




Morphology and Phylogeny of Two Novel Species within the Class Dothideomycetes Collected from Soil in Korea

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ABSTRACT

Two fungal strains (KNU-JJ-1827 and KNU-JJ-1829) belonging to the class Dothideomycetes were discovered from Jeju Island, Korea during this investigation of soil microfungi. Strain KNU-JJ-1827 showed fewer conidial septations, larger conidiogenous cells, and smaller conidia compared to the previously identified closest species of *Didymocyrtis*. Strain KNU-JJ-1829 revealed the similar characteristics of the nearest certain species of the genus *Parathyridaria* with the production of conidiogenous cells and conidia, because no asexual morphs were detected from the closest type strain *Parathyridaria rosae*. The novelty of the strains was also confirmed by analyzing molecular data using internal transcribed spacer regions and 28S rDNA. The molecular phylogeny also strongly support the detailed description and illustration for each proposed species as *Didymocyrtis septata* sp. nov. (KNU-JJ-1827) and *Parathyridaria ellipsoidea* sp. nov. (KNU-JJ-1829) isolated from soil in Korea.

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1. Introduction

Dothideomycetes are the largest and one of the most important groups of Ascomycota, including more than 23 orders, 110 families, 1261 genera, and 19,000 species at present [1]. The genus *Didymocyrtis* within class Dothideomycetes was recently readdressed for the species lichenicolous which was previously assigned to *Diederichia*, *Diederichomyces*, *Leptosphaeria*, and *Phoma* [2]. Several species of *Didymocyrtis* have been shown to produce *Phoma* anamorphs during their lifecycle. The genera *Diederichia* and *Diederichomyces* have been synonymized with *Didymocyrtis* [3]. The new combinations, *Didymocyrtis bryonothae*, *Didymocyrtis cladoniicola*, *Didymocyrtis foliaceiphila*, *Didymocyrtis infestans*, *Didymocyrtis kaernefeltii*, *Didymocyrtis melanelixiae*, *Didymocyrtis pseudeverniae*, *Didymocyrtis ramalinae*, *Didymocyrtis slaptoniensis*, and *Didymocyrtis xanthomendozae* were created, and the new name *Didymocyrtis epiphyscia* was introduced for *Phoma physciicola* [3]. Several anamorphic-teleomorphic relationships were resolved, such as *D. ramalinae* (*Phoma ficuzzae*) and *Didymocyrtis consimilis* (*P. caloplacae*), with single ascospore cultures endorsing phylogenetic results leading to the asexual stage where pycnidia and conidia develop in nature [3].

The family Thyridariaceae was introduced to accommodate the genus *Thyridaria* Sacc., with type species *Thyridaria broussonetiae* [4]. The inclusion criteria for this family were stromatic, pigmented, prosenchymatous tissues and ostioles, with a disc-like ostiolar tube, solitary or gregarious, immersed to erumpent, globose, coriaceous ascomata, in valloid configurations. According to Jaklitsch and Voglmayr (2016), *Cyclothyrium* removed from Thyridariaceae and classified the genus as Pleosporales, and genera incertae sedis was tentatively defined [5]. Furthermore, they also synonymized Roussoellaceae under Thyridariaceae, and accepted *Neorousoella*, *Thyridaria*, *Rousoella*, *Rousoellopsis*, and *Parathyridaria*. The family Thyridariaceae was circumscribed having two to three septate ascospore forms, and Jaklitsch and Voglmayr (2016) included *Parathyridaria* in Thyridariaceae which rarely forms muriform ascospores [4]. Beyond that, the genus differs from *Thyridariella* produces pale to grayish brown ascospores that are occasionally muriform, and the upper part is slightly wider than the lower part. It has the common features for both genera - guttulate ascospores with lateral thickening of the ostiolar neck [6].

The purpose of screening this microflora was to identify and classify the novel species of fungi isolated from soil in Korea. The novelty of the strains

was confirmed through molecular phylogenetic analyses along with their cultural and morphological characteristics. A detailed description and illustration were studied as a novel fungal species from Korea in this study.

2. Material and methods

2.1. Soil sample collection and fungal isolation

The soil samples were collected from Jeju Island (33°25'22.9"N, 126°33'01.6"E), Korea, in 2018. The soil samples were collected randomly from a depth of 15–30 cm using a pre-autoclaved sterile spatula and transferred immediately into plastic Zip-lock bags and stored at 4 °C until use. The collected soil (1 g) was suspended in 10 mL of sterile distilled water, vortexed gently and diluted serially, then spread on potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates and incubated for 2–3 days at 25 °C [7]. The single colonies on the plates were then transferred to new PDA plates and incubated at 25 °C for 5–7 days. Subsequently, the strains were selected according to their different cultural characteristics for further molecular analyses. Fungal strains were maintained in 20% glycerol at –80 °C for further study.

2.2. Culture-related and morphological observations

Cultural characteristics and morphological observations were studied using different media according to genus and species. The strains were transferred to PDA, oatmeal agar (OA), malt extract agar (MEA), and synthetic nutrient agar (SNA), and incubated at 25 °C for 14 days [8,9]. The characteristics of the colonies were then recorded, and the mycological characteristics were observed by examining the fungal structures under a light microscope (BX-50; Olympus, Tokyo, Japan).

2.3. Genomic DNA extraction, PCR amplification, and sequencing

The fungal mycelia were grown on PDA plates for 5–7 days at 25 °C. The mature mycelia were scraped off from the surface of the PDA plates with a sterile blade. Genomic DNA was extracted using a HiGene Genomic DNA prep kit (BIOFACT, Daejeon, Korea), according to the manufacturer's instructions, and DNA extracts were stored at –20 °C prior to use. The PCR amplification process was carried out using the fragment of internal transcribed spacer regions (ITS) regions [10,11] and large subunit (LSU) using primers LR0R/LR7 and LSU1Fd/LR5 [12,13]. The yield of the PCRs was verified by

running the products on a 0.8% agarose gel using ethidium bromide. Subsequently, the amplified PCR products were purified with EXOSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced by Solgent Co. Ltd. (Daejeon, Korea). The sequenced data obtained in this study were adjusted using SeqMan Lasergene software (DNASTar Inc., Madison, Wisconsin, USA).

2.4. Molecular phylogenetic analyses

The phylogenetic analyses were performed with the sequences retrieved from the National Center for Biotechnology Information. Ambiguous regions were deleted from the alignments and the evolutionary distance matrices for the neighbor-joining algorithm were calculated according to Kimura's two-parameter model [14]. To determine the exact taxonomic position of each strain, neighbor-joining [15], maximum likelihood [16], and maximum parsimony [17], trees were also constructed. The neighbor-joining method was inferred by tree topology using the MEGA7 program with bootstrap values based on 1000 replications [18].

3. Results

3.1. Taxonomical analysis of *Didymocyrtis septata*

3.1.1. Taxonomy

Strain KNU-JJ-1827 showed different morphological characteristics compared with other allied *Didymocyrtis* species. Therefore, it is proposed as a new species.

***Didymocyrtis septata* K. Das, S.Y. Lee and H.Y. Jung, sp. nov.** (Figure 1)

Mycobank: MB 835967

Etymology: The specific name "septata" refers to the number of septa in the conidia.

Typus: Jeju Island (33°25'22.9"N 126°33'01.6"E), isolated from soil. The stock culture (NIBRFGC000502248) was deposited in the National Institute of Biological Resources (NIBR), as a metabolically inactive culture.

Ecology and Distribution: Several members of this genus were isolated from the hard, leathery leaves of *Banksia* and from the surface of the leaves. Some of the species were found on *Brachylaena discolor* from South Africa and *Cladonia cervicornis* from Spain. Moreover, several members were also isolated from *Cladonia arbuscula*, *C. uncialis*, and *C. uliginosa* from Finland and Russia. The proposed novel species, namely *D. septata*, was isolated from soil obtained in Jeju Island. The soil contained plant debris, was sandy, and had a lower moisture capacity.

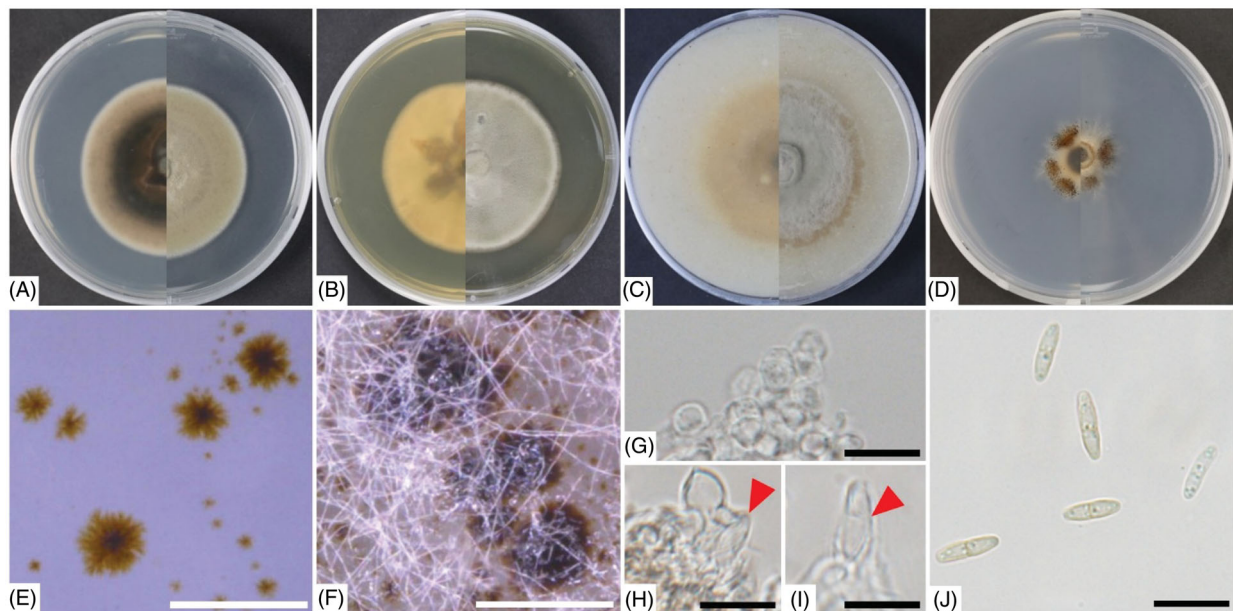


Figure 1. Cultural and morphological characteristics of KNU-JJ-1827. The colony on potato dextrose agar (A); malt extract agar (B); oatmeal agar (C); synthetic nutrient agar (D) incubated for 14 days at 25 °C. Conidiomata on SNA (E,F); Conidiogenous cells (G–I); Conidia (J). Arrows indicated conidiogenous cells. Scale bars: E,F = 500 µm; G–J = 10 µm.

Cultural characteristics: The colonies were flat, spreading with moderate aerial mycelium, with round, smooth, lobate margins, reaching 31.3 to 34.2 mm in diameter after 7 days at 25 °C. Reverse colonies were brown to dark olive-green associated with white mycelia growing on the edge of the Petri dish containing PDA medium (Figure 1(A)). On MEA, the colony surface was brown to olive green reaching 30.1 to 34.2 mm in diameter after 7 days at 25 °C with reverse dirty white to irregular brown patches (Figure 1(B)). On OA, the colony surface was brown, measuring 31.2 to 36.3 mm in diameter after 7 days at 25 °C with reverse brownish patches (Figure 1(C)). On SNA, the colonies were white to light brown and measured 12.2 to 15.1 mm in diameter after 7 days at 25 °C; the reverse colonies were also white to light brown (Figure 1(D)).

Morphological characteristics: The micromorphological structures were studied using the colonies and conidiomata abundantly produced on SNA media. The conidiomata were pycnidial, immersed, dark brown to black, sparse, irregular, solitary, or aggregated, and measured up to 500 µm. The vegetative mycelium was immersed, hyaline to light brown, smooth-walled, and mostly 1.8–2.0 µm thick (Figure 1(E,F)). There were also conidiogenous cells lining the inner wall of the pycnidial cavity, globose to subglobose, also ampulliform to doliiform, hyaline, non-proliferating, aggregated, smooth-walled, and measuring 7.0–10.9 × 2.2–3.3 µm (Figure 1(G–I)). Conidia were abundant, arising singly, hyaline, greenish to light yellowish, blunted at both apices, fusiform, clavate to subcylindrical, (0–)1 septate, guttulate, diameter of 7.0–9.5 × 1.9–2.9 µm

($n=100$), with an average size of 8.2×2.3 µm (Figure 1(J)).

Note: Conidiomata of the strain KNU-JJ-1827 were pycnidial, immersed, dark brown to black, sparse, irregular, solitary, or aggregated, and with a diameter of up to 500 µm. However, the closest type strain, *Didymocyrtis brachylaenae*, produced conidiomata that were pycnidial, globose, brown, measuring 200–350 µm in diameter, with central ostiole (Table 1). Moreover, another closest strain, *D. cladoniicola*, produced conidiomata that were immersed in pale necrotic areas of the thallus or partially erumpent, black, subspherical to pyriform, with a diameter of 50–100 µm (Table 1). Furthermore, the conidiomata were larger than those of strains *D. xanthomendozae* (140–160), *D. slaptoniensis* (80–120), and *D. banksiae* (200–300). The size of the conidiogenous cells also differed from that of the closest species, with the larger diameter (7.0–10.9 × 2.2–3.3 µm) than the closest species *D. brachylaenae* (5.0–7.0 × 2.5–3.5). The conidiogenous cells were also larger than those of *D. cladoniicola* (2.5–4.5 × 2.5–4.0 µm), but very close in size to *D. xanthomendozae* (5.0–10.0 × 2.5–3.5 µm) and *D. slaptoniensis* (8.0–10.0 × 5.0–6.0 µm; Table 1). The strain, *D. banksiae* produced larger (5.0–13.0 × 3.0–4.0) conidiogenous cells than strain KNU-JJ-1827. The diameter of conidia of the strain KNU-JJ-1827 was 7.0–9.5 × 1.9–2.9 µm, whereas *D. brachylaenae* produced conidia with diameter of 9.0–10.0 × 2.0–3.0 µm. The conidia were also comparatively larger than those of *D. xanthomendozae* (5.6–7.1 × 3.3–4.3 µm), *D. cladoniicola* (4.7–5.9 × 2.4–3.0), and *D. slaptoniensis* (6.0–8.0 × 2.5–3.5 µm; Table 1). The strain, *D. banksiae*, produced larger conidia (10.0–11.0 × 3.0–4.0), whereas

Table 1. Morphological comparison between *D. septata* and the closest species of *Didymocyrtis*.

| Sl. No. | Name of strain | Conidiomata (μm) | Conidiogenous cells (μm) | Conidia (μm) | References |
|---------|---|-------------------------------|---------------------------------------|----------------------------|---------------|
| 1 | <i>D. septata</i> KNU-JJ-1827 ^T | up to 500 | 7.0–10.9 \times 2.2–3.3 | 7.0–9.5 \times 1.9–2.9 | In this study |
| 2 | <i>D. brachylaenae</i> CPC 32651 ^T | 200–350 | 5.0–7.0 \times 2.5–3.5 | 9.0–10.0 \times 2.0–3.0 | [8] |
| 3 | <i>D. cladoniicola</i> PRM 907325 ^T | 50–100 | 2.5–4.5 \times 2.5–4.0 | 4.7–5.9 \times 2.4–3.0 | [19] |
| 4 | <i>D. xanthomendozae</i> CBS 129666 | 140–160 | 5.0–10.0 \times 2.5–3.5 | 5.6–7.1 \times 3.3–4.3 | [3] |
| 5 | <i>D. slaptoniensis</i> IMI 359711 | 80–120 | 8.0–10.0 \times 5.0–6.0 | 6.0–8.0 \times 2.5–3.5 | [3] |
| 6 | <i>D. banksiae</i> CBS 142523 ^T | 200–300 | 5.0–13.0 \times 3.0–4.0 | 10.0–11.0 \times 3.0–4.0 | [20] |

the proposed novel strain KNU-JJ-1827 produced conidia 7.0–9.5 \times 1.9–2.9 μm in diameter. Furthermore, there was a difference in the number of septate conidia with that of the closest species. Moreover, the closest species, *D. brachylaenae*, produced 1(–3) septate conidia, but strain KNU-JJ-1827 developed (0–)1 septate conidia. A comparison with the nearest certain species of the genus showed a lower number of septate conidia, larger size of conidiogenous cells and conidomatal size, and different conidial size. Thus, the morphology of strain KNU-JJ-1827 is totally different from previously identified closest species of *Didymocyrtis*.

3.1.2. Molecular phylogeny of strain KNU-JJ-1827

The nucleotide sequences of the ITS regions and 28S rDNA (LSU) were analyzed to determine the evolutionary relationships with the strains obtained from GenBank (Table 2). After analyzing the nucleotide sequences, 495 and 731 bp sequences were obtained from the ITS regions and 28S rDNA, respectively. The strain KNU-JJ-1827 showed a maximum of 98.99 and 98.93% similarities from the BLAST results of ITS regions with the strains of *D. cladoniicola* UTHSC DI16-313 and *Parastagonospora nodorum* NRRL 62782, respectively. The partial 28S rDNA sequences revealed the maximum similarities with the different strains of *D. cladoniicola* UTHSC DI16-313 (100%), *D. brachylaenae* CPC 32651^T (99.59%), *D. cladoniicola* UTHSC DI16-330 (99.59%). The phylogenetic analysis was constructed based on the combination of ITS regions with the partial sequences of the 28S rDNA indicated by the node in the neighbor-joining phylogenetic tree along with the filled nodes in the maximum likelihood and maximum parsimony trees (Figure 2). The corresponding nodes were also generated using the maximum likelihood or maximum parsimony algorithms, as shown by the open circles. A phylogenetic analysis was also conducted based on a combination of sequences with maximum-parsimony (tree length = 229, consistency index = 0.69, retention index = 0.72, and composite index = 0.59) to determine the exact taxonomic position of the strain. The phylogenetic tree showed that the phylogenetic position of strain KNU-JJ-1827 is distinct from the other

Table 2. GenBank accession numbers used for the phylogenetic analyses in this study.

| Species | Strain numbers | GenBank accession numbers | |
|------------------------------|--------------------------------|---------------------------|-----------------|
| | | ITS | LSU |
| <i>Didymocyrtis banksiae</i> | CBS 142523 ^T | NR154037 | KY979812 |
| <i>D. cladoniicola</i> | UTHSC DI16-330 | LT796886 | LN907473 |
| <i>D. foliaceiphila</i> | CBS 129141 | KP170648 | JQ238640 |
| <i>D. xanthomendozae</i> | CBS 129666 | KP170651 | JQ238634 |
| <i>D. caloplacae</i> | CBS 129338 | KP170639 | JQ238643 |
| <i>D. aff. consimilis</i> | Berger 26876 | KT383814 | KT383798 |
| <i>D. brachylaenae</i> | CPC 32651 ^T | MH327821 | MH327857 |
| <i>D. consimilis</i> | Gardiennet 12041 | KT383812 | KT383796 |
| <i>D. melanelixiae</i> | Isolate 552 | KT383826 | KT383800 |
| <i>D. pseudeverniae</i> | Diederich 17338 | KT383834 | KT383801 |
| <i>D. ramalinae</i> | Ertz 16399 | KT383838 | KT383802 |
| <i>D. slaptoniensis</i> | MoraA (BR) | KT383841 | KT383803 |
| <i>D. cf. epiphyscia</i> | Ertz 17411 (BR) | KT383820 | KT383799 |
| <i>D. septata</i> | KNU-JJ-1827^T | LC552949 | LC552951 |
| <i>P. percutanea</i> | CBS 868.95 ^T | KF322118 | KF366449 |
| <i>P. philadelphia</i> | CBS 143432 ^T | MH107905 | NG063958 |
| <i>P. ramulicola</i> | CBS 141479 ^T | KX650565 | KX650565 |
| <i>P. robiniae</i> | MFLUCC 14-1119 ^T | NR168161 | KY511141 |
| <i>P. rosae</i> | MFLU 17-0623 ^T | NR157530 | NG059873 |
| <i>Rousoella chiangraina</i> | MFLUCC 10-0556 ^T | NR155712 | KJ474840 |
| <i>R. hysteroioides</i> | CBS 546.94 | KF443405 | KF443381 |
| <i>R. intermedia</i> | CBS 170.96 | KF443407 | KF443382 |
| <i>R. pustulans</i> | KT 1709 | KJ474830 | AB524623 |
| <i>R. siamensis</i> | MFLUCC 11-0149 ^T | NR155716 | KJ474845 |
| <i>T. broussonetiae</i> | CBS 141481 ^T | KX650568 | KX650568 |
| <i>T. mangrovei</i> | NFCCI4213 | MG020434 | MG020437 |
| <i>Torula hollandica</i> | CBS 220.69 ^T | KF443406 | KF443384 |
| <i>P. ellipsoidea</i> | KNU-JJ-1829^T | LC552950 | LC552952 |

The newly generated sequences were indicated in bold.

identified species of *Didymocyrtis* (Figure 2). Consequently, strain KNU-JJ-1827 is proposed as a new species in the fungal flora under the genus *Didymocyrtis*.

3.2. Taxonomical analysis of *Parathyridaria ellipsoidea*

3.2.1. Taxonomy

Strain KNU-JJ-1829 showed different morphological characteristics compared with the other species of *Parathyridaria*. Therefore, it is proposed as a new species.

***Parathyridaria ellipsoidea* K. Das, S.Y. Lee and H.Y. Jung, sp. nov.** (Figure 3)

MycoBank: MB 835968

Etymology: The Latin word “ellipsoidea” refers to the shape of the conidia.

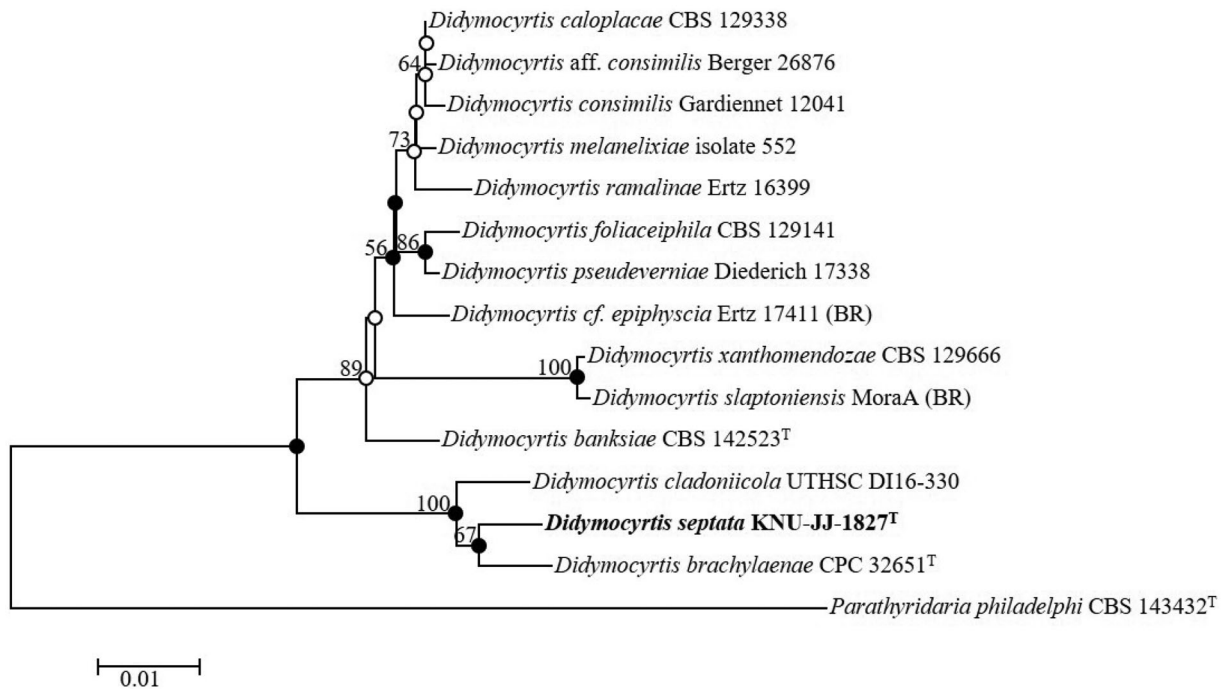


Figure 2. Neighbor-joining phylogenetic tree of KNU-JJ-1827 based on combined sequences (ITS + LSU), showing the phylogenetic position among the related strains in the genus *Didymocyrtis*. The tree was rooted using *Parathyridaria philadelphi* CBS 143432^T as an outgroup. Bootstrap values greater than 50% (percentage of 1000 replications) are shown at the branching points. Bar, 0.01 substitutions per nucleotide position.

Typus: Jeju Island (33°25′22.9″N, 126°33′01.6″E), isolated from soil. The stock culture (NIBRFGC000502247) was deposited in the National Institute of Biological Resources (NIBR), as a metabolically inactive culture.

Ecology and Distribution: Members of this genus are known to be saprobes and human pathogens. Recently, the members of this genus were isolated from *Robinia pseudoacacia* in Italy and from twigs of *Philadelphus coronarius* (Hydrangeaceae) in Germany, and also as a saprobic fungus on dead twigs of *Ribes rubrum* and *Sambucus nigra* in France and Germany. The isolated species proposed as a novel species, namely *P. ellipsoidea*, was isolated from soil in Jeju Island. The soil contained plant debris, was sandy, and had lower moisture capacity.

Cultural characteristics: The colonies were flat, with moderate aerial mycelia, irregular fungal growth, and reached 26.3 to 28.1 mm in diameter after 14 days at 25 °C. The reverse colonies were brown to blackish on PDA media (Figure 3(A)). On MEA, the colonies were white to brown reaching 29.3 to 31.1 mm diameter after 14 days at 25 °C, with the reverse colonies brown to yellowish at the center (Figure 3(B)). On OA, the colonies were brown, appearing as thick white patches around the edges to center reaching 32.2 to 34.3 mm in diameter after 14 days at 25 °C, with the reverse colonies brownish (Figure 3(C)).

Morphological characteristics: The pycnidial conidiomata cultured on PDA were separate,

pycnidial, brown, globose, black, solitary, spherical to subspherical, with thin wall, with pycnidia observed after 8 weeks, and measuring up to 500 μm (Figure 3(D,E)). The hyphae were branched, septate, hyaline becoming dark brown with age, and 1.8–2.4 μm wide. The conidiophores were limited to the conidiogenous cells lining the inner cavity, and were hyaline, smooth, round, oval to ampulliform, proliferating percurrently near the phialidic apex, and measuring 4.5–5.7 × 2.5–3.7 μm (Figure 3(F,G)). The conidia were aseptate, solitary, with obtuse apex and base bluntly rounded, broadly ellipsoidal to ellipsoidal, brown, smooth, at times slightly granular, measuring 3.4–4.8 × 1.7–3.6 μm ($n = 100$), with an average diameter of 4.0 × 2.3 μm (Figure 3(H)).

Note: Strain KNU-JJ-1829 developed pycnidial conidiomata cultured on PDA and measured up to 500 μm after 8 weeks, whereas the closest type strain, *Parathyridaria percutanea*, produced 59–102 × 54–96 μm. Type strain, *P. philadelphi*, developed smaller conidiomata (on OA media), 250–300 μm in diameter, but the conidiomatal structures were not detected in the closest type strains determined by phylogeny, such as *P. ramulicola*, *P. robiniae*, and *P. rosae* (Table 3). The conidiogenous cells of the strain KNU-JJ-1829 were hyaline, smooth, round, oval to ampulliform, with a diameter of 4.5–5.7 × 2.5–3.7 μm. However, the conidiogenous cells of the closest species, *P. rosae*, were not detected (Table 3). Even other closest species, *P. ramulicola* and *P. percutanea*, did not produce any

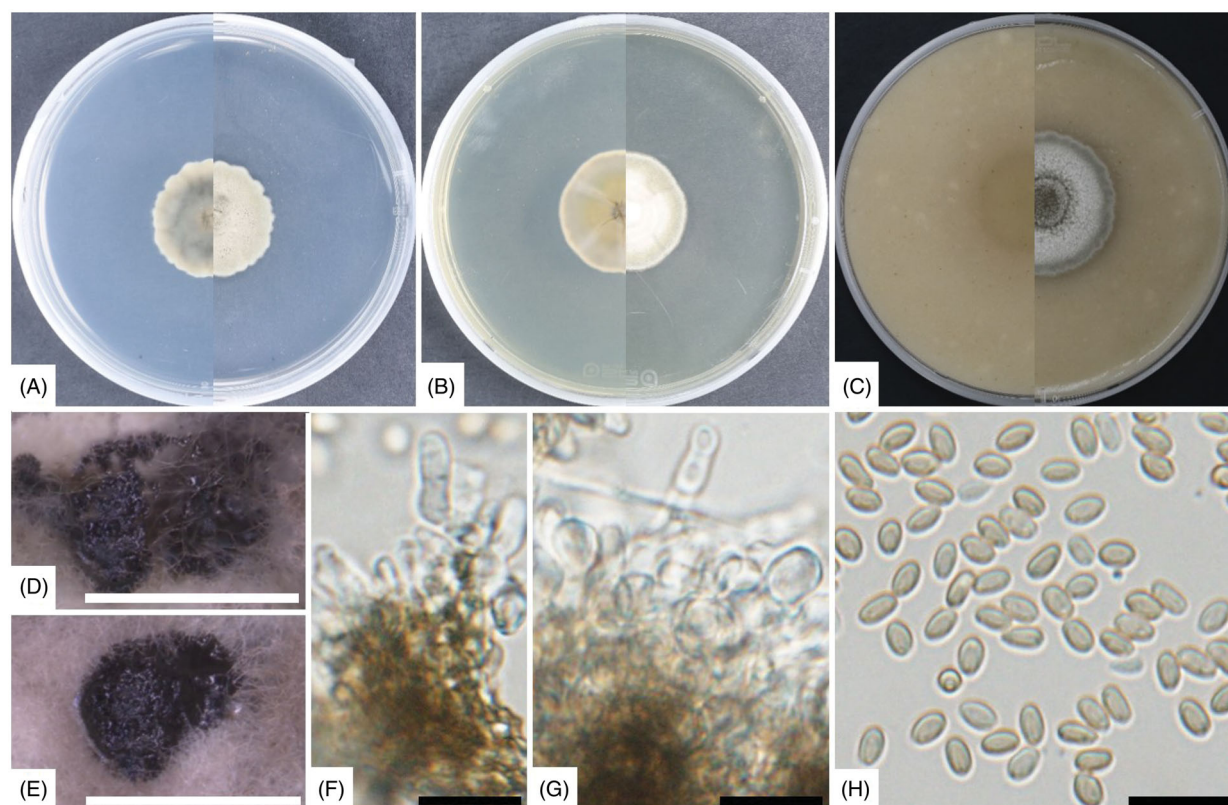


Figure 3. Cultural and morphological characteristics of KNU-JJ-1829. The colony growing on potato dextrose agar (A); malt extract agar (B); oatmeal agar (C) for 14 days incubation at 25 °C. Conidiomata on PDA after 8 weeks (D,E); Conidiogenous cells (F,G); Conidia (H). Scale bars: D,E = 500 μ m, F–H = 10 μ m.

Table 3. Morphological comparison between *P. ellipsoidea* and the closest species of *Parathyridaria*.

| Sl. No. | Name of strain | Conidiomata (μ m) | Conidiogenous cells (μ m) | Conidia (μ m) | References |
|---------|---|------------------------|--------------------------------|--------------------------|---------------|
| 1 | <i>P. ellipsoidea</i> KNU-JJ-1829 ^T | up to 500 | 4.5–5.7 \times 2.5–3.7 | 3.4–4.8 \times 1.7–3.6 | In this study |
| 2 | <i>P. rosae</i> MFLUCC 17-0800 ^T | N/D | N/D | N/D | [21] |
| 3 | <i>P. ramulicola</i> CBS 141479 ^T | N/D | N/D | N/D | [6] |
| 4 | <i>P. percutanea</i> CBS 868.95 ^T | 59–102 \times 54–96 | N/A | 1.2–2.0 \times 0.7–0.9 | [22] |
| 5 | <i>P. philadelphia</i> CBS 143432 ^T | 250–300 | 4.0–7.0 \times 3.0–4.0 | 4–6 \times 2.0. | [9] |
| 6 | <i>P. robiniae</i> MFLUCC 14-1119 ^T | N/D | N/D | N/D | [23] |

N/A: not available; N/D: not determined.

conidiogenous cells either, whereas *P. philadelphia* produced cells with a diameter measuring 4.0–7.0 \times 3.0–4.0 μ m (Table 3). Strain KNU-JJ-1829 produced numerous conidia, with diameter of 3.4–4.8 \times 1.7–3.6 μ m. Conversely, the closest species, *P. rosae* and *P. ramulicola*, did not produce any conidia, and another nearest species, *P. philadelphia* (4.0–6.0 \times 2.0 μ m), was larger but *P. percutanea* (1.2–2.0 \times 0.7–0.9) produced smaller conidia (Table 3). A comparison of the nearest certain species within the genus showed the production of conidiogenous cells and conidia, because the production of any asexual morph was not detected in the closest type strain *P. rosae*. The next closest type strain produced very similar conidiogenous cells and conidia, and it is not clustered together phylogenetically with strain KNU-JJ-1829. Therefore, the morphology of

strain KNU-JJ-1829 is distinct from previously identified species of *Parathyridaria*.

3.2.2. Molecular phylogeny of strain KNU-JJ-1829

A phylogenetic tree of strain KNU-JJ-1829 was constructed to determine the phylogenetic relationship with its allied species (Table 2). Amplification of the ITS regions and partial 28S rDNA loci yielded 520 and 1287-bp fragments, respectively. The BLAST search results revealed that the ITS regions showed a maximum of 99.79 and 94.06% similarities with the strains of *P. ramulicola* isolate N7 and *P. ramulicola* CBS 141479^T, respectively. Other strains of *P. rosae* MFLU 17-0623^T and *P. philadelphia* CBS 143432^T displayed 94.27 and 94.03% similarities with the ITS regions, respectively. Partial 28S rDNA sequences had maximum similarities with strains *P.*

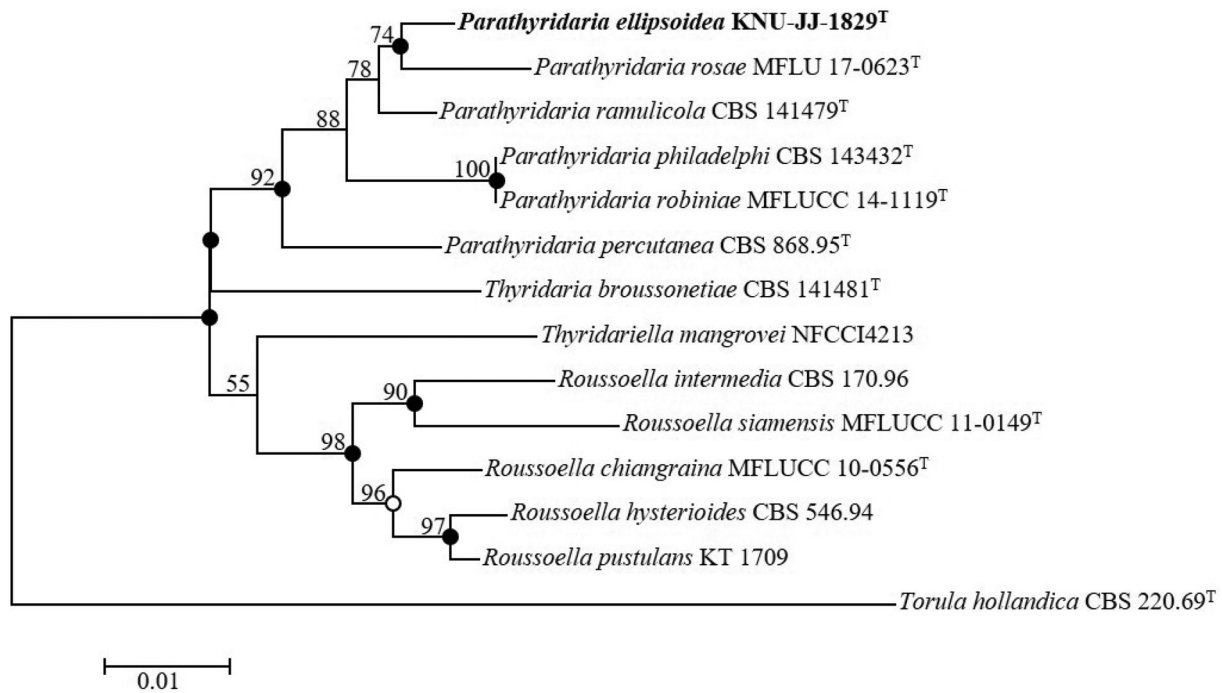


Figure 4. Neighbor-joining phylogenetic tree of KNU-JJ-1829 based on the combined sequences (ITS+LSU), showing the phylogenetic position among related strains within genus *Parathyridaria*. The tree was rooted using *Torula hollandica* CBS 220.69^T as an outgroup. Bootstrap values greater than 50% (percentage of 1000 replications) are shown at the branching points. Bar, 0.01 substitutions per nucleotide position.

ramulicola MUT ITA 4397 (99.38%), *P. robiniae* MUT ITA 4893 (98.99%), and *P. percutanea* CBS 128203 (98.29%). A phylogenetic analysis was conducted based on a combination of ITS regions and partial sequences of 28S rDNA with the filled circles indicated in the neighbor-joining tree along with the maximum likelihood and maximum parsimony algorithms (Figure 4). Open circles indicate that the corresponding nodes were also generated from the tree with the maximum likelihood or maximum parsimony algorithms. The phylogenetic analysis was also performed based on a combination of sequences with maximum-parsimony (tree length = 273, consistency index = 0.56, retention index = 0.62, and composite index = 0.46) to determine the strain's exact taxonomic position. The phylogenetic tree shows that the phylogenetic position of strain KNU-JJ-1829 is distinct from those of the other identified species of *Parathyridaria* (Figure 4). Therefore, phylogenetic relationship determined through sequence analyses indicated that strain KNU-JJ-1829 was distinct from each of the strains identified within the species *Parathyridaria*.

4. Discussion

Morphologically distinct strains, KNU-JJ-1827 and KNU-JJ-1829, were isolated from soil samples obtained from Jeju Island, Korea, in this investigation. The strains were morphologically different from each of the identified closely related species, as

is supported by descriptions of the latter in previous reports (Tables 1 and 3).

D. banksiae appears to be a non-lichenicolous species isolated from the hard, leathery leaves of *Banksia* and also from the surfaces of leaves in previous studies [20]. Furthermore, *D. brachylaenae* was found on the leaves of *B. discolor* from South Africa in 2010 [8]. Although *D. cladoniicola* was reported from *Cladonia subturgida* as a new host species and was also found on *C. cervicornis* from Spain in 2002 for the first time in Asia [24], *D. foliaceiphila* was also previously known to come from Asia based on an uncertain report from Turkey [25]. Subsequently, this species was reported from Finland and Russia with the association of *C. arbuscula*, *C. uncialis*, and *C. uliginosa*, which are also new host species [24]. Moreover, *D. xanthomendozae* (previously known as *Phoma xanthomendozae*) was found on fallen *Salix* and a sexual morph was found on *Xanthomendoza hasseana* from Canada in 2010 and 2011, respectively [23]. The proposed novel species *D. septata* (KNU-JJ-1827) was isolated from soils containing plant debris, sands, or even lower moisture capacity from Jeju Island, Korea.

Genus *Parathyridaria* was recently introduced to accommodate *P. percutanea* and *P. ramulicola* which were recorded as saprobes and human pathogens, respectively [6]. *P. philadelphi* is phylogenetically related to *P. robiniae*, a teleomorph species recently found in Italy on *R. pseudoacacia* [23]. *P. philadelphi* was found on the twigs of *P. coronarius*

(Hydrangeaceae) from Germany, near Berlin in 2016 [9] and *P. ramulicola* was identified as saprobic on dead twigs of *R. rubrum* and *S. nigra* from France and Germany in 2013 [6]. *P. percutanea* (previously *Rousoella percutanea*), has also recently been described from subcutaneous mycetoma-like infections in humans, although grain and sinus tract formation has not been reported to date [6,22]. Moreover, *P. percutanea* was also identified as the cause of subcutaneous phaeohyphomycosis in India [26] and Aruba [27] and *P. rosae* found on a host, namely *Rose* sp. from UK [21]. The novel species proposed as *P. ellipsoidea* (KNU-JJ-1829) was isolated from soil samples collected from Jeju Island, Korea.

In conclusion, there were diversified host ranges for each species belonging to the genera studied. According to cultural, morphological, and phylogenetic analyses, the strains are especially distinct from previously identified strains of the genera *Didymocyrtis* and *Parathyridaria*. Thus, these two species are proposed as *D. septata* and *P. ellipsoidea*. Therefore, considering all the aspects of these two new species, further investigation is essential to explore the etiology as well as their pathogenicity along with their ecological importance based on Korean soils and environmental conditions.

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