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Nigrospora humicola (Apiosporaceae, Amphisphaeriales), a New Fungus from Soil in China

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Abstract: The fungal genus *Nigrospora* is known to be a plant pathogen, endophyte, and saprobe, and it is usually isolated from various substrates like soil and air. During the surveys of soil fungi in Hebei Province of China, two isolates of *Nigrospora* were obtained. A multi-locus phylogeny of combined loci of the 5.8S nuclear ribosomal gene with the two flanking transcribed spacers (ITS), part of the translation elongation factor 1-alpha (*tef1*), and the beta-tubulin (*tub2*) loci, in conjunction with morphological characters were used to identify the newly collected isolates. *Nigrospora humicola* sp. Nov. is described and proposed herein, which differs from its phylogenetically close species *N. chinensis* and *N. globosa* by the sequences of ITS, *tef1*, and *tub2*.

Keywords: Ascomycota; new species; phylogeny; systematics; taxonomy

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1. Introduction

Nigrospora was proposed based on *N. panici*, collected from dead leaves of *Panicum amphibium* in Indonesia, which had spherical to subspherical conidiogenous cells and black and globose to subglobose conidia [1]. Members of *Nigrospora* were traditionally distinguished by comparing the morphological features, especially the conidial dimensions [2]. However, a recent study showed that the conidial dimensions frequently overlapped among phylogenetically distinct species [3]. *Nigrospora* was currently classified in the family Apiosporaceae within Amphisphaeriales evidenced by the phylogeny of molecular data [4,5]. Subsequently, several novel species of *Nigrospora* were revealed on the basis of the molecular and morphological evidence [6–13].

Nigrospora is a cosmopolitan genus on various substrates, including multifarious plants, soil, and air [2,3,7]. In addition, some *Nigrospora* species were considered to be important plant pathogens [14,15]. For example, *N. sphaerica* causes *Camellia sinensis* leaf blight diseases in China [16], *N. lacticolonia* and *N. sphaerica* are associated with the reddish brown spot disease of *Hylocereus polyrhizus* [17], and *N. oryzae* results in the leaf spot of *Hibiscus mutabilis* [18]. In addition, species of *Nigrospora* are also commonly discovered in an indoor environment and sometimes from the soil [3,9].

Nigrospora taxa are considered as a source of natural products due to their industrial applications [19–21]. For example, *N. sacchari* produces metabolites that have remarkable herbicidal activity in greenhouse-grown plants [22]; *N. spherical* can produce phomalactone against mosquitoes [23]. Hence, species of this genus are worth studying to develop related natural products. In the present study, new isolates were obtained from the forest soil and identified using a combined method of morphology and phylogeny.

2. Materials and Methods

2.1. Isolation

Strains of *Nigrospora* in the present study were isolated from forest soils in the Hebei Province of China in July 2021. Soil samples were divided into 1 g per portion and spread on 15 cm petri dishes containing potato dextrose agar medium (PDA; 200 g potato, 20 g glucose, 16 g agar per liter) with streptomycin sulfate and ampicillin 100 mg/mL in each dish. Plates were incubated at 25 °C for 2 d, the colonies were obtained, and then, they were transferred to the new PDA plates. The cultures were deposited in the China Forestry Culture Collection Center and the specimens in the herbarium of the Chinese Academy of Forestry.

2.2. Morphology

Isolates obtained in the present study were observed and described in terms of the colony color and appearance, based on the colonies grown on PDA medium. Plates were incubated for a week in the dark at 25 °C. Micro-morphological features were observed and recorded by a Nikon Eclipse 80i compound microscope equipped with a Nikon digital sight DS-Ri2 high-definition color camera. A total of 50 conidiogenous cells and conidia were randomly selected, observed, and measured.

2.3. DNA Extraction, PCR Amplification, and Phylogenetic Analyses

The fungal DNA was extracted from cultures grown on PDA plates overlaid with cellophane using a CTAB method [24]. The primer pair ITS1/ITS4 was used to amplify the internal transcribed spacer region and intervening 5.8S nrRNA gene (ITS) [25]. The primer pair EF-688F/EF2 was used to amplify part of the translation elongation factor 1-alpha (*tef1*) [26]. Bt2a/Bt2b was used to amplify part of the Beta-tubulin gene (*tub2*) [27]. The polymerase chain reaction (PCR) conditions were as performed. The resulting PCR products were visualized on a 1.4% agarose gel with ethidium bromide under UV light, and then the PCR positive products were sent to the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China) for sequencing reactions using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminater Kit v.3.1 (Invitrogen, Beijing, China).

Reference sequences were retrieved from the National Center for Biotechnology Information (NCBI) based on recent publications on the genus *Nigrospora* [3,6–13], and sequences from the present study were deposited in GenBank (Table 1). Sequences were aligned using MAFFT v. 7 [28] and manually edited using MEGA7 [29]. The phylogenetic analyses of the combined ITS, *tef1,* and *tub2* loci were conducted using both Maximum Likelihood (ML) and Bayesian Inference (BI) methods. ML was implemented on the website of CIPRES Science Gateway using RAxML-HPC BlackBox 8.2.10 [30], employing a GTRGAMMA substitution model with 1000 bootstrap replicates, while BI was performed by a Markov Chain Monte Carlo (MCMC) algorithm using MrBayes v. 3.0 [31]. Two MCMC chains, started from random trees for 1,000,000 generations and trees, were sampled every 100th generation, resulting in a total of 10,000 trees. The first 25% of trees were discarded as the burn-in of each analysis. Branches with significant Bayesian Posterior Probabilities (BPP) were estimated in the remaining 7500 trees. Phylogenetic trees were viewed and edited in FigTree v.1.3.1 and Adobe Illustrator CS5.

Table 1. Isolates and GenBank accession numbers used in the phylogenetic analyses of *Nigrospora*.

Species	Isolate	Host/Substrate	Origin	GenBank Accession Numbers		
				ITS	*tub2*	*tef1*
Apiospora qinlingensis	CFCC 52303 *	*Fargesia qinlingensis*	China	MH197120	MH236791	MH236795
A. vietnamensis	IMI 99670 *	*Citrus sinensis*	Vietnam	KX986096	KY019466	NA
Nigrospora aurantiaca	CGMCC 3.18130 *	*Nelumbo*	China	KX986064	KY019465	KY019295
N. aurantiaca	LC 7034	*Musa paradisiaca*	China	KX986093	KY019598	KY019394
N. bambusae	CGMCC 3.18327 *	Bamboo	China	KY385307	KY385319	KY385313

Table 1. *Cont.*

Species	Isolate	Host/Substrate	Origin	GenBank Accession Numbers		
				ITS	*tub2*	*tef1*
N. bambusae	LC 7244	Bamboo	China	KY385306	KY385320	KY385314
N. brasiliensis	CMM 1214 *	*Nopalea cochenillifera*	Brazil	KY569629	MK720816	MK753271
N. camelliae-sinensis	CGMCC 3.18125 *	*Camellia sinensis*	China	KX985986	KY019460	KY019293
N. camelliae-sinensis	LC 4460	*Castanopsis*	China	KX986015	KY019538	KY019353
N. chinensis	CGMCC 3.18127 *	*Machilus breviflora*	China	KX986021	KY019544	KY019442
N. chinensis	LC 4593	*Machilus duthiei*	China	KX986023	KY019462	KY019422
N. cooperae	BRIP 72531c *	*Senna* sp.	Australia	OP035049	OP039542	OP039541
N. covidalis	CGMCC 3.20538 *	*Lithocarpus* sp.	China	OK335209	OK431479	OK431485
N. falsivesicularis	CGMCC 3.19678 *	*Saccharum officinarum*	China	MN215778	MN329942	MN264017
N. globosa	CGMCC 3.19633 *	Soil	China	MK329121	MK336134	MK336056
N. globospora	CGMCC 3.20539 *	Petasites hybridus	China	OK335211	OK431481	OK431487
N. gorlenkoana	CBS 480.73 *	*Vitis vinifera*	Kazakhstan	KX986048	KY019456	KY019420
N. guangdongensis	CFCC 53917 *	*Cunninghamia lanceolata*	China	MT017509	MT024495	MT024493
N. guilinensis	LC 7301	*Nelumbo*	China	KX986063	KY019608	KY019404
N. guilinensis	CGMCC 3.18124 *	*Camellia sinensis*	China	KX985983	KY019459	KY019292
N. hainanensis	CGMCC 3.18129 *	*Musa paradisiaca*	China	KX986091	KY019464	KY019415
N. hainanensis	LC 6979	*Musa paradisiaca*	China	KX986079	KY019586	KY019416
N. humicola	**CFCC 56884 ***	**Soil**	**China**	**ON555686**	**ON557392**	**ON557394**
N. humicola	**CFCC 56885**	**Soil**	**China**	**ON555687**	**ON557393**	**ON557395**
N. lacticolonia	CGMCC 3.18123 *	*Camellia sinensis*	China	KX985978	KY019458	KY019291
N. lacticolonia	LC 7009	*Musa paradisiaca*	China	KX986087	KY019594	KY019454
N. macarangae	MFLUCC 19-0141 *	*Macaranga tanarius*	China	MW114318	NA	NA
N. magnoliae	MFLUCC 19-0112 *	*Magnolia candolli*	China	MW285092	MW438334	NA
N. magnoliae	LC 6704	*Camellia sinensis*	China	KX986047	KY019571	KY019373
N. musae	CBS 319.34 *	*Musa paradisiaca*	Australia	KX986076	KY019455	KY019419
N. musae	LC 6385	*Camellia sinensis*	China	KX986042	KY019567	KY019371
N. oryzae	LC 6759	*Oryza sativa*	China	KX986054	KY019572	KY019374
N. oryzae	LC 6760	*Oryza sativa*	China	KX986055	KY019573	KY019375
N. osmanthi	CGMCC 3.18126 *	*Osmanthus*	China	KX986010	KY019461	KY019421
N. osmanthi	LC 4487	*Hedera nepalensis*	China	KX986017	KY019540	KY019438
N. philosophiae-doctoris	CGMCC 3.20540 *	*Disporum sessile*	China	OK335213	OK431483	OK431489
N. pyriformis	CGMCC 3.18122 *	*Citrus sinensis*	China	KX985940	KY019457	KY019290
N. pyriformis	LC 2688	*Lindera aggregata*	China	KX985941	KY019468	KY019297
N. rubi	CGMCC 3.18326 *	*Rubus*	China	KX985948	KY019475	KY019302
N. saccharicola	CGMCC 3.19362 *	*Saccharum officinarum*	China	MN215788	MN329951	MN264027
N. sacchari-officinarum	CGMCC 3.19335 *	*Saccharum officinarum*	China	MN215791	MN329954	MN264030
N. singularis	CGMCC 3.19334 *	*Saccharum officinarum*	China	MN215793	MN329956	MN264032
N. sphaerica	LC 7294	*Nelumbo*	China	KX985932	KY019602	KY019397
N. sphaerica	LC 7295	*Nelumbo*	China	KX985933	KY019603	KY019398
N. vesicularifera	CGMCC 3.19333 *	*Saccharum officinarum*	China	MN215812	MN329975	MN264051
N. vesicularis	LC 0322	NA	Thailand	KX985939	KY019467	KY019296
N. vesicularis	CGMCC 3.18128 *	*Musa paradisiaca*	China	KX986088	KY019463	KY019294
N. zimmermanii	CBS 167.26	NA	NA	KY385308	KY385318	KY385312
N. zimmermanii	CBS 290.62 *	*Saccharum officinarum*	Ecuador	KY385309	KY385317	KY385311

Note: NA, not applicable. Ex-type strains are marked with *, and strains from the present study are marked in bold.

3. Results

3.1. Phylogeny

The resulting phylogram based on a combined analysis of ITS, *tef1*, and *tub2* loci was used to reveal the species relationship of the newly collected isolates within *Nigrospora*. The dataset consisted of 45 sequences including two outgroup taxa, namely *Apiospora qinglingensis* (CFCC 52303) and *A. vietnamensis* (IMI 99670). The dataset comprised 1511 characters after alignment including the gaps (547 for ITS, 535 for *tef1*, and 429 for *tub2*), which were included in the phylogenetic analysis. Of these, 876 characters were constant, 166 variable characters were parsimony uninformative, and 467 characters were parsimony informative.

The topologies resulting from the ML and BI analyses of the concatenated dataset were congruent (Figure 1). Two isolates from the present study clustered into a distinct clade from the other species of this genus, which represents an undescribed *Nigrospora* species.

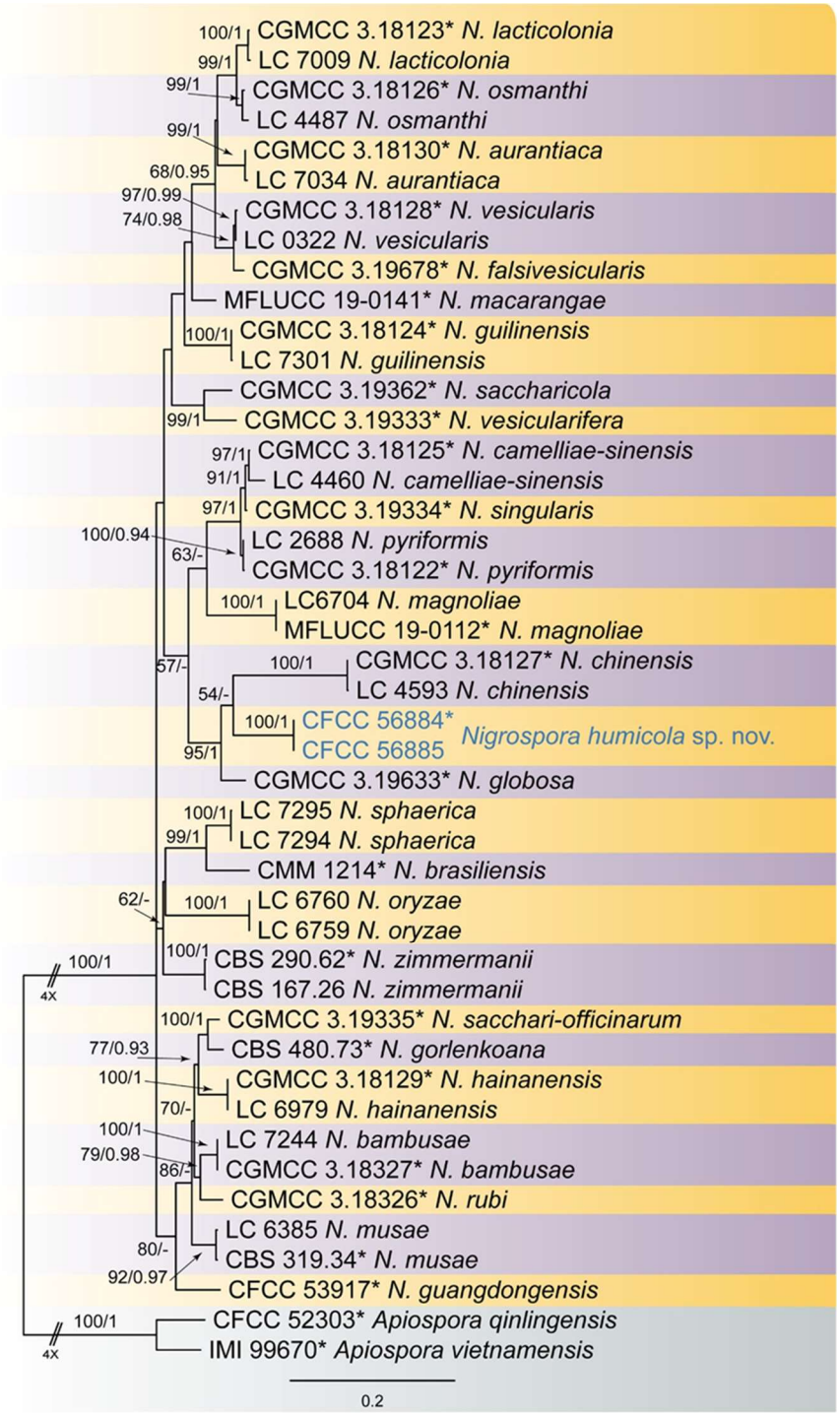

Figure 1. Phylogenetical tree of *Nigrospora* of ML analysis on basis of combined ITS, *tef1,* and *tub2* loci. Numbers above the branches indicate ML bootstraps (left, ML BS $\geq$ 50%) and Bayesian Posterior Probabilities (right, BPP $\geq$ 0.90). The tree is rooted with *Apiospora qinlingensis* (CFCC 52303) and *A. vietnamensis* (IMI 99670). New species from the present study are marked in blue, and ex-type strains are marked with *.

3.2. Taxonomy

Nigrospora humicola Q. Yang & Ning Jiang, sp. nov.
Figure 2.

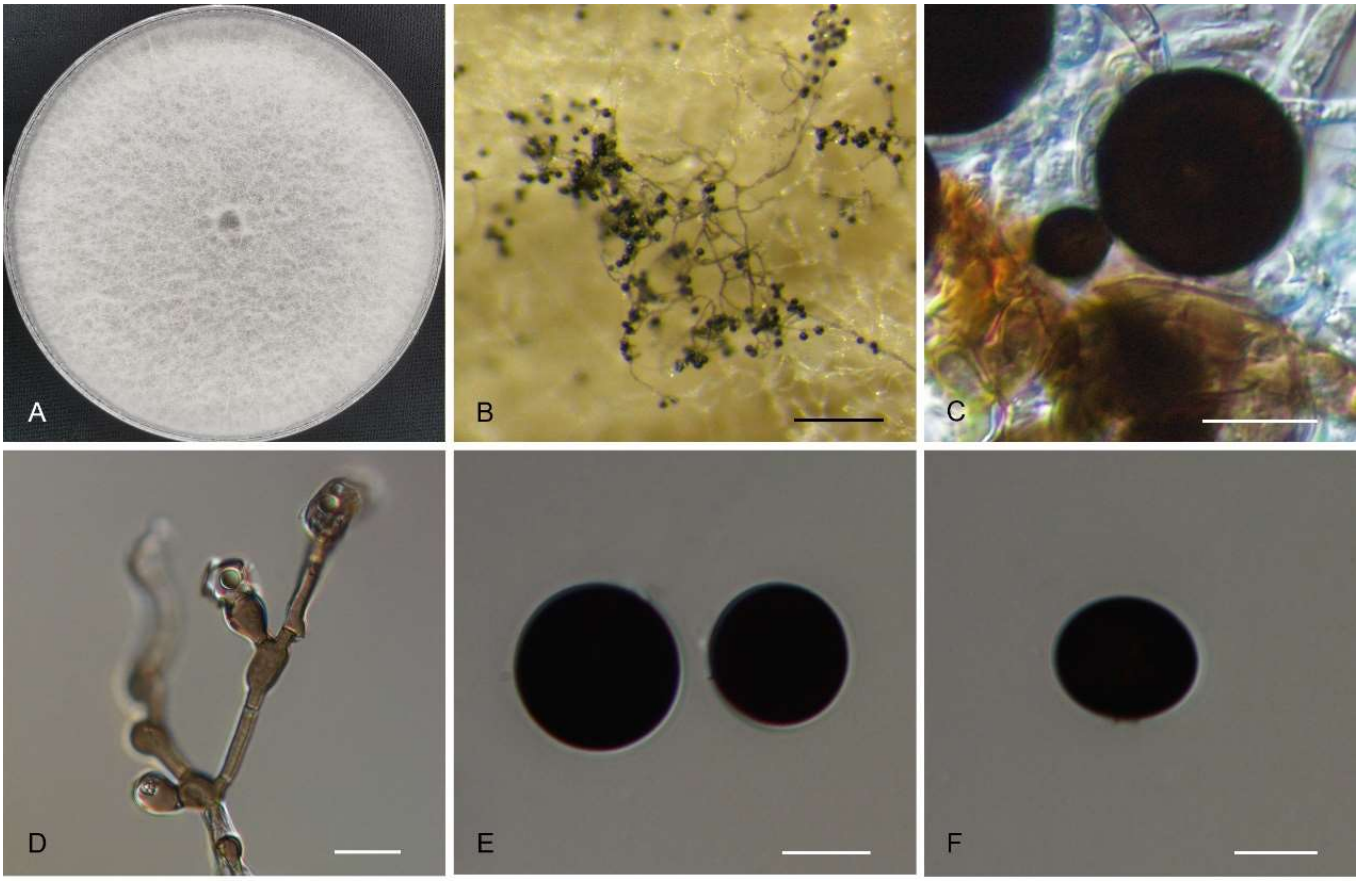

Figure 2. Morphology of *Nigrospora humicola* (CFCC 56884). (**A**) Colony on PDA. (**B**) Conidiomata formed in culture. (**C**,**D**) Conidiogenous cells giving rise to conidia. (**E**,**F**) Conidia. Scale bars: (**B**) = 200 μm; (**C**–**F**) = 10 μm.

MycoBank no: 844103

Etymology: Referring to the substrate soil, where the type of strain originated.

Sexual morph undetermined. Asexual morph on PDA: Hyphae 2.5–6 μm diam., smooth, hyaline to brown, branched, septate. Conidiophores smooth, hyaline to brown, branched, septate, sometimes reduced to conidiogenous cells. Conidiogenous cells 4.5–15.5 × 2.5–12 μm, aggregated in clusters on hyphae, pale brown, subglobose to ampulliform. Conidia 12.5–23.5 × 9.5–16 μm (av. = 16.3 ± 3.4 × 13.2 ± 2.7 μm) solitary, globose to subglobose, black, shiny, smooth, aseptate.

Cultural characteristics: Colonies on PDA at 25 °C floccose, edge entire, initially white, becoming grey to brown with age, reaching 9 cm diam in 10 d, reverse smoke-grey with black patches.

Material examined: CHINA, Hebei Province, Chengde City, Tongshan Garden, from soil, Q. Yang, 5 July 2021 (holotype CAF 800052; ex-type culture CFCC 56884); *ibid*. (culture CFCC 56885).

Notes: Two saprophytic isolates in the soil obtained in this study clustered into a well-supported clade distinguished from the other known species (Figure 1). *Nigrospora humicola* is phylogenetically close to *N. chinensis* and *N. globosa*. Morphologically, these three species share similar conidial morphology and size (12.5–23.5 × 9.5–16 μm in *N. humicola* vs. 10–14.5 × 7.5–11 μm in *N. chinensis* vs. 11–14.5 × 9–13 μm in *N. globosa*). However,

N. humicola differs from *N. chinensis* (ITS: 28/518; *tef1*: 110/481; *tub2*: 40/392) and *N. globosa* (ITS: 19/486; *tub2*: 38/392) by sequence data [3,9].

4. Discussion

Nigrospora is a recently redefined monophyletic genus, and the species were well distinguished based on the combined loci of ITS, *tef1*, and *tub2* sequence data [3,7]. Currently, the type species of *Nigrospora*, *N. panici* from *Panicum amphibium* in Indonesia, is not available in molecular data, and the holotype has been lost [1]. Hence, new collections from the original region and host *P. amphibium* are necessary to improve the genus concept.

Nigrospora species are common during fungal investigations; however, the sexual morph is rarely observed. From the asexual morph, all members have spherical to subspherical conidiogenous cells and black and globose to subglobose conidia [3]. In recent publications, species were distinguished mainly by conidial sizes [6–13,32]. However, the new species from the present study, *N. humicola*, is difficult to distinguish from its related species *N. chinensis* and *N. globosa*. Hence, molecular data (ITS, *tef1* and *tub2*) are necessary for the species identification and delimitation of *Nigrospora*.

Two other phylogenetically distinct genera within Apiosporaceae, *Arthrinium* and *Apiospora*, are morphologically similar to *Nigrospora* in producing deeply pigmented conidia [33–35]. The distinction between these three genera is obscure, but the most characteristic difference is the production of a single conidium produced on each conidiogenous cell in *Nigrospora*, while conidia are usually produced in clusters in *Arthrinum* and *Apiospora* [33–35].

A new *Arthrinum*-like genus named *Neoarthrinium* was recently proposed in Amphisphaeriales based on *Neo. lithocarpicola*, *Neo. moseri* (syn. *Wardomyces moseri*), *Neo. trachycarpi* (syn. *A. trachycarpi*), and *Neo. urticae* (syn. *A. urticae*) [36]. This paper further confirmed the classification of *Arthrinum*, *Apiospora*, *Neoarthrinium,* and *Nigrospora* in the order Amphisphaeriales [36]. More strains of *Nigrospora* are needed from different ecosystems to improve the phylogram of this genus and related genera in the future.

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References

1. Zimmerman, A. Ueber einige an tropischen Kulturpflanzen beobachtete Pilze III. *Zentralblatt Für Bakteriol. Parasitenkd.* **1902**, *8*, 216–221.
2. Mason, E.W. On species of the genus *Nigrospora zimmermann* recorded on monocotyledons. *Trans. Br. Mycol. Soc.* **1927**, *12*, 152-IN6. [CrossRef]
3. Wang, M.; Liu, F.; Crous, P.W.; Cai, L. Phylogenetic reassessment of *Nigrospora*: Ubiquitous endophytes, plant and human pathogens. *Persoonia* **2017**, *39*, 118–142. [CrossRef]
4. Hyde, K.D.; Norphanphoun, C.; Maharachchikumbura, S.S.N.; Bhat, D.J.; Jones, E.B.G.; Bundhun, D.; Chen, Y.J.; Bao, D.F.; Boonmee, S.; Calabon, M.S.; et al. Refined families of Sordariomycetes. *Mycosphere* **2020**, *11*, 305–1059. [CrossRef]
5. Wijayawardene, N.N.; Hyde, K.D.; Al-Ani, L.K.; Tedersoo, L.; Haelewaters, D.; Rajeshkumar, K.C.; Zhao, R.L.; Aptroot, A.; Leontyev, D.V.; Saxena, R.K.; et al. Outline of Fungi and fungus-like taxa. *Mycosphere* **2020**, *11*, 1060–1456. [CrossRef]
6. Crous, P.W.; Carnegie, A.J.; Wingfield, M.J.; Sharma, R.; Mughini, G.; Noordeloos, M.E.; Santini, A.; Shouche, Y.S.; Bezerra, J.D.; Dima, B.; et al. Fungal Planet description sheets: 868–950. *Persoonia* **2019**, *42*, 291–473. [CrossRef]
7. Raza, M.; Zhang, Z.F.; Hyde, K.D.; Diao, Y.Z.; Cai, L. Culturable plant pathogenic fungi associated with sugarcane in southern China. *Fungal Divers* **2019**, *99*, 1–104. [CrossRef]

8. Tian, L.Y.; Zhang, Y.F.; Liao, T.; Qin, C.S.; Xu, J.Z. *Nigrospora guangdongensis* sp. nov. from the needle of *Cunninghamia lanceolata* in China. *Phytotaxa* **2020**, *449*, 181–187. [CrossRef]
9. Zhang, Z.F.; Zhou, S.Y.; Eurwilaichitr, L.; Ingsriswang, S.; Raza, M.; Chen, Q.; Zhao, P.; Liu, F.; Cai, L. Culturable mycobiota from Karst caves in China II, with descriptions of 33 new species. *Fungal Divers* **2020**, *106*, 29–136. [CrossRef]
10. De Silva, N.I.; Maharachchikumbura, S.S.; Thambugala, K.M.; Bhat, D.J.; Karunarathna, S.C.; Tennakoon, D.S.; Phookamsak, R.; Jayawardena, R.S.; Lumyong, S.; Hyde, K.D. Morpho-molecular taxonomic studies reveal a high number of endophytic fungi from *Magnolia candolli* and *M. garrettii* in China and Thailand. *Mycosphere* **2021**, *12*, 163–237. [CrossRef]
11. Tennakoon, D.S.; Kuo, C.H.; Maharachchikumbura, S.S.; Thambugala, K.M.; Gentekaki, E.; Phillips, A.J.; Bhat, D.J.; Wanasinghe, D.N.; de Silva, N.I.; Promputtha, I.; et al. Taxonomic and phylogenetic contributions to *Celtis formosana*, *Ficus ampelas*, *F. septica*, *Macaranga tanarius* and *Morus australis* leaf litter inhabiting microfungi. *Fungal Divers* **2021**, *108*, 1–215. [CrossRef]
12. Tan, Y.P.; Bishop-Hurley, S.L.; Shivas, R.G.; Cowan, D.A.; Maggs-Kölling, G.; Maharachchikumbura, S.S.; Pinruan, U.; Bransgrove, K.L.; De la Peña-Lastra, S.; Larsson, E.; et al. Fungal Planet description sheets: 1436–1477. *Persoonia* **2022**, *49*, 261–350. [CrossRef] [PubMed]
13. Chen, Q.; Bakhshi, M.; Balci, Y.; Broders, K.D.; Cheewangkoon, R.; Chen, S.F.; Fan, X.L.; Gramaje, D.; Halleen, F.; Jung, M.H.; et al. Genera of phytopathogenic fungi: GOPHY 4. *Stud. Mycol.* **2022**, *101*, 417–564. [CrossRef] [PubMed]
14. Palmateer, A.J.; Mclean, K.S.; Van Santen, E.; Morgan-Jones, G. Occurrence of *Nigrospora* lint rot caused by *Nigrospora oryzae* on Cotton in Alabama. *Plant Dis.* **2003**, *87*, 873. [CrossRef] [PubMed]
15. Xu, Y.M.; Liu, Y.J. First report of *Nigrospora sphaerica* causing leaf blight on *Cunninghamia lanceolata* in China. *Plant Dis.* **2017**, *101*, 389. [CrossRef]
16. Liu, Y.J.; Tang, Q.; Fang, L. First report of *Nigrospora sphaerica* causing leaf blight on *Camellia sinensis* in China. *Plant Dis.* **2016**, *100*, 221. [CrossRef]
17. Kee, Y.J.; Hafifi, A.B.; Huda-Shakirah, A.R.; Wong, K.L.; Jin, X.L.; Nordahliawate, M.S.; Zakaria, L.; Mohd, M.H. 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. [CrossRef]
18. Han, S.; Yu, S.; Zhu, T.; Li, S.; Qiao, T.; Liu, Y.; Lin, T.; Yang, C. *Nigrospora oryzae* causing black leaf spot disease of *Hibiscus mutabilis* in China. *Plant Dis.* **2021**, *105*, 2255. [CrossRef] [PubMed]
19. Metwaly, A.M.; Kadry, H.A.; El-Hela, A.A.; Mohammad, A.I.; Ma, G.; Cutler, S.J.; Ross, S.A. Nigrosphaerin A a new isochromene derivative from the endophytic fungus *Nigrospora sphaerica*. *Phytochem. Lett.* **2014**, *7*, 1–5. [CrossRef]
20. Ibrahim, D.; Chong, C.L.; Tong, W.Y.; Zakaria, L.; Sheh-Hong, L. Effect of the extract of endophytic fungus, Nigrospora sphaerica CL-OP 30, against the growth of methicillin-resistant Staphylococcus aureus (MRSA) and Klebsiella pneumonia cells. *Trop. J. Pharm. Res.* **2015**, *14*, 2091–2097. [CrossRef]
21. Zhong, J.; Zhao, S.Q.; Li, G.F.; Pang, X.D.; Deng, X.J.; Zhu, H.J.; Da Gao, B.; Zhou, Q. A novel fusarivirus isolated from the phytopathogenic fungus *Nigrospora oryzae*. *Virus Genes* **2016**, *52*, 891–895. [CrossRef]
22. Fukushima, T.; Tanaka, M.; Gohbara, M.; Fujimori, T. Phytotoxicity of three lactones from *Nigrospora sacchari*. *Phytochemistry* **1998**, *48*, 625–630. [CrossRef]
23. Meepagala, K.M.; Becnel, J.J.; Estep, A.S. Phomalactone as the active constituent against mosquitoes from Nigrospora spherica. *Agric. Sci.* **2015**, *6*, 1195–1201. [CrossRef]
24. Doyle, J.J.; Doyle, J.L. Isolation of plant DNA from fresh tissue. *Focus* **1990**, *12*, 39–40.
25. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protoc. Guide Methods Appl.* **1990**, *18*, 315–322.
26. Carbone, I.; Kohn, L.M. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **1999**, *3*, 553–556. [CrossRef]
27. Glass, N.L.; Donaldson, G.C. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microbiol.* **1995**, *61*, 1323–1330. [CrossRef] [PubMed]
28. Katoh, K.; Rozewicki, J.; Yamada, K.D. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* **2019**, *20*, 1160–1166. [CrossRef] [PubMed]
29. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. [CrossRef] [PubMed]
30. Stamatakis, A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. [CrossRef]
31. Ronquist, F.; Huelsenbeck, J.P. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **2003**, *19*, 1572–1574. [CrossRef] [PubMed]
32. Lee, W.; Kim, D.; Perera, R.H.; Kim, J.S.; Cho, Y.; Lee, J.W.; Seo, C.W.; Lim, Y.W. Diversity of Nigrospora (Xylariales, Apiosporaceae) species identified in Korean macroalgae including five unrecorded species. *Mycobiology* **2023**, *51*, 401–409. [CrossRef] [PubMed]
33. Jiang, N.; Liang, Y.M.; Tian, C.M. A novel bambusicolous fungus from China, *Arthrinium chinense* (Xylariales). *Sydowia* **2020**, *72*, 77–83. [CrossRef]
34. Pintos, A.; Alvarado, P.; Planas, J.; Jarling, R. Six new species of *Arthrinium* from Europe and notes about *A. caricicola* and other species found in *Carex* spp. hosts. *MycoKeys* **2019**, *49*, 15–48. [CrossRef]

35. Pintos, A.; Alvarado, P. Phylogenetic delimitation of *Apiospora* and *Arthrinium*. *Fungal Syst. Evol.* **2021**, *7*, 197–221. [CrossRef]
36. Jiang, N.; Voglmayr, H.; Ma, C.Y.; Xue, H.; Piao, C.G.; Li, Y. A new *Arthrinium*-like genus of Amphisphaeriales in China. *MycoKeys* **2022**, *92*, 27–43. [CrossRef]