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RESEARCH ARTICLE

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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. lacticolonia	Pathogenic	Reddish brown spots	*Hylocereus polyrhizus*	Malaysia	Kee et al. [32]
N. musae	Endophytic	NA	*Musa accuminata*	Australia	Brown et al. [33]
N. oryzae	Pathogenic	Lint rot	*Gossypium hirsutum*	Alabama	Palmateer et al. [34]
N. oryzae	Pathogenic	Stem blight	*Brassica juncea*	India	Sharma et al. [35]
N. oryzae	Pathogenic	Leaf spot	*Aloe vera*	China	Zhai et al. [36]
N. oryzae	Pathogenic	Leaf spot	*Dendrobium candidum*	China	Wu et al. [37]
N. oryzae	Pathogenic	Brown/black spot disease	*Actinidia deliciosa*	China	Li et al. [38]
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. oryzae	Pathogenic	Leaf spot	*Pearl millet*	Iran	Kalati et al. [40]
N. oryzae	Pathogenic	Leaf spot	*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	Saprobic	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. osmanthi	Pathogenic	Leaf blight	*Ficus pandurata*	China	Liu et al. [45]
N. sacchari	Endophytic	NA	*Bauhinia phoenicea*	India	Raviraja et al. [46]
N. sphaerica	Pathogenic	Leaf blight	*Sesamum indicum*	China	Zhao et al. [47]
N. sphaerica	Pathogenic	Leaf blight	*Saccharum*	China	Cui et al. [48]
N. sphaerica	Pathogenic	Leaf blight	*Camellia sinensis*	India	Dutta et al. [49]
N. sphaerica	Pathogenic	Shot hole disease	*Morus alba*	India and China	Chen et al. [50], Arunakumar et al. [51]
N. sphaerica	Pathogenic	Leaf spots, twigs, and shoot blight	*Vaccinium corymbosum*	Buenos Aires, Entre Ríos	Wright et al. [31]
N. sphaerica	Pathogenic	Leaf and stem black spot disease	*Phoenix dactylifera*	Iraq	Abass [41], Abass et al. [52]
N. sphaerica	Pathogenic	Reddish brown spots	*Hylocereus polyrhizus*	Malaysia	Kee et al. [32]
N. sphaerica	Pathogenic	Black end and squirter disease	*Musa* sp.	Australia	Allen [53], Simmonds [54]
N. sphaerica	Pathogenic	Leaf spots	*Actinidia* sp.	China	Chen et al. [55]
N. sphaerica	Pathogenic	Postharvest rot	*Actinidia* sp.	China	Li et al. [56]
N. sphaerica	Pathogenic	Leaf spots	*Lagenaria siceraria*	Georgia	Li et al. [57]
N. sphaerica	Pathogenic	Leaf blight	*Camellia sinensis*	China	Liu et al. [58]
N. sphaerica	Pathogenic	Leaf blight	*Cunninghamia lanceolata*	China	Xu et al. [59]
N. sphaerica	Pathogenic	Leaf spots	Kinnow Mandarin	Pakistan	Alam et al. [60]
N. sphaerica	Pathogenic	Leaf spots	*Phoenix dactylifera*	Pakistan	Alam et al. [61]
N. sphaerica	Pathogenic	Leaf spots	*Mangiferra indica*	India	Pandey et al. [62]
N. sphaerica	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. lacticolonia* Mei Wang & L. Cai, *N. osmanthi* Mei Wang & L. Cai, *N. pyriformis* Mei Wang & L. Cai, *N. rubi* Mei Wang & L. Cai, *N. vesicularis* 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

Table 2. Primers used in the study, with sequences and references.

Gene abbreviation	Definition	Primer	Sequence (5′-3′)	References
ITS1-5.8S-ITS2	Internal transcribed spacer	ITS 4	TCCTCCGCTTATTGATATGC	White et al. [12]
		ITS 5	GGAAGTAAAAGTCGTAACAAGG	
TEF 1	Partial translation elongation factor 1- α	TEF1-728F	CATCGAGAAGTTCGAGAAGG	Carbone et al. [64]
		EF-2	GGA(G/A)GTACCAGT(G/C)ATCATGTT	O'Donnell et al. [65]
TUB2	β-Tubulin	BT-2F	AACATGCGTGAGATTGTAAGT	O'Donnell et al. [66]
		BT-4R	TAGTGACCCTTGGCCCAGTTG	

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 with the primer pairs given in Table 2. Polymerase chain reactions (PCR) were conducted in an Applied Biosystems C1000 Touch^{TM} Thermal Cycler with the following PCR conditions for ITS, TEF1, and TUB2 regions [12]: initial denaturation for 3 min at 95 °C followed by 34 cycles of denaturation 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 H_2O. 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 (BYPP) 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 3. Strains of the *Nigrospora* species and related GenBank accession numbers of taxa included in this study.

Taxa	Culture collection Number[a,b]	Host[c]	GenBank Accession numbers[d]		
			ITS	TUB2	TEF1
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)	KY385307	KY385319	KY385313
N. bambusae	LC 7244	Bamboo (leaf)	KY385306	KY385320	KY385314
N. bambusae	LC 7245	Bamboo (leaf)	KY385305	KY385321	KY385315
N. camelliae-sinensis	LC 2710	*Castanopsis* sp.	KX985957	KY019484	KY019310
N. camelliae-sinensis	LC 3287	*Camellia sinensis*	KX985975	KY019502	KY019323
N. camelliae-sinensis	LC 3496	*Camellia sinensis*	KX985985	KY019510	KY019327
N. camelliae-sinensis	CGMCC 3.18125* = LC 3500	*Camellia sinensis*	KX985986	KY019460	KY019293
N. camelliae-sinensis	LC 6684	*Camellia sinensis*	KX986046	KY019570	KY019449
N. chinensis	LC 2696	*Lindera aggregata*	KX985947	KY019474	KY019424
N. chinensis	LC 3493	*Camellia sinensis*	KX985984	KY019509	KY019434
N. chinensis	LC 4433	*Castanopsis* sp.	KX986013	KY019536	KY019436
N. chinensis	LC 4558	Unknown host plant	KX986020	KY019543	KY019441
N. chinensis	CGMCC 3.18127* = LC 4575	*Machilus breviflora*	KX986023	KY019462	KY019422
N. chinensis	LC 4660	*Quercus* sp.	KX986026	KY019548	KY019445
N. chinensis	LC 6631	*Camellia sinensis*	KX986043	KY019569	KY019448
N. chinensis	LC 6851	Unknown host plant	KX986049	KY019579	KY019450
N. gorlenkoana	CBS 480.73*	*Vitis vinifera*	KX986048	KY019456	KY019420
N. gorlenkoana	**JZB 3230001**	***Cirsium setosum*** **	**MN495939**	**MN549381**	**MN544645**
N. guilinensis	LC 7301	Vitis vinifera	KX986063	KY019608	KY019404
N. guilinensis	CGMCC 3.18124* = LC 3481	*Nelumbo* sp. (stem)	KX985983	KY019459	KY019292
N. hainanensis	CGMCC 3.18129* = LC 7030	*Musa paradisiaca* (leaf)	KX986091	KY019464	KY019415
N. hainanensis	LC 6979	*Musa paradisiaca* (leaf)	KX986079	KY019586	KY019416
N. hainanensis	LC 7031	*Musa paradisiaca* (leaf)	KX986092	KY019597	KY019417
N. hainanensis	LC 7042	*Musa paradisiaca* (leaf)	KX986094	KY019599	KY019418
N. lacticolonia	CGMCC 3.18123* = LC 3324	*Camellia sinensis*	KX985978	KY019458	KY019291
N. lacticolonia	LC 7009	*Musa paradisiaca* (leaf)	KX986087	KY019594	KY019454
N. musae	CBS 319.34*	*Musa paradisiaca* (fruit)	KX986076	KY019455	KY019419
N. musae	LC 6385	*Camellia sinensis*	KX986042	KY019567	KY019371
N. oryzae	LC 6761	*Oryza sativa*	KX986056	KY019574	KY019376
N. oryzae	LC 7297	*Nelumbo* sp. (leaf)	KX985936	KY019605	KY019400
N. oryzae	LC 2693	*Neolitsea* sp.	KX985944	KY019471	KY019299
N. oryzae	LC 2707	*Rhododendron simiarum*	KX985954	KY019481	KY019307
N. oryzae	LC 4338	*Camellia* sp.	KX986008	KY019532	KY019349
N. oryzae	LC 4961	*Pittosporum illicioides*	KX986031	KY019553	KY019358
N. oryzae	LC 5243	Submerged wood	KX986033	KY019555	KY019360
N. oryzae	LC 6923	*Oryza sativa* L.	KX986051	KY019581	KY019383
N. oryzae	**JZB 3230002**	***Phyllostachys nigra*** **	**MN495940**	**–**	**MN544639**
N. oryzae	**JZB 3230003**	***Rudbeckia hirta*** **	**MN495941**	**–**	**MN544640**
N. oryzae	**JZB 3230004**	***Scirpus* sp.** **	**MN495942**	**MN549382**	**MN544641**
N. osmanthi	CGMCC 3.18126* = LC 4350	*Osmanthus sp.*	KX986010	KY019461	KY019421
N. osmanthi	LC 4487	*Hedera nepalensis*	KX986017	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 3230008**	***Cirsium setosum*** **	**MN495946**	**MN549386**	**MN508182**
N. osmanthi	**JZB 3230009**	***Phyllostachys nigra*** **	**MN495947**	**MN549387**	**MN508183**
N. osmanthi	**JZB 3230010**	***Phyllostachys nigra*** **	**MN495948**	**MN549388**	**MN508184**
N. osmanthi	**JZB 3230011**	***Rudbeckia hirta*** **	**MN495949**	**MN549389**	**MN508185**
N. pyriformis	CGMCC 3.18122* = LC 2045	*Citrus sinensis*	KX985940	KY019457	KY019290
N. pyriformis	LC 2688	*Lindera aggregate*	KX985941	KY019468	KY019297
N. pyriformis	LC 2694	*Rubus reflexus*	KX985945	KY019472	KY019300
N. pyriformis	LC 3099	*Camellia sinensis*	KX985971	KY019498	KY019322
N. pyriformis	LC 3292	*Camellia sinensis*	KX985976	KY019503	KY019324
N. rubi	CGMCC 3.18326* = LC 2698	*Rubus* sp.	KX985948	KY019475	KY019302
N. rubi	**JZB 3230012**	***Fraxinus* sp.** **	**MN495950**	**–**	**MN544646**
N. sphaerica	LC 7312	*Nelumbo* sp. (leaf)	KX985935	KY019618	KY019414
N. sphaerica	LC 7298	*Nelumbo* sp. (leaf)	KX985937	KY019606	KY019401
N. sphaerica	LC 2840	*Harpullia longipetala*	KX985965	KY019492	KY019318
N. sphaerica	LC 3477	*Camellia sinensis*	KX985982	KY019508	KY019326
N. sphaerica	LC 4264	*Rhododendron arboretum*	KX985993	KY019517	KY019334
N. sphaerica	LC 4307	*Rhododendron arboretum*	KX986005	KY019529	KY019346
N. sphaerica	LC 5901	Submerged wood	KX986034	KY019556	KY019361
N. sphaerica	LC 6294	*Camellia sinensis*	KX986044	KY019565	KY019369
N. sphaerica	LC 6996	*Musa paradisiaca* (leaf)	KX986085	KY019592	KY019390
N. sphaerica	**JZB 3230013**	***Cirsium setosum*** **	**MN495951**	**MN549390**	**MN544642**
N. sphaerica	**JZB 3230014**	***Phragmites australis*** **	**MN495952**	**MN549391**	**MN544643**
N. sphaerica	**JZB 3230015**	***Fraxinus* sp.** **	**MN495953**	**MN549392**	**MN544644**
Nigrospora sp. 1	LC 2725	*Symplocos zizyphoides*	KX985960	KY019487	KY019313
Nigrospora sp. 1	LC 4566	*Lithocarpus* sp.	KX986022	KY019545	KY019354
Nigrospora sp. 2	LC 6704	*Camellia sinensis*	KX986047	KY019571	KY019373
N. vesicularis	LC 0322	Unknown host plant	KX985939	KY019467	KY019296
N. vesicularis	CGMCC 3.18128* = LC 7010	*Musa paradisiaca* (leaf)	KX986088	KY019463	KY019294
N. zimmermanii	CBS 167.26	Unknown	KY385308	KY385318	KY385312

(*continued*)

Table 3. Continued.

Taxa	Culture collection Number[a,b]	Host[c]	GenBank Accession numbers[d] ITS	TUB2	TEF1
N. zimmermanii	CBS 290.62*	*Saccharum officinarum* (leaf)	KY385309	KY385317	KY385311
N. zimmermanii	CBS 984.69	*Saccharum officinarum* (leaf)	KY385310	KY385322	KY385316
Arthrinium obovatum	LC 4940		KY494696	KY705166	KY705095
Arthrinium malaysianum	CBS 102053		NR120273	KF144988	KF145030

[a]CGMCC: China General Microbiological Culture Collection, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; CBS: Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; JZB: Beijing Academy of Agriculture and Forestry Sciences Culture Collection, China; LC: working collection of Lei Cai, housed at the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.
[b,*]Ex-type culture.
[c,**]Novel host associations.
[d]ITS: internal transcribed spacer region (ITS1-5.8S-ITS2); TUB2: β-tubulin; TEF1: translation elongation factor 1-α.
Sequences generated in this study are in bold type face.

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 + I + 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 α = 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: Khuskia H.J. Huds., Trans. Br. mycol. Soc. 46:358 (1963),

Nigrospora gorlenkoana 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. ($\overline{x}$ = 8.4 × 6 μm, n = 30), monoblastic, solitary, discrete, determinate, doliiform to ampulliform, and pale brown. *Conidia* 10.3–14 × 13.3–17.2 μm diam. ($\overline{x}$ = 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.

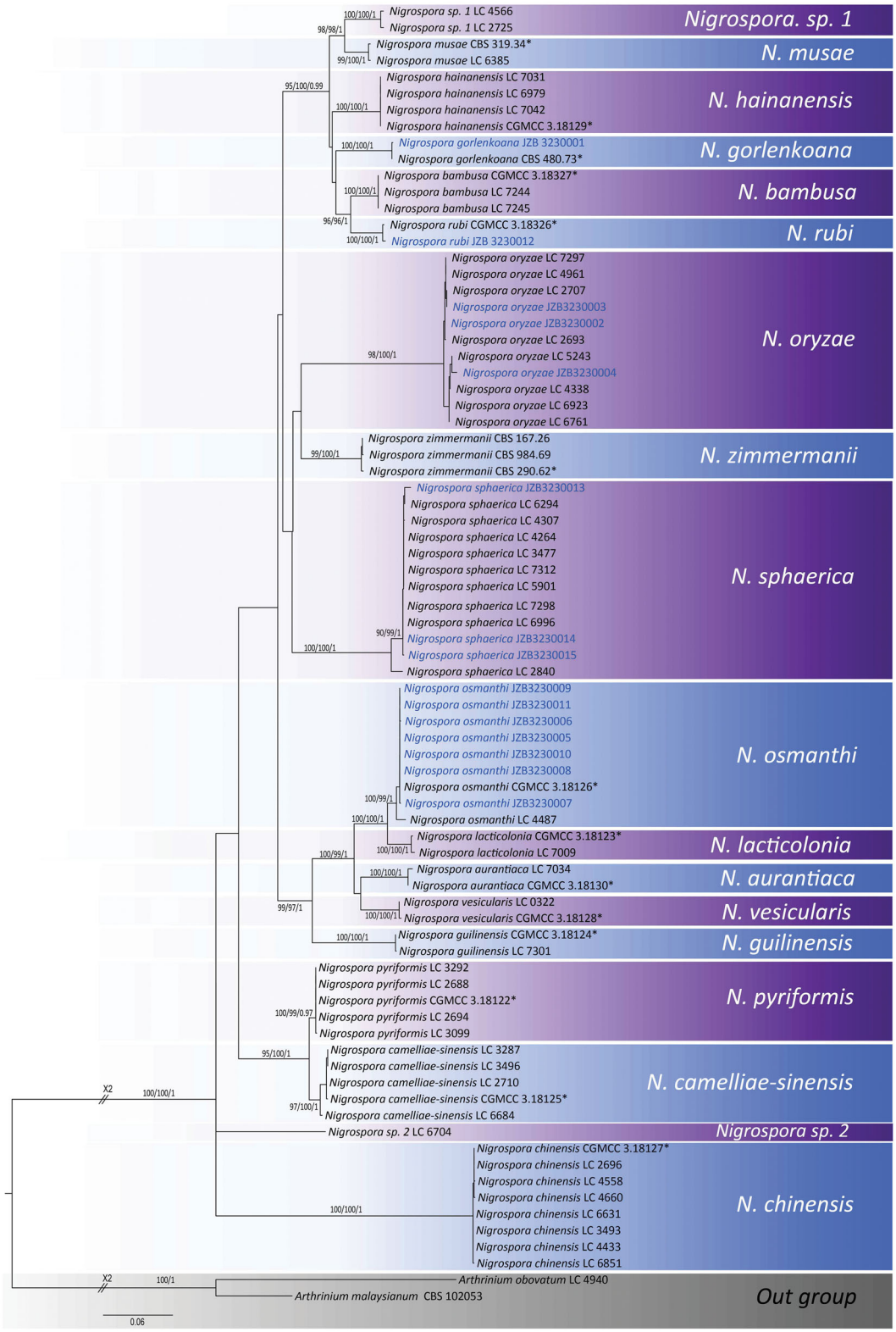

Figure 1. Multilocus phylogenetic tree based on the combined ITS, TEF1, and TUB2 sequences alignment generated from a maximum likelihood phylogenetic analysis. Bootstrap support values for ML, MP (> 70%), and posterior probabilities (> 0.9) are given at the nodes (ML/MP/PP). The tree is rooted with *Arthrinium malaysianum* (CBS 102053) and *Arthrinium obovatum* (LC 4940). (*indicates the ex-type isolates).

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

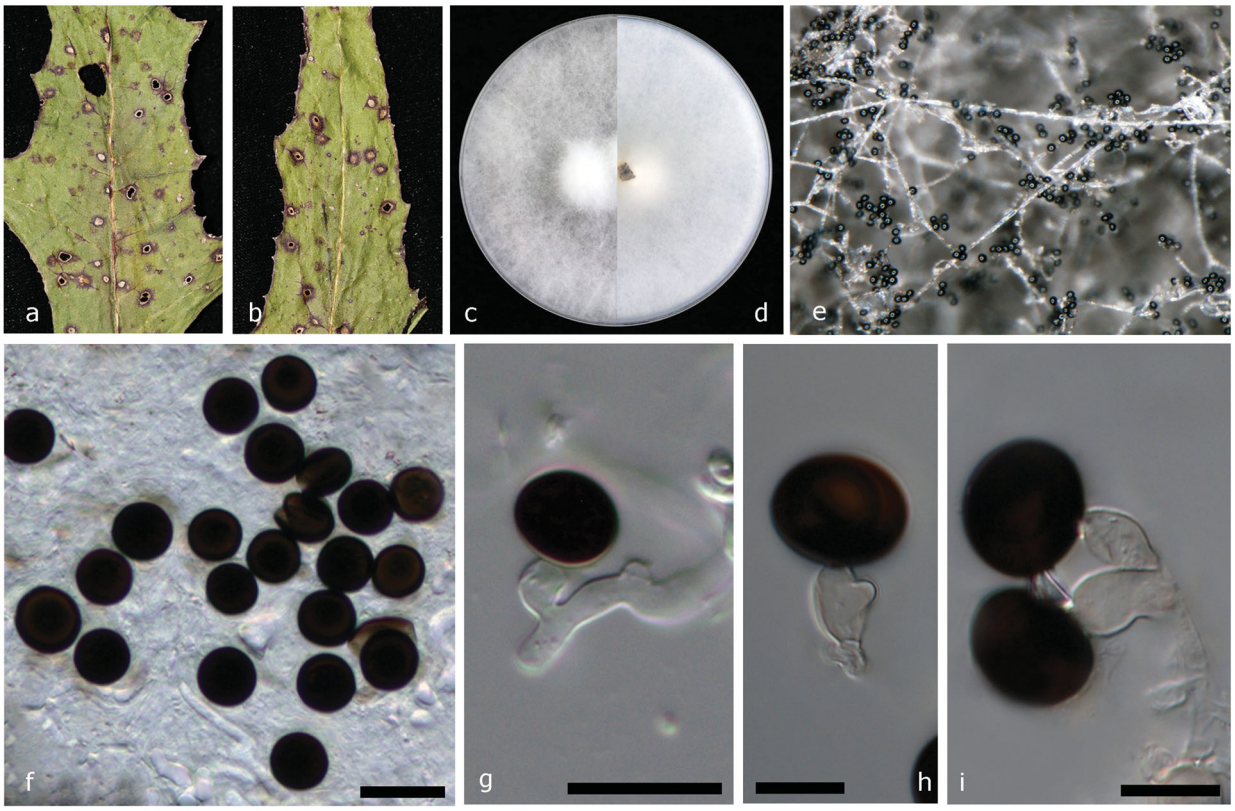

Figure 2. *Nigrospora gorlenkoana* (JZB 3230001). (a and b) Appearance of leaf spots on the host substrate; (c and d) Upper view (c) and reverse view (d) of culture on PDA; (e) Conidia on aerial mycelia on PDA; (f) Mature conidia; (g–i) Mature conidia attached to conidiogenous cells. Scale bars f, $g = 20\,\mu m$, h, and $i = 10\,\mu m$.

ring 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.

Nigrospora oryzae (Berk. & Broome) Petch, J. Indian Bot. Soc. 4:24 (1924),

Facesoffungi number: FoF 06596 (Figure 3).

Basionym: *Monotospora oryzae* Berk. & Broome, J. Linn. Soc., Bot. 14: 99 (1873) [1875]

≡ *Khuskia oryzae* H.J. Huds., Trans. Br. mycol. Soc. 46(3): 358 (1963)

≡ *Apiospora oryzae* (H.J. Huds.) Arx, *Gen. Fungi Sporul. Cult.*, Edn 2: 129 (1974).

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. ($\overline{x}$ = 11.18 × 7.98 μm, $n = 30$), aggregated in clusters on hyphae, monoblastic, determinate, ampulliform or doliiform, and hyaline to pale brown. *Conidia* 9.0–13.2 × 12.6–15.8 μm diam. ($\overline{x}$ = 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

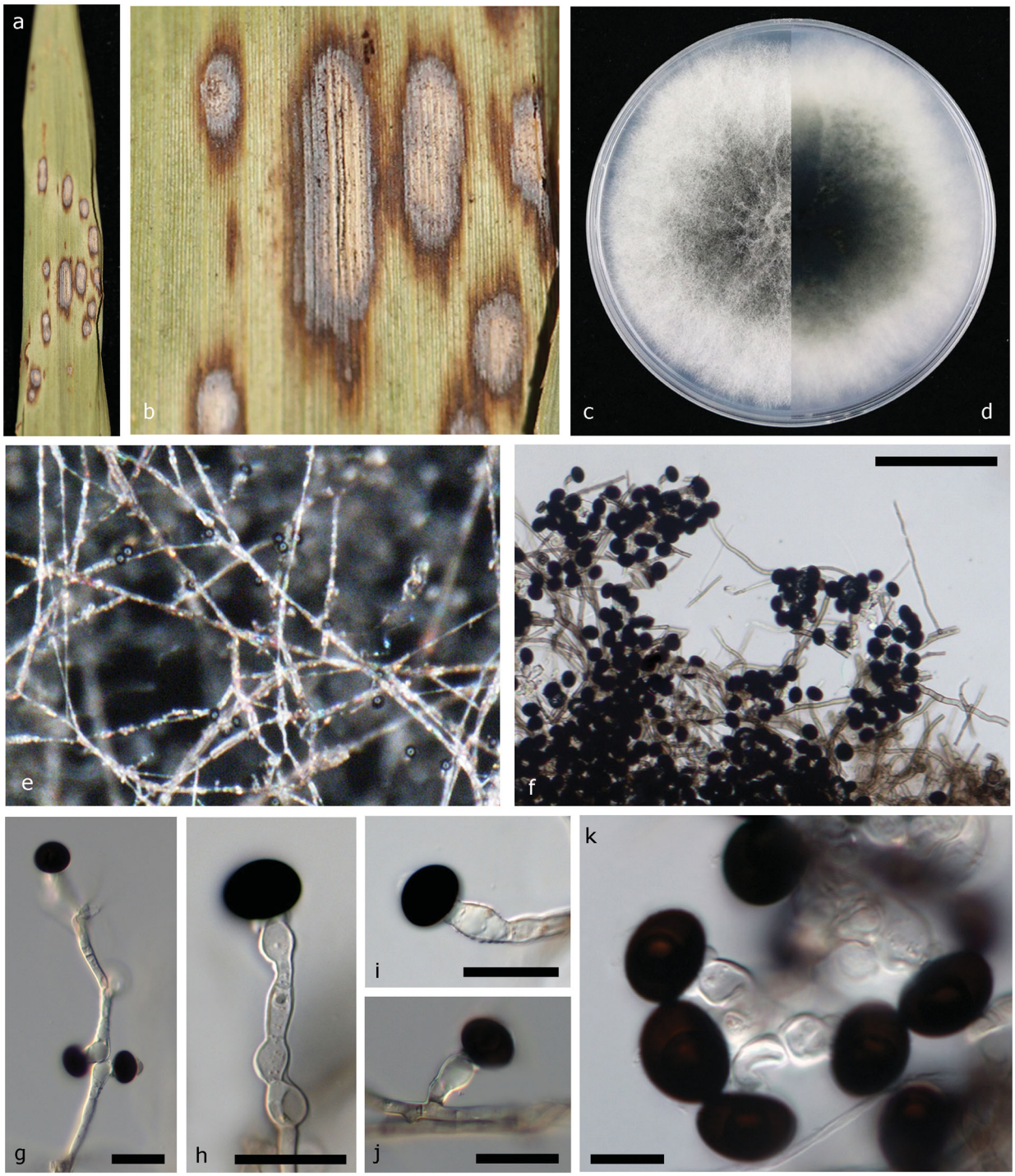

Figure 3. *Nigrospora oryzae* (JZB 3230004). (a and b) Appearance of leaf spots on the host substrate; (c and d) Upper view (c) and reverse view (d) of culture on PDA; (e) Surface view of the colony on PDA; (f) Colony on PDA; (g–k) Mature conidia attached *to* conidiogenous cells. Scale bars f, $g = 20\,\mu m$, h, and $i = 10\,\mu m$.

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

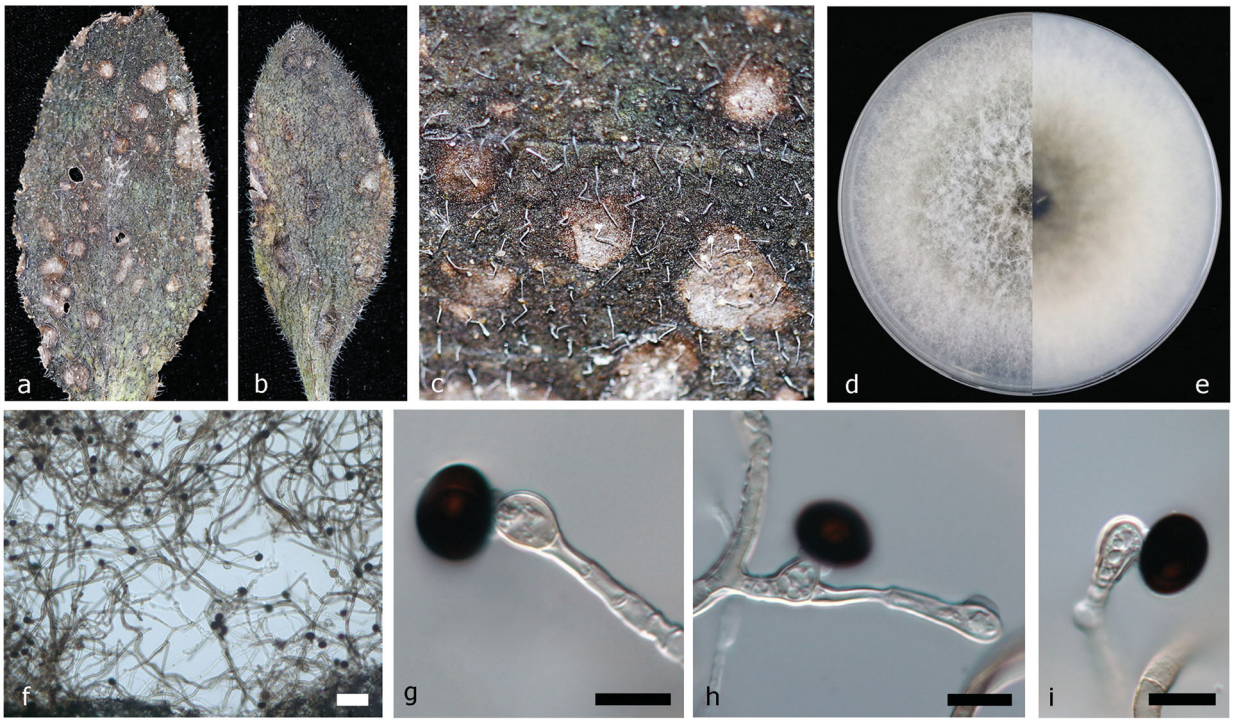

Figure 4. *Nigrospora osmanthi* (JZB 3230011). (a and b) Appearance of leaf spots on host substrate; (c) Enhanced view of leaf spot on the host substrate; (d and e) Upper view (c) and reverse view (d) of culture on PDA; (f) Colony on PDA; (g–i) Mature conidia attached to conidiogenous cells. Scale bars *f* = 50 µm, g–*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. ($\overline{x}$ = 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. ($\overline{x}$ = 10 × 13.2 µm, *n* = 50), discrete on aerial mycelia, solitary, globose or oblate, dark brown to black, shiny, smooth-walled, and aseptate. 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.*, 07 October 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.*

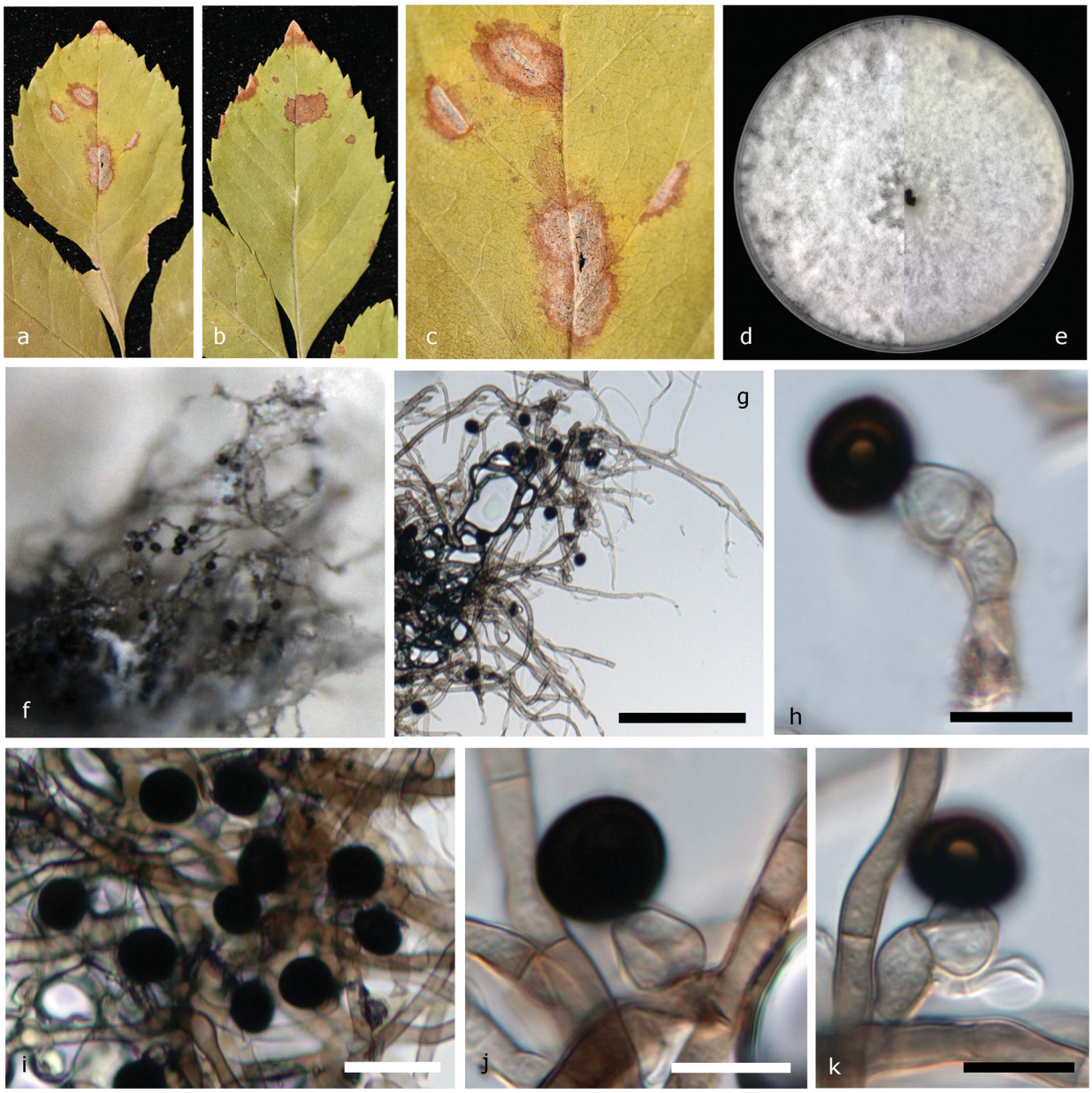

Figure 5. *Nigrospora rubi* (JZB 3230012). (a and b) Appearance of leaf spots on host substrate; (c) Enhanced view of leaf spot on the host substrate; (d and e) Upper view (c) and reverse view (d) of culture on PDA; (f) Surface view of the colony on PDA; (g) Colony on PDA (h, j, and k) Mature conidia attached to conidiogenous cells; (i) Mature conidia. Scale bars $g = 100$ µm, $h = 10$ µm, $i = 20$ µm, j, and $k = 10$ µm.

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*, which is a Chinese herb, *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 ($\overline{x}$ = 6.7 × 6.9 µm, $n = 30$), clustered on hyphae, unbranched, ampulliform, short, and squat pale brown. *Conidia*

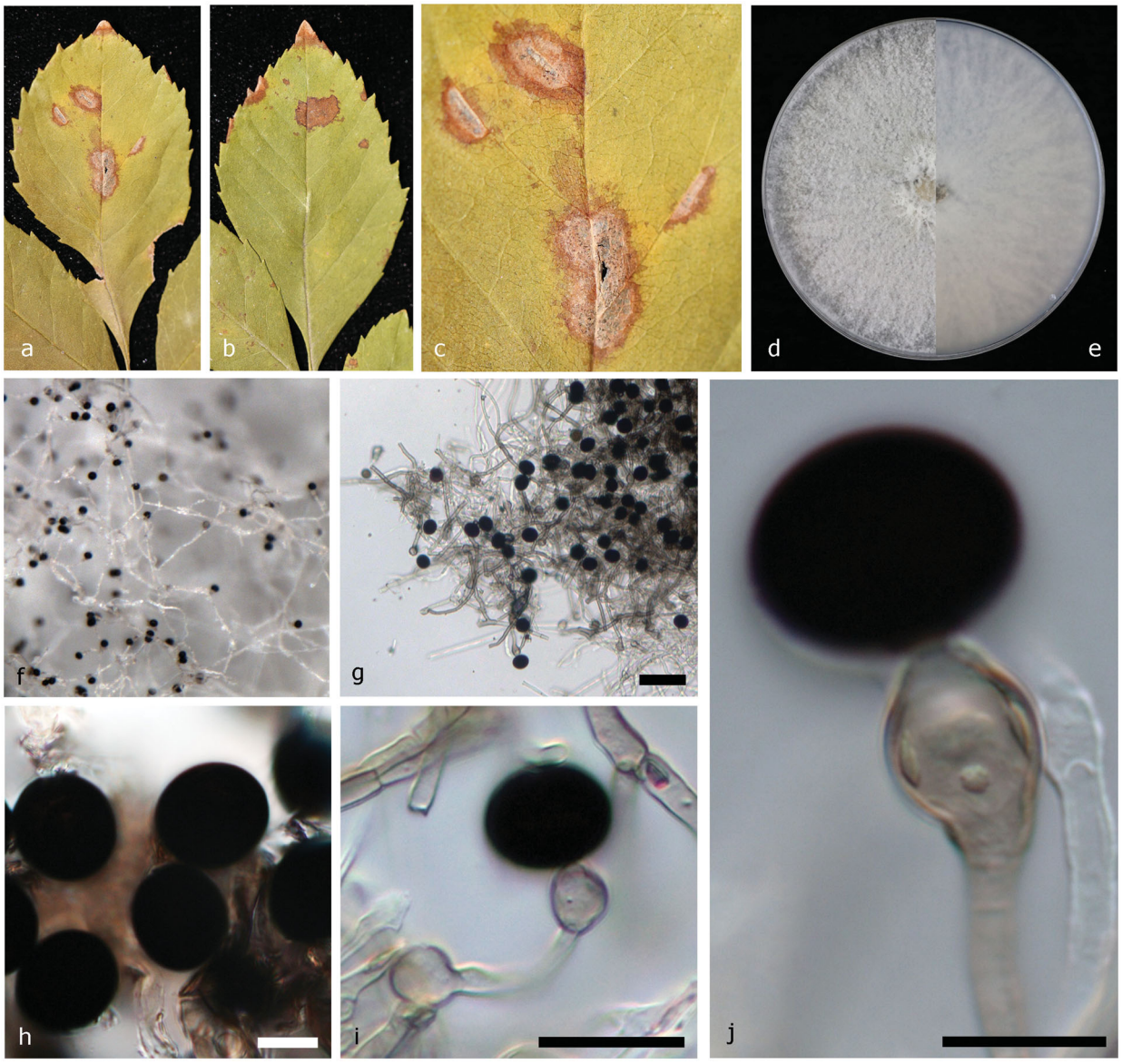

Figure 6. *Nigrospora sphaerica* (JZB 3230015). (a and b) Appearance of leaf spots on host substrate; (c) Enhanced view of leaf spot on the host substrate; (d and e) Upper view (c) and reverse view (d) of culture on PDA; (f) Surface view of the colony on PDA; (g) Colony on PDA; (h) Mature conidia. (i and j) Mature conidia attached to conidiogenous cells. Scale bars $g = 50\,\mu m$, $h = 10\,\mu m$, $i = 20\,\mu m$, and $j = 10\,\mu m$.

7.9–10.7 × 10–12.1 μm diam. ($\overline{x}$ = 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 conidiogenous cells. *Conidiogenous cells* 9.5–16.5 × 7.4–9.8 μm diam. ($\overline{x}$ = 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. ($\overline{x}$ = 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. gallarum*, *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. Kohlmerer & R.V. Gessner, *Corollospora maritima* Werderm., *Dryosphaera navigans* Jørg. Koch & E.B.G. Jones, *Halosphaeriopsis mediosetigera* (A.B. Cribb & J.W. Cribb) T.W. Johnson, *Lignincola laevis Höhnk*, *Monosporascus cannonballus* Pollack & Uecker, *Natantispora retorquens* (C.A. Shearer & J.L. Crane) J. Campb., J.L. Anderson & C.A. Shearer, *Pleospora betae* Björl., *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 achrasporum* 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. Sutherl. 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., *Agaricus silvicola* (Vittad.) Peck, *Ganoderma lingzhi* Sheng H. Wu, Y. Cao & Y.C. Dai, *Grifola frondosa* (Dicks.) Gray, *Lactarius deliciosus* L., *Lactarius subvellereus* Peck, *Perenniporia fraxinea* (Bull.) Ryvarden, *Pholiota adipose* (Batsch) P. Kumm., *Schizophyllum commune* Fr., *Suillus bovinus* (L.) Roussel, *Suillus granulatus* (L.) Roussel, *Xerocomellus chrysenteron* (Bull.) Šutara, and *Xerula radicata* (Relhan) Dörfelt, are among edible fungi [25]. Further, *Leptosphaeria agnita* (Desm.) Ces. & De Not., *L. dumetorum* 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 public repositories. The GenBank accession numbers and the TreeBase submission number are given within the article.

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