensis*                            | LC 6631                     | *Camellia sinensis*          | KX860643 KY019569 KY019448 |
| *N. chinensis*                            | LC 8433                     | *Unciaria tomentosa*         | KX860623 KY019567 KY019402 |
| *N. oryzae*                               | JZB 3230001                 | *Cirsium setosum**           | MN495939 MN549381 MN546465 |
| *N. oryzae*                               | JZB 3230002                 | *Rudbeckia hirta*            | MN495941 – MN546460 |
| *N. oryzae*                               | JZB 3230004                 | *Scirpus sp.*                | MN495942 MN549382 MN546461 |
| *N. osmanthi*                             | CGMCC 3.18126 = LC 4350     | *Osmanthus sp.*              | KX860610 KY019561 KY019421 |
| *N. osmanthi*                             | LC 4487                     | *Hedera nepalensis*          | KX860617 KY019540 KY019438 |
| *N. osmanthi*                             | JZB 3230005                 | *Rosa chinensis**            | MN495943 MN549383 MN508179 |
| *N. osmanthi*                             | JZB 3230006                 | *Rosa chinensis**            | MN495944 MN549384 MN508180 |
| *N. osmanthi*                             | JZB 3230007                 | *Phragmites australis**      | MN495945 MN549385 MN508181 |
| *N. osmanthi*                             | JZB 3230009                 | *Cirsium setosum**           | MN495946 MN549386 MN508182 |
| *N. osmanthi*                             | JZB 3230010                 | *Phyllostachys nigra**       | MN495947 MN549387 MN508183 |
| *N. osmanthi*                             | JZB 3230011                 | *Rudbeckia hirta*            | MN495949 MN549389 MN508185 |
| *N. pyriformis*                           | CGMCC 3.18122 = LC 2045     | *Citrus sinensis*            | KX859890 KY019457 KY019290 |
| *N. pyriformis*                           | LC 2689                     | *Lindera aggregata*          | KX859891 KY019468 KY019427 |
| *N. pyriformis*                           | LC 2694                     | *Rubus reflexus*             | KX859894 KY019472 KY019300 |
| *N. pyriformis*                           | LC 3099                     | *Camellia sinensis*          | KX859897 KY019498 KY019322 |
| *N. pyriformis*                           | LC 3292                     | *Camellia sinensis*          | KX859897 KY019503 KY019324 |
| *N. rubi*                                 | JZB 3230012                 | *Rhus sp.* **                | MN495950 – MN546446 |
| *N. sphaerica*                            | LC 7312                     | *Nelumbo sp.* (leaf)         | KX859895 KY019618 KY019414 |
| *N. sphaerica*                            | LC 7298                     | *Nelumbo sp.* (leaf)         | KX859893 KY019606 KY019401 |
| *N. sphaerica*                            | LC 2640                     | *Harpullia longipetala*      | KX859895 KY019492 KY019318 |
| *N. sphaerica*                            | LC 3477                     | *Camellia sinensis*          | KX859892 KY019508 KY019326 |
| *N. sphaerica*                            | LC 2645                     | *Rhododendron arborescens*   | KX859933 KY019517 KY019334 |
| *N. sphaerica*                            | LC 4307                     | *Rhododendron arborescens*   | KX860005 KY019529 KY019346 |
| *N. sphaerica*                            | LC 5901                     | *Submerged wood*             | KX860634 KY019556 KY019361 |
| *N. sphaerica*                            | LC 6294                     | *Camellia sinensis*          | KX860644 KY019565 KY019369 |
| *N. sphaerica*                            | LC 6605                     | *Nigrospora sp.* (leaf)      | KX859892 KY019506 KY019326 |
| *N. sphaerica*                            | JZB 3230013                 | *Cirsium setosum**           | MN495951 MN549390 MN546462 |
| *N. sphaerica*                            | JZB 3230014                 | *Phragmites australis**      | MN495952 MN549391 MN546463 |
| *N. sphaerica*                            | JZB 3230015                 | *Fraxinus sp.*               | MN495953 MN549392 MN546444 |
| *Nigrospora sp.* 1                        | LC 2725                     | *Symphlocos zizyphoides*     | KX859860 KY019487 KY019313 |
| *Nigrospora sp.* 1                        | LC 4566                     | *Lithocarpus sp.*            | KX860622 KY019545 KY019354 |
| *Nigrospora sp.* 2                        | LC 6704                     | *Camellia sinensis*          | KX860647 KY019571 KY019373 |
| *N. vesicularis*                          | LC 0322                     | Unknown host plant           | KX859893 KY019467 KY019296 |
| *N. vesicularis*                          | CGMCC 3.18128 = LC 7010     | *Musa paradisiaca* (leaf)    | KX860688 KY019463 KY019294 |
| *N. zimmermannii*                         | CBS 167.26                  | Unknown                      | KY853308 KY853118 KY853122 |

(continued)
Bayesian analysis was executed in MrBayes version 3.1.2 [20] through Markov Chain Monte Carlo (MCMC) sampling to calculate the posterior probabilities (PP) [18,21]. Partitioning of data was initially done by locus and then the parameters of the nucleotide substitution models for every partition were selected independently using MrModeltest version 2.3 [22] under the Akaike information criterion (AIC) executed in PAUP version 4.0b10. The models GTR+G for ITS and HKY+1+G for TEF1 and TUB2 were set for their respective genes in the analysis. Six Markov chains were run in parallel for 3 million generations with trees being sampled at every 1000th generation. Twenty-five percent of the trees were discarded representing the burn-in phase. Generated trees were used to calculate the PP in the majority rule consensus tree. The resulting trees were viewed in FigTree version 1.4.0 (Institute of Evolutionary Biology, University of Edinburgh, UK) [23] and annotated in Adobe Illustrator CC 2017 version 21.0.0 (Adobe Systems Incorporated, Seattle, WA). All the sequence data generated in this study were deposited in NCBI GenBank (Table 3). The sequence alignment generated in this study was deposited in TreeBase under the accession number of 25396.

### 3. Results

#### 3.1. Phylogenetic analysis

The combined ITS, TEF1, and TUB2 gene data set comprised 64 sequences from *Nigrospora* including isolates from this study, *Arthrinium malaysianum* (CBS 102053) and *Arthrinium obovatum* (LC 4940) were considered as outgroup taxa (Figure 1). The combined alignment of three gene regions was analyzed and the best scoring RAXML tree is shown in Figure 1 with a final ML optimization likelihood value of –9176.491460. The matrix had 605 distinct alignment patterns, with 8.57% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.209857, C = 0.308100, G = 0.240726, and T = 0.241318; substitution rates AC = 0.968792, AG = 2.885236, AT = 0.956737, CG = 0.911966, CT = 4.642164, and GT = 1.000000; proportion of invariable sites I = 0.401481; gamma distribution shape parameter x = 0.808089. The MP analysis with combined ITS, TEF1, and TUB2 gene data comprised 1344 total characters including gaps, of which 759 characters were constant, 498 characters were parsimony-informative, while 87 variable characters are parsimony-uninformative. In the most parsimonious tree, TL = 1621, CI = 0.570, RI = 0.907, RCI = 0.517, and HI = 0.430. The Bayesian analysis resulted in 15,000 trees after 3,000,000 generations. All trees (ML, MP, and BYPP) were similar in topology and did not differ significantly (data not shown). At the generic level, relationships are in agreement with the previous study based on multi-gene phylogeny [3]. Our phylogenetic analyses resulted in 18 clades corresponding to species in *Nigrospora* similar to the study conducted by Wang et al. [3]. Isolates from this study clustered within five clades corresponding to known species and thus confirmed their identities.

#### 3.2. Taxonomy

*Nigrospora* Zimm., Centbl. Bakt. ParasitKde, Abt. I 8:220 (1902),

*Synonym:* *Khusia* H.J. Huds., Trans. Br. mycol. Soc. 46:358 (1963),

*Nigrospora golenkoana* Novobr., Nov. sist. Niz. Rast. 9:180 (1972),

*Facesoffungi number:* FoF 06595 (Figure 2).

Pathogenic or saprobic on leaves of *Cirsium setosum* (Willd.) Besser ex M.Bieb (Asteraceae). Asexual morph: *Hyphae* smooth, branched, septate, and hyaline. *Conidiophores* mostly reduced to conidiogenous cells. *Conidiogenous cells* 6.9–10 × 4.2–8 μm diam. (x = 8.4 × 6 μm, n = 30), monoblastic, solitary, discrete, determinate, doliform to ampulliform, and pale brown. *Conidia* 10.3–14 × 13.3–17.2 μm diam. (x = 12.5 × 15.2 μm, n = 50), solitary, globose or oblate, dark brown to black, shiny, sparse, discrete on aerial mycelia, and smooth-walled. Sexual morph: Undetermined.
**Culture characteristics** – Colonies on PDA, reach 9 cm diam. after 5 d at 25 °C, circular shaped, entire margined, floccose with aerial mycelium, surface initially white, turning grayish when mature and reverse initially white, turning smoke gray when mature.

**Material examined** – China, Shandong Peninsula, on living leaves of *Cirsium setosum*, 07 October 2017, Yuanyuan Hao (JZBH 3230001), living culture JZB 3230001, and KUMCC 19-0222.

**Leaf spot symptoms** – Leaf spots irregularly scattered and composed of a dark brown circular outer boundary.
Nigrospora gorlenkoana (JZB 3230001) is a fungus with leaf spot symptoms characterized by randomly scattered and elliptical shaped leaf spots. The symptoms are composed of dark brown to black, shiny, smooth, and aseptate conidia attached to conidiogenous cells. The fungus is identified with a light brown inner ring, margined by apparently healthy leaf tissues.

Notes – Based on the phylogenetic analysis of combined ITS, TEF1, and TUB2 sequence data of Nigrospora species (Figure 1), our strain Nigrospora gorlenkoana (JZB 3230001) clustered with the ex-type strain of N. gorlenkoana (CBS 480.73) with strong bootstrap support and Bayesian probabilities (100% ML, 100% MP, and 1.00 BYPP) (Figure 1). The base pair difference comparison of ITS, TEF1, and TUB2 gene regions between our strain (JZB 3230001) and ex-isotype strain of N. gorlenkoana (CBS 480.73) reveal less than 1% difference and the two specimens share similar morphological characters confirming both strains are conspecific. In contrast to the ex-type strain (CBS 480.73), an equatorial slit on conidia was not observed in our strain (JZB 3230001) [3]. Nigrospora gorlenkoana has not frequently been identified as a plant pathogen and it was previously reported to be isolated from leaves and fruits of Vitis vinifera [3]. This is the first report of Nigrospora gorlenkoana from Cirsium setosum.

Pathogenic or saprobic on leaves of Scirpus sp. (Cyperaceae). Asexual morph: Hyphae smooth, branched, septate, hyaline or pale brown. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 8.6–14 × 6.4–11.9 μm diam. (x = 11.18 × 7.98 μm, n = 30), aggregated in clusters on hyphae, monoblastic, determinate, ampulliform or doliform, and hyaline to pale brown. Conidia 9.0–13.2 × 12.6–15.8 μm diam. (x = 10.95 × 14 μm, n = 50), formed abundantly, solitary, globose or oblate, dark brown to black, shiny, smooth, and aseptate. Sexual morph: Undetermined.

Culture characteristics – Colonies on PDA reach 9 cm diam. in 6 d at 25°C, circular, entire margined, floccose, filiform, surface and reverse initially white, becoming dark gray, or black toward the center with age.

Material examined – China, Shandong Peninsula, on living leaves of Scirpus sp., October 7 2017, Yuanyuan Hao (JZBH 3230004), living culture JZB 3230004, and KUMCC 19-0225.

Leaf spot symptoms – Randomly scattered and elliptical shaped leaf spots are composed of dark brown to black, shiny, smooth, and aseptate conidia attached to conidiogenous cells. Scale bars f, g = 20 μm, h, and i = 10 μm.
brick, slightly dispersed outer halo with light brown inner core, and margined by healthy leaf tissues.

**Other materials examined** – China, Shandong Peninsula, on living leaves of *Phyllostachys nigra* (Lodd. ex Lindl.) Munro (Poaceae), October 7 2017, Yuanyuan Hao (JZBH 3230002), living culture JZB 3230002, KUMCC 19-0223; China, Shandong Peninsula, on living leaves of *Rudbeckia hirta* L. (Asteraceae), October 7 2017, Yuanyuan Hao (JZBH 3230003), living culture JZB 3230003, and KUMCC 19-0224.

Notes – *Nigrospora gorlenkoana* and *N. oryzae* are reported to have the same synonym of *Basisporium gallarum* in Mycobank. However, in our phylogenetic analysis, *N. oryzae* and *N. gorlenkoana* are placed in two distinct clades. *Khuskia oryzae* was introduced as the teleomorph of *N. oryzae*. The multi-gene phylogeny generated herein indicates that our strains of *Nigrospora oryzae* form a strongly supported lineage (98% ML, 100% MP, and 1.00 BYPP) in *N. oryzae* cluster (Figure 1). Base pair comparison of ITS, TEF1, and TUB2 gene regions.

Figure 3. *Nigrospora oryzae* (JZB 3230004). (a and b) Appearance of leaf spots on the host substrate; (c and d) Upper view (c) and reverse view (d) of culture on PDA; (e) Surface view of the colony on PDA; (f) Colony on PDA; (g–k) Mature conidia attached to conidiogenous cells. Scale bars f, g = 20 μm, h, and i = 10 μm.
between our strain (JZB 3230004) and reference strain of *N. oryzae* (LC 5243) reveal less than 1% difference. The morphological characters, such as conidiogenous cells, conidial dimensions, and culture characteristics also overlap confirming that the two strains are the same species [3]. This is the first time *N. oryzae* has been reported from *Scirpus* sp., which is an aquatic grass-like plant species, *Phyllostachys nigra* commonly known as black bamboo and *Rudbeckia hirta*, a garden plant belongs to the sunflower family.

*Nigrospora osmanthi* Mei Wang, F. Liu, P.W. Crous & L. Cai. Persoonia 39:135 (2017), Facesoffungi number: FoF 06597 (Figure 4).

Pathogenic or saprobic on leaves of *Rudbeckia hirta* L. Asexual morph: Hyphae smooth, branched, septate, hyaline, or pale brown. Conidiophores mostly reduced to conidiogenous cells. Conidiogenous cells 6.8–12.6 × 5.3–7.4 µm diam. (x = 9.3 × 6.3 µm, n = 30), discrete, solitary, monoblastic, determinate, ampulliform to subglobose, straight or curved, hyaline. Conidia 9–11.5 × 12.5–14.6 µm diam. (x = 10 × 13.2 µm, n = 50), discrete on aerial mycelia, solitary, globose or oblate, dark brown to black, shiny, smooth-walled, and asetate. Sexual morph: Undetermined.

Culture characteristics – Colonies on PDA reach 9 cm diam. in 5 d at 25°C, circular, entire margined, flat with aerial mycelium, floccose, filiform, surface initially white turning dark gray when mature and reverse initially white, and turning leek green when mature.

**Material examined** - China, Shandong Peninsula, on living leaves of *Rudbeckia hirta* L., October 07 2017, Yuanyuan Hao (JZBH 3230011), living culture JZB 3230011, KUMCC 19-0229.

Leaf spot symptoms and characters – Irregularly scattered and free-form shaped leaf spots are composed of dark brown outer border with light brown inner core, margined by apparently healthy leaf tissues.

**Other materials examined** – China, Shandong Peninsula, on living leaves of *Cirsium setosum*, October 7 2017, Yuanyuan Hao (JZBH 3230008), living culture JZB 3230008, KUMCC 19-0227; China, Shandong Peninsula, on living leaves of *Phyllostachys nigra*, October 07 2017, Yuanyuan Hao (JZBH 3230009), living culture JZB 3230009, KUMCC 19-0228; China, Shandong Peninsula, on living leaves of *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae), October 7 2017, Yuanyuan Hao (JZBH 3230007), living culture JZB 3230007; China, Shandong Peninsula, on living leaves of *Rosa chinensis* Jacq. (Rosaceae), October 7 2017, Yuanyuan Hao (JZBH 3230005), living culture JZB 3230005, and KUMCC 19-0226.

Notes – Based on the phylogenetic analysis of combined ITS, TEF1, and TUB2 sequence data of *Nigrospora* species (Figure 1), our strains of *N.
Osmanthi (JZB 3230005, JZB 3230006, JZB 3230007, JZB 3230008, JZB 3230009, JZB 3230010, and JZB 3230011) form a strongly supported lineage (100% ML, 99% MP, and 1.00 BYPP) with the ex-type strain *N. osmanthi* (CGMCC 3.18126) (Figure 1). The base pair comparison shows 100% similarity in all three gene regions of ITS, TEF1, and TUB2 between our strain (JZB 3230011) and ex-type strain (CGMCC 3.18126). The two specimens share similar morphological characters except for culture characteristics where our strain (JZB 3230011) has an entire margin and reference strain (CGMCC 3.18126) has a lobate margin [3]. This is the first time *N. osmanthi* has been isolated from *Rudbeckia hirta* L., *Cirsium setosum*, *Phyllostachys nigra*, *Phragmites australis* which is a perennial grass species found in wetlands, and *Rosa chinensis*.

*Nigrospora rubi* Mei Wang, F. Liu, P.W. Crous & L. Cai. Persoonia 39:135 (2017), Facesoffungi number: FoF 06598 (Figure 5).

Pathogenic or saprobic on leaves of *Fraxinus* sp. (Oleaceae). Asexual morph: Hyphae smooth, branched, septate, and hyaline. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 5.2–7.4 × 6.6–7.3 μm diam (μ = 6.7 × 6.9 μm, n = 30), clustered on hyphae, unbranched, ampulliform, short, and squat pale brown. Conidia

![Image of Nigrospora rubi](image-url)
7.9–10.7 × 10–12.1 μm diam. (X = 9.58 × 11.17 μm, n = 50), solitary, spherical or subglobose, black, shiny, smooth, and aseptate. Sexual morph: Undetermined.

Culture characteristics – Colonies on PDA reach 9 cm diam. after 6 d at 25 °C, circular, entire margined, velvety to lanose, surface initially white, becoming dark olive-green to gray with age and reverse initially white, and turning leek green when mature.

Material examined – China, Shandong Peninsula, on living leaves of Fraxinus sp., October 7 2017, Yuanyuan Hao (JZBH 3230012), living culture JZB 3230012, and KUMCC 19-0242.

Leaf spot symptoms and characters – Irregularly scattered and free-form shaped leaf spots are composed of dark brick outer border with light brown inner core, margined by healthy leaf tissues.

Notes – Based on multi-locus molecular phylogeny, our isolate of N. rubi (JZB 3230012) forms a strongly supported lineage (100% ML, 100% MP, and 1.00 BYPP) with N. rubi as the type species (CGMCC 3.18326) (Figure 1) and the base pair comparison between these two strains exhibit 100% similarity in ITS and 98.8% similarity in TEF1 gene region. The TUB2 gene sequence could not be obtained for our strain (JZB 3230012). The conidial measurements were slightly larger (11.5–16.5 μm) in type specimen (CGMCC 3.18326), compared to our strain (JZB 3230012, 9.58 × 11.17 μm) [3]. The culture characteristics slightly deviate in color; the ex-type culture (CGMCC 3.18326) was initially white, becoming black with age and reverse smoke-gray in patches, where our strain shows initially white surface becoming dark olive-green to gray with age and initially white reverse turning leek.
green when mature (JZB 3230012). Nigrospora rubi has been previously isolated from 
Rubus species [3]. This is the first time N. rubi has been isolated from
Fraxinus sp.

Nigrospora sphaerica (Sacc.) E.W. Mason, Trans. Br. Mycol. Soc. 12: 158 (1927),
Facesoffungi number: FoF 06599 (Figure 6).
Basionym: Trichosporum sphaericum Sacc., Michelia 2 (no. 8): 579 (1882).

Pathogenic or saprobic on leaves of Fraxinus sp. Asexual morph: Hyphae smooth, branched, septate, hyaline, or pale brown. Conidiophores mostly reduced to conidigenous cells. Conidiogenous cells 9.5–16.5 × 7.4–9.8 μm diam. (x = 12.7 × 8.4 μm, n = 30), discrete, monoblastic, determinate, unbranched, and ampulliform to subglobose hyaline to pale brown. Conidia 11.5–15.7 × 13.3–19.6 μm diam. (x = 14 × 16.7 μm, n = 50), sparse, discrete, globose or subglobose, black, shiny, smooth, and aseptate. Sexual morph: Undetermined.

Culture characteristics – Colonies on PDA reach 9 cm diam. in 5 d at 25°C, circular, entire margined, floccose or suede-like texture, surface initially white, becoming dark gray with age and reverse initially white, and turning smoke gray when mature.

Material examined – China, Shandong Peninsula, on living leaves of Fraxinus sp., October 7 2017, Yuanyuan Hao (JZBH 3230015), living culture JZB 3230015, and KUMCC 19-0232.

Leaf spot symptoms – Leaf spots irregularly scattered and free-form shaped, composed of dark brick outer border with light brown inner core, and margined by healthy leaf tissues.

Other materials examined – China, Shandong Peninsula, on living leaves of Cirsium setosum, October 7 2017, Yuanyuan Hao (JZBH 3230013), living culture JZB 3230013, KUMCC 19-0230; China, Shandong Peninsula, on living leaves of Phragmites australis, October 7 2017, Yuanyuan Hao (JZBH 3230014), living culture JZB 3230014, and KUMCC 19-0231.

Notes – Nigrospora sphaerica is identified as a widely distributed plant pathogen on a diverse range of host species worldwide. Since the DNA sequence data of N. sphaerica type specimen was not available, Wang et al. [3] determined a collection of Nigrospora isolates from their study as N. sphaerica by comparing morphological characters of vesicular structures and conidial dimensions to the original description. In combined phylogenetic analysis, our isolates of N. sphaerica (JZB 3230013, JZB 3230014, and JZB 3230015) clustered with strong bootstrap support and posterior probability values (90% ML, 99% MP, and 1.00 BYPP). Less than 1% base pair difference was observed in base pair comparison of ITS, TEF1, and TUB2 gene regions between our strain (JZB 3230015) and reference N. sphaerica (LC 6996) strain. Also, similar morphologies were observed between the two strains confirming these two strains as conspecific. This is the first time N. sphaerica has been isolated from Fraxinus sp., Cirsium setosum and Phragmites australis.

4. Discussion

This study illustrates five different Nigrospora species isolated from various hosts in Shandong Peninsula, China. Nigrospora gorlenkoana, N. oryzae, N. osmanthi, N. rubi and N. sphaerica are reported from this study. Thirteen novel host associations (Table 3) were revealed on hosts such as Fraxinus sp., Phragmites australis, Scirpus sp. and including economically important plant varieties, such as Cirsium setosum, Phyllostachys nigra, Rosa chinensis, and Rudbeckia hirta.

Nigrospora is a monophyletic genus in Apiosporaceae (Xylariales) [3]. The phylogenetic construction of the DNA sequences of combined ITS, TEF1, and TUB2 gene regions provide robust confirmation and resolution for species delimitation by separating different species of the genus with high bootstrap support (Figure 1).

Currently, there are 15 records of Nigrospora species in MycoBank and 16 in GenBank but sequence data are not available for Nigrospora aerophila, N. arundinacea, N. canescens, N. gellarum, N. gossypii, N. javanica, N. maydis, N. padwickii, and N. panici. Therefore, epitypification of these species must be carried out and further studies based on molecular phylogeny are needed on these species.

There are few studies conducted on the fungal ecology of the Shandong peninsula. A study on aquatic fungi in China revealed various fungal species isolated from different hosts from Shandong province; Arenariomyces trifurcata Höhnk, Buergenerula spartinae J. Kohlmer & R.V. Gessner, Corollospora maritima Werderm., Dryosphaera navigans Jörg. Koch & E.B.G. Jones, Halosphaeriopsis mediosetigera (A.B. Cribb & J.W. Cribb) T.W. Johnson, Lignincola laevis Höhnk, Monosporascus cannonballus Pollack & Uecker, Natantiospora retorquens (C.A. Shearer & J.L. Crane) J. Campb., J.L. Anderson & C.A. Shearer, Pleospora betae Björk., Pleospora spartinae (J. Webster & M.T. Lucas) Apinis & Chesters, Pleospora vitalbae (De Not.) Berl., Tetraploa aristata Berk. & Broome, Torula herbarum (Pers.) Link, Torpedospora radiata Meyers, Trichocladium achorasporum Meyers & R.T. Moore) M. Dixon ex Shearer & J.L. Crane, Zalerion maritimum (Linder) Anastasiou, Zalerion varium Anastasiou from driftwood; Ceriosporopsis halima
Linder from bamboo; *Passeriniella obiones* (P. Crouan & H. Crouan) K.D. Hyde & Mouzouras from straw; *Torpedospora radiata* Meyers from drift bamboo as marine Ascomycetes [24], and *Nia vibrissa* R.T. Moore & S. P. Meyers from driftwood as marine Basidiomycetes [24], and *Alternaria maritima* G.K. Sutherland from driftwood as marine Hyphomycetes [24]. Shandong province is also famous for economically important fungal resources, 182 taxa of wild edible and medicinal fungi belong to 39 families, and 80 genera are reported [25]. *Agaricus silvaticus* Schaeff., *Cao & Y.C. Dai*, *Grifola frondosa* (Vittad.) Peck, *Ganoderma lingzhi* L. *Sutara*, and *Xerula radicata* (L.) Roussel, *Xerocomellus chrysenteron* (L.) Roussel, *Fr.*, *Suillus bovinus* pose (Batsch) P. Kumm., *Perenniporia fraxinea* (Bull.) Ryvarden, *Pholiota adipose* (Batsch) P. Kumm., *Schizophyllum commune* Fr., *Suillus bovinus* (L.) Roussel, *Suillus granulatus* (L.) Roussel, *Xerocomellus chrysenteron* (Bull.) Štarta, and *Xerula radicata* (Relhan) Dörfelt, are among edible fungi [25]. Further, *Leptosphaeria agnita* (Desm.) Ces. & De Not., *L. dometororum* Niessl, *L. eustomoides* Sacc., and *L. solani* Romell ex Berl. were isolated from deadwood materials as saprophytic fungi from Shandong Peninsula [26]. There are no previous records on the occurrence of *Nigrospora* species from the Shandong peninsula.

Among the five *Nigrospora* species reported in this study, *N. oryzae*, *N. osmanthi*, and *N. sphaerica* were recorded frequently as pathogenic on a broader range of host plants (Table 1). Even though the pathogenic behavior of *N. oryzae* is prominent, in most cases it is identified as a weak pathogen [27, 28]. Spore dispersal of *Nigrospora* is aided by the wind, rain splash and insect vectors [29] supporting a rapid spread of the disease. The presence of a sticky mucilaginous substance was observed on discharged spores [30]. It has been hypothesized that this mucilaginous substance facilitates adherence to the host substrate or to a vector, such as mites as a successful spore dispersal mechanism. Since *Nigrospora* infections occur easily on weakened or wounded plants, spore dispersal through vectors is an added advantage on disease establishment. *Nigrospora sphaerica* isolated from Blueberry (*Vaccinium corymbosum*) leaf spots, twigs and shoot blight was identified as a pathogen that penetrates the host plant through wounds caused by insects or abiotic frost damages [31]. Previously, it was believed that *Nigrospora* was limited to monocotyledonous hosts [9], but later studies revealed it can occur on a diverse range of hosts and the pathogenicity of *Nigrospora* alerts the concerns on agronomy and forestry management. Molecular phylogeny guided species identification would be essential in developing effective bio-control measures against these species. Here, we extend the known host range of five species in *Nigrospora*.

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**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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**Data availability**

The data that supports the findings of this study are openly available in GenBank and TreeBase repositories. The GenBank accession numbers and the TreeBase submission number are given within the article.

**References**

[1] Zimmerman A, Ueber einige an tropischen Kulturpflanzen beobachtete Pilze III. Zentralblatt Für Bakteriol Parasitenkd. 1902:8:216–221.

[2] Hyde K, Norphanphoun C, Maharachchikumbura S, et al. Refined families of Sordariomycetes. Mycosphere. 2020;11(1):305–1059.

[3] Wang M, Liu F, Crous PW, et al. Phylogenetic reassessment of *Nigrospora*: ubiquitous endophytes, plant and human pathogens. Persoonia. 2017; 39(1):118–142.

[4] Rashmi M, Kushveer J, Sarma V. A worldwide list of endophytic fungi with notes on ecology and diversity. Mycosphere. 2019;10(1):798–1079.

[5] Sun X, Guo L-D, Hyde KD. Community composition of endophytic fungi in *Acer truncatum* and their role in decomposition. Fungal Divers. 2011; 47(1):85–95.

[6] Metwalay AM, Kadry HA, El-Hela AA, et al. Nigrosphaerin A a new isochromene derivative from the endophytic fungus *Nigrospora sphaerica*. Phytochem Lett. 2014;7:1–5.

[7] Zhao JH, Zhang YL, Wang LW, et al. Bioactive secondary metabolites from *Nigrospora* sp. LLGLM003, an endophytic fungus of the medicinal plant *Moringa oleifera* Lam. World J Microbiol Biotechnol. 2012;28(5):2107–2112.

[8] Zhang SP, Huang R, Li FF, et al. Antiviral anthraquinones and azaphilones produced by an
endophytic fungus *Nigrospora* sp. from *Aconitum carmichaeli*. Fitoterapia. 2016;112:85–89.

[9] Mason EW. On species of the genus Nigro-spora Zimmermann recorded on monocotyledons. Trans Br Mycol Soc. 1927;12(2–3):152–165.

[10] Jayasiri SC, Hyde KD, Ariyawansa HA, et al. The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. Fungal Divers. 2015;74(1):3–18.

[11] Guo LD, Hyde KD, Liew E. Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. New Phytol. 2000;147(3):617–630.

[12] White TJ, Bruns T, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols. Amsterdam, Netherlands: Elsevier; 1990. p. 315–322.

[13] Katoh K, Rozewicki J, Yamada KD. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform. 2019;20(4):1160–1166.

[14] Stamatakis A. RAxML version 8: a tool for phylogenetic inference. Bioinformatics. 2014;30(9):1312–1313.

[15] Miller MA, Pfeiffer W, Schwartz T. Creating the CIPRES science gateway for inference of large phylogenetic trees. 2010 gateway computing environments workshop (GCE). Piscataway (NJ): IEEE; 2010. p. 1–8.

[16] Swofford DL. PAUP: phylogenetic analysis using parsimony [Internet]. Vol. 42. Sunderland (MA): Sinauer Associates; 2002. p. 294–307.

[17] Wright ER, Folgado M, Rivera MC, et al. Preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia. Fungal Divers. 1998;1:27–51.

[18] Kim SR. Phylogenetic analysis of *Nigrospora* species complex in Hong Kong and *Musa acuminata* Caes. Genetica. 2000;100:39–51.

[19] Zhang LX, Li SS, Tan GJ, et al. First report of *Nigrospora oryzae* in China. Plant Dis. 2013;97(9):1256–1258.

[20] Zheng L, Shi F, Kelly D, et al. First report of leaf blight caused by *Nigrospora sphaerica* in Malaysia. Crop Prot. 2019;122:165–170.

[21] Wright ER, Folgado M, Rivera MC, et al. *Nigrospora sphaerica* causing leaf spot and twig and shoot blight on blueberry: a new host of the pathogen. Plant Dis. 2008;92(1):171–171.

[22] Kei Yi, Hafifi ABM, Huda-Shakirah AR, et al. First report of reddish brown spot disease of red-fleshed dragon fruit (*Hylocereus polyrhizus*) caused by *Nigrospora lacticolonia* and *Nigrospora sphaerica* in Malaysia. Crop Prot. 2019;122:165–170.

[23] Brown KB, Hyde KD, Guest DI. Preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia. Fungal Divers. 1998;1:27–51.

[24] Swofford DL. PAUP: phylogenetic analysis using parsimony [Internet]. Vol. 42. Sunderland (MA): Sinauer Associates; 2002. p. 294–307.

[25] Nigrospora oryzae (Berk. & Broome) Petch causing stem bight on *Brassica juncea* in India. J Phytopathol. 2013;161(6):439–441.

[26] Zhao LF, Liu J, Zhang MX, et al. The first report of leaf spots in *Aloe vera* caused by *Nigrospora oryzae* in China. Plant Dis. 2013;97(9):1256–1256.

[27] Wu JB, Zhang CL, Mao PP, et al. First report of leaf spot caused by *Nigrospora oryzae* on *Dendrobium candidum* in China. Plant Dis. 2014;98(7):996–996.

[28] Shinmura T, Tanaka M, Suzuki Y, et al. First report of *Nigrospora oryzae* causing brown/black spot disease of kiwifruit in China. Plant Dis. 2018;102(1):243–243.

[29] Zheng L, Shi F, Kelly D, et al. First report of leaf spot of *Kentucky Bluegrass* (*Poa pratensis*) caused by *Nigrospora oryzae* in Ontario. Plant Dis. 2012;96(6):909–909.

[30] Kalati TH, Jahani M, Zare R, et al. First report of *Nigrospora oryzae* leaf spot on *Pennisetum americanum* in Iran. J Plant Pathol. 2014;96(3):606.

[31] Abbas MH. Morphological, molecular and pathological study on *Nigrospora oryzae* and *Nigrospora sphaerica*, the leaf spot fungi of date palm abstract. Basra J Date Palm Res. 2014;13(1):26–38.

[32] Rathod D, Dar M, Gade A, et al. Griseofulvin producing endophytic *Nigrospora oryzae* from Indian *Emblica officinalis* Gaertn: a new report. Austin J Biotechnol Bioeng. 2014;1(3):1–5.

[33] Cossoevanu A. Fungi as endophytes in Chinese *Artemisia spp.*: juxtaposed elements of phylogeny, diversity and bioactivity. Mycosphere. 2016;7(2):102–117.

[34] Mei SS, Wang ZY, Zhang J, et al. First report of leaf blight on *Stenotaphrum secundatum* caused by...
Nigrospora osmanthi in China. Plant Dis. 2019; 103(7):1783–1783.

[45] Liu J, Yang L, Miao P, et al. First report of leaf blight on Ficus pandurata caused by Nigrospora osmanthi in China. Plant Dis. 2019;103(10): 2685–2685.

[46] Raviraja NS, Maria GL, Sridhar KR. Antimicrobial evaluation of endophytic fungi inhabiting medicinal plants of the Western Ghats of India. Eng Life Sci. 2006;6(5):515–520.

[47] Zhao H, Liu HY, Yang XS, et al. First report of Nigrospora leaf blight on sesame caused by Nigrospora sphaerica in China. Plant Dis. 2014; 98(6):842–842.

[48] Cui YP, Wu B, Peng AT, et al. First report of Nigrospora leaf blight on sugarcane caused by Nigrospora sphaerica in China. Plant Dis. 2018; 102(4):824–824.

[49] Dutta J, Gupta S, Thakur D, et al. First report of Nigrospora leaf blight on tea caused by Nigrospora sphaerica in India. Plant Dis. 2015;99(3):417–417.

[50] Chen J, Xiang TT, Liu XY, et al. First report of Nigrospora sphaerica causing shot hole disease on mulberry in China. Plant Dis. 2018;102(1):245.

[51] Arunakumar GS, Gnanesh BN, Supriya M, et al. First report of Nigrospora sphaerica causing shot hole disease on Mulberry in India. Plant Dis. 2019; 103(7):1783.

[52] Abass MH, Hameed MA, Ahmed AN. First report of Nigrospora sphaerica (Sacc.) Mason as a potential pathogen on date palm (Phoenix dactylifera L.). Can J Plant Pathol. 2013;35(1):75–80.

[53] Allen R. Control of black-end and squirter diseases in bananas with benzimidazole and salicylanilide compounds. Aust J Exp Agric. 1970;10(45):490.

[54] Simmonds JH. Banana leaf spot progress report. Queensl Agric J. 1933;39(1):21–40.

[55] Chen Y, Yang X, Zhang A-F, et al. First report of leaf spot caused by Nigrospora sphaerica on kiwifruit in China. Plant Dis. 2016;100(11):2326.

[56] Li L, Pan H, Liu YF, et al. First report of Nigrospora sphaerica causing kiwifruit postharvest rot disease in China. Plant Dis. 2018;102(8):1666.

[57] Li YG, Huang MH, Sun LP, et al. Occurrence of leaf spot of calabash caused by Nigrospora sphaerica in Georgia. Plant Dis. 2016;100(7):1506–1506.

[58] Liu YJ, Tang Q, Fang L. First report of Nigrospora sphaerica causing leaf blight on Camellia sinensis in China. Plant Dis. 2016;100(1):221.

[59] Xu YM, Liu YJ. First report of Nigrospora sphaerica causing leaf blight on Cunninghamia lanceolata in China. Plant Dis. 2017;101(2):389.

[60] Alam MW, Rehman A, Gleason ML, et al. First report of Nigrospora sphaerica causing leaf spot of Kinnow mandarin in Pakistan. J Plant Pathol. 2017;99(1):295.

[61] Alam MW, Rehman A, Ahmad S, et al. First report of Nigrospora sphaerica causing leaf spot of Kinnow mandarin in Pakistan. J Plant Pathol. 2020;102(1):223–223.

[62] Pandey A, Pandey S, Awasthi AK, et al. A new host record of Nigrospora sphaerica on Mangifera indica from Jabalpur, India. J Mycol Plant Pathol. 2013;43(2):255–256.

[63] Wu SH, Chen YW, Shao SC, et al. Two new Solanapyrone analogues from the endophytic fungus Nigrospora sp. YB-141 of Azadirachta indica. Chem Biodivers. 2009;6(1):79–85.

[64] Carbone I, Kohn LM. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia. 1999;91(3):553–556.

[65] O’Donnell K, Kistler HC, Cigelnik E, et al. 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(5):2044–2049.

[66] O’Donnell K, Cigelnik E. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are nonorthologous. Mol Phylogenet Evol. 1997;7(1):103–116.