Taxonomy of Arthrinium minutisporum sp. nov., Pezicula neosporulosa, and Acrocalymma pterocarpi: New Records from Soil in Korea

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ABSTRACT
The strains 17E-042, 17E-039, and NC13-171 belong to Ascomycota and were isolated from soil collected from Sancheong-gun and Yeongam-gun, Korea. The strain 17E-042 produced white mycelial colonies that developed a sienna color with a round margin on potato dextrose agar (PDA), and the reverse side developed a light sienna color. Morphologically, this strain was similar to the strains of Arthrinium phragmites and A. hydei, but the shorter conidial size of the newly identified strain (17E-042) was distinct. The strain 17E-039 produced macroconidia that were pale yellow to orange-brown, elongated-ellipsoid to oblong, round at both ends, primarily straight but sometimes slightly curved, 0-septate, thin-walled, and filled with numerous droplets, having diameters of 20.4–34.3 × 8.0–12.0 μm. And the strain NC13-171 formed hyaline to light brown chlamydospores, solitary or in a chain. Multigene phylogenetic analyses were conducted using sequence data obtained from internal transcribed spacer (ITS) regions, 28S rDNA large subunit (LSU), β-tubulin (TUB2), translation elongation factor 1-alpha (TEF1-α), and RNA polymerase II large subunit (RPB2) genes. The results of molecular phylogeny, the detailed descriptions and illustrations of each species strongly support our proposal that these strains from soil in Korea be designated as Arthrinium minutisporum sp. nov. and two new records of Pezicula neosporulosa and Acrocalymma pterocarpi.

1. Introduction
The genus Arthrinium (sexual morph Apiospora) is widespread and ecologically diverse; some species were associated with plants as endophytes or saprobies, and some were pathogenic to plants, such as ornamentals [1,2]. Currently, there were more than 80 recognized species in Arthrinium [3]. Notably, Arthrinium species show a distinct preference for growing on two graminaceous families, Poaceae and Cyperaceae. Bambusa (Poaceae) and Cwerex (Cyperaceae) were two of the most common host genera for Arthrinium species [4]. The asexual morph of Arthrinium species can be easily recognized by its dark, aseptate, lenticular conidia with a hyaline rim or germ slit [5]. Because of their relatively conserved morphology, the identification of Arthrinium to the species level is difficult when only the asexual morph is available. The Arthrinium species can produce hyphomyceteous fruiting bodies in culture or coelomycetous structures on host substrates [6]. A detailed reevaluation of Arthrinium based on multigenic data added eight new species and presented genetic data from several other taxa strains [7]. Therefore, molecular data and phylogenetic analysis have been used to classify Arthrinium species, allowing closely related taxa to be differentiated [6,7].

Genus Pezicula Tul. and C. Tul. (Leotiomycetes, Helotiales, Dermateaceae) was introduced as a widespread Discomycetes with the type strain Peziza carpinea and the members of Pezicula were characterized by their brightly colored apothecia, which occur on the bark of woody plants [8]. Verkley monographed Pezicula and resolved the taxonomy of the common species P. cinnamomea, which includes P. livida and P. eucrita [9]. He also introduced a new species, P. sporulosa which was morphologically distinct from the two other species [9]. Subsequently, two new species and nine new combinations have been added to the genus [10]. The phylogeny of these genera using sequence data from internal transcribed spacer (ITS) sequences,
which provided the foundation for later studies on this generic complex [11]. In 1912, Cryptosporiopsis scutellata was introduced as the asexual morph; the sexual morph is Pezicula ocellata. Since then, many species have been described under Cryptosporiopsis and Pezicula [12–14].

The genus Acrocalymma belongs to the Dothideomycetes class, which was introduced as a pathogen that causes reddening of the internal root and crown tissue of Lucerne (Medicago sativa) in Australia [15,16]. The genus Rhizopycnis is nestled among Acrocalymma species and is reduced to synonymy based on its morphology and phylogeny of DNA. Acrocalymmaceae was introduced into the Dothideomycetes class as a new family to accommodate members of this group. The family Acrocalymmaceae was also named after the genus Acrocalymma [17]. Acrocalymma represents an undefined lineage of Dothideomycetes that has Massarina-like sexual morphs in culture, establishing asexual or sexual propagation [18]. At present, seven species have been identified in different countries from diverse sources and habitats under the family of Acrocalymmaceae: A. aquatica, A. cycadis, A. fici, A. medicaginis, A. pterocarpi, A. vagum, and A. walker [19]. Among these seven species, two were identified based on their sexual morph: A. pterocarpi and A. walker from Thailand and Australia, respectively [17,19]. A. medicaginis was described as a sexual morph of Massarina walkeri [18]. Although some species were identified in different countries, there has not been a sexual or asexual morph recorded under this genus in Korea until now.

The aim of the overall study from which this report has emerged is to investigate the diversity of native fungal species in Korea based on cultural and morphological characteristics along with their molecular phylogenetic relationships. These previously undescribed fungal species of Ascomycetes were identified during this study.

2. Material and methods

2.1. Soil sample collection and fungal isolation

In 2017, the fungal candidates were obtained from soil samples collected from Sancheong-gun (35°20′15.0″N, 127°47′32.4″E), and Yeongam-gun (34°46′14.9″N, 126°39′21.1″E) in Korea. The soil samples were collected by using pre-autoclaved sterile spatula and shovel to randomly select composite soil samples and then removed the waste materials and debris. The collected soil was thoroughly mixed, transferred immediately into sterile plastic bags, and then transported to the laboratory. A serial dilution was performed, and 0.1 mL of sample plated on potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates [20]. To avoid bacterial contamination, ampicillin 100 μg/mL was added to the media before pouring into Petri dishes. The plates were incubated at 25°C for 3 or 4 days. Single colonies were transferred to fresh PDA plates and incubated at 25°C to obtain pure cultures. The fungal candidates were selected from among multiple candidates based on different cultural and morphological characteristics for further molecular analyses. Each pure fungal candidate was stored in 20% glycerol at −80°C until further use.

2.2. Morphological characterizations

Cultural characteristics and morphological observations were performed with different media depending on the genus and species. The strains 17E-042 and 17E-039 were transferred onto potato dextrose agar (PDA), oatmeal agar (OA), or malt extract agar (MEA) and incubated at 25°C temperature for 7 days [7,10]. Similar cultures of the strain 17E-039 were also incubated for 14–21 days under near-ultraviolet light [10]. The strain NC13-171 was sub-cultured on PDA, OA, and MEA at 25°C for 14 days [17,19]. Colony characteristics were recorded, and the mycological characteristics were observed by light microscopy (BX-50; Olympus, Tokyo, Japan).

2.3. Genomic DNA extraction, PCR amplification, and DNA sequencing

For molecular identification, the genomic DNA of each fungal strain was extracted using a HiGene Genomic DNA prep kit (BIOFACT, Daejeon, Korea), according to the manufacturer’s instructions. The ITS regions of rDNA genes were amplified using the primers ITS1 and ITS4 [21]. For 17E-042, a portion of the translation elongation factor 1-alpha (TEFI-α) gene was amplified and sequenced using primers EF1-728F [22] and EF-2 [23]. A portion of the β-tubulin (TUB2) gene was also amplified using primers T1 [24] and B2b [25]. In 17E-039, primers rRPB2-5F2 and rRPB2-7cR [26] were used to amplify RNA polymerase II large subunit (RPB2) along with ITS regions [27] and the partial 28S rDNA large subunit (LSU) gene using primer pairs LROR and LR5 [28]. In NC13-171, a portion of the nuclear 28S rDNA was also amplified using the primer pairs LROR and LR5 [28] along with the ITS regions. PCR amplicons were sequenced using the same primers as were used for the amplification reactions, purified with EXOSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA), and sequenced by Solgent (Daejeon, Korea). SeqMan Lasergene software
(DNASeq Inc., Madison, WI, USA.) was used to align the sequence data obtained in this study.

### 2.4. Phylogenetic analysis

The sequences generated in this study were supplemented with additional sequences obtained from the National Center for Biotechnology Information (NCBI) (Table 1). The sequences were selected from NCBI using BLAST searches to identify members closest to the strains isolated in this study. The evolutionary distance matrices for the neighbor-joining (NJ) algorithm were calculated using Kimura’s two-parameter model [29]. Phylogenetic relationships were also inferred by tree topology using maximum-likelihood (ML), and maximum-parsimony (MP) methods with MEGA7.0 software with bootstrap values based on 1000 replications [30].

#### Table 1. List of species used in this study and their GenBank accession numbers for phylogenetic analysis.

| Species Strain numbers | ITS | LSU | TUB2 | TEF1-α | RPB2 |
|-------------------------|-----|-----|------|--------|------|
| Arthrinium arundinis    | CBS 106.12 | KF144883 | – | – | – |
| A. aurantiaca           | CBS 244.83 | KF149025 | – | – | – |
| A. balearicum           | CBS 1451291 | MK014870 | – | – | – |
| A. bambusae             | LC7106 | KY494718 | – | – | – |
| A. camelliae-sinensis   | LC5007 | KY494704 | – | – | – |
| A. deschampsii          | CBS 1451301 | MK014870 | – | – | – |
| A. dichomantii          | LC4509 | KY494697 | – | – | – |
| A. gaoyouense           | CFCC52301 | MH179124 | – | – | – |
| A. guizhouense          | LC5318 | KY494708 | – | – | – |
| A. hydei                | CBS 114990 | KF144890 | – | – | – |
| A. langiense            | LC4577 | KY494693 | – | – | – |
| A. kogelbergense        | CBS 113332 | KF144891 | – | – | – |
| A. locutum-pollienis    | LC1683 | MF939595 | – | – | – |
| A. malaysianum          | CBS 201.46 | KF145025 | – | – | – |
| A. marinii              | CBS 497.90 | KF144905 | – | – | – |
| A. minutusporum         | 17E-0421 | LC517882 | – | – | – |
| A. obovatum             | LC4940 | KY494696 | – | – | – |
| A. phaeospermum         | CBS 114314 | KF144904 | – | – | – |
| A. phragmites           | CBS 135458 | KF144990 | – | – | – |
| A. pseudoparenchymaticum | LC7234 | KY494743 | – | – | – |
| A. pseudopogoniniii     | CBS 1020521 | KF144911 | – | – | – |
| A. pteroperum           | CPC 20193T | KF144913 | – | – | – |
| A. qinlingense          | CFCC 52303 | MH179120 | – | – | – |
| A. rhizosclerophora     | LC7115 | KY494721 | – | – | – |
| A. saccharicola         | CBS 191.73 | KF144920 | – | – | – |
| A. subrostrum           | LC7291 | KY494751 | – | – | – |
| A. thailandicum         | CBS 244.83 | KF145013 | – | – | – |
| A. xerocordellense      | CBS 595.66 | KF144920 | – | – | – |
| Sordaria phyletica      | CPC 19965 | LT853240 | – | – | – |
| Pezicula acercola       | CBS 245.97 | KR85909 | KR858889 | – | – |
| P. aurantiacus          | CBS 201.46 | KR859102 | KR858893 | – | – |
| P. carpinea             | CBS 921.96 | KR859107 | KR858898 | – | – |
| P. chlamydospora        | MFLUCC 15-01701 | KU310622 | KU310622 | – | – |
| P. cinnamomea           | CBS 626.96 | KF859152 | KR858894 | – | – |
| P. cornina              | CBS 285.39 | KF859163 | KR858895 | – | – |
| P. crylina              | CBS 249.97 | KF859168 | KR858890 | – | – |
| P. eucrita              | CBS 259.97 | KF859179 | KR858897 | – | – |
| P. foagarum             | CBS 112400T | KR855654 | KR858893 | – | – |
| P. microspore           | CBS 124641 | KR85909 | KR859004 | – | – |
| P. neocinnamomea        | CBS 100248T | KR855656 | KR859005 | – | – |
| P. neoheterochroma       | CBS 1273881 | KR855657 | NG58178 | – | – |
| P. neoparulae           | CBS 101.96T | KR853603 | MH847192 | – | – |
| P. neoparulae           | 17E-039 | LC517883 | – | – | – |
| P. occidentale          | CBS 949.97 | KF859233 | KR859025 | – | – |
| P. pseudocinnamomea     | CBS 101000 | KR855658 | KR859027 | – | – |
| P. rubi                 | CBS 253.97 | KF859320 | KR859042 | – | – |
| P. sporulosa            | CBS 224.96T | KR853603 | MH847199 | – | – |
| Rhizodermea velvensis   | CBS 110605T | KR853603 | RH859076 | – | – |
| Acrocalymma aquatic     | MFLUCC 11-0281 | JX226951 | GN042698 | – | – |
| A. crassipes            | CPC 17345 | KJ869124 | KJ869181 | – | – |
| A. lycites              | CBS 317.76T | KP170619 | KP170712 | – | – |
| A. medinicipiens        | CPC 243400 | KP170620 | KP170713 | – | – |
| A. pterocarpus          | MFLUCC 17-0926T | MK34773 | MK347949 | – | – |
| A. pterocarpus          | NC13-171 | LC517880 | – | – | – |
| A. vagnicola            | REF096 | JN859316 | JN859474 | – | – |
| A. walkeri              | CBS 257.93 | AF839365 | FJ795454 | – | – |
| Boeremia exigua         | CBS 431.74 | FJ427001 | JX681074 | – | – |

ITS: internal transcribed spacer regions; LSU: 28S rDNA gene; TUB2: β-tubulin; TEF1-α: translation elongation factor 1-alpha; RPB2: RNA polymerase II. The newly generated sequences were indicated in bold.
3. Results

3.1. Taxonomical analysis of Arthrinium minutisporum 17E-042

3.1.1. Taxonomy

The collected strain 17E-042 exhibited distinct morphology compared with other allied species. Therefore, it is described as a new species.

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

MycoBank: MB835039

Etymology: The specific epithet “minutisporum” refers to the small size of the conidia.

Typus: Sancheong-gun, Korea (35°20’15.0”N, 127°47’32.4”E), isolated from mountain soil. The stock culture (ZEVCFG0000000088 = KCTC 56425) was deposited in the National Institute of Biological Resources (NIBR) and Korean Collection for Type Cultures (KCTC) as a metabolically inactive culture.

Specimen examined: Sancheong-gun, Korea, isolated from mountain soil, deposited in NIBR Oct. 2017, H.Y. Jung, (holotype ZEVCFG0000000088, dried and living culture, ex-holotype living culture KCTC 56425).

Ecology and distribution: Several members of this genus have been recorded from different hosts as endophytes and as pathogens to plants, such as barley, wheat, lichens, and humans. However, there are so many members of this genus were also associated with the hosts and plant species in many countries such as *Hordeum vulgare* (Iran); *Phragmites australis* (Harderbois); leaf of bamboo, *Camellia sinensis*, *Brassica capestris*, *Phragmites australis*, air in karst cave (China); gut of a grasshopper, *Jatropha podagrica* (India); *Carex despalata* (Japan); plant species of Restionaceae family (South Africa); *Bambusa sp.* (Bangladesh); air and maritime sand (Spain); culms of *Bambusa tuldoides*, *Arundinaria hindsii* (Hong Kong); and also from leaf and culms of bamboo (The Netherlands, Thailand). Whereas, the present studied strain 17E-042 was collected from mountain soil in Korea. The soil contained plant debris, was yellowish brown, and had a low moisture capacity.

Cultural characteristics: Mycelia were superficial and immersed in media, with branched, septate, smooth hyphae. The colonies were flat, spreading with profuse aerial mycelium when cultured at 25°C for 7 days. The colonies were initially white and then developed a sienna color with a round margin on PDA, and the reverse side displayed a light sienna color. On MEA, the colonies were white.
with a light brown color on the reverse side. On OA, colonies produced a light sienna color in the center. At higher magnification on OA, colonies were dirty white with patches of olivaceous-gray. The growth extent of colonies is similar in all media tested: 79.0–84.0, 80.0–84.0, and 80.0–83.0 mm on PDA, OA, and MEA, respectively. Colonies also develop a surrounding light-yellow pigment with age on PDA.

**Morphological characteristics:** The micromorphological structures were studied by culturing on PDA. Mycelia were produced profusely and were hyaline, branched, smooth, septate, and 1.8–4.0 μm wide. Conidiophores were reduced to conidiogenous cells. The conidiogenous cells were erect, ellipsoid to ovoid, hyaline, pale brown to umber in color, finely roughened, sometimes curved or shrunk in the middle or at the edge of cells, and ellipsoid to subcylindrical. Diameter (length/width) ranges were 5.0–7.5 × 3.6–6.0 μm with an average diameter of 6.0 × 5.0 μm. The strain also forms sterile cells that were solitary or sometimes aggregated with the conidia, light brown to brown in color, finely roughened, sometimes curved or shrunk in the middle or at the edge of cells, and ellipsoid to subcylindrical. Diameter ranges (n = 10) were 10.2–14.2 × 3.8–4.8 μm with an average diameter of 12.1 × 4.3 μm. The strain produced numerous conidia that were brown, smooth to finely roughened, ellipsoid to ovoid, thick-walled, solitary to aggregated, with irregular dot-like structures inside the conidia. Diameter ranges (n = 100) were 5.7–8.2 × 4.6–7.0 μm with an average diameter of 6.7 ± 6.1 μm.

**Note:** The strain 17E-042 produced white colonies that developed a sienna color with a round margin on PDA; the reverse side displayed a light sienna color. However, *Arthrinium phragmites* displayed a dirty white color with pale luteous patches, and the reverse side was luteous. The colonies of *A. hydei* also produced pale luteous colonies on PDA (Table 2). The strain 17E-042 produced smaller conidiogenous cells with diameters of 5.0–7.5 × 3.6–6.0 μm. However, *A. phragmites* produced conidiogenous cells with diameters of 12.0–15.0 × 3.0–5.0 μm, *A. hydei* also produced conidiogenous cells with the diameters of 5.0–8.0 × 4.0–5.0 μm, and *A. aureum* was described with the integrated, polyblastic, and denticulate conidiogenous cells. The conidia of 17E-042 were also smaller (5.7–8.2 × 4.6–7.0 μm) than those of *A. phragmites* (9.0–10.0 (12.0) × (5.0–6.0 (7.0)) μm), *A. aureum* (10.0–30.0 × 10.0–15.0) μm and *A. hydei* (17.0(15.0)–(19.0(10.0)) × 11.0(10.0)–12.0(14.0–)) μm (Table 2). Sterile cells of *A. phragmites* were solitary loci on hyphae, brown, finely roughened, ellipsoid to clavate, with diameters of 13.0–15.0 (17.0) × (5.0–6.0) μm. However, the
strain 17E-042 produced sterile solitary cells that were aggregated with the conidia, light brown to brown, sometimes curved or shrunken in the middle or at the edge of cells, ellipsoid to subcylindrical, with diameters \( n = 10 \) of 10.2–14.2 \( \times \) 3.8–4.8 \( \mu \text{m} \) (Table 2). Therefore, the cultural and morphological characteristics supported that the strain 17E-042 is distinct from previously known species of \textit{Arthrinium}.

### 3.2. Phylogenetic analysis of the strain 17E-042

The BLAST search results of ITS regions sequences from the NCBI database reflected similarities between 17E-042 and \textit{Arthrinium hydei} G1863 (96%), \textit{A. phaeospermum} N.SBA34 (95%), and \textit{A. aureum} CBS 244.83\(^{\text{T}}\) (96%). The \textit{TEF1-\(\alpha\)} gene revealed similarities with \textit{A. camelliae-sinensis} LC3181 (90%), \textit{A. hydei} CBS 114990\(^{\text{T}}\) (89%), \textit{A. aureum} CBS 244.83\(^{\text{T}}\) (88%), and \textit{A. phaeospermum} CBS 114315 (85%). The \textit{TUB2} gene reflected similarities with \textit{A. raskravindrii} LC7129 (90%), \textit{A. phaeospermum} MAFF 410785 (89%), \textit{A. hydei} LC7103 (88%), and \textit{A. aureum} CBS 244.83\(^{\text{T}}\) (87%). A combination of ITS regions, \textit{TEF1-\(\alpha\)} and \textit{TUB2} genes was used to perform the phylogenetic analysis. Three phylogenetic trees were constructed using NJ, ML, and MP statistical method from concatenated sequences and the results were same. The ML and MP method results were also indicated in NJ tree with open and filled circles. Filled circles indicated that the corresponding nodes were recovered in trees generated with both the ML and MP algorithms. The tree positions generated with either the ML or MP algorithm were indicated with open circles (Figure 2). The boundaries within the \textit{Arthrinium} were defined by a combined alignment of ITS regions, \textit{TEF1-\(\alpha\)}, and \textit{TUB2} sequences to clarify the species that exhibited the highest sequence similarities and these boundaries contained 29 strains. The phylogenetic analysis was also conducted based on a combination of the sequences with MP (tree length = 1161, consistency index = 0.55, retention index = 0.71, and composite index = 0.43) to determine the exact taxonomic position of the strain. The phylogenetic tree showed that the phylogenetic position of the strain 17E-042 is distinct from the other identified species of \textit{Arthrinium} (Figure 2). Consequently, the strain 17E-042 is proposed as a new species in the fungal flora under the genus of \textit{Arthrinium}.

### 3.2.1. Taxonomy

The strain 17E-039 was isolated from mountain soil in Sancheong-gun, Korea (35°20′15.00″N, 127°47′32.4′′E) exhibited the cultural and morphological characteristics (Table 3) that were similar to the identified \textit{Pezicula neosporulosa}, which has not been reported in Korea previously.

**Specimen examined:** Sancheong-gun, Korea (35°20′15.00″N, 127°47′32.4′′E), isolated from mountain soil, deposited in NIBR July, 2017, H.Y. Jung, (NIBRFG0000501934, dried and living culture, National Institute of Biological Resources).

**Ecology and distribution:** The members of the genus \textit{Pezicula} reported previously from temperate regions, where they occur as endophytes or as saprobes on recently dead branches, twigs, and herbaceous plants such as wood bark in Northern Thailand. They were also the causal agent of bull’s-eye rot on Japanese pears, even responsible for stem canker disease on apples and pears. Moreover, there are so many members of this genus were also associated with the plant species in many countries such as \textit{Acer spicatum}, \textit{Gaultheria shallon} (Canada), \textit{Eucalyptus} sp. (USA), \textit{Carpinus betulus} (Netherlands, Germany), \textit{Abies balsamea} (Norway), \textit{Fagus sylvatica} (France), and also from \textit{Juniperus communis} (Switzerland). Whereas, the strain 17E-039 was isolated from mountain soil in Korea. The soil contained plant debris, was dark brown to yellowish brown, and had a low moisture capacity.

**Cultural characteristics:** The colonies on PDA were flat, white to brown, with aerial mycelium, and dark brown pigment developed on the agar media; the colonies extended to 29.0–32.0 mm in diameter when cultured at 25 °C for 14 days. On the reverse side, colonies were brown to black, with a dark yellow margin at the edge. Colonies on MEA were flat, white to pale brown, with submerged mycelium, pale yellow to dark yellow; the colonies extended to 28.0–30.0 mm diameter. On the reverse side, colonies were white at the edge and brown in the center. Colonies grow faster on OA than on other media, reaching 44.0–69.0 mm diameter under identical incubation conditions. Colonies were flat and white, and on the reverse side, colonies were pale brown to brown in the center.

**Morphological characteristics:** The asexual morph was studied \textit{in vitro} on OA media to describe the morphology. Conidiomata were produced profusely on the agar surface, globose to subglobose, initially closed with white to gray hyphae. The structures then became disc-like with a diameter range of 0.5–1.2 mm; these structures produced water droplets, with white to pale yellow conidial slime that contained micro- and macroconidia. Macroconidiogenous cells were hyaline, flask-shaped.
to cylindrical, simple, or branched. The macroconidiogenous cells proliferate percurrently, achieving a diameter (length/width) range of $7.2 - 23.1 \times 4.5 - 9.1 \mu m$, with an average diameter of $16.4 \times 7.3 \mu m$. Microconidiogenous cells were hyaline, phialidic, with a minute periclinal thickening, having a diameter range of $7.5 - 14.7 \times 1.5 - 2.5 \mu m$ with an average diameter of $10.2 \times 2.1 \mu m$.

Macroconidia were pale yellow to orange-brown, elongated-ellipsoid to oblong, round at both ends, primarily straight but sometimes slightly curved, 0-septate, thin-walled, and filled with numerous droplets, having diameters ($n = 50$) of $20.4 - 34.3 \times 8.0 - 12.0 \mu m$ with an average diameter of $26.5 \times 10.0 \mu m$. Microconidia were cylindrical, straight, sometimes slightly bent but typically wider.

**Figure 2.** Neighbor-joining phylogenetic tree of 17E-042 based on combined sequences data (ITS+TUB2+TEF1) showing the relationship between *Arthrinium minutisporum* sp. nov., and the closest *Arthrinium* spp. The tree was rooted using *Seiridium phylicae* CPC 19965 as an outgroup. The numbers above the branches represent the bootstrap values (ML/MP/NJ) obtained for 1000 replicates (values smaller than 50% were not shown). The isolated strain of this study is indicated in bold. Bar, 0.02 substitutions per nucleotide position.
in the middle than near the rounded top, base truncate, hyaline, thin-walled, having diameters \( n = 30 \) of \( 7.6-15.0 \times 1.2-1.7 \) \( \mu \)m with an average diameter of \( 12.0 \times 1.4 \) \( \mu \)m.

### 3.2.2. Phylogenetic analysis of strain 17E-039

Nucleotide sequences data encompassing 536 bp of the ITS regions, 830 bp of 28S rDNA, and 912 bp of \( RPB2 \) were obtained to investigate the evolutionary
relationships between the strain 17E-039 and previously described species of *Pezicula neosporulosa*. ITS sequences from BLAST search results revealed the maximum similarity (99.81%) with the different strains of *P. neosporulosa* (CBS 101.96ᵀ, CBS 724.95, CBS 636.96, CBS 102.96, CBS 100416). The 28S rDNA displayed the maximum similarity (100%) with the strains of *P. neosporulosa* (CBS 101.96ᵀ, CBS 635.96, CBS 100416) and also showed high similarity (99.88%) with the strains of *P. neosporulosa* (CBS 636.96, CBS 660.95, CBS 724.95, CBS 102.96). The highest similarities with RPB2 displayed with *P. eucrita* CBS 259.97 (98.53%), *P. sporulosa* CBS 225.96 (98.53%), *P. sporulosa* CBS 224.96ᵀ (98.42%), and *P. cinnamomea* CBS 626.96 (97.97%). A phylogenetic analysis was conducted based on a combination of the ITS regions, partial sequences of the 28S rDNA, and RPB2 genes by using the NJ method (Figure 4). The taxonomic position of the strain 17E-039 was same in each three phylogenetic tree methods. Filled circles indicated that the corresponding nodes were also recovered in trees generated with both the MP and MP algorithms. The tree positions generated with either the ML or MP algorithm were indicated with open circles (Figure 4). Phylogenetic analyses using the NJ method revealed that the strain 17E-039 clustered with the previously identified strain *P. neosporulosa* CBS 101.96ᵀ (Figure 4). Consequently, the strain 17E-039 is identified as *Pezicula neosporulosa*, a previously undescribed fungal species in Korea.

### 3.3. Taxonomical analysis of *Acrocalymma pterocarpi* NC13-171

*Acrocalymma pterocarpi* Jayasiri SC, Jones EBG and Hyde KD, Mycosphere 10 (1): 20 (2019) (Figure 5)

#### 3.3.1. Taxonomy

The strain NC13-171 was isolated from field soil in Yeongam-gun (34°46′14.9″N, 126°39′21.1″E) exhibited similar cultural and morphological characteristics to the identified *Acrocalymma pterocarpi*, which has not been reported in Korea previously.

**Specimen examined:** Yeongam-gun, Korea (34°46′14.9″N, 126°39′21.1″E), isolated from field soil, deposited in NIBR May, 2017, H.Y. Jung, (ZEVCLC00000000002, dried and living culture, in the National Institute of Biological Resources).

**Ecology and distribution:** The member of the genus *Acrocalymma* associated with a root and

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**Figure 4.** Neighbor-joining phylogenetic tree of 17E-039 based on combined sequences data (ITS + LSU + RPB2) showing the relationships between *P. neosporulosa* and the closest *Pezicula* spp. The tree was rooted using *Rhizoderma veluwensis* CBS 110605ᵀ as an outgroup. The numbers above the branches represent the bootstrap values (ML/MP/NJ) obtained for 1000 replicates (values smaller than 60% were not shown). The isolated strain of this study is indicated in bold. Bar, 0.01 substitutions per nucleotide position.
Crown rot disease of lucerne (*Medicago sativa*), found from freshwater in Thailand, leaves of *Cycas calcicola* in Australia, on *Medicago sativa* from Australia, and also from *Pterocarpus indicus* seed pod in Thailand. Whereas, the present studied strain NC13-171 was collected from field soil in Korea. The soil contained plant debris, was dark brown to blackish, and had a medium moisture capacity.

**Cultural and morphological characteristics of the strain NC13-171**

The mycelial colonies were white to pale gray, circular, and dense, with a smooth edge. On the reverse side, colonies were light brown to dark brown in the center on PDA. The colonies were circular, dense, white, rough in the middle with a smooth edge, and slightly undulate. On the reverse side, colonies were slightly yellow on MEA and OA. Fungal growth was circular with diameters of 42.0–51.0, 48.0–52.0, and 55.0–58.0 mm on PDA, MEA, and OA, respectively, when incubated at 25 °C for 14 days. The strain NC13-171 developed structures on the MEA surface after 4–5 weeks of incubation at 25 °C. The strain NC13-171 also produced compact chlamydospores, solitary or in a chain, and hyaline to light brown. Previous studies reported that the only species with a teleomorph is *Acrocalymma walker*, while the other five species were coelomycetous [18]. Recently, another strain identified as a teleomorph of *A. pterocarpi* was isolated from a decaying *Pterocarpus indicus* seed pod in Thailand. However, *A. pterocarpi* failed to produce anamorphic structures in culture, and only chlamydospores were observed [19]. The strain NC13-171 was isolated from the soil and cultured on different media to assess the cultural and morphological characteristics. However, NC13-171 failed to develop teleomorphic structures in culture but produced anamorphic structures, such as chlamydospores, like those of *A. pterocarpi*. Therefore, the cultural and morphological characteristics supported that the strain NC13-171 was *Acrocalymma pterocarpi*, and is reported in Korea for the first time.

### 3.3.2. Phylogenetic analysis of strain NC13-171

Nucleotide sequences data encompassing 489 bp of the ITS regions and 837 bp of the 28S rDNA were obtained to determine the evolutionary relationships between the strain NC13-171 and related strains obtained from GenBank (Table 1). The BLAST results from the ITS regions sequence data showed 99.16% similarity with the recently identified type strain *Acrocalymma pterocarpi* (MFLUCC 17-0926T) from Thailand. The 28S rDNA sequences revealed the maximum similarity (99.76%) with the type strain *A. pterocarpi* (MFLUCC 17-0926T). The phylogenetic tree was constructed using NJ method based on the combination of ITS regions and the partial sequences of 28S rDNA. NC13-171 was
clustered together with the previously identified A. pterocarpi (MFLUCC 17-0926T) with strong boot-strap values (99%). The taxonomic position of the strain NC13-171 was same in each three-NJ, ML, and MP statistical method from concatenated sequences. Filled circles indicated that the corre-sponding nodes were recovered in trees generated with both the ML and MP algorithms. Tree posi-tions generated with open circles either the ML or MP methods in the MP algorithm (Figure 6). The phylogenetic results strongly supported the identifi-cation of NC13-171 as Acrocalymma pterocarpi. To our best knowledge, this is the first report of Acrocalymma pterocarpi isolated from soil in Korea.

4. Discussion

The morphologically distinct strain 17E-042 and two unreported strains, 17E-039 and NC13-171, were obtained in 2017 from the soil at Sancheong-gun and Yeongam-gun in Korea, respectively. The strains exhibited morphological differences and similarities with the identified and closely related species; these findings were supported by the descriptions of closely related species in previously published reports.

Arthrinium is also an endophyte in plant tissues [33], lichens [34], and soils [5] and can also cause infections in humans [35]. Many Arthrinium species produce bioactive compounds with pharmacological and medicinal applications, such as A. arundinis and A. saccharicola isolated from a brown alga Sargassum sp. [36], A. saccharicola, A. sacchari and A. phaeospermum isolated from Miscanthus sp. produce industrially important enzymes [37]. A. marii occurs most frequently on Poaceae grasses Ampelodesmos mauritanicus and Phragmites australis, and A. phragmitis is commonly found on Phragmites australis and less commonly on Arundo donax [7]. Recently, six new species of Arthrinium have been proposed: A. balearicum, A. descalsii, A. esporlense, A. ibericum, A. italicum, and A. pipta-theri. Five of these species were found in the Mediterranean biogeographical region; the exception was A. ibericum, which was found in the Atlantic areas of Spain associated with host plants of the Poaceae family, such as Arundo donax and Piptatherum miliaceum [3]. Moreover, Arthrinium species were also isolated from soil in different places such as A. rasikravindrii (karst cave); A. arundinis (karst cave and roadway); A. xenocordella (roadway) [4]. Even, some species were also found from soil in different countries including A. xenocordella (Zimbabwe), A. phaeospermum (Japan), A. sacchari (Netherlands), and A. xenocordella (Austria).
from freshwater in Thailand [44], and Pezicula Pseudotsuga menziesii from Netherlands. The member identified as the causal agent of bull’s-eye rot on Japanese pears, P. cinnamomea was isolated from stem canker disease on apples and pears [40]. Pezicula species also have pharmaceutical importance as antibiotics such as mellein, mycorrhizin, cryptosporiopsin, cryptocandin, and cryptocin have been isolated from endophytic Pezicula and Cryptosporiopsis species [41]. Moreover, P. neosporulosa was isolated from diversified host plants such as Amelanchier lamarkii, Abies alba, Larix decidua, Pseudotsuga menziesii from Netherlands. The member of Pezicula (Pezicula ocellata) was found from the plant species of Salix sp. in Germany, P. melanigena (asexual morph Cryptosporiopsis melanigena) from Quercus petraea in Austria, and P. microspora from an endophyte called Berberis vulgaris in Italy [42]. From the previous studies, the members of the Pezicula species were hardly found from soil, whereas, the newly identified undescribed species called Pezicula neosporulosa was found in soil during the investigation of native fungal diversity in Korea.

The genus Acrocalymma was established for A. medicaginis, a species associated with a root and crown rot disease of lucerne (Medicago sativa) [15]. Stagonospora meliloti is also responsible for lucerne crown and root rot disease and is prevalent in southern New South Wales [16]. Both pathogens (A. medicaginis and Stagonospora meliloti) cause redening of the internal root and crown tissue of lucerne, and they can be distinguished by the symptoms they induce [43]. A. aquatica was isolated from freshwater in Thailand [44], and A. cycadis was isolated from the leaves of Cycas calcicola in Australia [45]. A. walkeri is the only species with a sexual morph found on Medicago sativa from Australia [17]. Recently, another sexual morph of A. pterocarpi was identified from a Pterocarpus indicus seed pod in Thailand [19]. There are different members of the genus Acrocalymma were isolated from submerged wood in a freshwater stream, leaf litter of Cycas calcicola, Ficus sp., Medicago sativa, Amaranthus sp., Citrullus lanatus, Cucumis melo, C. sativus, Cucurbita rootstock, and also from Vitis vinifera in different countries [19]. To date, there are seven species identified including some of their sexual morph from different countries with the diversified host plants, but there has not been a sexual or asexual morph recorded under this genus in Korea until now. Although members of the genus Acrocalymma occur in many habitats worldwide, but the identified strain NC13-171 was isolated from soil in Korea.

Microorganisms are receiving more attention in recent years because of their harmful effects, their important role in our ecosystems, and their production of compounds that are used in pharmaceuticals. In this study, the identification of the Arthrinium species, Pezicula neosporulosa, and Acrocalymma pterocarpi was reported: cultural, morphological characteristics, and molecular phylogenetic relationships. Further investigation is necessary to reveal the pharmaceutical importance and potential pathogenicity to the range of host species present in the ecological and environmental conditions of Korea. To our best knowledge, this is the first report of Pezicula neosporulosa and Acrocalymma pterocarpi in Korea, and Arthrinium minutisporum is proposed as a novel species.

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Disclosure statement

The authors declare that they have no potential conflicts of interest.

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