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Short Article

# *Nigrospora camelliae-sinensis*: a new record for the funga of Iran, with an endophytic association in *Chenopodium quinoa*

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## ABSTRACT

During an investigation of endophytic fungi associated with quinoa plants (*Chenopodium quinoa* Willd.) in May 2024, samples were collected from the research farm of the Seed and Plant Improvement Institute (SPII), located in Mohammadshahr, Karaj, Iran. Several fungal isolates were obtained from healthy leaves of quinoa line Q4. Based on morphological characteristics, one of the isolates was assigned to the genus *Nigrospora*. For phylogenetic analysis of the recovered isolate (ABRIICC 10490), the partial nucleotide sequence of the beta-tubulin (*tub2*) gene was analyzed using the maximum likelihood (ML) and Bayesian inference (BI) methods, and a phylogenetic tree was subsequently constructed. The phylogenetic analyses resolved the recovered isolate at the species level and placed it separately from other closely related species of *Nigrospora*, supporting its correct morphological identification. Based on the morphological (conidia and conidiophores) and phylogenetic analyses, the recovered isolate was identified as *Nigrospora camelliae-sinensis*. This study represents the first report of *N. camelliae-sinensis* as a new species for funga of Iran, and also the first report of its occurrence as an endophytic fungus associated with quinoa worldwide. Furthermore, *Chenopodium quinoa* is reported here as a new host (*matrix nova*) for *N. camelliae-sinensis* in the world.

## KEYWORDS

Biodiversity, Morphology, Phylogeny, *tub2* gene, Quinoa.

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## INTRODUCTION

*Chenopodium quinoa* Willd., belonging to the family *Amaranthaceae* Juss., is an herbaceous annual plant. This plant contains various important nutritional compounds, including proteins, lipids, vitamins, fibers, and minerals. Therefore, it is widely recognized as a pseudocereal. The high genetic diversity of this species has enabled it to adapt to harsh climatic conditions, including drought, salinity, and extreme temperatures (Angeli et al. 2020).

The genus *Nigrospora* Zimm. belonging to the phylum *Ascomycota* Cava.-Sm. (*Sordariomycetes*, *Amphisphaeriales*, *Apiosporaceae*), was first introduced by Zimmermann in 1902, with *N. panici* Zimm., isolated from dead leaves of *Panicum amphibium* Steud. in Java, Indonesia, and designated as the type species (Zhang et al. 2025). The genus was originally described based on its asexual morph, characterized by the production of globose, dark-colored, opaque conidia arising solely from grey conidiophores (Zhang et al. 2025). Mason (1927) classified four *Nigrospora* species occurring on monocotyledons and considered *N. javanica* to be a synonym of *N. panici*. Like other fungi, species of the genus *Nigrospora* were initially distinguished based on morphological characteristics. However, subsequent studies have shown that certain morphological characteristics, such as conidium size, overlap among phylogenetically distinct species, thereby limiting their reliability for species delimitation (Zhang et al. 2025). According to the MycoBank database (https://www.mycobank.org), 59 accepted species are currently recognized in the genus *Nigrospora*. The genus *Nigrospora* was revised by Seifert et al. (2011), and subsequently placed in the family *Apiosporaceae* by Wang et al. (2017) based on morphological characteristics and multigene phylogenetic analyses. As

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of August 2026, 64 records of *Nigrospora* are available in major fungal databases, including Index Fungorum (www.indexfungorum.org). Species of *Nigrospora* occur as endophytes within plant tissues and as saprobes on various types of dead organic matter (Mason 1927, Wu et al. 2009, Thalavaipandian et al. 2011). Species of *Nigrospora* are also important plant pathogens affecting crops, fruits, and ornamental plants, with *N. oryzae* (Berk. & Broome) Petch causing stem blight, *N. sphaerica* (Sacc.) E.W. Mason causing leaf blight, and *N. musae* McLennan & Hoëtte causing squirter disease in bananas (Sharma et al. 2013, Liu et al. 2015). Additionally, *N. sphaerica* has been reported as an opportunistic human pathogen associated with onychomycosis and corneal ulcers (De Hoog et al. 2000, Fan et al. 2009, Kindo et al. 2014). *Nigrospora camelliae-sinensis* M. Wang & L. Cai was first isolated and described by Wang et al. (2017) from *Camellia sinensis* (L.) Kuntze based on morphological characteristics and a multilocus phylogenetic analysis of the ITS rDNA, *tef1*-α, and *tub2* gene regions.

In Iran, species of the genus *Nigrospora* have been reported from different host plants, either as disease-causing agents or as new fungal taxa for funga of Iran or the world. For example, *N. oryzae* (Berk. & Br.) Petch was isolated from leaf spot symptoms of *Cyperus rotundus* L. and *C. esculantus* L. in Guilan, Mazandaran, and Zanjan provinces (Farzaneh et al. 2009), and was reported as a leaf spot pathogen of *Rosmarinus officinalis* L. in Kerman Province (Zarandi et al. 2012). The association of *N. oryzae* with cotton boll and lint rot has also been reported from South Khorasan Province, Iran (Mirzaee et al. 2013). *Nigrospora sphaerica* was isolated from *Cynodon dactylon* (L.) Pers. as an endophytic fungus in Mazandaran Province of Iran by Goodarzian et al. (2021). Safi et al. (2025) reported *Nigrospora lacticolonia* Mei Wang & L. Cai from *Tamarix passerinoides* Delile ex Decne.; *N. oryzae* from *Suaeda vermiculata* Forssk. ex J. F. Gmel., *Arthrocnemum macrostachyum* (Moric.) K. Koch, *Anthoxanthum monticola* (Bigelow) Veldkamp and *Aeluropus lagopoides* (L.) Thwaites; *N. osmanthi* Mei Wang & L. Cai from *Suaeda vermiculata* Forssk. ex J. F. Gmel.; *N. pernambucoensis* A.C.Q. Brito & A.R. Machado from *Tamarix passerinoides*, *Allenrolfea occidentalis* (S. Watson) Kuntze, *Arthrocnemum macrostachyum*, *Cressa cretica* L., and a novel taxon, *N. shadeganensis* Safi, M. Mehrabi & Arzanlou from *Aeluropus lagopoides*, *Phragmites australis* (Cav.) Trin. ex Steud., *Seidlitzia rosmarinus* Bunge ex Boiss., *Halocnemum strobilaceum* (Pall.) M. Bieb., *Aeluropus lagopoides*, and *Tamarix passerinoides*. These species were reported as endophytic fungi associated with healthy plants collected from the Shadegan wetland and its surrounding salt marshes. Based on previous studies conducted in Iran, the main aim of the present study was to characterize a *Nigrospora* strain recovered from *Chenopodium quinoa* using morphological and phylogenetic approaches.

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## MATERIALS AND METHODS

### Fungal isolate

In May 2024, a study was conducted to investigate the diversity of endophytic fungi associated with quinoa plants. Samples were collected from the research farm of the Seed and Plant Improvement Institute (SPII) in Mohammadshahr, Karaj, Iran. Leaves of the line Q4 of the plant were used for the isolation of endophytic fungi. The leaf samples were washed under running tap water for five minutes to remove surface contaminants. Subsequently, surface disinfection was performed according to the method described by González et al. (2017), with slight modifications, to eliminate microbial contaminants. The leaves were cut into small pieces (0.5 × 0.5 cm) and sequentially immersed in 0.5% sodium hypochlorite solution, then in 70% ethanol, and 0.5% sodium hypochlorite solution, with each treatment lasting one minute. Subsequently, the leaf pieces were rinsed three times with sterile distilled water, for one minute each time. The leaf pieces were then placed on sterile filter paper to remove excess moisture and subsequently transferred onto water-agar (WA) culture medium. The Petri dishes were incubated in complete darkness at 25 °C to allow the growth of endophytic fungi from the leaf pieces. Each grown endophytic fungus was transferred to a new WA culture medium, and the inoculated Petri dishes were incubated in complete darkness at 25 °C to allow further colony growth. For purification of fungal colonies, the hyphal tip method was used. Each hyphal tip was transferred to potato dextrose agar (PDA) culture medium, and the Petri dishes were incubated in complete darkness at 25 °C until pure colonies developed. The growing colony of the recovered isolate was deposited in the Agricultural Microbial Collection of the Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj.

### Morphology

The morphology of the recovered isolate was investigated using PDA culture medium (Ibresco Life Science, Iran). A disc of the culture medium containing pure fungal mycelium was transferred onto fresh culture medium, and the inoculated plates were incubated at 25 °C in complete darkness for seven to 15 days. Macro- and micromorphological characteristics, including colony features and size, shape and color of conidia and conidiogenous cells, were examined using a BH2 light microscope (Olympus, Japan) and microscopic slide mounts prepared in lactophenol solution. The morphological characteristics of the recovered fungal strain were measured and compared with the descriptions provided by Wang et al. (2017) and Zhang et al. (2025).

**Phylogeny**

For phylogenetic analysis, genomic DNA of the recovered fungal strain was extracted using mycelia grown on potato dextrose agar (PDA) culture medium, following the protocol described by Zhong and Stephenson (2001). The partial region of the beta-tubulin (*tub2*) gene was selected for amplification. The partial fragment of the *tub2* gene was amplified using primer pair Bt2a (GGTAACCAAATCGGTGCTGCTTTC) and Bt2b (ACCCTCAGTGTAGTGACCCTTGGC) (Glass and Donaldson 1995). PCR amplification was carried out in a final volume of 25 µL containing 10 µL of *Taq* DNA polymerase Mix Red-MgCl2, 11 µL of deionized water, 1 µL of each primer (10 pmol), and 2 µL of template DNA. PCR amplification was performed in a Palm-Cycler thermocycler (Corbett Research, Australia) under the following thermal cycling conditions. The PCR cycling program began with an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 5 min. The PCR product was evaluated by gel electrophoresis in a 1.5% agarose gel using 1X Tris-Boric acid-EDTA (TBE) buffer. PCR product was subsequently sent to Codon Genetic Group (Tehran, Iran) for sequencing. The resulting sequence (386 bp for *tub2*) was subjected to a BLAST search (Altschul et al. 1990) to find similar sequences in the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov, Sayers et al. 2025) database. Thirty-five *tub2* gene sequences from different *Nigrospora* species, most of which were obtained from type strains, were selected for phylogenetic analyses (Table 1). *Apiospora obovata* (M. Wang & L. Cai) Pintos & P. Alvarado strain LC4940 (KY705166) was included as the outgroup taxon. The sequence generated in the present research, together with sequences retrieved from GenBank (NCBI) were aligned using the MAFFT v. 7 online server (https://mafft.cbrc.jp/alignment/server/index.html). The resulting alignment was manually edited using BioEdit v.7.0.0. (Hall 2004). Phylogenetic analyses were conducted using the maximum likelihood (ML) and Bayesian inference (BI) methods. The ML analysis was performed using the IQ-TREE software (Nguyen et al. 2015), whereas the BI analysis was performed using MrBayes 3.2.7 software (Ronquist et al. 2012).

**RESULTS**

Samplings were conducted from quinoa line Q4 in May 2024. An isolate of *Nigrospora* which was obtained only once from healthy leaf tissue was identified as *Nigrospora camelliae-sinensis* based on an integrated approach combining morphological characterization, and phylogenetic analyses based on the partial sequence of the *tub2* gene.

**Phylogeny**

To investigate the phylogenetic relationships of the *Nigrospora* strain obtained in this study with other species of the genus, a phylogenetic tree was constructed based on the partial nucleotide sequences of the *tub2* gene using the maximum likelihood, and Bayesian inference methods. Following the construction of the trees using both methods, the maximum likelihood phylogenetic tree was selected. The support values for the clades and subclades are presented on the branches from left to right as ML and BI values, respectively (Fig. 1).

The ML analysis was performed using IQ-TREE, and the best-fit model, TPM2u+F+I, was selected according to the Akaike information criterion (AIC). The final ML tree was constructed based on this model, with a maximum log-likelihood value of -1866.498258. For the Bayesian inference method, posterior probabilities (PP) were calculated using the selected evolutionary model. A total of 202 phylogenetic trees were generated, of which 50 trees (25%) were discarded as burn-in, and the remaining 152 trees were used to construct the consensus tree. The average standard deviation of split frequencies was 0.029647. In the resulting phylogenetic tree, 36 strains were assigned to 16 *Nigrospora* species. The clade highlighted in orange comprises the strains of *N. camelliae-sinensis* and *N. singularis* Raza & L. Cai, which were clearly separated from each other with strong ML and BI support (100/1). The placement of these two species within the same clade indicates their close taxonomic relationship. However, phylogenetic analysis based on the *tub2* gene sequence using ML and BI methods was able to differentiate these two species. Furthermore, the newly recovered strain of *Nigrospora camelliae-sinensis* (ABRIICC 10490) clustered with other *N. camelliae-sinensis* isolates retrieved from NCBI, with strong values of 100/1 (ML/BI), confirming the correct identification based on morphological characteristics.

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**Table 1.** The *tub2* sequences used in the phylogenetic analyses. [T] refers to type material sequences.

| Species | Strain | Host | Country | GenBank accession no. *tub2* | Reference |
|---|---|---|---|---|---|
| *Nigrospora aurantiaca* | CGMCC3.18130[T] | *Nelumbo* sp. | China | KY019465 | Wang et al. (2017) |
| | LC7034 | *Musa paradisiaca* | China | KY019598 | Wang et al. (2017) |
| *N. bambusae* | LC7114[T] | Bamboo leaf | China | KY385319 | Wang et al. (2017) |
| | LC7245 | Bamboo leaf | China | KY385321 | Wang et al. (2017) |
| | LC7244 | Bamboo leaf | China | KY385320 | Wang et al. (2017) |
| *N. camelliae-sinensis* | CGMCC3.18125[T] | *Camellia sinensis* | China | KY019460 | Wang et al. (2017) |
| | **ABRIICC 10490** | ***Chenopodium quinoa*** | **Iran** | **PX583147** | **This Study** |
| | LC3496 | *Camellia sinensis* | China | KY019510 | Wang et al. (2017) |
| | LC2710 | *Castanopsis* sp. | China | KY019484 | Wang et al. (2017) |
| *N. gorlenkoana* | CBS 480.73[T] | Unknown | China | KY019456 | Wang et al. (2017 |
| | JZB 3230001 | *Cirsium setosum* | China | MN549381 | Hao et al. (2020) |
| *N. guilinensis* | CGMCC3.18124[T] | *Camellia sinensis* | China | KY019459 | Wang et al. (2017) |
| | LC7301 | *Nelumbo* sp. | China | KY019608 | Wang et al. (2017) |
| *N. hainanensis* | CGMCC3.18129[T] | *Musa paradisiaca* | China | KY019464 | Wang et al. (2017) |
| | LC6979 | *Musa paradisiaca* | China | KY019586 | Wang et al. (2017) |
| | LC7042 | *Musa paradisiaca* | China | KY019599 | Wang et al. (2017) |
| *N. lacticolonia* | CGMCC3.18123[T] | *Camellia sinensis* | China | KY019458 | Wang et al. (2017) |
| | LC7009 | *Musa paradisiaca* | China | KY019594 | Wang et al. (2017) |
| *N. musae* | CBS 319.34[T] | Unknown | China | KY019455 | Wang et al. (2017) |
| | LC6385 | *Camellia sinensis* | China | KY019567 | Wang et al. (2017) |
| *N. oryzae* | LC6923 | *Oryza sativa* | China | KY019581 | Wang et al. (2017) |
| | LC6760 | *Oryza sativa* | China | KY019573 | Wang et al. (2017) |
| | LC4338 | *Camellia* sp. | China | KY019532 | Wang et al. (2017) |
| *N. osmanthi* | JZB 3230010 | *Phyllostachys nigra* | China | MN549388 | Hao et al. (2020) |
| | JZB 3230005 | *Rosa chinensis* | China | MN549383 | Hao et al. (2020) |
| | JZB 3230008 | *Cirsium setosum* | China | MN549386 | Hao et al. (2020) |
| *N. rubi* | LC2698[T] | *Rubus* sp. | China | KY019475 | Wang et al. (2017) |
| *N. shadeganensis* | SCUA-Saf-N27[T] | *Halocnemum strobilaceum* | Iran | PP263821 | Safi et al. (2024) |
| *N. sphaerica* | LC3477 | *Camellia sinensis* | China | KY019508 | Wang et al. (2017) |
| | JZB 3230015 | *Fraxinus* sp. | China | MN549392 | Hao et al. (2020) |
| *N. singularis* | CGMCC3.19334[T] | *Saccharum officinarum* | China | MN329956 | Raza et al. (2019) |
| | LC12068 | *Saccharum officinarum* | China | MN329957 | Raza et al. (2019) |
| *N. vesicularis* | CGMCC3.18128[T] | *Musa paradisiaca* | China | KY019463 | Wang et al. (2017) |
| | LC0322 | Unknown | Thailand | KY019467 | Raza et al. (2019) |
| *N. zimmermanii* | CBS 290.62[T] | *Saccharum officinarum* | Ecuador | KY385317 | Wang et al. (2017) |
| | CBS 984.69 | *Saccharum officinarum* | Brazil | KY385322 | Wang et al. (2017) |
| *Apiospora obovata* | LC4940[T] | *Lithocarpus* sp. | China | KY705166 | Wang et al. (2018) |

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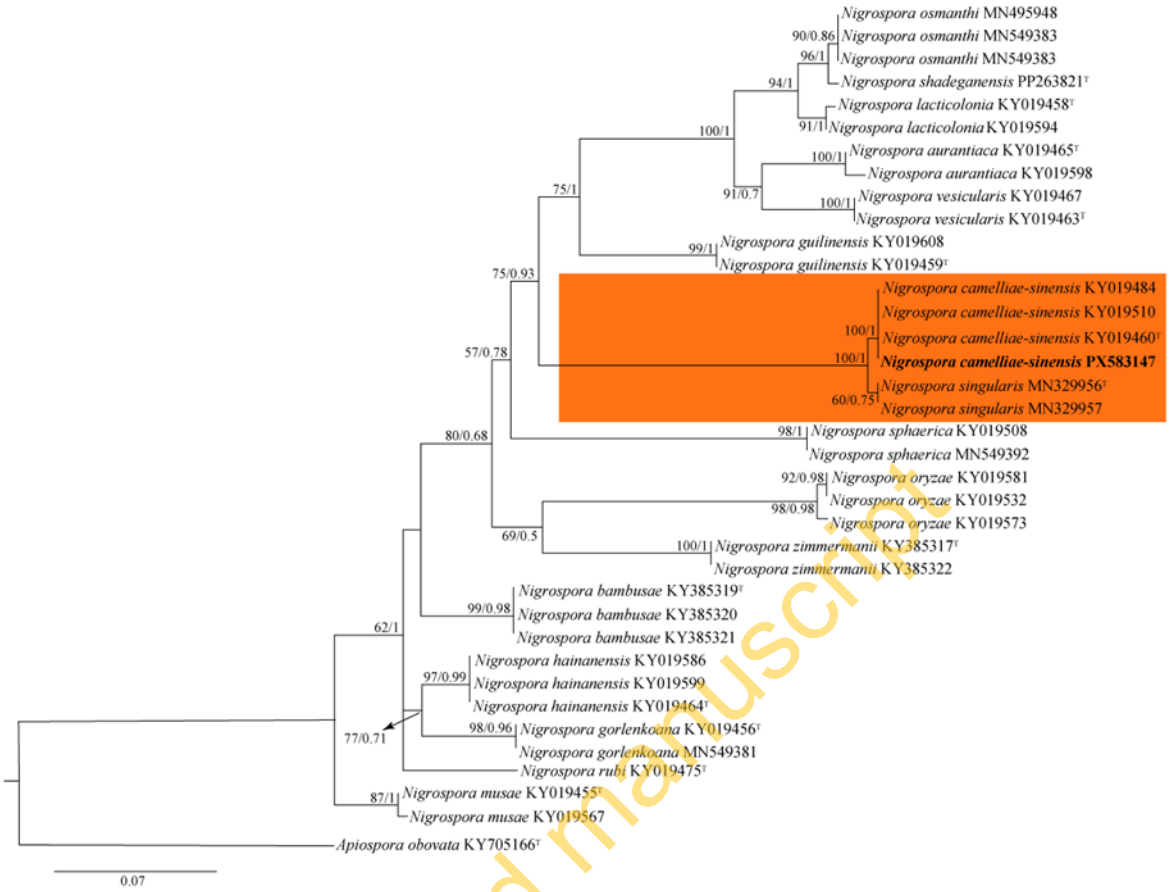


**Fig. 1.** The phylogenetic tree constructed based on partial nucleotide sequences of the *tub2* gene using maximum likelihood and Bayesian inference methods, comprising 36 strains representing 16 species of the genus *Nigrospora*. Bootstrap support was assessed with 1000 replicates. Bootstrap supports above 50 and the posterior probabilities (PP) were shown at the nodes from left to right, respectively. The tree was rooted with *Apiospora obovata* LC4940.

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## Taxonomy

***Nigrospora camelliae-sinensis*** M. Wang & L. Cai, Persoonia 39: 127 (2017)

The colony of isolate ABRIICC 10490 was cultured on PDA medium at 25 °C under continuous dark conditions. Initially, the colony appeared white during vegetative growth and gradually changed to grey and black with sporulation and aging. The colony reached a diameter of 80 mm after 10 days of incubation. The hyphae were smooth, pale brown to dark brown, branched, and septate. The conidiophores were micronematous, mononematous, solitary, and unswollen. They were typically aseptate or, occasionally, contained a single transverse septum. The conidiophores were frequently integrated with conidiogenous cells. Conidiogenous cells were pale brown, globose to flask-shaped, and measured as 6–13 × 4–7 µm (av. = 9.3 ± 1.5 × 5.2 ± 0.6). Conidia were solitary, black, smooth, and aseptate occurring in two forms: spherical, measuring 14–19 µm in diameter (av. = 16.8), and slightly ellipsoidal, measuring 13–17 × 10–14 µm in diameter (av. = 14.5± 1.2 × 11± 1.24) (Fig. 2). The morphological features of the investigated isolate were consistent with the description of *N. camelliae-sinensis* provided by Wang et al. (2017) and Zhang et al. (2025). The growing colony of the recovered isolate was deposited in the Agricultural Microbial Collection of the Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, under accession no. ABRIICC 10490.

Specimen examined: IRAN, Alborz Province, Mohammadshahr, Karaj, N 35°47'46.42" E 50°56'23.46", isolated as an endophytic fungus from a healthy leaf of quinoa plant (*Chenopodium quinoa* Willd.), Q4 line, May 2024, A. Yaghoubi Hassanali-Deh (ABRIICC 10490), GenBank accession no. for *tub2*: PX583147.

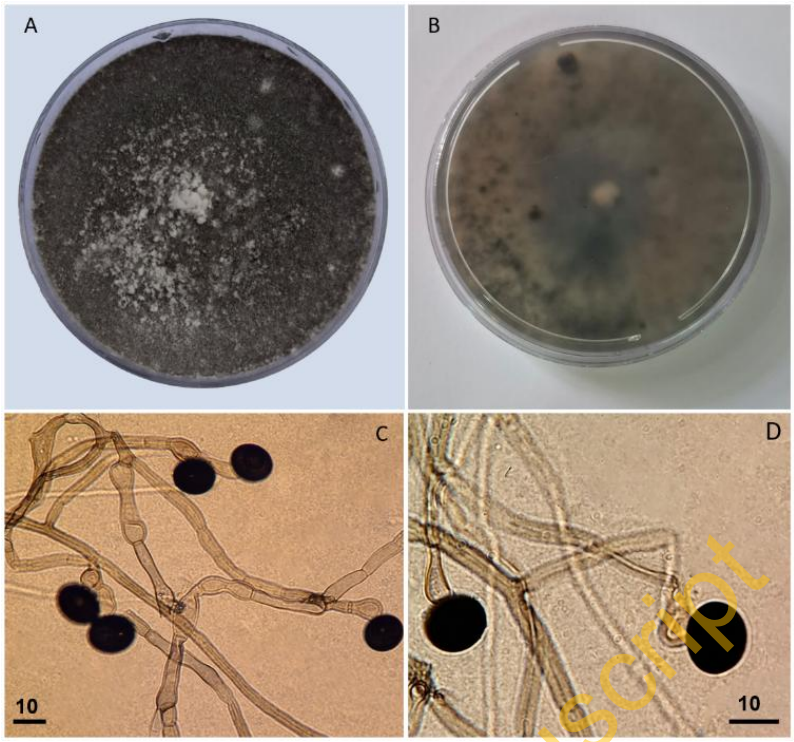


**Fig. 2**. *Nigrospora camelliae-sinensis* isolate ABRIICC 10490. (A, B) Obverse and reverse of the colony after 14 days of incubation at 25 °C under continuous dark conditions, (C, D) conidiophores, conidiogenous cells, and conidia. Scale bars (C, D) = 10 µm.

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## DISCUSSION

One strain of *Nigrospora camelliae-sinensis*, recovered from healthy leaf of *Chenopodium quinoa*, was morphologically investigated following Wang et al. (2017) and Zhang et al. (2025). Due to the considerable overlap in morphological characters among species of the genus *Nigrospora*, species identification and delimitation based solely on morphological criteria can be challenging and sometimes controversial (Wang et al. 2017). Therefore, in the present study, a molecular approach based on sequencing of the partial *tub2* gene was also employed. For species delimitation and introduction of new species within the genus *Nigrospora*, the *tub2* gene is commonly used as one of the main markers, together with sequences of the ITS regions and *tef1-α* gene (Liu et al. 2024). In the present study, partial sequences of the *tub2* gene were analyzed using ML and BI methods, which provided strong support for the inferred phylogenetic relationships and demonstrated the effectiveness of this marker in resolving closely related species within the genus *Nigrospora*. In the resulting phylogenetic tree, *N. camelliae-sinensis* isolate ABRIICC 10490 (PX583147) clustered with other *N. camelliae-sinensis* isolates retrieved from NCBI and formed a fully supported clade (Fig. 1). Morphologically, *N. camelliae-sinensis* closely resembles *N. singularis*, as indicated by phylogenetic analyses. However, the two species are morphologically distinct. *Nigrospora singularis* can be distinguished from *N. camelliae-sinensis* by its larger conidia (9.5–13 × 11–15 µm), and conidiogenous cells (7–12 × 5–10 µm) (Raza et al. 2019). *Nigrospora camelliae-sinensis* can be distinguished from *N. oryzae*, *N. osmanthi*, and *N. shadeganensis* primarily by the combination of conidiogenous cell morphology and conidial dimensions (Safi et al. 2025). *Nigrospora camelliae-sinensis* produces relatively large, solitary, black, smooth, aseptate, globose to ellipsoidal conidia [15–18 (–19) × 12–16 (–17) µm], whereas the conidia of *N. oryzae* are globose to subglobose and smaller [12.5–16 (mostly 12–14) µm]. In addition, *N. oryzae* is characterized by branched and clustered conidiophores, in contrast to *N. camelliae-sinensis*, in which the conidiophores are mostly reduced to conidiogenous cells. In comparison, *N. shadeganensis* differs more markedly from *N. camelliae-sinensis*, particularly in conidial size. *Nigrospora shadeganensis* produces globose to subglobose conidia (11.5–17.5 × 14–14.5 µm), and subglobose to ellipsoidal conidia (13–17 × 10–15.5 µm), which are considerably smaller than those of *N. camelliae-sinensis*. The conidiophores of *N. shadeganensis* are discrete and solitary, mostly reduced to form conidiogenous cells, and are ampulliform to cylindrical or subspherical, (3.8–)5–25(–26.9) × (1.47–)2–3.5(–4.4) µm. However, the substantially smaller conidia and markedly narrower conidiogenous cells of *N. shadeganensis* provide important morphological differences between the two species (Wang et al. 2017, Bashiri et al. 2024, Safi et al. 2025). *Nigrospora camelliae-sinensis* also shows considerable phylogenetic and morphological affinity with *N. guilinensis* Mei Wang & L. Cai, but can be distinguished from the latter by conidial size [spherical, 11.5–15 µm diam (av.= 12.91 ± 0.7), and ellipsoidal, 10.5–14 × 8–12 µm (av. = 12.46 ± 0.62 × 9.69 ± 0.71)] and the production of red pigments in synthetic nutrient-poor agar medium (SNA) (Wang et al. 2017).

*Nigrospora camelliae-sinensis* was first introduced by Wang et al. (2017) from a dead leaf of the tea plant, *Camellia sinensis*, in China. This species has also been isolated from dead leaves of *Camellia sinensis* by Manawasinghe et al. (2021). Subsequently, Yan et al. (2025) reported this species as a pathogen associated with leaf spot symptoms on tea plants in China. In addition, *N. camelliae-sinensis* was isolated as an endophytic fungus from the medicinal plant, *Dicranopteris pedata* (Houtt.) Nakaike, in China (Bao et al. 2025). Zhang et al. (2025) also reported this species as an endophytic fungus from a new host, *Aquilaria sinensis* (Lour.) Spreng., in China. Wang et al. (2017) investigated the phylogenetic relationships of species within the genus *Nigrospora* using morphological and molecular data based on *tef1*-α, *tub*, and ITS sequences, and introduced 12 new species, including *N. camelliae-sinensis*. Also, *Nigrospora camelliae-sinensis* has been reported from other host plants in China, including *Camellia sinensis* (Wang et al. 2017, Raza et al. 2019), *Castanopsis* sp. and *Musa*×*paradisiaca* L. (Wang et al. 2017), and *Saccharum officinarum* L. (Raza et al. 2019). To the best of our knowledge, this is the first report of *N. camelliae-sinensis* as a new record for funga of Iran, as well as the first report of this species as an endophytic fungus associated with the quinoa plant (*Chenopodium quinoa*), worldwide. Furthermore, *Chenopodium quinoa* is reported here as a new host (matrix nova) for *N. camelliae-sinensis* in the world.

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## AUTHOR CONTRIBUTION

Khalil-Berdi Fotouhifar planned and supervised the work; prepared the final manuscript; Ali Yaghoubi Hassanali-Deh and Abbas Atashi Khalilabad performed DNA sequencing; performed the phylogenetic analysis; provided the drawings; prepared the first manuscript draft; All authors reviewed the manuscript and commented on the manuscript.

## DATA AVAILABILITY

All datasets generated during this study are available from the corresponding author upon request.

## FUNDING

The authors declare that no financial support was received during this research.

## DECLARATION

The authors declare no competing interests.

## ETHICAL APPROVAL

This article does not contain any studies with human participants or animals by any of the authors.

## REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology. 215: 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Angeli V, Miguel Silva P, Crispim Massuela D, Khan MW, Hamar A, … et al. 2020. Quinoa (*Chenopodium quinoa* Willd.): An Overview of the Potentials of the "Golden Grain" and Socio-Economic and Environmental Aspects of Its Cultivation and Marketization. Foods. 9(2): 216. https://doi.org/10.3390/foods9020216.

Bashiri S, Abdollahzadeh J. 2024. Identification and pathogenicity of *Nigrospora shadeganensis* on oak trees in Zagros forests, Iran. Mycologia Iranica. 11(1): 93-100. https://doi.org/10.22092/mi.2024.367119.1288.

Bao DF, Zhang JY, Lu YZ, Tian XG, Hyde KD, ... et al. 2025. Endophytic fungi associated with medicinal ferns in Guizhou Province, China I: Morpho-molecular characterization of culturable endophytic fungi associated with *Dicranopteris* spp. Mycosphere. 16(1): 2259-2455. https://doi.org/10.5943/mycosphere/16/1/13.

De Hoog SG, Guarro J, Gene J, Gené J, Ahmed S, … et al. 2000. Atlas of clinical fungi. 4th edition. Westerdijk Fungal Biodiversity Institute, Netherlands.

Fan YM, Huang WM, Li W, Zhang GX. 2009. Onychomycosis caused by *Nigrospora sphaerica* in an immunocompetent man. Archives of Dermatology. 145(5): 611-612. https://doi.org/10.1001/archdermatol.2009.80.

Farzaneh M, Javan-Nikkhah M, Fotouhifar KB, and Karim-Mojeni H. 2009. Fungi associated with leaf spots of nutsedge plants in north of Iran. Rostaniha. 10(1): 68-79.

Glass NL, Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology. 61(4): 1323-1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995.

Accepted manuscript

Goodarzian K, Tajick Ghanbary MA, Babaeizad V, Mojerlou S. 2021. Identification of root endophytic fungi from rangeland plants in Mazandaran province. Iranian Journal of Forest and Range Protection Research. 18(2): 216-232.

González-Teuber M, Vilo C, Bascuñán-Godoy L. 2017. Molecular characterization of endophytic fungi associated with the roots of *Chenopodium quinoa* inhabiting the Atacama Desert, Chile. Genomics Data. 11: 109-112. https://doi.org/https://doi.org/10.1016/j.gdata.2016.12.015.

Hall T. 2004. BioEdit version 7.0.0. Department of Microbiology, North Carolina State University, USA.

Hao Y, Aluthmuhandiram JV, Chethana KT, Manawasinghe IS, Li X, … et al. 2020. *Nigrospora* species associated with various hosts from Shandong Peninsula, China. Mycobiology. 48(3): 169-183. https://doi.org/10.1080/12298093.2020.1761747.

Kindo AJ, Subramanian A, Suresh K. 2014. *Nigrospora sphaerica* causing corneal ulcer in an immunocompetent woman: a case report. International Journal of Case Reports and Images (IJCRI). 5: 675-679. https://doi.org/10.5348/ijcri-2014119-CR-10430.

Liu M, Liu Y, Zhang X, Wu Y, Wang Y, … et al. 2024. *Nigrospora weininensis* sp. nov., a new potent nematicidal fungus isolated from *Globodera rostochiensis* in Guizhou, China. Phytotaxa. 675 (2): 135-146. https://doi.org/10.11646/phytotaxa.675.2.4.

Liu YJ, Tang Q, Fang L. 2015. First report of *Nigrospora sphaerica* causing leaf blight on *Camellia sinensis* in China. Plant Disease. 100: 221. https://doi.org/10.1094/PDIS-04-15-0493-PDN.

Manawasinghe IS, Jayawardena RS, Li HL, Zhou YY, Zhang W, … et al. 2021. Microfungi associated with *Camellia sinensis*: A case study of leaf and shoot necrosis on Tea in Fujian, China. Mycosphere. 12(1): 430-518. https://doi.org/10.5943/mycosphere/12/1/6.

Mason EW. 1927. On species of the genus *Nigrospora* Zimmermann recorded on monocotyledons. Transactions of the British Mycological Society. 12: 152-165. https://doi.org/10.1016/S0007-1536(27)80011-2.

Mirzaee MR, Heydari A, Zare R, Naraghi L, Sabzali F, … et al. 2013. Fungi associated with boll and lint rot of cotton in Southern Khorasan province of Iran. Archives of Phytopathology and Plant Protection. 46(11): 1285-1294. https://doi.org/10.1080/03235408.2013.765135.

Nguyen LT, Schmidt HA, Von Haeseler A, Minh B. Q. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution. 32(1): 268-274. https://doi.org/10.1093/molbev/msu300.

Raza M, Zhang ZF, Hyde KD, Diao YZ, Cai L. 2019. Culturable plant pathogenic fungi associated with sugarcane in southern China. Fungal Diversity. 99: 1-104. https://doi.org/10.1007/s13225-019-00434-5.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, … et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic biology. 61(3): 539-542. https://doi.org/10.1093/sysbio/sys029.

Safi A, Mehrabi-Koushki M, Arzanlou M. 2024. Endophytic species of *Nigrospora* from grasses and shrubs of Shadegan International Wetland, with new species and records from Iran. Antonie van Leeuwenhoek. 117(1): 77. https://doi.org/10.1007/s10482-024-01976-8.

Sayers EW, Beck J, Bolton EE, Brister JR, Chan J, … et al. 2025. Database resources of the National Center for Biotechnology Information in 2025. Nucleic Acids Research. 6: 53 (D1): D20-D29. https://doi.org/10.1093/nar/gkae979.

Seifert KA, Morgan-Jones G, Gams W, Kendrick B. 2011. The genera of Hyphomycetes. CBSKNAW Fungal Biodiversity Centre, Utrecht, Netherlands. https://doi.org/10.3767/003158511X617435.

Sharma P, Meena PD, Chauhan JS. 2013. First report of *Nigrospora oryzae* (Berk. & Broome) Petch causing stem blight on *Brassica juncea* in India. Journal of Phytopathology. 161: 439-441. https://doi.org/10.1111/jph.12081.

Thalavaipandian A, Ramesh V, Bagyalakshmi, Muthuramkumar S, Rajendran A. 2011. Diversity of fungal endophytes in medicinal plants of Courtallam hills, Western Ghats, India. Mycosphere. 2(5): 575-582. https://doi.org/10.5943/mycosphere/2/5/7.

Wang M, Liu F, Crous PW, Cai L. 2017. Phylogenetic reassessment of *Nigrospora*: Ubiquitous endophytes, plant and human pathogens. Persoonia. 39: 118-142. https://doi.org/10.3767/persoonia.2017.39.06.

Wu SH, Chen YW, Shao SC, Wang LD, Yu Y, … et al. 2009. Two new solanapyrone analogues from the endophytic fungus *Nigrospora* sp. YB-141 of *Azadirachta indica*. Chemistry & Biodiversity. 6: 79-85. https://doi.org/10.1002/cbdv.200700421.

Yan L, Pan W, Zhou X. 2025. *Nigrospora* species causing leaf disease on *Camellia* plants. Journal of Phytopathology. 173(1): 1-4. Article e70020. https://doi.org/10.1111/jph.70020.

Zarandi DM, Aminaee MM, Sharzei A, Rezaee S. 2014. First report of rosemary leaf spot caused by *Nigrospora oryzae* in Iran. New Disease Reports. 30(1): 27-27. https://dx.doi.org/10.5197/j.2044-0588.2014.030.027.

Zhang S, Li J, Jiang H, Ye S, Mapook A, … et al. 2025. The polyphasic approach reveals two new species and two new records of *Nigrospora (Apiosporaceae*, *Amphisphaeriales*) associated with *Aquilaria sinensis* from China. MycoKeys. 121: 1-20. https://doi.org/10.3897/mycokeys.121.154055.

Zhong S, Steffenson BJ. 2001. Genetic and molecular characterization of mating type genes in *Cochliobolus sativus*. Mycologia. 93(5): 852-863. https://doi.org/10.1080/00275514.2001.12063220.

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مقاله کوتاه

# گونه *Nigrospora camelliae-sinensis* یک آرایه جدید برای فهرست قارچ‌های ایران همراه با ارتباط اندوفیتی با گیاه *Chenopodium quinoa*

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## چکیده

طی بررسی قارچ‌های اندوفیت گیاه کینوآ (*Chenopodium quinoa* Willd.) در اردیبهشت ماه سال ۱۴۰۳، نمونه‌برداری از مزرعه تحقیقاتی مؤسسه تحقیقات اصلاح و تهیه نهال و بذر، واقع در محمدشهر، کرج، استان البرز، انجام شد و جدایه‌های قارچی متنوعی از برگ‌های سالم این گیاه متعلق به لاین Q4 به دست آمد. بر اساس ویژگی‌های ریخت‌شناختی، یکی از جدایه‌های قارچی به جنس *Nigrospora* تعلق داشت. به‌منظور تعیین جایگاه تبارزایی جدایه به‌دست‌آمده، توالی نوکلئوتیدی بخشی از ژن بتا-توبولین با استفاده از روش‌های بیشینه درست‌نمایی (maximum likelihood) و استنباط بیزی (Bayesian inference) مورد تجزیه و تحلیل قرار گرفت و در نهایت درخت تبارزایی بازسازی شد. نتایج تحلیل تبارزایی، جدایه مورد مطالعه را در سطح گونه‌ای با حمایت زیاد مشخص و آن را از سایر گونه‌های مرتبط با جنس *Nigrospora* که توالی‌های آن‌ها از بانک ژن (NCBI) بازیابی شده بودند، تفکیک نمود و همچنین شناسایی ریخت‌شناختی این گونه را نیز تائید کرد. بر اساس بررسی‌های ریخت‌شناختی (کنیدیوم‌ها و کنیدیوفورها) و تبارزایی مولکولی، جدایه به‌دست‌آمده به‌عنوان گونه *Nigrospora camelliae-sinensis* شناسایی شد. این نخستین گزارش از گونه *N. camelliae-sinensis* به‌عنوان گونه جدید برای فهرست قارچ‌های ایران است و همچنین نخستین گزارش این گونه به‌عنوان یک قارچ اندوفیت مرتبط با گیاه کینوآ در جهان می‌باشد. همچنین، در مطالعه حاضر، *Chenopodium quinoa* به‌عنوان میزبان جدیدی (*matrix nova*) برای گونه *N. camelliae-sinensis* در جهان گزارش می‌شود.

**واژگان کلیدی:** تبارزایی، تنوع زیستی، ریخت شناسی، ژن *tub2*، کینوآ.

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