MYCOBIOLOGY
2024, VOL. 52, NO. 6, 477–485
https://doi.org/10.1080/12298093.2024.2435096

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Four Unrecorded Species of Endophytic *Diaporthe* (Sordariomycetes) in Korea

Jae-Eui Cha[a], Ju-Kyeong Eo[b] and Ahn-Heum Eom[a]

[a]Department of Biology Education, Korea National University of Education, Cheongju, Republic of Korea; [b]Ecological Technology Research Team, Division of Ecological Applications Research, National Institute of Ecology, Seocheon, Republic of Korea

ABSTRACT

Endophytic fungi associated with four plant species in Korea were isolated and characterized using morphological and molecular analyses. Phylogenetic analyses of the internal transcribed spacer (ITS) region, β-tubulin (TUB) gene, and translation elongation factor 1-α (EF1-α) gene revealed four previously unrecorded species of *Diaporthe* in Korea: *D. caryae*, *D. phoenicicola*, *D. stewartii*, and *D. unshiuensis*. Detailed descriptions of colony morphology and conidial characteristics are provided for each species. This study expands our knowledge of the diversity and distribution of endophytic *Diaporthe* in Korea and highlights the importance of these fungi in the ecosystem.

ARTICLE HISTORY
Received 13 November 2024
Revised 22 November 2024
Accepted 22 November 2024

KEYWORDS
Diaporthe; endophytic fungi; fungal biodiversity

1. Introduction

Diaporthe Nitschke (1870) is a genus within the class Sordariomycetes, containing numerous species with ecological roles ranging from pathogens to endophytes. It has a wide host range and is distributed global [1]. As of 2024, over 1,200 taxa under *Diaporthe* and approximately 1000 taxa under *Phomopsis* are registered in Index Fungorum (www.indexfungorum.org) and MycoBank (www.mycobank.org). *Diaporthe* is characterized by ostiolate, black conidiomata, containing elongate, cylindrical phialides that may produce two types of hyaline, non-septate conidia, namely alpha and beta. The alpha conidia are aseptate, generally hyaline, fusiform, and the beta conidia are also aseptate, hyaline, but are filiform, straight or more often hamate [2].

The classification of *Diaporthe* has increasingly relied on multi-gene phylogenetic methods based on the internal transcribed spacer (ITS) region and partial sequences of the translation elongation factor 1-α (EF1-α), β-tubulin (TUB), histone H3 (HIS3), and calmodulin (CAL) genes, reflecting the link between morphological features and host adaptation. Given the limitations of morphological variation in distinguishing many fungal species, an integrated approach combining genetic data with morphological characteristics is necessary, a perspective often described as reverse taxonomy [3,4].

Endophytic fungi reside within plant tissues without causing visible symptoms, representing a polyphyletic group with high biodiversity [5]. Traditional studies on endophytic fungal diversity involved isolation and culturing of endophytes from plant tissues, followed by morphological characterization. However, advancements in molecular biology have introduced DNA sequence-based analysis methods that do not require culturing, leading to the estimation that over a million endophytic species may coexist with plants [6]. Nonetheless, traditional methods of isolating and culturing endophytes continue to be important, as secondary metabolites produced by endophytes hold potential for the development of new bioactive compounds, such as antibiotics, antioxidants, and anticancer agents [7,8]. Endophytic fungal diversity research offers immense potential applications. For instance, as many as 90 endophytic species have been found on a single tropical plant leaf, and endophytes are known to occupy diverse ecological niches, even within the same region and across taxonomically unrelated host plants. Therefore, broad exploration of endophytic diversity is necessary to uncover this hidden biodiversity [9,10].

During the course of isolating and characterizing endophytic fungi from various host plants, we discovered four previously unrecorded species belonging to the genus *Diaporthe*. Herein, we provide

CONTACT Ahn-Heum Eom eomah@knue.ac.kr

Table 1. Information on host plant collection.

Collection date	Species	Location
April 22, 2021	*Quercus dentata* Thunb.	Gyeongju, Gyeongsangbuk-do, Korea, 35°41′17.8"N 129°20′60.0"E
September 2, 2021	*Callicarpa japonica* Thunb.	Gyeongju, Gyeongsangbuk-do, Korea, 35°41′16.8"N 129°20′45.7"E
	Phytolacca americana L.	Pohang, Gyeongsangbuk-do, Korea, 35°56′34.3"N 129°30′18.0"E
July 13, 2023	*Trachelospermum asiaticum* (Siebold & Zucc.) Nakai	Jeungdo Island, Sinan, Jeollanam-do, Korea, 34°59′11.9"N 126°08′07.9"E

detailed morphological and molecular descriptions of these novel endophytic species, contributing to a better understanding of fungal diversity in Korea.

2. Materials and methods

2.1. Sample collection

Plant tissues were collected from various locations across Korea for the isolation of endophytic fungi (Table 1). The samples were collected from the 1-year-old branches, and plant species were chosen as a host plant due to their relatively high frequency of occurrence in the area. From the perspective of plant individual, healthy plants, free from any visible damage or disease symptoms, were specifically selected. The leaf samples were collected from 1-year-old branches, and host plants with relatively high occurrence rates in the region were selected, ranging from forested to island areas. Healthy plants with no damage or visible disease symptoms were chosen for sampling. For herbaceous plants, leaves and stems were collected, while leaves and twigs were gathered from woody plants. The collected plant tissues were placed in sterile polyethylene bags and transported to the laboratory within 24 h for further processing and endophyte isolation.

2.2. Fungal isolation and culturing

The plant tissues were washed under running tap water to remove surface debris and subsequently surface-sterilized by immersion in 1% sodium hypochlorite (NaClO) solution for 1 min, followed by a rinse in 70% ethanol for 2 min. The sterilized tissues were then cut into approximately 1.5 cm pieces and placed on potato dextrose agar (PDA; Difco Lab., Detroit, MI). The plates were incubated in the dark at 25 °C, and any hyphal growth emerging from the plant tissues was transferred to fresh media to obtain pure fungal cultures.

2.3. Morphological characterization

Colony morphology of the isolated fungi was observed by culturing them on PDA and malt extract agar (MEA; Kisan Bio, Seoul, Korea) at 25 °C in the dark for 7 d. To induce the formation of conidiomata and conidia, the isolates were cultured on pine needle agar (PNA; 2% water agar with sterilized pine needles) under a 12-h light/12-h dark cycle for 3 weeks [11]. The conidiomata were examined using a stereomicroscope (Olympus SZX7, Olympus, Tokyo, Japan), and conidia were observed using a light microscope (Axio Imager A2, Carl Zeiss, Oberkochen, Germany) at 1000× magnification. Conidial structures were mounted in lactic acid and observed at 1000× magnification. Measurements of conidia were taken using Zeiss ZEN 3.8 software and reported as minimum and maximum values, along with the mean and standard deviation. All isolated strains were deposited in the National Institute of Biological Resources (NIBR).

2.4. DNA extraction, amplification, and sequencing

Genomic DNA was extracted from mycelia using the HiGene Genomic DNA Prep Kit (BioFACT, Daejeon, Korea) according to the manufacturer's protocol. For phylogenetic analysis, three regions were targeted: the ITS region, the TUB gene, and the EF1-α gene. The following primer sets were used for PCR amplification: ITS1F/ITS4 for ITS [12,13], Bt2a/Bt2b for TUB [14], and EF1-728F/EF2 for EF1-α [15,16]. PCR reactions were performed under the following conditions: an initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 20 s, annealing at 50 °C (ITS), 52 °C (EF1-α), or 55 °C (TUB) for 40 s, and extension at 72 °C for 1 min. A final extension step was conducted at 72 °C for 5 min. The amplified DNA fragments were visualized on a 1.5% agarose gel to confirm product size. Sequencing of the PCR products was performed by SolGent Co., Ltd. (Daejeon, Korea) using Sanger sequencing.

2.5. Phylogenetic analyses

Sequences obtained from the fungal isolates were compared with those in the GenBank database using the Basic Local Alignment Search Tool (BLAST) provided by the National Center for Biotechnology Information (NCBI). Reference sequences from closely related species were retrieved from GenBank for phylogenetic analysis. Individual alignments for ITS, TUB, and EF1-α regions were performed using MEGA

version 7 software [17], and the aligned sequences were concatenated for a comprehensive phylogenetic analysis. The maximum-likelihood method was employed to construct phylogenetic trees, with the Kimura 2-parameter model selected as the substitution model. Bootstrap support values were calculated based on 1000 replicates. In addition, Bayesian inference was conducted using BEAST 10.5.0 software to estimate posterior probabilities [18]. The sequences of the unrecorded species were deposited in GenBank for future reference.

3. Results and discussion

3.1. Phylogenetic analysis

The sequenced regions from the isolates were approximately 560 bp for ITS, 360 bp for TUB, and 310 bp for EF1-α. BLAST comparison of KNUE 21E419 with reference sequences of *Diaporthe caryae* showed sequence similarity of 98.92% for ITS (OP218121), 99.44% for TUB (ON221770), and 99.36% for EF1-α (ON049538), and it was grouped together in the phylogenetic tree, confirming its identification as *D. caryae*. Similarly, KNUE 21E019 showed sequence similarity of 99.28% for ITS (ON035557), 100% for TUB (ON221762), and 99.67% for EF1-α (PQ296176) when compared to *Diaporthe phoenicicola* reference sequences, and it was also grouped together in the phylogenetic tree, confirming its identification as *D. phoenicicola*. KNUE 21E558 was identified as *Diaporthe stewartii* based on sequence similarity of 99.63% for ITS (NR111417), 97.27% for TUB (PP056743), and 99.68% for EF1-α (GQ250324) compared to reference sequences, and it was similarly grouped in the phylogenetic analysis. Finally, KNUE 23P353 was identified as *Diaporthe unshiuensis* with sequence similarities of 99.46% for ITS (OP218165), 100% for TUB (ON221808), and 100% for EF1-α (KJ490466), and it clustered with *D. unshiuensis* in the phylogenetic tree (Figure 1).

3.2. Taxonomy

Diaporthe caryae C.M. Tian & Q. Yang, MycoKeys 39:124 (2018) [MB#824706]

Description. On MEA, the colony covered the entire plate in 7 d at 25 °C, light grey in the center and white at the margin, with felty mycelium, and light beige on the reverse. On PDA, the colony covered the entire plate in 7 d at 25 °C, with white, cottony mycelium, and light beige on the reverse. Conidiomata were pycnidial, scattered on pine needle, black, subglobose or oval, and solid, occasionally with beige mucus. Alpha conidia were ellipsoidal, aseptate, smooth-walled, obtuse at both ends, measuring 4.65–7.20 × 2.41–3.15 µm ($\bar{x}$ ± SD = 6.14 ± 0.64 × 2.74 ± 0.20 µm). Beta conidia were not observed (Figure 2, Table 2).

Specimen examined. Gyeongju, Gyeongsangbuk-do, Korea, 35°41′16.8″N 129°20′45.7″E, September 2, 2021, *Diaporthe caryae*, isolated from the twig of *Callicarpa japonica* Thunb., strain KNUE 21E419, NIBRFGC000509068, GenBank No. PQ533147 (ITS), PQ539961 (TUB), and PQ539959 (EF-1α).

Notes: *D. caryae* was first described in 2018 from dieback lesions on *Carya illinoensis* in China, and it was reported as a new species based on ITS, TUB, and EF-1α sequences [11]. The isolate KNUE 21E419 from this study clustered with *D. caryae* in phylogenetic analysis and were morphologically similar, with alpha conidia that were ellipsoidal, obtuse at both ends and similar in size. *D. caryae* has been reported as the causal agent of pear canker in 2020 and stem blight on chili peppers in 2023 [19,20]. It has also been isolated from citrus lesions, although its pathogenicity in citrus has not been confirmed [21]. This is the first report of *D. caryae* as an endophyte.

Diaporthe phoenicicola (Traverso & Spessa) Udayanga, Crous & K.D. Hyde, fungal diversity 56:166 (2012) [MB#800699]

Description. On MEA, the colony covered the entire plate in 7 d at 25 °C, with white, felty mycelium and light beige on the reverse. On PDA, the colonies covered the entire plate in 7 d at 25 °C, white and felty in the center with a flat margin, and the reverse was beige and grey at the center with a beige margin. Conidiomata were pycnidial, scattered on pine needle, black, covered with white hyphae, oval or irregular, and solid. Alpha conidia were ellipsoidal or clavate, aseptate, smooth-walled, measuring 6.98–9.10 × 2.37–3.15 µm ($\bar{x}$ ± SD = 8.06 ± 0.50 × 2.71 ± 0.22 µm). Beta conidia were not observed (Figure 3, Table 3).

Specimen examined. Gyeongju, Gyeongsangbuk-do, Korea, 35°41′17.8″N 129°20′60.0″E, April 22, 2021, *Diaporthe phoenicicola*, isolated from the twig of *Quercus dentata* Thunb., strain KNUE 21E019, NIBRFGC000509075, GenBank No. PQ533146 (ITS), PQ539963 (TUB), and PQ539957 (EF-1α).

Notes: *D. phoenicicola* was first reported in 2012 as a combination of *Phomopsis phoenicicola* and *Subramanella arecae* through phylogenetic analysis of ITS, TUB, EF-1α, and CAL regions [22,23]. The morphological characteristics of KNUE 21E019 were found to be similar to the ex-isotype CBS

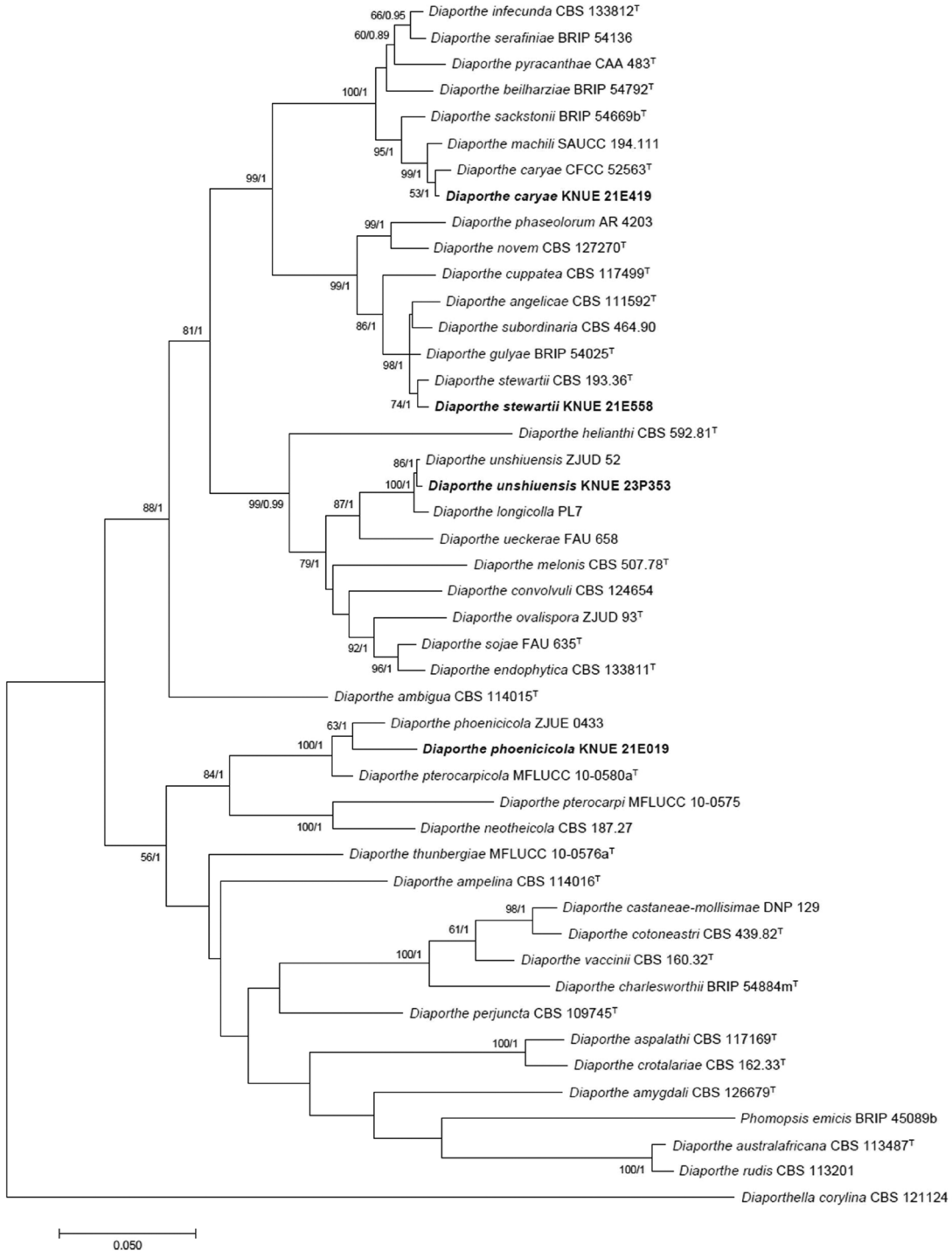

Figure 1. Maximum-likelihood phylogenetic tree based on concatenated sequences of internal transcribed spacer (ITS), β-tubulin (TUB) and translation elongation factor-1-α (EF1-α) regions. *Diaporthella corylina* was used as an outgroup. The numbers on the nodes represent maximum likelihood bootstrap values greater than 50% (left, 1000 replicates) and Bayesian posterior probability greater than 0.70 (right). Strains described in this study are in bold. T indicates ex-type culture.

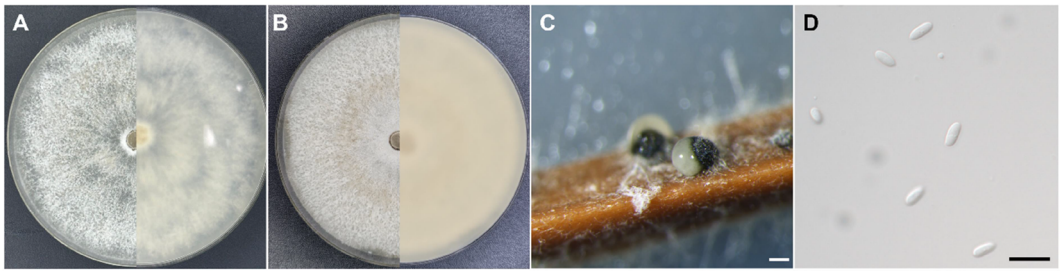

Figure 2. Morphology of *Diaporthe caryae* KNUE 21E419. (A) Colony on malt extract agar (MEA), left side is front side and right side is reverse side after 7 d at 25 °C, (B) Colony on potato dextrose agar (PDA), left side is front side and right side is reverse side after 7 d at 25 °C, (C) Conidiomata, (D) Alpha conidia. Scale bars: C = 200 µm, D = 10 µm.

Table 2. Morphological characteristics of strains KNUE 21E419 and CFCC 52563 of *Diaporthe caryae*.

Characteristics	*Diaporthe caryae* KNUE 21E419	*Diaporthe caryae* CFCC 52563 [10]
Colony	MEA, PDA, dark, 25 °C, 7 d MEA: covering the entire plate, light grey in the center, white at the margin, felty mycelium, reverse light beige. PDA: covering the entire plate, white, cottony mycelium, reverse light beige.	PDA, 25 °C, dark, 10 d First flat with white felty mycelium, becoming black in the center and black at the marginal area with age.
Conidiomata	Pycnidial, scattered on pine needle, black, subglobose or oval, solid, occasionally with beige mucus.	Pycnidial, immersed in bark, scattered, slightly erumpent through the bark surface, nearly flat, discoid, with a solitary undivided locule.
Conidia	Alpha conidia: ellipsoidal or fusirom, aseptate, smooth-walled, hyaline, 4.65–7.20 × 2.41–3.15 µm ($\bar{x}$ ± SD = 6.14 ± 0.64 × 2.74 ± 0.20 µm). Beta conidia: not observed.	Alpha conidia: Hyaline, aseptate, ellipsoidal of fusiform, eguttulate, obtuse at both ends, 7–8.5 × 2.1–2.5 µm ($\bar{x}$ = 8 × 2.3 µm). Beta conidia: Hyaline, aseptate, filiform, straight or hamate, eguttulate, base subtruncate, tapering toward on apex, 15.5–34 × 1.1–1.4 µm ($\bar{x}$ = 27.5 × 1.2 µm).

MEA: malt extract agar, PDA: potato dextrose agar

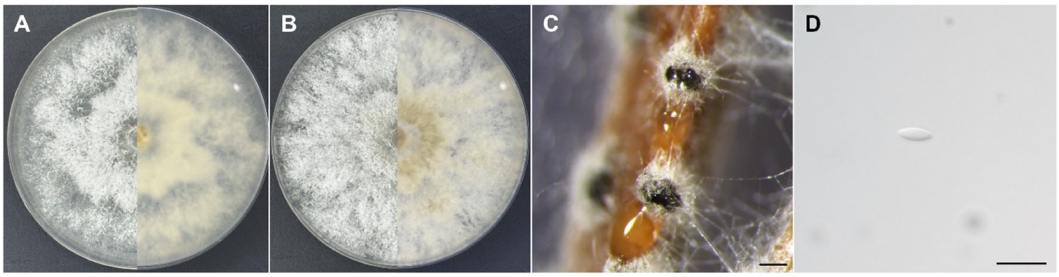

Figure 3. Morphology of *Diaporthe phoenicicola* KNUE 21E019. (A) Colony on malt extract agar (MEA), left side is front side and right side is reverse side after 7 d at 25 °C, (B) Colony on potato dextrose agar (PDA), left side is front side and right side is reverse side after 7 d at 25 °C, (C) Conidiomata, (D) Alpha conidia. Scale bars: C = 200 µm, D = 10 µm.

Table 3. Morphological characteristics of strains KNUE 21E019 and CBS H-7808 of *Diaporthe phoenicicola*.

Characteristics	*Diaporthe phoenicicola* KNUE 21E019	*Diaporthe phoenicicola* CBS H-7808 [22]
Colony	PDA, MEA, 25 °C, 7 d MEA: covering the entire plate, white, felty mycelium, reverse light beige. PDA: covering the entire plate, white, felty mycelium in the center and flat at the edge, reverse beige and grey in the center, beige at the margin.	Not observed.
Conidiomata	Pycnidial, scattered on pine needle, black, covered white hyphae, oval or irregular, solid.	Pycnidial, formed along the cortex, sometimes lobed at the bottom, sometimes coalescing.
Conidia	Alpha conidia: Ellipsoidal or clavate, aseptate, smooth-walled, hyaline, 6.98–9.10 × 2.37–3.15 µm. ($\bar{x}$ ± SD = 8.06 ± 0.50 × 2.71 ± 0.22 µm). Beta conidia: not observed.	Alpha conidia: Hyaline, unicellular, elliptic, 7.2–9.6 × 2.4 µm. Beta conidia: Slightly curved, needle-shaped, hyaline, unicellular, 14.4–24 × 1.2 µm.

MEA: malt extract agar, PDA: potato dextrose agar

H-7808, with alpha conidia that were ellipsoidal and comparable in size. Since its description, *D. phoenicicola* has been reported as the causal agent of leaf spot on *Vaccinium virgatum* in 2023 and leaf brown spot on *Pachira glabra* in 2024 [24,25]. This is the first report of *D. phoenicicola* as an endophyte.

Diaporthe stewartii A.L. Harrison, Mycologia 27: 525 (1935) [MB#278875]

Description. On MEA and PDA, the colony covered the entire plate, with white aerial mycelium, raised and cottony in the center, and flat at the margin, with light beige on the reverse. Conidiomata were pycnidial, scattered on pine needle, black, covered with white hyphae, oval or irregular, and solid. Alpha conidia were ellipsoidal or fusiform, aseptate, smooth-walled, hyaline, measuring 7.52–10.30 × 2.85–3.94 µm ($\bar{x}$ ± SD = 9.32 ± 0.71 × 3.37 ± 0.33 µm). Beta conidia were not observed (Figure 4, Table 4).

Specimen examined. Pohang, Gyeongsangbuk-do, Korea, 35°56′34.3"N 129°30′18.0"E, September 2, 2021, *Diaporthe stewartii*, isolated from the stem of *Phytolacca americana* L., strain KNUE 21E558, NIBRFGC 000509070, GenBank No. PQ533150 (ITS), PQ539964 (TUB), and PQ539958 (EF-1α).

Notes: *D. stewartii* was first reported in 1935 as a new species isolated from stem blight lesions on

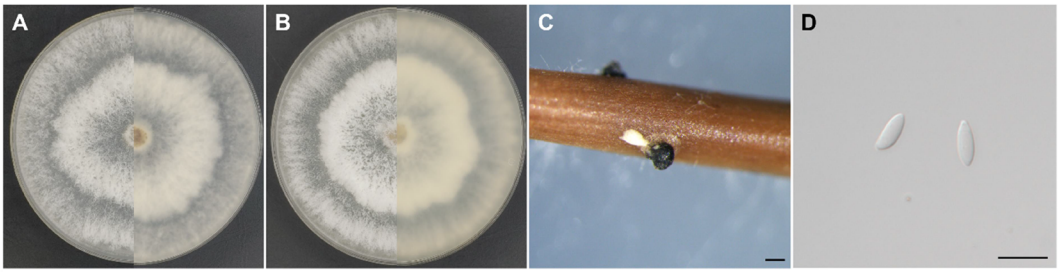

Figure 4. Morphology of *Diaporthe stewartii* KNUE 21E558. (A) Colony on malt extract agar (MEA), left side is front side and right side is reverse side after 7 d at 25 °C, (B) Colony on potato dextrose agar (PDA), left side is front side and right side is reverse side after 7 d at 25 °C, (C) Conidiomata, (D) Alpha conidia. Scale bars: C = 200 µm, D = 10 µm.

Table 4. Morphological characteristics of strains KNUE 21E558 and CUP 21993 of *Diaporthe stewartii*.

Characteristics	*Diaporthe stewartii* KNUE 21E558	*Diaporthe stewartii* CUP 21993 [25]
Colony	PDA, MEA, 25 °C, 7 d MEA: covering the entire plate, white aerial mycelium, raised, cottony around the center, flat at the margin, reverse light beige. PDA: same at the MEA.	PDA 7–10 d The mycelium covered the petri dish, abundant whitish cottony aerial mycelium, certain isolates produced a darkening of the agar.
Conidiomata	Pycnidial, scattered on pine needle, black, ordinarily subglobose, occasionally irregular, solid.	Numerous, scattered, occasionally gregarious, simple or chambered, at first subepidermal, later erumpent.
Conidia	Alpha conidia: ellipsoidal or fusiform, aseptate, smooth-walled, hyaline, 7.52–10.30 × 2.85–3.94 µm ($\bar{x}$ ± SD = 9.32 ± 0.71 × 3.37 ± 0.33 µm). Beta conidia: not observed.	Alpha conidia: oblong to subfusiform, hyaline, unicellular, 4.6–12.5 × 2.3–3.0 µm ($\bar{x}$ = 6.6–9.2 × 2.3–3 µm). Beta conidia: Filiform, curved, flexuous or uncinate, hyaline 11.7–28.4 × 1.0–1.5 µm ($\bar{x}$ = 16.7–21.7 × 1.0–1.5 µm).

MEA: malt extract agar, PDA: potato dextrose agar

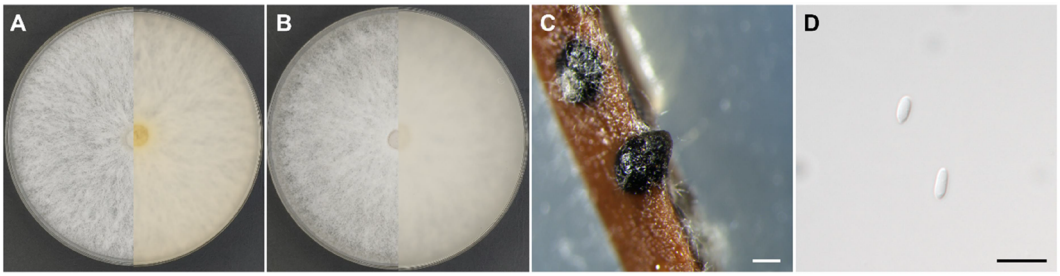

Figure 5. Morphology of *Diaporthe unshiuensis* KNUE 23P353. (A) Colony on malt extract agar (MEA), left side is front side and right side is reverse side after 7 d at 25 °C, (B) Colony on potato dextrose agar (PDA), left side is front side and right side is reverse side after 7 d at 25 °C, (C) Conidiomata, (D) Alpha conidia. Scale bars: C = 200 µm, D = 10 µm.

Table 5. Morphological characteristics of strains KNUE 23P353 and of *Diaporthe unshiuensis*.

Characteristics	*Diaporthe unshiuensis* KNUE 23P353	*Diaporthe unshiuensis* ZJUD 49 [27]
Colony	PDA, MEA, 25 °C, 7 d MEA: covering the entire plate, white, felty mycelium, reverse beige. PDA: covering the entire plate, white, felty mycelium, reverse light beige.	PDA, 25 °C, 12 h fluorescent light/12 h dark, 30 d PDA: white and turning to grey with aging, white aerial mycelium; reverse off white to grey with dark grey at the center, growth rate 14.4 mm diam per day.
Conidiomata	Pycnidial, scattered on pine needle, black, subglobose or irregular, solid.	Pycnidial, globose, subglobose, or irregular, bark brown, parenchymatous walls, 152 × 84 µm.
Conidia	Alpha conidia: ellipsoidal or clavate, aseptate, smooth-walled, hyaline, 5.53–7.66 × 2.08–2.88 µm ($\bar{x}$ ± SD = 6.35 ± 0.50 × 2.48 ± 0.22 µm). Beta conidia: not observed.	Alpha conidia: ellipsoidal or clavate, base truncate, aseptate, smooth, biguttulate, 5.2–7.5 × 2–3.9 µm ($\bar{x}$ ± SD = 6.5 ± 0.6 × 2.8 ± 0.4 µm). Beta conidia: not observed.

MEA: malt extract agar, PDA: potato dextrose agar

Cosmos bipinnatus [26]. KNUE 21E558, isolated in this study, was identified as *D. stewartii* based on phylogenetic analysis using ITS, TUB, and EF-1α sequences, as well as the similarity of its fusiform alpha conidia in size compared to the original description. *D. stewartii* has also been reported as the pathogen responsible for *Phomopsis* stem canker on *Helianthus annuus* in Minnesota [27].

***Diaporthe unshiuensis* F. Huang, K.D. Hyde & H.Y. Li, fungal biology 119 (5): 344 (2015) [MB#810845]**

Description. On MEA, the colony covered the entire plate, with white, felty mycelium, and beige on the reverse. On PDA, the colony covered the entire plate with white and felty, with light beige on the reverse. Conidiomata were pycnidial, scattered on pine needle, black, subglobose or irregular, and solid. Alpha conidia were ellipsoidal or clavate, aseptate, smooth-walled, measuring 5.53–7.66 × 2.08–2.88 µm ($\bar{x}$ ± SD = 6.35 ± 0.50 × 2.48 ± 0.22 µm). Beta conidia were not observed (Figure 5, Table 5).

Specimen examined. Jeungdo Island, Sinan, Jeollanam-do, Korea, 34°59′11.9"N 126°08′07.9"E, July 13, 2023, *Diaporthe unshiuensis*, isolated from the stem of *Trachelospermum asiaticum* (Siebold & Zucc.) Nakai, strain KNUE 23P353, NIBRFGC000510706, GenBank No. PQ533151 (ITS), PQ539962 (TUB), and PQ539960 (EF-1α).

Notes: *D. unshiuensis* was first described as a new species isolated from *Citrus unshiu* in 2015 and later reported as an endophyte from *Morinda officinalis* in 2022 [28,29]. Compounds isolated from a *D. unshiuensis* strain found in the leaves of *Caesalpinia sepiaria* exhibited antimicrobial and cytotoxic activity against cancer cells [30]. However, *D. unshiuensis* has also been reported as the causal agent of top blight on *Cunninghamia lanceolata*, leaf spot on *Sapindus mukorossi*, fruit rot of post-harvest *Gannan Navel Orange*, and it has been associated with grapevine dieback and stem blight of soybean [31–35]. KNUE 23P353 was identified as *D. unshiuensis* based on molecular phylogenetic analysis and morphological similarity of alpha conidia, which were ellipsoidal or clavate in shape and comparable in size.

Recent advances in multi-gene phylogenetic analysis have significantly improved the accuracy of species identification within the genus *Diaporthe*, leading to the discovery of new species and reevaluation of the taxonomic positions of various *Diaporthe* species [22,36]. This has prompted the investigation of *Diaporthe* across a wide range of host plants in various countries [37–40]. In Brazil, 14 endophytic *Diaporthe* species were identified from three host plants, among which seven were reported as new species. Among the previously known species, *D. ueckeri* was found in 23 plant species across 22 genera [38]. Furthermore, according to a global list of endophytic fungi compiled by Rashmi M et al. in 2019, *Diaporthe/Phomopsis* species have been reported as endophytes in host plants from 58 families and 76 genera, including herbs, shrubs, and trees [9]. *D. caryae*, *D. phoenicicola*, and *D. stewartii* in this study represent newly identified endophytic cases, while *D. unshiuensis*, originally described as an endophyte, has since been identified as a pathogen, highlighting the need for more detailed ecological investigations of *Diaporthe* species.

The discovery of four previously unrecorded endophytic *Diaporthe* species in Korea enriches our understanding of fungal biodiversity in this region and highlights the ecological adaptability of the genus. These findings underscore the importance of comprehensive studies on endophytic fungi, not only for taxonomic clarification but also for exploring their potential roles in host interactions and applications in biotechnology. As these *Diaporthe* species exhibit diverse ecological niche, including endophytic and pathogenic phases, further study into their ecological and genetic adaptations is warranted, particularly to understand the factors that trigger transitions between these phases, such as environmental stress or host susceptibility. Expanding our knowledge of endophytic *Diaporthe* could reveal insights into the mechanisms driving their ecological roles and provide valuable resources for developing novel biocontrol strategies, discovering new bioactive compounds with agricultural or medicinal applications, and improving plant disease management strategies.

Disclosure statement

The authors declare that there are no conflicts of interest.

Funding

This work was supported by a grant from the National Institute of Biological Resources (NIBR202402104) and National Institute of Ecology (NIE-B-2024-18) funded by the ministry of Environment (MOE) of the Republic of Korea.

ORCID

Ahn-Heum Eom http://orcid.org/0000-0002-6821-1088

References

[1] Gomes R, Glienke C, Videira S, et al. *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia. 2013;31(1):1–41. doi: 10.3767/003158513X666844.

[2] Udayanga D, Liu X, McKenzie EH, et al. The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. Fungal Divers. 2011;50(1):189–225. doi: 10.1007/s13225-011-0126-9.

[3] Pereira DS, Hilário S, Gonçalves MF, et al. *Diaporthe* species on palms: molecular re-assessment and species

boundaries delimitation in the *D. arecae* species complex. Microorganisms. 2023;11(11):2717. doi: 10.3390/microorganisms11112717.
[4] Eo JK, Choi JW, Eom AH. Diversity, distribution, and host plant of endophytic fungi: a focus on Korea. Mycobiology. 2022;50(6):399–407. doi: 10.1080/12298093.2022.2154044.
[5] Arnold AE. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fungal Biol Rev. 2007;21(2–3):51–66. doi: 10.1016/j.fbr.2007.05.003.
[6] Sun X, Guo LD. Endophytic fungal diversity: review of traditional and molecular techniques. Mycology. 2012;3(1):65–76. doi: 10.1080/21501203.2012.656724.
[7] Hashem AH, Attia MS, Kandil EK, et al. Bioactive compounds and biomedical applications of endophytic fungi: a recent review. Microb Cell Fact. 2023;22(1):107. doi: 10.1186/s12934-023-02118-x.
[8] Dos Reis JBA, Lorenzi AS, do Vale HMM. Methods used for the study of endophytic fungi: a review on methodologies and challenges, and associated tips. Arch Microbiol. 2022;204(11):675. doi: 10.1007/s00203-022-03283-0.
[9] Rashmi M, Kushveer J, Sarma V. A worldwide list of endophytic fungi with notes on ecology and diversity. Mycosphere. 2019;10(1):798–1079. doi: 10.5943/mycosphere/10/1/19.
[10] Suryanarayanan TS, Devarajan P, Girivasan K, et al. The host range of multi-host endophytic fungi. Curr Sci. 2018;115(10):1963–1969. doi: 10.18520/cs/v115/i10/1963-1969.
[11] Yang Q, Fan X-L, Guarnaccia V, et al. High diversity of *Diaporthe* species associated with dieback diseases in China, with twelve new species described. MycoKeys. 2018;39(39):97–149. doi: 10.3897/mycokeys.39.26914.
[12] Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol. 1993;2(2):113–118. doi: 10.1111/j.1365-294x.1993.tb00005.x.
[13] White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR Protocols: A guide to methods and applications. San Diego, CA, USA: Academic Press; 1990. p. 315–322.
[14] Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol. 1995;61(4):1323–1330. doi: 10.1128/aem.61.4.1323-1330.1995.
[15] Carbone I, Kohn LM. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia. 1999;91(3):553–556. doi: 10.1080/00275514.1999.12061051.
[16] O'Donnell K, Kistler HC, Cigelnik E, et al. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc Natl Acad Sci USA. 1998;95(5):2044–2049. doi: 10.1073/pnas.95.5.2044.
[17] Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870–1874. doi: 10.1093/molbev/msw054.
[18] Suchard MA, Lemey P, Baele G, et al. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol. 2018;4(1):vey016. doi: 10.1093/ve/vey016.
[19] Guo Y, Crous PW, Bai Q, et al. High diversity of *Diaporthe* species associated with pear shoot canker in China. Persoonia. 2020;45(1):132–162. doi: 10.3767/persoonia.2020.45.05.
[20] Zhu QH, He JP, Cheng SY, et al. First report of *Diaporthe caryae* causing stem blight of chilli pepper (*Capsicum frutescens* L.) in China. J Phytopathol. 2024;172:e13239.
[21] Xiao X, Liu Y, Zheng F, et al. High species diversity in *Diaporthe* associated with citrus diseases in China. Persoonia. 2023;51(1):229–256. doi: 10.3767/persoonia.2023.51.06.
[22] Udayanga D, Liu X, Crous PW, et al. A multi-locus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). Fungal Divers. 2012;56(1):157–171. doi: 10.1007/s13225-012-0190-9.
[23] Srivastava H, Banu Z, Govindarajan V. Fruit rot of arecanut caused by a new fungus. Mycologia. 1962;54(1):5–11. doi: 10.1080/00275514.1962.12024974.
[24] Lai J, Liu B, Xiong G, et al. First report of *Diaporthe phoenicicola* causing leaf spot on blueberry (*Vaccinium virgatum*) in China. Plant Dis. 2022;107(2):569. doi: 10.1094/PDIS-05-22-1007-PDN.
[25] Deng C, Wang H, Wen B, Gu J, Peng X, Zhang Z-F. First report of leaf brown spot caused by *Diaporthe phoenicicola* on *Pachira glabra* in China. Plant Dis 2024;108:2220.
[26] Harrison AL. The perfect stage of *Phomopsis stewartii* on Cosmos. Mycologia. 1935;27(5):521–526. doi: 10.1080/00275514.1935.12017096.
[27] Olson T, Kontz B, Markell S, et al. First report of *Diaporthe stewartii* causing *Phomopsis* stem canker of sunflower (*Helianthus annuus*) in Minnesota. Plant Dis. 2017;101(2):382–382. doi: 10.1094/PDIS-08-16-1122-PDN.
[28] Huang F, Udayanga D, Wang X, et al. Endophytic *Diaporthe* associated with Citrus: a phylogenetic reassessment with seven new species from China. Fungal Biol. 2015;119(5):331–347. doi: 10.1016/j.funbio.2015.02.006.
[29] Luo M, Guo W, Zhao M, et al. Endophytic *Diaporthe* associated with *Morinda officinalis* in China. JoF. 2022;8(8):806. doi: 10.3390/jof8080806.
[30] Khan B, Li Y, Wei W, et al. Chemical investigation of endophytic *Diaporthe unshiuensis* YSP3 reveals new antibacterial and cytotoxic agents. JoF. 2023;9(2):136. doi: 10.3390/jof9020136.
[31] Liao YCZ, Sun JW, Li DW, et al. First report of top blight of *Cunninghamia lanceolata* caused by *Diaporthe unshiuensis* and *Diaporthe hongkongensis* in China. Plant Dis. 2022;107(3):962. doi: 10.1094/PDIS-06-22-1467-PDN.
[32] Si YZ, Li DW, Zhu LH. First report of *Diaporthe eres* and *D. unshiuensis* causing leaf spots on *Sapindus mukorossi* in China. Plant Dis. 2022;107(4):1224. doi: 10.1094/PDIS-05-22-1176-PDN.

[33] Yuan X, Li Z, Wen L, et al. First report of postharvest fruit rot on Gannan navel orange caused by *Diaporthe unshiuensis* in Jiangxi Province, China. Plant Dis. 2023;107(9):2869. doi: 10.1094/PDIS-04-23-0652-PDN.

[34] Manawasinghe IS, Dissanayake AJ, Li X, et al. High genetic diversity and species complexity of *Diaporthe* associated with grapevine dieback in China. Front Microbiol. 2019;10:1936. doi: 10.3389/fmicb.2019.01936.

[35] Zhao X, Li K, Zheng S, et al. *Diaporthe* diversity and pathogenicity revealed from a broad survey of soybean stem blight in China. Plant Dis. 2022;106(11):2892–2903. doi: 10.1094/PDIS-12-21-2785-RE.

[36] Dissanayake AJ, Zhu JT, Chen YY, et al. A re-evaluation of *Diaporthe*: refining the boundaries of species and species complexes. Fungal Divers. 2024;126(1):1–125. doi: 10.1007/s13225-024-00538-7.

[37] Huang S, Xia J, Zhang X, et al. Morphological and phylogenetic analyses reveal three new species of *Diaporthe* from Yunnan, China. MycoKeys. 2021;78:49–77. doi: 10.3897/mycokeys.78.60878.

[38] Ferro L, Bezerra J, Da Silva T, De Oliveira C, dos S Nascimento S, Paiva L, Fan X, Crous P, Souza-Motta C. Endophytic *Diaporthe* species from Brazil. Fungal Syst Evol 2024;14:251–269.

[39] Jiang N, Voglmayr H, Piao CG, et al. Two new species of *Diaporthe* (Diaporthaceae, Diaporthales) associated with tree cankers in the Netherlands. MycoKeys. 2021;85:31–56. doi: 10.3897/mycokeys.85.73107.

[40] Hilário S, Santos L, Alves A. Diversity and pathogenicity of *Diaporthe* species revealed from a survey of blueberry orchards in Portugal. Agriculture. 2021;11(12):1271. doi: 10.3390/agriculture11121271.