

9-25-2024

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WANG, JING; LIU, ZHONG-XUAN; YU, WEI; KANG, CHAO; and YANG, YI HUA (2024) "A new species of *Nigrospora* (Apiosporaceae) in *Corylus heterophylla* in China," *Turkish Journal of Botany*: Vol. 48: No. 5, Article 6. <https://doi.org/10.55730/1300-008X.2815>

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A new species of *Nigrospora* (Apiosporaceae) in *Corylus heterophylla* in China

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Received: 05.04.2024 • Accepted/Published Online: 21.08.2024 • Final Version: 25.09.2024

Abstract: *Corylus heterophylla*, the majestic hazelnut tree, stands as one of the esteemed quartets of grand nuts reigning across the landscapes of temperate Asia, Europe, and North America. However, its relationship with endophytic fungi remains thinly explored. This study successfully identified a new species designated as *Nigrospora coryli* through conjoint analysis of morphology and systematics. The genetic regions examined encompassed ITS, TUB2, and TEF1- α . The detailed morphological description, colour photographs of Macro- and microcharacters are presented.

Key words: Isolate, taxonomy, endophytes, phylogeny

1. Introduction

Nigrospora, a fungal species belonging to the genus within the Ascomycota, Sordariomycetes, Xylariales, and Apiosporaceae, exhibits a diverse range of hosts and can be encountered in various ecological roles, including as endophytes, saprobes, and pathogens (Mason 1927, Wu et al. 2009, Thalavaipandian 2011, Sun et al. 2014, Uzor et al. 2015, Wang et al. 2017, Lee et al. 2019, Tripathi & Joshi 2019, Ukwatta et al. 2019, Oh et al. 2020, Ola et al. 2021, Huang 2021, Lee et al. 2023). The genus *Nigrospora* contains a variety of species, characterized by black conidia and coniangia (Zimmerman et al. 1902). The distinctive features of these fungi include dark-colored conidia and mycelium, hence the name “*Nigrospora*” with “*Nigro*” indicating black (Zimmerman 1902). According to Index Fungorum records, only 46 taxa and 43 species have been recognized since the establishment of *Nigrospora* (<https://www.indexfungorum.org/Names/Names.asp>, last accessed Jan. 2024). Zimmerman (1902) established *Nigrospora* Zimm for *N. panici*. Following morphological investigations, various new species were identified and published, namely *N. canescens*, *N. aerophila*, *N. musae*, *N. gorlenkoana*, *N. oryzae*, *N. padwickii*, *N. panici*, *N. sacchari*, and *N. vietnamensis*. In 2017, a phylogenetic analysis that employed internal transcribed spacers (ITS), the large subunit (LSU), a fragment of beta-tubulin (TUB2), and the translation elongation factor 1-alpha (TEF1- α) confirmed the classification of *Nigrospora* within the family Apiosporaceae of the order Xylariales. Additionally, it identified a novel species, *Nigrospora vesicularis*, as an

endophyte isolated from an unidentified host plant (Wang et al., 2017). de Queiroz Brito et al. (2023) contributed three new species—*Nigrospora endophytica*, *Nigrospora manihoticola*, and *Nigrospora pernambucoensis*—isolated as endophytes from the stem of *Manihot esculenta*. These findings further underscore the significance of *Nigrospora* in the realm of endophytic fungi.

Hazelnuts, one of the world's four major nuts, boast a wealth of nutrients, including abundant proteins, vitamin E, folic acid, eight essential amino acids for the human body, and resveratrol (Yu 2019, Li et al. 2008). The genus *Corylus*, within the Fagale and Betulaceae, encompasses approximately 20 species globally. Wild *Corylus* species predominantly thrive in temperate regions across Asia, Europe, and North America, with major hazelnut production occurring in countries such as Türkiye, Italy, Spain, the United States, and Portugal (Özenç & Bender - Özenç 2014). In China, nine wild *Corylus* species exist, namely *C. heterophylla* Fisch. ex Trautv. var. *sutchuenensis* Franch., *C. wangii* Hu., *C. ferox* Wall., *C. yunnanensis* (Franchet) A. Camus, *C. fargesii* Schneid., *C. chinensis* Franch., *C. mandshurica* Maxim., *C. heterophylla* Fisch., and *C. wulingensis* Q. X. Liu et C. M. Zhang. Ectomycorrhizal fungi establish symbiotic relationships with *Corylus* species, enhancing the trees' absorption of soil minerals and water, elevating photosynthetic rates, and bolstering stress resistance, thereby fostering the growth and development of *Corylus* species (Mamoun & Olivier 1993). Furthermore, numerous studies indicate that endophytic fungi can generate a diverse array of

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bioactive secondary metabolites, playing a pivotal role in promoting mycorrhizal formation (Long et al. 2007, Li et al. 2017).

Endophytic fungi, abundant in resources and diverse metabolites with unique chemical structures, exhibit notable antibacterial activity, making them valuable reservoirs for discovering novel antibiotics (Porras et al. 2020). Fan (2012) isolated 8 strains of endophytic fungi from hazelnut bark, which were preliminarily identified as *Penicillium*, *Fusarium*, *Neurospora*, *Gibberella*, *Cladosporium*, *Hypocrea* and *Paraphaeosphaeria* by morphological identification. Yang et al. (2019) discovered a substantial number of endophytic fungi within the roots of *Corylus avellana*. Küngas et al. (2020) used high-throughput sequencing techniques to compare the diversity and community structure of fungi residing in the leaves, branches, and trunks of *Corylus avellana*, revealing a rich abundance of endophytic fungi. Mohammadi Ballakuti et al. (2022) identified 18 endophytic fungi able to produce taxane compounds from the stem, leaves, and inflorescences of *Corylus avellana*. Fungal metabolites play a pivotal role in stimulating host growth, enhancing development, and fortifying resistance against both biotic and abiotic stresses (Brundrett 2006). *Nigrospora*, when cocultured with plants, produces metabolites that significantly inhibit various plant pathogens such as *Gloeosporium musarum*, *Penicillium citri*, *Fusarium oxysporum*, *Fusarium grammani*, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Plasmodium falciparum*, *Mycobacterium tuberculosis*, and others (Luo et al. 2017; Shang et al. 2012; Trisuwan et al. 2009; Wu et al. 2018; Kornsakulkarn et al. 2018; Masayasu et al. 1997; Wang et al. 2013; Ding et al. 2020; Ukwatta et al. 2019). This makes *Nigrospora* a promising candidate in agricultural plant protection and fungicide development research, showcasing high development potential.

2. Materials and methods

2.1. Sampling and fungal isolation

Samples of healthy and vibrant *Corylus heterophylla* stems were collected from the Mycorrhizal Seedling Cultivation Center at the Guizhou Provincial Institute of Biology in 2023. Fungal endophytes were isolated through the methodologies outlined by Sun et al. (2011) and de Queiroz Brito et al. (2023). Collected fragments underwent sterilization steps: 75% ethanol for 30 s, rinsing in sterile water, 5% NaClO for 30 s, rinsing again, and another 75% ethanol treatment for 30 s, followed by three rinses. Fragments were then dried with sterilized paper and plated on Potato Dextrose Agar (PDA). Cultures are meticulously maintained in PDA and sterile distilled water at the Guizhou Institute of Biology, Guizhou Academy of Sciences.

2.2. Morphological characterization

After 3 days of growth, a hyphal tip was collected and transferred onto PDA, where it was incubated at 25 °C in darkness for 7 days, following the methodology outlined by Brito et al. (2020). Conidia and hyphae were observed and captured using a Nikon H550S light microscope. The characterization of colony and microscopic structures adhered to the methods described by Rayner (1970) and Wang et al. (2017).

2.3. DNA extraction, PCR amplification

DNA extraction and PCR protocols followed Guo et al. (2000) and Wang et al. (2015). ITS5(GGAAGTAAAAGT CGTAACAAGG)+ITS4(TCCTCCGCTTATTGATATGC), 526F(GTCGTYGTYATYGGHCAYGT)+EF2(GGARGTACCAGTSATCATGTT) and T1(AACATGCGTGAGATTGTAAGT)+BT2B(ACCCTCAGTG TAGTGACCCTTGGC) were used to amplify the ITS, *tef1- α* (Carbone & Kohn 1999, O'Donnell et al. 1998) and *tub2* (Glass & Donaldson 1995).

2.4. Phylogenetic analysis

Sequences of *Nigrospora* and related genera (outgroup) were obtained from Wang et al. (2017), and incorporated into new sequences (Table 1). For alignment, we used the online MAFFT v7 with the L-INS-I strategy (Kato and Toh 2008). Manual adjustments were made using BioEdit v.7.1.3.0 to ensure alignment accuracy (Hall 1999).

The best model test and phylogenetic tree were investigated in the MEGA X software (Kumar et al. 2018). The most likely tree generated was visualized in FigTree v1.3.1. Nodes with ML-BP \geq 75% were considered significantly supported for monophyly.

3. Taxonomy

Nigrospora coryli J. Wang & Y.H. Yang, sp. nov. (Figure 1)

Mycobank no: MB852554

Etymology:—isolated from *Corylus heterophylla* stems.

Typification:—CHINA. Guizhou Province, Guiyang City, in Mycorrhizal Seedling Cultivation Center at the Guizhou Provincial Institute of Biology, isolated from the stem of *Corylus heterophylla* as an endophyte, May 2023, W18, holotype.

Description:—Hyphae 1.4–9 μ m diam, hyaline to pale brown, smooth, septate, branched. Conidiophores similar to vegetative hyphae, short, rare, predominantly reduced to conidiogenous cells. Conidiogenous cells globose to subglobose, 6.0–9.5 \times 5.5–9.5 μ m (av. 7.71 \times 7.25 μ m), pale brown, smooth, monoblastic, determinate. Conidia globose or subglobose, 13–20.5 μ m diam (av. = 15.91 \pm 1.72 μ m), acrogenous, smooth, solitary, shiny, aseptate, black; ellipsoidal, 13.5–17.5 \times 11.5–14 μ m (av. = 16.00 \pm 1.34 \times 13.09 \pm 0.94).

Culture characteristics:—On PDA, colonies flat, loose, floccose, surface and reverse creamy white, reaching 7 cm diam in 4 d at 25 °C.

Table 1. Taxa and collections are used for multigene phylogenetic analyses in this study. Sequences produced in the present study are in bold. The other sequences are from Wang et al. (2017) and new species published in the last five years.

Species	Accession numbers	Host	ITS	tub2	tefl- α
<i>Nigrospora anhuiensis</i>	YL-2024a	Rice	OP677969	PP103614	PP103590
<i>N. aurantiaca</i>	CGMCC 3.18130* = LC 7302	<i>Nelumbo sp.</i> (leaf)	KX986064	KY019465	KY019295
<i>N. aurantiaca</i>	LC 7034	<i>Musa paradisiaca</i>	KX986093	KY019598	KY019394
<i>N. bambusae</i>	CGMCC 3.18327* = LC 7114	Bamboo (leaf)	KY385307	KY385319	KY385313
<i>N. bambusae</i>	LC 7244	Bamboo (leaf)	KY385306	KY385320	KY385314
<i>N. camelliae-sinensis</i>	CGMCC 3.18125* = LC 3500	<i>Camellia sinensis</i>	KX985986	KY019460	KY019293
<i>N. camelliae-sinensis</i>	LC 6304	<i>Camellia sinensis</i>	KX986045	KY019566	KY019370
<i>N. coryli</i>	W18	<i>Corylus heterophylla</i>	PP218065	PP320372	PP461302
<i>N. cooperae</i>	SFC20230324-M03	<i>Heteropogon sp.</i>	OQ726361	OQ735179	OQ735196
<i>N. cooperae</i>	BRIP 72408b	<i>Heteropogon sp.</i>	OP035047	OP039537	OP039538
<i>N. cooperae</i>	BRIP 72440a	<i>Heteropogon sp.</i>	NR185745	OP039540	OP039539
<i>N. covidalis</i>	CGMCC 3.20538	<i>Lithocarpus sp.</i>	NR177177	OK431479	OK431485
<i>N. covidalis</i>	SFC20230324-M04	<i>Lithocarpus sp.</i>	OQ726371	OQ735180	OQ735197
<i>N. chinensis</i>	LC 2696	<i>Lindera aggregata</i>	KX985947	KY019474	KY019424
<i>N. chinensis</i>	LC 3085	<i>Camellia sinensis</i>	KX985970	KY019497	KY019427
<i>N. endophytica</i>	ARM973	<i>Manihot esculenta</i>	OM265233	OP572420	OP572416
<i>N. endophytica</i>	ARM687	<i>Manihot esculenta</i>	OM265226	OP572418	OP572415
<i>N. falsivesicularis</i>	CGMCC3.19678	<i>Saccharum officinarum</i>	MN215778	MN329942	MN264017
<i>N. ficuum</i>	ZHKUCC 22-0143	<i>Ficus sp.</i>	OR164911	OR166318	-
<i>N. globospora</i>	LC15839	<i>Petasites hybridus</i>	OK335212	OK431482	OK431488
<i>N. globospora</i>	CGMCC3.20539	<i>Petasites hybridus</i>	OK335211	OK431481	OK431487
<i>N. gorlenkoana</i>	CBS 480.73*	<i>Vitis vinifera</i>	KX986048	KY019456	KY019420
<i>N. guilinensis</i>	LC 7301	<i>Nelumbo sp.</i> (stem)	KX986063	KY019608	KY019404
<i>N. guangdongensis</i>	CFCC 53917	<i>Cunninghamia lanceolata</i>	NR174814	MT024495	MT024493
<i>N. guangdongensis</i>	Tly068	<i>Cunninghamia lanceolata</i>	MT017510	MT024496	MT024494
<i>N. guilinensis</i>	CGMCC 3.18124* = LC 3481	<i>Camellia sinensis</i>	KX985983	KY019459	KY019292
<i>N. hainanensis</i>	CGMCC 3.18129* = LC 7030	<i>Musa paradisiaca</i> (leaf)	KX986091	KY019464	KY019415
<i>N. hainanensis</i>	LC 6979	<i>Musa paradisiaca</i> (leaf)	KX986079	KY019586	KY019416
<i>N. lacticolonia</i>	CGMCC 3.18123* = LC 3324	<i>Camellia sinensis</i>	KX985978	KY019458	KY019291
<i>N. lacticolonia</i>	LC 7009	<i>Musa paradisiaca</i> (leaf)	KX986087	KY019594	KY019454
<i>N. macarangae</i>	ZHKUCC23-0003	-	PP091035	PP646185	PP646182
<i>N. magnoliae</i>	MFLUCC 19-0112	<i>Magnolia liliifera</i>	NR172443	MW438334	-
<i>N. musae</i>	CBS 319.34*	<i>Musa paradisiaca</i> (fruit)	KX986076	KY019455	KY019419
<i>N. musae</i>	LC 6385	<i>Camellia sinensis</i>	KX986042	KY019567	KY019371
<i>N. oryzae</i>	LC6759	<i>Oryza sativa</i>	KX986054	KY019572	KY019374
<i>N. oryzae</i>	LC 6760	<i>Oryza sativa</i>	KX986055	KY019573	KY019375
<i>N. oryzae</i>	LC 6761	<i>Oryza sativa</i>	KX986056	KY019574	KY019376
<i>N. oryzae</i>	LC 2693	<i>Neolitsea sp.</i>	KX985944	KY019471	KY019299
<i>N. oryzae</i>	LC 2695	<i>Rubus reflexus</i>	KX985946	KY019473	KY019301

Table 1. (Continued.)

<i>N. oryzae</i>	LC 2699	<i>Hamamelis mollis</i>	KX985949	KY019476	KY019303
<i>N. osmanthi</i>	CGMCC 3.18126* = LC 4350	<i>Osmanthus sp</i>	KX986010	KY019461	KY019421
<i>N. osmanthi</i>	LC 4487	<i>Hedera nepalensis</i>	KX986017	KY019540	KY019438
<i>N. pernambucoensis</i>	SCUA-Saf-N16	<i>Arthrocaulon macrostachyum</i>	PP256498	PP263820	PP263806
<i>N. philosophiae-doctoris</i>	CGMCC 3.20540	<i>Disporum sessile</i>	NR177178	OK431484	OK431489
<i>N. pyriformis</i>	CGMCC 3.18122* = LC 2045	<i>Citrus sinensis</i>	KX985940	KY019457	KY019290
<i>N. pyriformis</i>	LC 2688	<i>Lindera aggregata</i>	KX985941	KY019468	KY019297
<i>N. pyriformis</i>	LC 3099	<i>Camellia sinensis</i>	KX985971	KY019498	KY019322
<i>N. rubi</i>	CGMCC 3.18326* = LC 2698	<i>Rubus sp.</i>	KX985948	KY019475	KY019302
<i>N. saccharicola</i>	LC12057	<i>Saccharum officinarum</i>	MN215789	MN329952	MN264028
<i>N. sacchari-officinarum</i>	CGMCC 3.19335	<i>Saccharum officinarum</i>	NR165926	MN329954	MN264030
<i>N. singularis</i>	LC12068	<i>Saccharum officinarum</i>	MN215794	MN329957	MN264033
<i>N. sphaerica</i>	LC 7294	<i>Nelumbo sp.</i> (leaf)	KX985932	KY019602	KY019397
<i>N. sphaerica</i>	LC 7295	<i>Nelumbo sp.</i> (leaf)	KX985933	KY019603	KY019398
<i>N. sphaerica</i>	LC 7298	<i>Nelumbo sp.</i> (leaf)	KX985937	KY019606	KY019401
<i>N. sphaerica</i>	LC 4372	<i>Rhododendron arboreum</i>	KX986012	KY019535	KY019351
<i>N. sphaerica</i>	LC 4447	Unknown host plant	KX98601	KY019537	KY019352
<i>N. sphaerica</i>	LC 5901	Submerged wood	KX986034	KY019556	KY019361
<i>N. sp.</i>	LC 2725	<i>Symplocos zizyphoides</i>	KX985960	KY019487	KY019313
<i>N. sp.</i>	LC 4566	<i>Lithocarpus sp.</i>	KX986022	KY019545	KY019354
<i>N. sp.</i>	LC 6704	<i>Camellia sinensis</i>	KX986047	KY019571	KY019373
<i>N. stoneae</i>	BRIP 75022a	<i>Cyperus aromaticus</i>	OR608744	OR604067	OR604065
<i>N. vesicularifera</i>	MFLUCC:22-0014	<i>Litchi chinensis</i>	ON211313	ON622465	ON622464
<i>N. vesicularis</i>	LC 0322	Unknown host plant	KX985939	KY019467	KY019296
<i>N. vesicularis</i>	CGMCC 3.18128* = LC 7010	<i>Musa paradisiaca</i> (leaf)	KX986088	KY019463	KY019294
<i>N. zimmermanii</i>	CBS 167.26	Unknown	KY385308	KY385318	KY385312
<i>N. zimmermanii</i>	CBS 290.62*	<i>Saccharum officinarum</i> (leaf)	KY385309	KY385317	KY385311
<i>N. zimmermanii</i>	CBS 984.69	<i>Saccharum officinarum</i> (leaf)	KY385310	KY385322	KY385316
<i>Arthrimum vietnamensis</i>	IMI 99670*	<i>Citrus sinensis</i>	KX986096	KY019466	-

Habitat and Distribution:—*Nigrospora coryli* was discovered as an endophyte within the stem of *Corylus heterophylla* at Mycorrhizal Seedling Cultivation Center in Guizhou, China.

Notes:—Strain of *N. coryli* forms a distinct clade in concatenated gene trees with well-support and closely related to *N. chinensis* (Figure 2). The species can be distinguished from each other through the morphology of

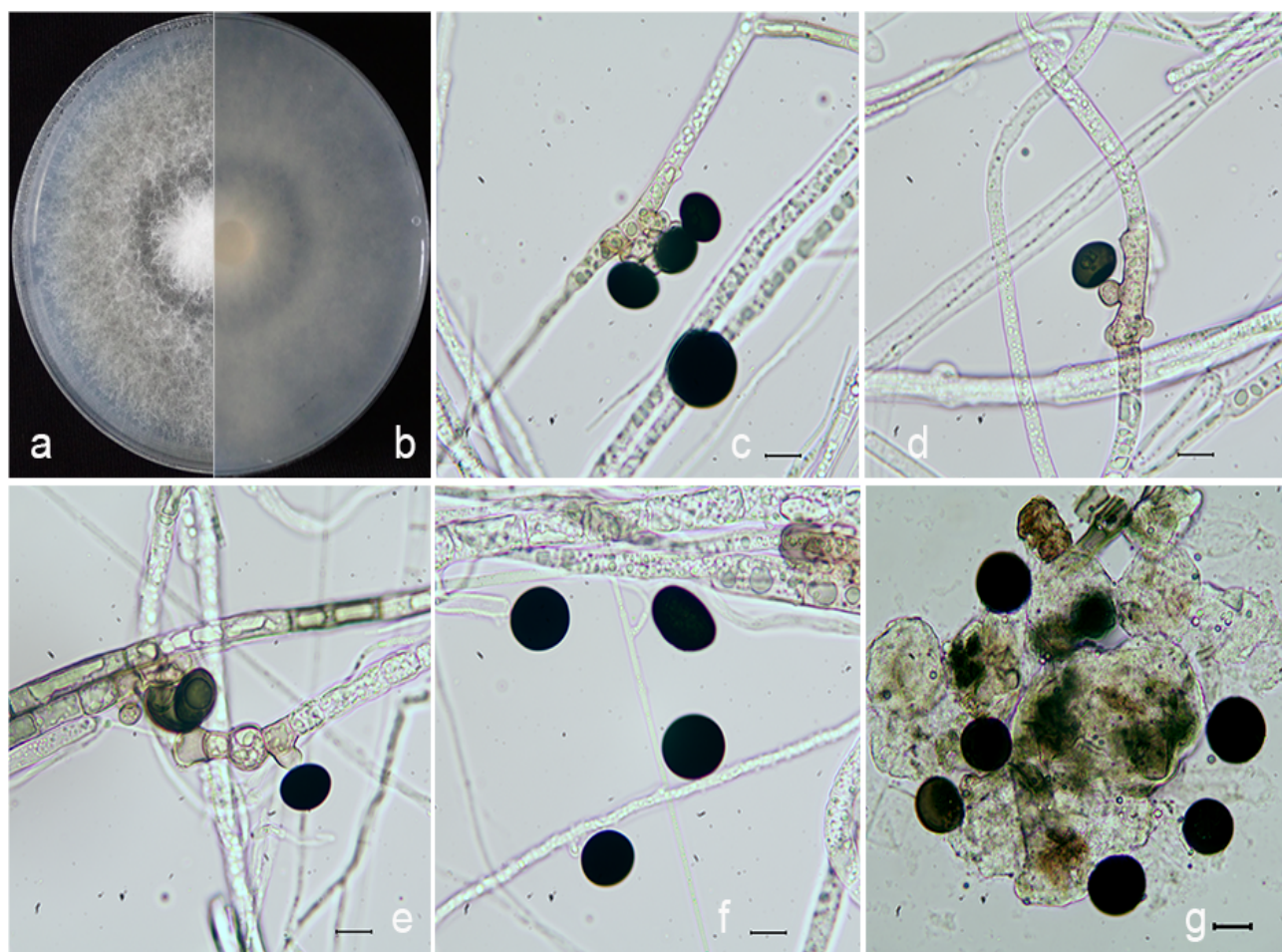


Figure 1. *Nigrospora coryli* (from ex-type strain W18). a-b. Upper surface and reverse overview of culture 7 d after inoculation on PDA medium. c-e. conidiogenous cells giving rise to conidia; f-g. conidia. -Scale bars: c-g = 10 μ m.

their hyphae and conidia. Specifically, *N. chinensis* exhibits finer hyphae only 2–5 μ m diam, as well as smaller spores measuring 10–14.5 \times 7.5–14 μ m (Wang et al. 2017).

4. Discussion

Nigrospora exhibit a versatile range of roles, functioning as saprobes, endophytes, and pathogens. *Nigrospora* has no host specificity, having already been isolated from *Castanopsis*, *Camellia*, *Citrus*, *Chenopodium*, *Guarea*, *Lindera*, *Napolea*, *Manihot*, *Musa*, *Nopalea*, *Oxalis*, *Rosa*, *Rubus*, *Saccharum* and so on (Wang et al. 2017; Raza et al. 2019; Chen et al. 2020; Conforto et al. 2019; Santos et al. 2021; Brito et al. 2020). This diversity in ecological niches suggests the potential for varied interactions with different host organisms, impacting plant health and ecosystem dynamics. In this paper, a new strain of *Nigrospora* was isolated from the healthy and vigorous stems of *Corylus heterophylla*. The strain was clustered with *N. chinensis* in phylogeny and then clustered with *N. magnoliae*, *N. globospora*, *N. camellinensis*, *N. singularis*,

and *N. pyriformis*. As endophytic fungus, *N. chinensis*, *N. magnoliae*, *N. camelliae-sinensis* were respectively isolated from *Geodorum densiflorum* labium (Rahayu et al. 2021), *Magnolia* (Silva et al. 2021), *Lumnitzera littorea* (Huang et al. 2021). It is similar to *N. saccharicola* and *N. gorlenkoana* in morphology, but The Conidia of *N. saccharicola* was smaller, 9.5–13.5 \times 11–17.5 μ m at ellipsoidal, and the Conidia of *N. gorlenkoana* was pale brown to black.

The continuous discovery of new *Nigrospora* species, along with research advancements in understanding their ecology and bioactivity, underscores the dynamic nature of fungal diversity and the importance of ongoing scientific exploration. These findings not only expand our knowledge of fungal biodiversity but also pave the way for innovative applications in agriculture and biotechnology. Besides, the study of endophytic fungi needs to go through a long process, from the discovery of endophytic fungi, classification, culture, and analysis of metabolites, to the relationship between endophytic fungi and hosts.

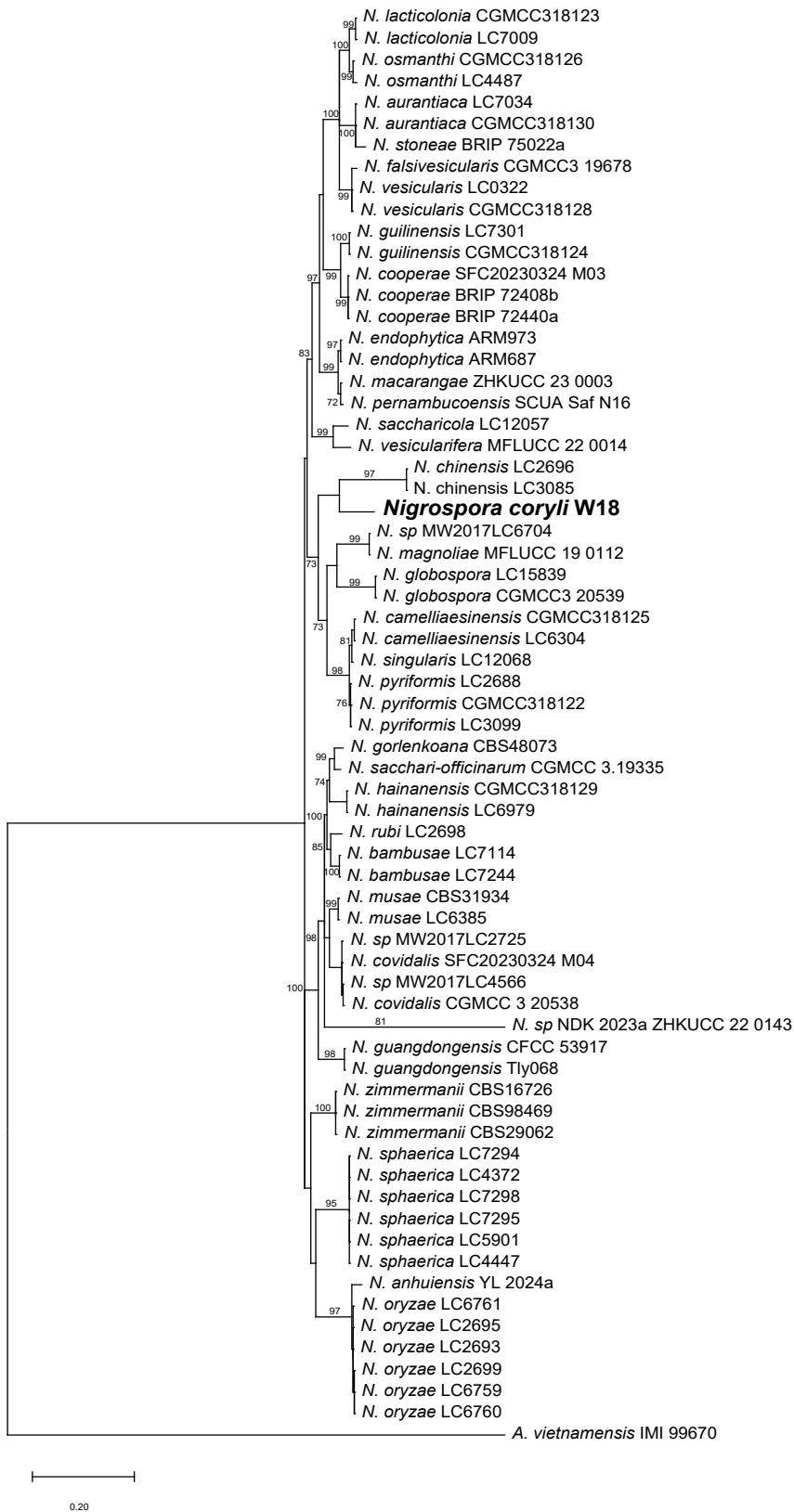


Figure 2. Multilocus phylogenetic tree based on the combined ITS, tub2, and tef1- α sequences alignment generated from a Maximum likelihood phylogenetic analysis.

5. Conclusion

This article introduces a newly discovered species, *Nigrospora coryli*, and documents a new host record for *Nigrospora* on a global scale. The defining characteristic of *Nigrospora coryli* is its creamy white colonies, both on the surface and reverse, which exhibit rapid growth on PDA. The hyphae measure 1.49 µm in diameter, and the conidia are either spherical or ellipsoidal in shape. The research results will provide a valuable reference for the isolation,

identification, and interaction of endophytic fungi in hazelnuts.

Acknowledgments

This work was financed by the Project of Science and Technology Programs of Guizhou Province [ZK(2022)283], [(2024)171], [(2023)204], the Project of Central Government Financial Fund for Forest Reform and Development [Gui(2024)TG12].

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