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Research article

New host and geographic records of *Epicoccum italicum* (Didymellaceae) and *Nigrospora gorlenkoana* (Apiosporaceae) from Egypt

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Abstract

Two endophytic fungi, *Epicoccum italicum* and *Nigrospora gorlenkoana*, are reported here for the first time from medicinal plants collected in Wadi Bir-El-Ain, Sohag Governorate, Egypt. *Epicoccum italicum* was recorded from healthy roots of *Zygophyllum coccineum*, whereas *Nigrospora gorlenkoana* was from healthy leaves of *Alhagi maurorum*. Phylogenetic analyses of the combined ITS and LSU rDNA sequence data, along with morphological characteristics, were used to identify the new collections. The conidia of *E. italicum* were multicellular-phragmosporous, subglobose-pyriform, verrucose, brown, with a basal cell, while those of *N. gorlenkoana* were aseptate, globose to sub-globose, pale brown to black, smooth with an equatorial slit. This is the first report of these species in Egypt and Africa. Detailed descriptions and illustrations are provided for both fungi. These findings expand the known geographic distribution and host range of these taxa. They further highlight the role of medicinal plants in arid ecosystems as reservoirs of endophytic fungi with important ecological functions and promising biotechnological potential.

Keywords

mitosporic fungi, endophytes, molecular phylogeny, medicinal plants, Wadi Bir-El-Ain

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Introduction

Endophytes are microorganisms that inhabit healthy plant tissue at least a part of their life cycles without producing any noticeable symptoms of disease (Wilson, 1995; Hardoim et al., 2015). Fungal endophytes are found in almost all plants on Earth, from the Arctic to the tropics (Aly et al., 2011). They play a crucial role in enhancing host plant resilience and improving tolerance to biotic and abiotic stresses (Udoukpo et al., 2024). Medicinal plants serve as a significant reservoir of novel endophytic fungi, representing a valuable source of bioactive natural products (Toghueo et al., 2017; Toppo et al., 2024).

The genus *Epicoccum* was established by Link (1815) to accommodate *E. nigrum* Link, which was observed on the dry stalks of plants. It is characterized by producing dark sporodochia with

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mono- or polyblastic conidiogenous cells that produce colored dictyoconidia (Seifert et al., 2011). The genus description was emended to accommodate species either hyphomycetous or phoma-like synanmorphs with aseptate conidia in pycnidial conidiomata (Aveskamp et al., 2010; Chen et al., 2015). Most *Epicoccum* species were identified from terrestrial habitats (i.e., as endophytes, saprobes, or plant pathogens), with few species found in aquatic environments (Chen et al., 2017; Ahumada-Rudolph et al., 2019; Voronin et al., 2021). Schol-Schwarz (1959) revised the species of *Epicoccum* and reduced these taxa to a single variable species, *E. nigrum*. Chen et al. (2017) proposed nine new species in the genus *Epicoccum*: four taxa with epicoccoid conidia and five with pycnidial conidiomata. Jayasiri et al. (2017) introduced the first sexual morph for this genus in nature; *E. mackenziei* Jayasiri, Camporesi & K.D. Hyde on the dead stem of *Ononis spinosa*. Thirty taxa were discovered in the genus between 2020 and 2024, reflecting the high diversity of this genus. Fifty-one species have molecular data in the *Epicoccum* genus (Tian et al., 2024; Manawasinghe et al., 2025). *Epicoccum* species produce diverse secondary metabolites with promising biological activities, including antimicrobial, anticancer, antioxidant, and anti-inflammatory (Braga et al., 2018). They are also used as biocontrol agents against phytopathogens and secrete pigments with potential industrial applications.

The genus *Nigrospora* was established by Zimmermann (1902), based on *N. panici* Zimm., which was isolated from the leaves of *Panicum amphibium* in Indonesia and has sub-globose, solitary, black, and aseptate conidia. Later, several new species were discovered or transferred to the genus *Nigrospora* with monoblastic, ampulliform, sub-cylindrical to clavate conidiogenous cells and ellipsoidal, pyriform, pale brown to black conidia (Wang et al., 2017; Raza et al., 2019; Chen et al., 2022; Zhang et al., 2024). Members of the genus *Nigrospora* were isolated from terrestrial habitats (i.e., endophytic, saprobic, or pathogenic on plants and humans), with many species reported from marine and freshwater environments (Zhang et al., 2015; Wang et al., 2017; Ukwatta et al., 2019; Chen et al., 2022; Huang et al., 2022; Lee et al., 2023; Choi et al., 2024). *Nigrospora* species are cosmopolitan with a wide host range (Chen et al., 2020). Wang et al. (2017) identified 12 new taxa in the genus *Nigrospora* and transferred *N. vietnamensis* Hol.-Jech. to *Arthrinium* Kunze. Based on morphology and phylogeny, they transferred *Nigrospora* to the family Apiosporaceae (Xylariales). Hyde et al. (2020) placed the genus within Apiosporaceae, Amphisphaeriales, and stated that the genus comprises 25 species with morphological data and 17 species have sequence data. In addition to their ecological and taxonomic significance, *Nigrospora* species are considered a source of various biologically active compounds with industrial applications (Metwaly et al., 2014; Chen et al., 2024).

During a survey of endophytic fungi from Egypt, two new host and geographic records, *Epicoccum italicum* Qian Chen, Crous & L. Cai and *Nigrospora gorlenkoana* Novobr. were established and illustrated in this article based on morphological and molecular data.

Materials and Methods

Sample collection and fungal isolation

Alhagi maurorum Medik. (Fabaceae) and *Zygophyllum coccineum* L. (Zygophyllaceae) plants were collected from Wadi Bir-EL-Ain (lat. 26°37'41.3" to 26°38'46.0" N, long. 31°48'11.9" to 31°50'28.7" E), in the eastern desert of Sohag Governorate, Egypt, in February 2022. The healthy plant samples were placed in clean plastic bags and returned to the laboratory in portable cool chambers (4 °C).

Digital photos were taken for the species studied. Identification of the plants have been achieved at the herbarium of Sohag (SHG).

The different plant parts (leaves, stems, and roots) were washed gently with running tap water. Isolation of endophytic fungi was performed according to the method outlined by Hallmann et al. (2006). A total of 54 plant segments (18 each from leaves, stems, and roots) were processed per species. *Zygophyllum coccineum* yielded 17 endophytic fungal isolates, corresponding to a colonization frequency of 31.48%. Among these, *E. italicum* was isolated from 10 of 18 root segments, with a colonization frequency of 55.55%. *Alhagi maurorum* yielded 15 endophytic fungal isolates (colonization frequency 27.78%), including 7 isolates of *N. gorlenkoana* from leaf tissue, with a colonization frequency of 38.88%.

The sterilized segments were seeded in Petri dishes containing malt extract agar (MEA; 1% w/v; Techno Pharmchem, India) and 1/4 Potato dextrose agar (PDA; HiMedia Laboratories, Mumbai, India) and supplemented with 50 mg L^{-1} chloramphenicol for suppressing bacterial growth. The plates were incubated at 24 °C and were examined daily for any fungal growth; single isolates grown out from plant tissues were sub-cultured on fresh medium and preserved in cryotubes containing 15% glycerol at -80 °C. Living cultures have been preserved in the Sohag University Microbial Culture Collection, Egypt (SUMCC).

Growth and morphological studies

Colonies characters were observed after 7 days of growth from those cultivated on PDA and MEA. Morphological characters were observed, and photomicrographs were taken by a Toup Tek XCAM1080PHA (Toup Tek, Zhejiang, China) digital camera mounted on an Olympus BX51 compound microscope. Measurements of all fungal characters were made in freshwater mounts.

DNA extraction, sequencing, and phylogenetic analyses

Total genomic DNA was extracted directly from freshly scraped fungal mycelium using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), in accordance with the manufacturer's protocol. The primer pairs LROR and LR7 (Vilgalys and Hester, 1990) and ITS1 and ITS4 (White et al., 1990) were used for PCR amplification and sequencing of the partial large subunit nuclear rDNA (LSU) and internal transcribed spacer (ITS) regions, respectively. The PCR was performed in a 25 µL reaction using Solgent EF-Taq as follows: 2.5 µL of 10X EF-Taq buffer, 0.5 µL of 10 mM dNTPs (T), 1 µL of each primer, 0.25 µL of EF-Taq (2.5 U), 1 µL of DNA template, and distilled water to a final volume of 25 µL. The cycling parameters were as follows: an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 20 s at 94 °C, 40 s at 56 °C, and 1 min at 72 °C, with a final extension of 5 min at 72 °C. The amplified PCR products were sequenced by Solgent Co., Ltd (South Korea). Sequences were assembled using Sequencher 4.2.2 (Gene Codes Corporation) and aligned using ClustalX (Thompson et al., 1997) with relevant ones retrieved from GenBank (Table 1–2). The combined sequence data were used to conduct Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian inference (BI) analyses. The MP analysis was implemented in PAUP*4 (Swofford, 2003), the ML analysis by RAxMLGUI v. 2.0.13 (Edler et al., 2021) and the BI analysis was conducted with MrBayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003) with details outlined by Bakhit and Abdel-Wahab (2022, 2025). Newly generated sequences were deposited in GenBank (Table 1–2).

Results

This study presents the isolation and identification of two endophytic fungal species, *E. italicum* and *N. gorlenkoana*, from roots of *Z. coccineum* and leaves of *A. maurorum*, respectively. The following sections provide a detailed identification of the isolated species, incorporating morphological descriptions and phylogenetic analyses to establish their taxonomic placement.

Molecular and morphological characteristics of E. italicum

Epicoccum italicum Qian Chen, Crous & L. Cai, in Chen et al., Stud. Mycol. 87:144 (2017) (Figs. 1–2).
GenBank numbers – ITS: PV268490; LSU: PV268682.

Phylogenetic analysis
The combined ITS and LSU rDNA dataset consisted of 41 taxa, of which 40 belong to the *Epicoccum*, with *Neoascochyta desmazieri* (CBS 297.69) as outgroup (Table 1). The maximum parsimony dataset consists of 1201 characters (ITS: 450, LSU: 751) after alignment that included: 1114 constant, 50 variable parsimony-uninformative, and 37 parsimony-informative characters. The best scoring RAxML tree with a final likelihood value of -2477.015254 is presented in Fig. 1. The matrix had 98 distinct alignment patterns with 2.33% undetermined characters or gaps. Estimated base frequencies were found to be A = 0.247143, C = 0.223508, G = 0.279733, T = 0.249616; substitution rates, AC = 8.556417, AG = 18.212641, AT = 12.904133, CG = 4.097420, CT = 69.887178, GT = 1.000000. The tree topologies derived from the three analyses were highly similar. Phylogenetic analysis placed our new strain (SUMCC 22007) within *Epicoccum* and clustered with the ex-type strain of *E. italicum* (CGMCC 3.18361) and strains (MFLU 23-0166, IPLA 35013, and IPLA 35014) with high statistical support (98/92/1.00 for ML/MP/BYPP, respectively) (Fig. 1).

Taxonomy
Endophytic on *Z. coccineum* roots. Sexual morph: undetermined. Asexual morph: growing well on MEA. Conidiomata sporodochial, mostly solitary, sometimes aggregated, superficial, clavate, yellowish brown to dark brown, produced on the agar surface. Mycelium composed of yellowish red to pale brown, septate, branched, 2.5–4.5 µm wide hyphae. Conidiophores mostly reduced to holoblastic conidiogenous cells. Conidia: 14–30 µm in diameter (mean = 19.6 µm, n = 34), multicellular-phragmosporous, subglobose-pyriform, verrucose, brown to dark brown, with a basal cell.
Culture characteristics
Colonies on MEA reached 40–55 mm diam., after 7 days at 24 °C, yellow to orange with a white, regular margin; with floccose aerial mycelia, the reverse side orange with a yellowish margin; Colonies on PDA reached 35–51 mm diam., after 7 days at 24 °C, white to pale yellow, with floccose aerial mycelia, the reverse side saffron to reddish brown with pale yellowish margin.

Table 1 – Taxa used in the phylogenetic analysis of *Epicoccum italicum*.

Species	**Voucher/Strains**	**GenBank accession no.**		**References**
		ITS	**LSU**	
Epicoccum anhuiense	YCW961	OP648058	OP837091	Wang et al. 2024
E. brahmansense	CBS 990.95	MN973514	MN943720	Hou et al. 2020
E. brasiliense	CBS 120105	GU237760	GU238049	Chen et al. 2015
E. camelliae	LC:4858	KY742091	KY742245	Chen et al. 2017
E. cedri	MFLUCC 17-1058	KY711170	KY711172	Hyde et al. 2017
E. dendrobii	LC:8145	KY742093	KY742247	Chen et al. 2017
E. draconis	CBS 186.83	GU237795	GU238070	Chen et al. 2015
E. duchesneae	LC:5139	KY742095	KY742249	Chen et al. 2017
E. endophyticum	JZB380043	MN654966	MN654935	Manawasinghe et al. 2020
E. henningsii	CBS 104.80	GU237731	GU238081	Chen et al. 2015
E. hordei	LC:8148	KY742097	KY742251	Chen et al. 2017
E. huancayense	CBS 105.80	MH861244	MH873016	Chen et al. 2015
E. italicum	**SUMCC 22007**	**PV268490**	**PV268682**	**This study**
E. italicum	CGMCC 3.18361; LC:8150	KY742099	KY742253	Chen et al. 2017
E. italicum	MFLU 23-0166	OR438337	OR438808	Tian et al. 2024
E. italicum	IPLA 35013	OQ570977	OQ568300	Rodríguez et al. 2023
E. italicum	IPLA 35014	OQ570978	OQ568301	Rodríguez et al. 2023
E. jingdongense	YCW1868	OP648072	OP837098	Wang et al. 2024
E. latusicollum	LC:5158	KY742101	KY742255	Chen et al. 2017
E. layuense	LC:8155	KY742107	KY742261	Chen et al. 2017
E. longiostiolatum	CBS:902.96	MN973507	MN943713	Hou et al. 2020
E. mackenziei	YCW1965	OP648085	OP837109	Wang et al. 2024
E. mezzettii	CBS 173.38	MN973496	MN943701	Hou et al. 2020
E. mezzettii	CBS 873.72	MH860625	MH872318	Hou et al. 2020
E. nigrum	CBS 173.73	FJ426996	GU237975	Aveskamp et al. 2010
E. nigrum	LC 8157	KY742110	KY742264	Chen et al. 2017
E. oryzae	CBS:174.34	MH855478	MH866957	Hou et al. 2020
E. oryzae	YCW2010	OP648087	OP837111	Wang et al. 2024
E. ovisporum	CBS 180.80	FJ427068	LT623212	Valenzuela-Lopez et al. 2018
E. pimprinum	CBS 246.60	FJ427049	GU237976	Aveskamp et al. 2010
E. plurivorum	CBS 558.81	MH861377	NG069783	Vu et al. 2019
E. polychromum	CBS 141502	NR170783	MN943712	Hou et al. 2020
E. proteae	CBS 114179	JQ044433	JQ044452	Crous et al. 2011
E. rosae	MFLU 15-3240	MG828899	MG829009	Wanasinghe et al. 2018
E. sorghinum	CBS 179.80	FJ427067	GU237978	Aveskamp et al. 2010
E. tobaicum	YCW336	OP648069	OP837096	Wang et al. 2024
E. tritici	MFLUCC 16-0276	KX926426	KX954391	Thambugala et al. 2017
E. variabile	CBS 119733	MN973501	MN943706	Hou et al. 2020
E. viticis	LC:5126	KY742118	KY742272	Chen et al. 2017
E. yunnanensis	MFLU 23 0165	OR438338	OR438809	Tian et al. 2024
Neoascochyta desmazieri	CBS 297.69	KT389508	KT389726	Chen et al. 2015

*Newly generated sequences are indicated in bold.

Materials examined: Egypt, Sohag Governorate, Wadi Bir-El-Ain (26°38'24.1"N 31°50'06.3"E), from healthy roots of *Zygophyllum coccineum* (Zygophyllaceae), Feb. 2022, coll. G. G. Faheem, SUMCC 22007.
Known distribution: Antarctic Peninsula, China, Italy, Poland, Spain, Thailand, and the USA.

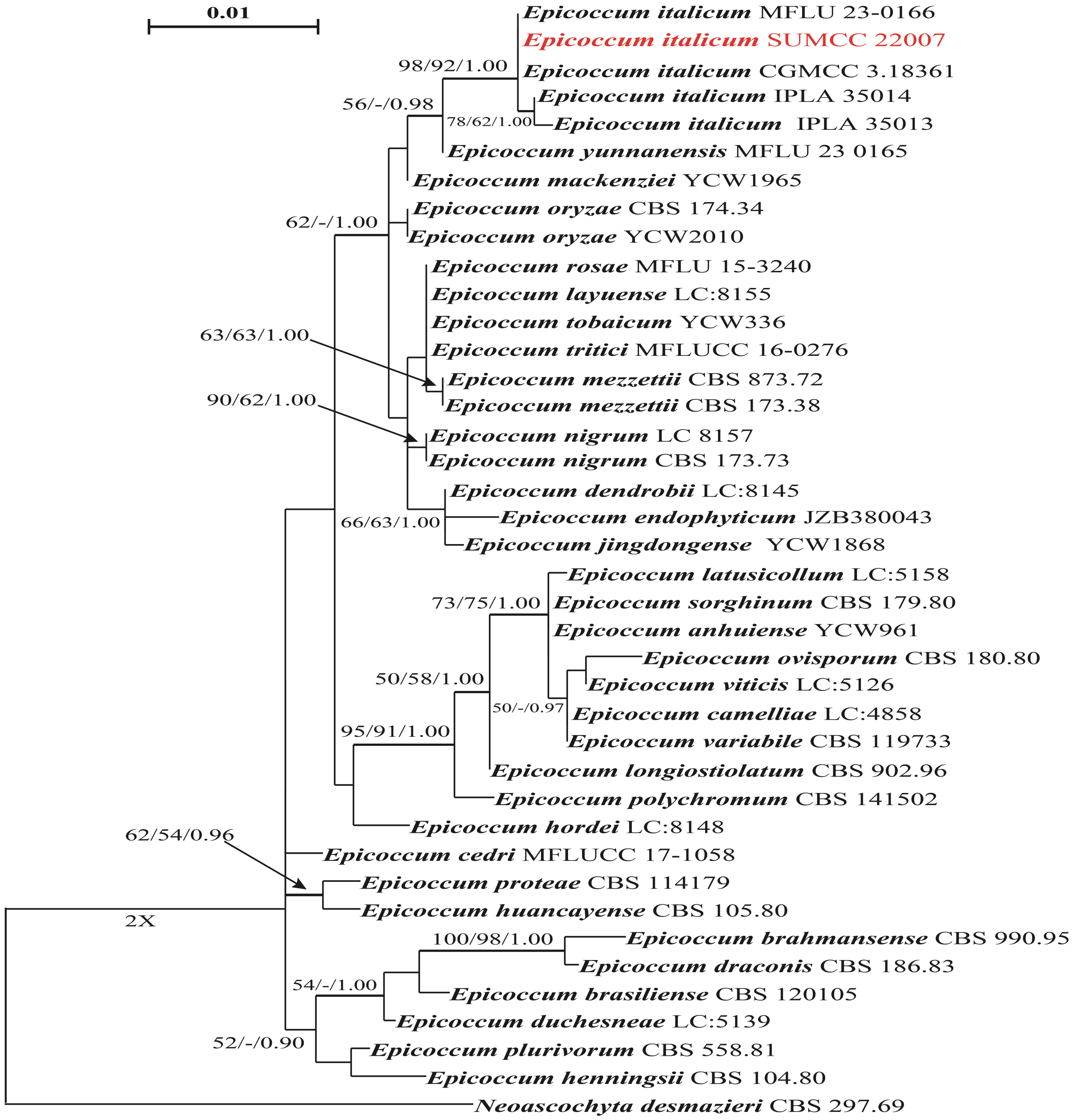

Fig. 1 – Phylogram generated from maximum likelihood analysis (RAxML) of a combined ITS and LSU sequence dataset for *Epicoccum italicum* (SUMCC 22007), along with other species of *Epicoccum*. Bootstrap support on the nodes represents ML, MP ≥ 50 %, and Bayesian pp ≥ 0.90. The newly generated sequences are indicated in red.

Notes: Phylogenetic analysis of combined ITS and LSU sequence data placed our new collection (SUMCC 22007) within *Epicoccum* and clustered with other strains of *E. italicum*, including the ex-type (CGMCC 3.18361). Our collection exhibits a similar colony morphology and conidial structure to those illustrated by Chen et al. (2017) and Tian et al. (2024). The conidial size of our collection

(14–30 µm) is also similar to the ex-type strain (12.5–28 µm) and to the Thailand collection (10−25 µm) (Chen et al., 2017; Tian et al., 2024). Furthermore, in pairwise nucleotide comparisons of our isolate (SUMCC 22007) and the ex-type strain of *E. italicum* (CGMCC 3.18361), no base pair differences were observed in both ITS and LSU rDNA, confirming that they are the same species. Based on morphological characteristics and phylogenetic analysis, we introduce our strain *E. italicum* (SUMCC 22007) as a new host and geographical record on *Zygophyllum coccineum* in Egypt. Based on our phylogenetic analysis, *E. italicum* strains cluster with *E. yunnanensis* and *E. mackenziei*.

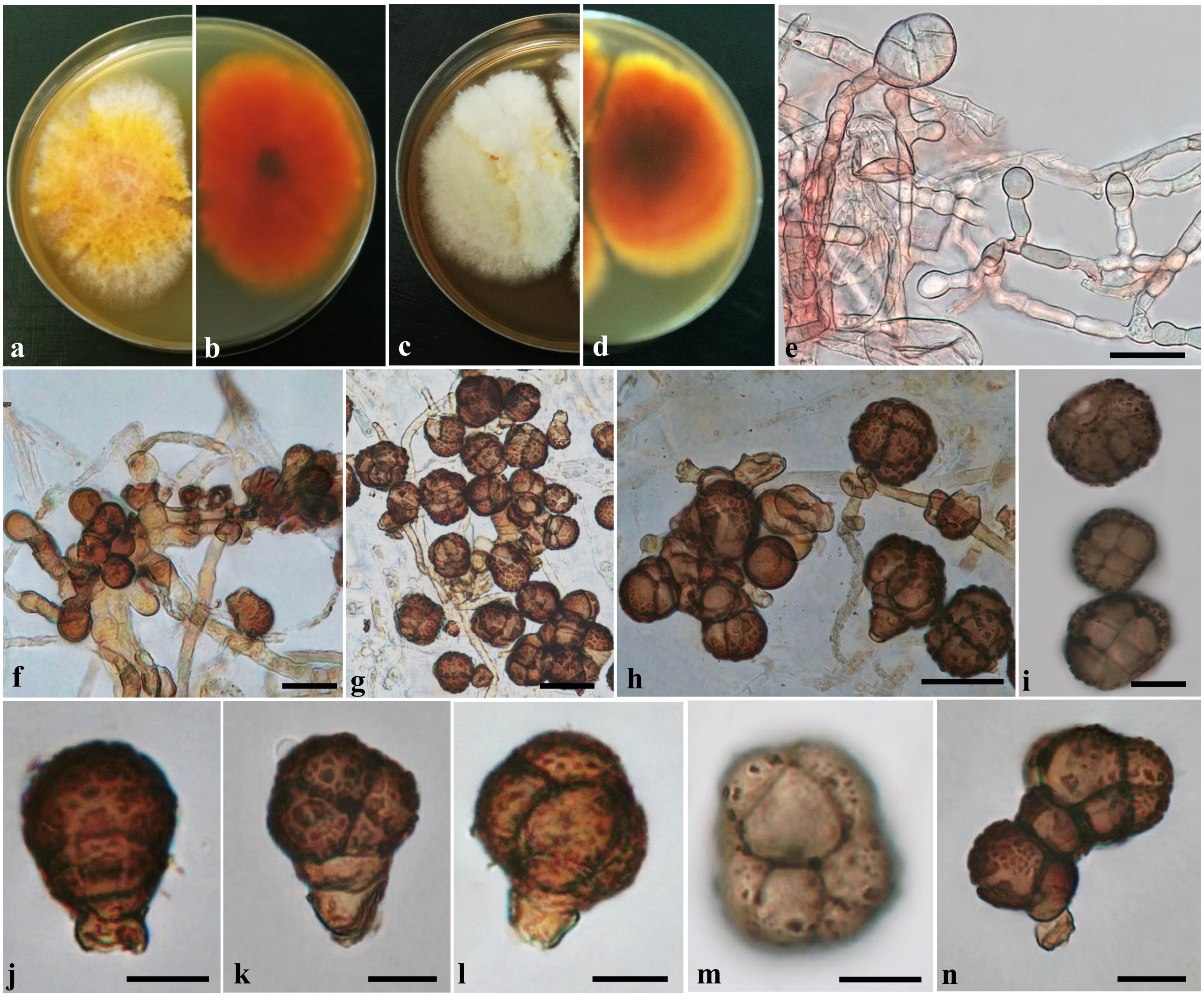

Fig. 2 – *Epicoccum italicum* (SUMCC 22007). (a, b) colony morphology on MEA (front and reverse), (c, d) colony morphology on PDA (front and reverse), (e) different developing stages of the conidia, (f–h) sporodochia, (i–n) conidia showing variation in size and morphology. Scale bars: e–h =20 µm, i–n=10 µm.

Molecular and morphological characteristics of N. gorlenkoana

Nigrospora gorlenkoana Novobr., Nov. sist. Niz. Rast. 9:180 (1972) (Figs. 3–4).
GenBank numbers – ITS: PV270000; LSU: PV270001.

Phylogenetic analysis

The combined ITS and LSU rDNA dataset consisted of 44 fungal strains, of which 42 belong to the genus *Nigrospora* with *Arthrinium obovatum* (LC4940), and *A. sacchari* (MFLU 19-2736) as an outgroup (Table 2). The maximum parsimony dataset consists of 1203 characters (ITS: 403, LSU: 800) after alignment that included: 1047 constant, 41 variable parsimony uninformative, and 115 parsimony-informative characters. The final ML optimization likelihood value of the best RAxML tree was - 3193.461662 (Fig. 3). The matrix had 166 distinct patterns with 31.67% undetermined characters or gaps. Estimated base frequencies were found to be A = 0.243845, C = 0.222189, G = 0.279239, T = 0.254728; substitution rates, AC = 2.924131, AG = 1.933748, AT = 0.978875, CG = 0.930996, CT 10.825504, GT = 1.0. Phylogenetic analysis placed our new strain of *N. gorlenkoana* (SUMCC 22008) within *Nigrospora* and close to the ex-isotype (CBS 480.73), and other strains of *N. gorlenkoana* (JZB 3230001, MFG 70051, MFG 70032, MFG 70044, P13LECIII, and KoRLI046079) with 80% ML and 0.99 PP bootstrap support.

Taxonomy

Endophytic on *A. maurorum* leaves. Sexual morph: undetermined. Asexual morph: growing well on PDA. Mycelium composed of 1.2 to 4 µm diameter, hyalin to pale brown, smooth, septate, and branched hyphae. Conidiophores 15–45 × 3–5 µm (mean = 26.5 × 4.1 µm, n = 11), smooth, pale brown, flexuous or straight, sometimes reduced to conidiogenous cells. Conidiogenous cells 6–11 × 5–10 µm (mean = 8.3 × 7.4 µm, n = 14), monoblastic, discrete, determinate, solitary, pale brown, doliiform to ampulliform. Conidia 11–14 x 14–18 µm (mean = 12.8 × 16.5 µm, n = 46), sparse, globose to sub-globose, aseptate, pale brown to black, smooth, discrete on aerial mycelia, shiny and with an equatorial slit.

Culture characteristics

Colonies on PDA reached 80–90 mm diameter after 7 days at 24 °C, flat, initially white, becoming greyish to black with age, and the reverse white, becoming greyish black, circular-shaped, with aerial, woolly mycelium.

Materials examined: Egypt, Sohag Governorate, Wadi Bir-El-Ain (26°38'27.6"N 31°50'07.9"E), from the healthy leaves of *A. maurorum* (Fabaceae), Feb. 2022, coll. G. G. Faheem, SUMCC 22008.

Known distribution: China, Croatia, Iran, Kazakhstan, Poland**,** Russia, and South Korea.

Notes: Based on molecular phylogenetic analysis, our collection clustered in the same clade with another seven strains of *N. gorlenkoana*, including the ex-isotype (CBS 480.73). Morphologically, our isolate is very similar to the isotype strain of *N. gorlenkoana* (Wang et al., 2017). It was characterized by globose or sub-globose, pale brown to black conidia with an equatorial slit (Wang et al., 2017; This study). Furthermore, no base pair difference in ITS was observed between our strain (SUMCC 22008) and the ex-isotype strain of *N. gorlenkoana* (CBS 480.73), while only 1 bp difference was observed across LSU (852 nucleotides), confirming that they are the same species. Based on morphological characteristics and phylogenetic analysis, we introduce SUMCC 22008 as a new host record of *N. gorlenkoana* from *A. maurorum* and a new geographic record in Egypt.

Table 2 – Taxa used in the phylogenetic analyses of *Nigrospora gorlenkoana*.

Species	**Voucher/Strains**	**GenBank accession no.**		**References**
		ITS	**LSU**	
Arthrinium obovatum	LC4940	KY494696	KY494772	Wang et al. 2018
A. sacchari	MFLU 19-2736	MW114317	MW114396	Tennakoon et al. 2021
Nigrospora aurantiaca	CGMCC 3.18130	KX986064	KX986098	Wang et al. 2017
N. bambusae	CGMCC 3.18327	KY385307	KY806271	Wang et al. 2017
N. brasiliensis	CMM 1214	KY569629		Crous et al. 2019
N. camelliae-sinensis	CGMCC 3.18125	KX985986	KX986103	Wang et al. 2017
N. chinensis	CGMCC 3.18127	KX986021	KX986107	Wang et al. 2017
N. cooperae	BRIP 72531c	OP035049		Tan et al. 2022
N. covidalis	CGMCC 3.20538	OK335209		Chen et al. 2022
N. endophytica	ARM687	OM265226		de Queiroz Brito et al. 2023
N. falsivesicularis	CGMCC 3.19678	MN215778		Raza et al. 2019
N. globosa	CGMCC 3.19633	MK329121	MK329027	Zhang et al. 2021
N. globospora	CGMCC3.20539	OK335211		Chen et al. 2022
N. gorlenkoana	**SUMCC 22008**	**PV270000**	**PV270001**	**This study**
N. gorlenkoana	CBS 480.73	KX986048	KX986109	Wang et al. 2017
N. gorlenkoana	MFG 70051	OK563250		Orina et al. 2023
N. gorlenkoana	MFG 70032	OK563238		Orina et al. 2023
N. gorlenkoana	JZB 3230001	MN495939		Hao et al. 2020
N. gorlenkoana	MFG 70044	OK563246		Orina et al. 2023
N. gorlenkoana	P13LECIII	OP999642		Petrović et al. 2023
N. gorlenkoana	KoRLI046079	MN341456		Oh et al. 2020
N. guangdongensis	CFCC 53917	MT017509		Tian et al. 2020
N. guilinensis	CGMCC 3.18124	KX985983	KX986113	Wang et al. 2017
N. hainanensis	CGMCC 3.18129	KX986091	KX986112	Wang et al. 2017
N. humicola	CFCC 56884	ON555686		Zhang et al. 2024
N. lacticolonia	CGMCC 3.18123	KX985978	KX986105	Wang et al. 2017
N. macarangae	MFLUCC 19-0141	MW114318	MW114397	Tennakoon et al. 2021
N. magnolia	MFLUCC 19-0112	MW285092		de Silva et al. 2021
N. manihoticola	ARM645	OM265224		de Queiroz Brito et al. 2023
N. musae	LC6385	KX986042	KY806260	Wang et al. 2017
N. musae	CBS 319.34	KX986076	MH867051	Wang et al. 2017
N. oryzae	LC3695	KX985988	KY806252	Wang et al. 2017
N. oryzae	LC6957	KX986053	KY806264	Wang et al. 2017
N. osmanthi	CGMCC 3.18126	KX986010	KX986106	Wang et al. 2017
N. pernambucoensis	ARM974	OM265234		de Queiroz Brito et al. 2023
N. philosophiae-doctoris	CGMCC 3.20540	OK335213		Chen et al. 2022
N. pyriformis	CGMCC 3.18122	KX985940	KX986100	Wang et al. 2017
N. rubi	CGMCC 3.18326	KX985948	KX986102	Wang et al. 2017
N. sacchari-officinarum	CGMCC 3.19335	MN215791		Raza et al. 2019
N. singularis	CGMCC 3.19334	MN215793		Raza et al. 2019
N. sphaerica	LC4264	KX985993	KY806253	Wang et al. 2017
N. vesicularifera	CGMCC 3.19333	MN215812	ON860837	Raza et al. 2019
N. vesicularis	CGMCC 3.18128	KX986088	KX986099	Wang et al. 2017
N. zimmermanii	CBS 290.62	KY385309	KY806276	Wang et al. 2017

*Newly generated sequences are indicated in bold.

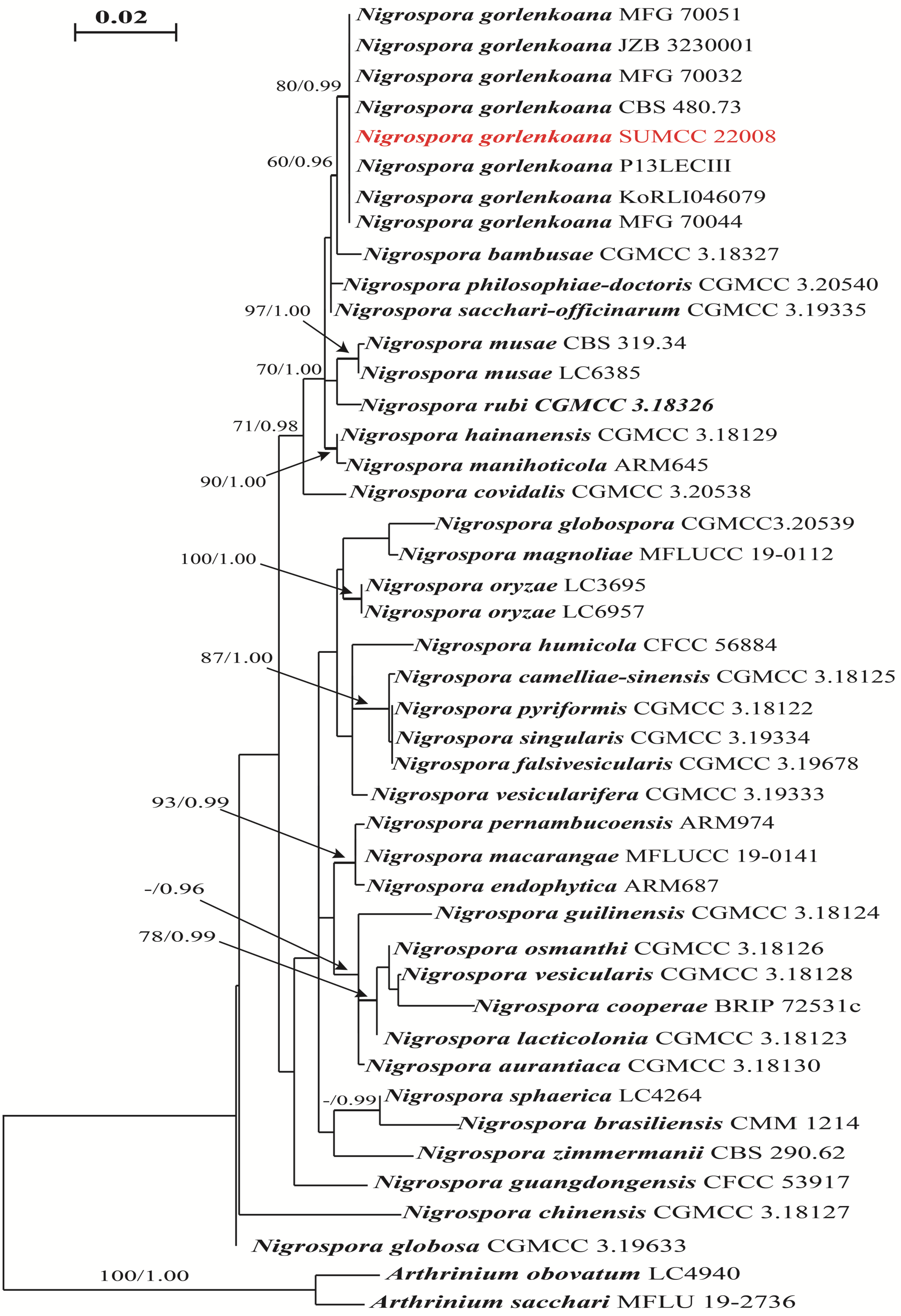

Fig. 3 – Phylogram generated from maximum likelihood analysis (RAxML) of a combined ITS and LSU sequence dataset for *Nigrospora gorlenkoana* (SUMCC 22008), along with other species of *Nigrospora*. Bootstrap support on the nodes represents ML ≥ 60 % and Bayesian pp ≥ 0.95. The newly generated sequences are in red.

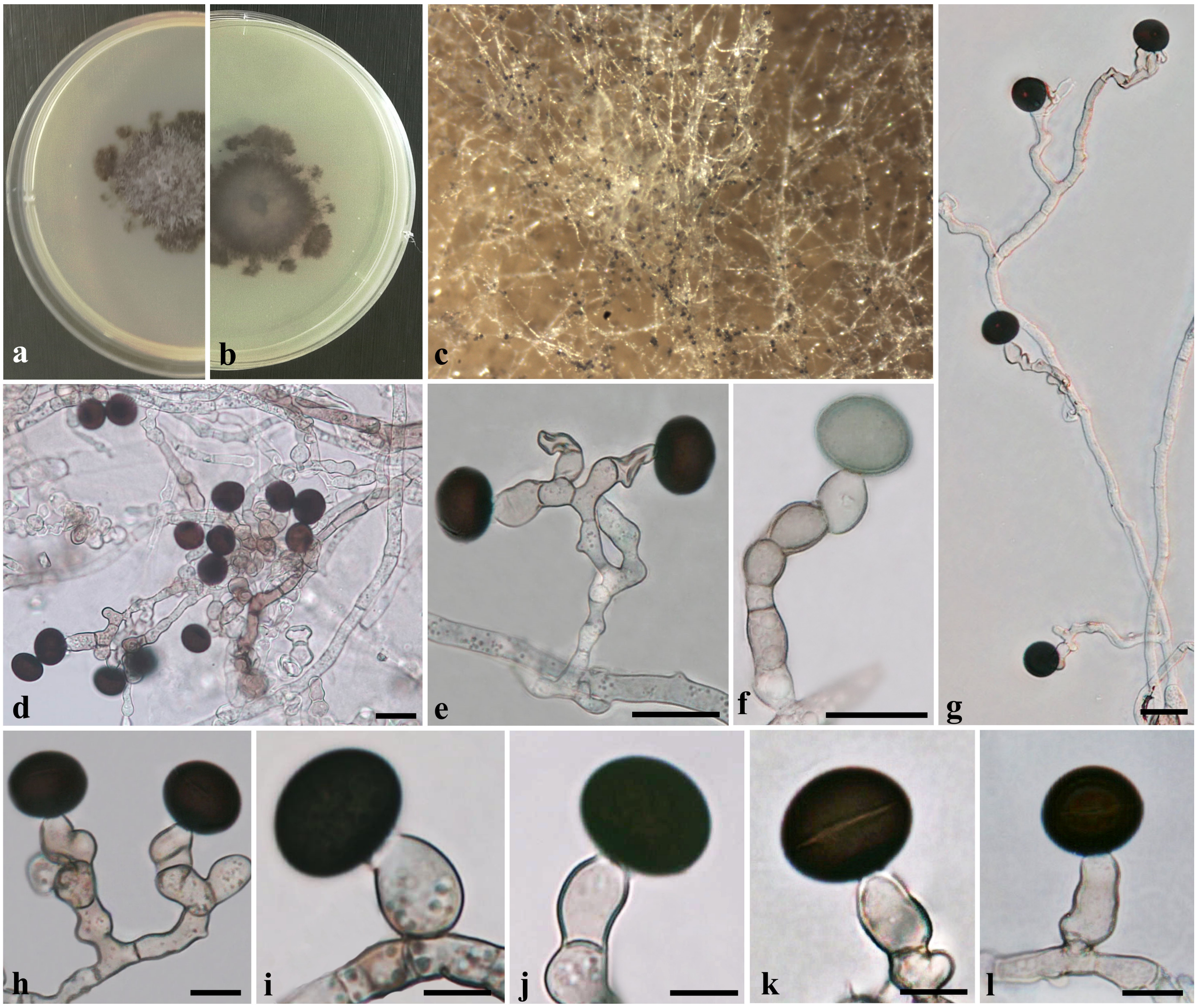

Fig. 4 – *Nigrospora gorlenkoana* (SUMCC 22008). (a–b) colony morphology on PDA (front and reverse), (c) conidia on aerial mycelia grown on PDA, (d–h) conidiophores and conidia, (i–l) conidiogenous cells giving rise to conidia. Scale bars: d–g=20 μm, h–l=10 μm.

Discussion

During the current study of endophytic fungi from Wadi Bir-El-Ain, Sohag governorate, Egypt, a total of 439 isolates were obtained from 918 tissue segments of 17 medicinal plant species. During this study, 16 genera, 30 fungal species, and two sterile mycelial forms were recovered (unpublished data). Medicinal plants are an invaluable resource for endophyte bioprospecting (Wiyakrutta et al., 2004; Rana et al., 2020; El deeb et al., 2025a,b). In this article, we describe and illustrate two new host records, *Epicoccum italicum* from healthy roots of *Zygophyllum coccineum*, and *Nigrospora gorlenkoana* from healthy leaves of *Alhagi maurorum* in Egypt. This represents the first report of the two species in Egypt and Africa.

Epicoccum italicum was first described from seedlings of *Acca sellowiana* in Italy (Chen et al., 2017). It was also reported in lake sediment in the Antarctic Peninsula (Ogaki et al., 2020), on *Quercus robur* in Poland (Rivera-Vega et al., 2022), on the leaf sheath of *Arundo donax* in the USA (Bon et al., 2023), and in the cheesemaking environment in Spain (Rodríguez et al., 2023).

Additionally, *E. italicum* was reported from healthy leaves of *Ananas comosus* in Thailand (Tian et al., 2024) and from tea plant leaves in China (Wang et al., 2024). This is the first report of *E. italicum* from *Zygophyllum coccineum*. *Epicoccum* species are cosmopolitan and found in diverse hosts and habitats. They frequently exhibit an endophytic lifestyle (Braga et al., 2018) on various plant parts in aquatic and terrestrial environments (Voronin et al., 2021; Barreto et al., 2022).

Epicoccum nigrum has been documented as an endophytic fungus in Egypt by several studies (Abdel-Hafez et al., 2016; Ali et al., 2024; Elkhateeb et al., 2024; Elnaggar et al., 2024; Mossa et al., 2024). In the present study, *E. italicum* was reported for the first time from *Zygophyllum coccineum*. *Zygophyllum coccineum* is a perennial herb belonging to the family Zygophyllaceae that thrives in saline coastal environments and is commonly found in arid and semi-arid regions (El-Afify et al., 2024). This species is well known for its medicinal properties, including anti-diabetic, antioxidant, anti-inflammatory, and antimicrobial activities, and has been traditionally used in managing hypertension (Mohamed et al., 2020). Endophytic fungi previously isolated from *Z. coccineum* include *Aspergillus terreus* Thom, *A. flavus* Link, *A. fumigatus* Fresen**.**, and *A. aculeatus* Iizuka from the Alexandria desert, Egypt (Soror et al., 2024), as well as *Cladosporium omanense* Halo, Maharachch., Al-Yahyai & Al-Sadi and *Talaromyces variabilis* (Sopp) Samson, N. Yilmaz, Frisvad & Seifert from desert regions in Oman (Halo et al., 2019, 2020).

Nigrospora gorlenkoana was first isolated from the leaf and fruit of *Vitis vinifera* from the Alma-Ata region, Kazakhstan (Novobranova, 1972; Wang et al., 2017). It was also recorded on living leaves of *Cirsium setosum* in China (Hao et al., 2020). Oh et al. (2020) isolated *N. gorlenkoana* as endolichenic from *Parmotrema reticulatum* in Jeju Island, South Korea. Aghyl et al. (2022) reported three species of genus *Nigrospora*, viz. *N. gorlenkoana*, *N. oryzae* (Berk. & Broome) Petch, and *N. osmanthi* Mei Wang & L. Cai in association with insects in Iran. Orina et al. (2023) studied the physiological and biochemical characteristics of fourteen *N. gorlenkoana* strains isolated from Barley, maize, oats, rape, and wheat grains in Russia. Petrović et al. (2023) reported *N. gorlenkoana*, *N. osmanthi*, and *N. philosophiae-doctoris* Raza, Qian Chen & L. Cai causing leaf spot on olive trees. This study provides the first record of this fungus from *A. maurorum*. *Alhagi maurorum* (Fabaceae) is a perennial thorny shrub, native to North Africa, the Middle East, and southeast Europe (Hammouda et al., 2005). Several phytochemical components, including alkaloids, flavonoids, glycosides, saponins, tannins, and steroids, have been found in this plant (Al-Snafi et al., 2019). Hegazy et al. (2019) isolated four endophytic fungi; *Aspergillus terreus*, *Penicillium crustosum* Thom, *P. chrysogenum* Thom, and *Alternaria alternata* (Fr.) Keissl., from *A. maurorum* samples collected from Sohag governorate, Egypt.

Three species of genus *Nigrospora* have been previously reported from Egypt, namely *N. oryzae* (Abdel-Hafez et al., 1977; Moubasher and Abdel-Hafez, 1978; Mohammed and Badawy, 2020; Ghanem et al., 2025; Salem et al., 2025), *N. panici* (El-Maghraby et al., 2014), and *N. sphaerica* (Sacc.) E.W. Mason (El-Morsy, 1999; Abou El-Kassem et al., 2019). Hence, the species *N. gorlenkoana* is a new record for Egypt.

Reports of *Nigrospora* species as endophytes have emerged in recent years. Wang et al. (2017) discovered *Nigrospora vesicularis* Mei Wang & L. Cai as an endophyte from an unknown host plant. Chua et al. (2022) reported *N. oryzae* and *N. pyriformis* as endophytic species from *Cymbidium* and *Dendrobium* orchids. De Queiroz Brito et al. (2023) introduced three new species, namely *Nigrospora endophytica* A.C.Q. Brito & A.R. Machado, *N. manihoticola* A.C.Q. Brito & A.R. Machado, and *N.*

pernambucoensis A.C.Q. Brito & A.R. Machado which were isolated as endophytes from the stem of *Manihot esculenta*. Wang et al. (2024) contributed a new endophytic species, *N. coryli* Jing Wang bis & Y.H. Yang from the stem of *Corylus heterophylla* in Guizhou, China. Five endophytic *Nigrospora* species, namely *N. lacticolonia* Mei Wang & L. Cai, *N. oryzae*, *N. osmanthi*, *N. pernambucoensis*, and a new species *N. shadeganensis* Safi, M. Mehrabi-Koushki & Arzanlou, associated with shrubs and grasses were reported in Iran (Safi et al., 2024).

The endophytic fungi associated with medicinal plants in arid environments likely play a key role in enhancing plant tolerance to environmental stress (Zuo et al., 2021). These fungi can facilitate host adaptation through multiple mechanisms, including improving water and nutrient uptake and modulating stress-responsive metabolites such as antioxidants (Aleynova et al., 2023). Additionally, endophytes can secrete bioactive compounds that mitigate oxidative, heat, and drought stress and protect the plant from disease through antimicrobial compounds, thereby enhancing survival under harsh, water-limited conditions (Wang et al., 2025). Such mutualistic interactions not only support the persistence of medicinal plants in arid habitats but may also contribute to the synthesis of secondary metabolites with therapeutic potential.

The identification of *E. italicum* and *N. gorlenkoana* as endophytes of *Z. coccineum* and *A. maurorum*, respectively, expands the known fungal diversity in Egypt and Africa. These findings underscore the need for further taxonomic, ecological, and phylogenetic studies to clarify their distribution and functional roles. Furthermore, given the growing biotechnological interest in endophytic fungi, exploring their secondary metabolites and bioactive properties is crucial. Their presence in arid-adapted medicinal plants suggests potential roles in stress tolerance and pathogen resistance, which warrants deeper investigation. Integrating molecular, biochemical, and ecological approaches will be key to unlocking their full potential for sustainable applications.

Conclusion

This study expands the knowledge of endophytic fungal diversity in Egypt and Africa by reporting *Epicoccum italicum* from *Zygophyllum coccineum* and *N. gorlenkoana* from *Alhagi maurorum*, establishing new host and geographic records. The findings highlight the broad ecological distribution of *Epicoccum italicum*, previously recorded in diverse environments, and reinforce the growing recognition of *N. gorlenkoana* as an endophyte. Morphological comparisons with previously reported strains confirmed species identity, while phylogenetic analyses based on combined ITS and LSU sequences placed the Egyptian collections within their respective clades. Their occurrence in arid-land medicinal plants underscores the importance of desert ecosystems as reservoirs of fungal diversity that may enhance host resilience under environmental stress. These findings offer important taxonomic and ecological insights into the distribution and adaptation strategies of these fungi, while also highlighting their potential biotechnological relevance. Future research should explore their bioactive potential and functional roles in plant health, stress tolerance, and symbiotic interactions.

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