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RESEARCH ARTICLE

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A Novel *Acremonium* Species Isolated from Air Samples in Korea

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ABSTRACT

The aim of this study was to characterize a new fungal species, *Acremonium conglutinatum*, isolated from air samples collected in Wando, South Korea. Phylogenetic analysis based on the internal transcribed spacer and large subunit regions revealed its unique position within the genus *Acremonium*. The isolated strain displayed distinct morphological characteristics, including ellipsoid or bent-ellipsoid conidia formed in clusters on the phialides. These features differentiate the new species from closely related species within the genus. This study describes the morphological and molecular characteristics of *A. conglutinatum* and emphasizes its phylogenetic relationships with other *Acremonium* spp. The identification of this novel species contributes to our understanding of the diversity and ecological role of *Acremonium*.

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Acremonium conglutinatum; air samples; hypocreales; morphological characteristics; phylogenetic analysis

1. Introduction

The genus *Acremonium* comprises fungi with various morphological traits such as septate hyphae, mostly tapered lateral phialides produced singly or in small groups, and unicellular conidia produced in mucoid heads or unconnected chains [1]. The taxonomic complexity of *Acremonium*, coupled with its morphological similarities to other genera, such as *Emericellopsis* and *Sarocladium*, makes the identification of species within this genus challenging [1–3]. *Acremonium* spp. are widely distributed in natural environments, including soil, air, and rocks [4]. Molecular phylogenetic analyses play crucial roles in classifying *Acremonium* spp. Glenn et al. [3] used a small subunit region in their analysis and revealed that *Acremonium* forms multiple clades and is distributed among at least three different orders of the phylum Ascomycota, along with species from other genera. Recent studies have employed both the large subunit (LSU) region and small subunit region to show that *Acremonium* spp. form two major clades within the order Hypocreales [1]. The purpose of the present study was to characterize a novel *Acremonium* species isolated from air samples in Korea using molecular phylogenetic analysis of the internal transcribed spacer (ITS) and LSU regions. By describing the morphological features and comparing the characteristics of this new *Acremonium* spp. with those of phylogenetically related species, we hope to provide a better understanding of *Acremonium* taxonomy and diversity.

2. Materials and methods

2.1. Fungal materials

Cultures were isolated from outdoor air collected in September 2022 from Gogeum-myeon (34.400644N, 126.857823E), Wando-gun, Jeollanamdo Province, South Korea. Fungal materials were collected by setting petri dishes containing malt extract agar (MEA; Kisan Bio, Seoul, South Korea) supplemented with chloramphenicol in outdoor air. The petri dishes were cultured at 25 °C in the dark. Next, the individual colonies were sub-cultured until pure strains were obtained. Cultures were deposited in the herbarium of the Korea National University of Education.

2.2. Morphological observation

Isolated strains were cultured on potato dextrose agar (PDA; Difco Laboratories Inc., Detroit, MI), MEA (Kisan Bio), oatmeal agar (OA; Kisan Bio) at 25 °C in the dark for seven days. After culturing, the diameter, color, margins, and texture of the colonies were recorded. The microstructure was

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observed using an optical microscope (Axio Imager A1; Carl Zeiss, Oberkochen, Germany).

2.3. Molecular analysis

DNA was extracted from mycelium of the isolates cultured on PDA using a HiGene Genomic DNA Prep Kit (BioFACT, Daejeon, South Korea) following the manufacturer's protocol. The ITS and LSU regions were amplified using primer sets ITS1F/ITS4 [5] and LR0R/LR16 [6], respectively. The PCR products were purified and sequenced by the company Solgent (Daejeon, South Korea).

2.4. Phylogenetic analysis

The obtained nucleotide sequences were identified using BLAST (https://www.ncbi.nlm.nih.gov/). Through phylogenetic analysis, the ITS and LSU sequences were compared with sequences from other phylogenetically related *Acremonium* spp. along with the outgroup *Pestalotiopsis thailandica* (Table 1). The sequences were aligned by ClustalW in MEGA7 software [7,8]. Phylogenetic trees were constructed with the neighbor-joining (NJ) and the maximum-likelihood (ML) methods utilizing either the ITS or LSU sequences separately or in combination. The Kimura-2-parameter model was employed with 1000 bootstrap replications to all phylogenetic tree constructions.

3. Results

3.1. Phylogenetic analysis

The ITS region of Y22O-20 showed the highest similarity (96.52%) to *Acremonium roseolum* CBS 289.62 (MH858153.1), and the LSU region also showed the highest similarity (99.32%) to *A. roseolum* CBS 289.62 (MH869748.1). In the phylogenetic trees constructed using each of the ITS region alone and the combined ITS-LSU regions, Y22O-20 was proven to belong to the same clade as *A. roseolum* (Figure 1). NJ and ML phylogenetic analyses of the LSU regions showed that Y22O-20 was grouped together with *A. roseolum* and *Acremonium inflatum* in the same clade but formed a separate clade with the sister groups of *A. roseolum* and *A. inflatum* (Figure 2).

3.2. Taxonomy

Acremonium conglutinatum sp. nov. A. H. Eom, J. M. Lee, J. E. Cha, Y. S. Yoon (Figure 3).

MycoBank: MB848316.

Etymology: The specific epithet refers to the cluster of conidia.

Typification: South Korea, Jeollanamdo Province, Wando-gun, Gogeum-myeon (34.400644N, 126.857823E), isolated from outdoor air in September 2022 by Y. S. Yoon. Holotype Y22O-20 (accession number: KACC410368).

DNA barcodes: ITS OQ048432, LSU OQ048428.

Cultural characteristics: Colonies were incubated on PDA, MEA, and OA at 25 °C for seven days. The colonies grown on PDA displayed a white

Table 1. Details of the strains used in phylogenetic analysis.

		GenBank accession numbers	
Species	Strain numbers	ITS	LSU
Acremonium sp.	Y22O-20	OQ048432	OQ048428
Acremonium biseptum	CBS 750.69 T	NR_159609	NG_056978
Acremonium borodinense	CBS 101148 T	HE608635	HQ232003
Acremonium citrinum	CBS 384.96 T	NR_154670	HF680217
Acremonium exiguum	CBS 587.73 T	NR_159619	NG_056981
Acremonium fusidioides	CBS 840.68 T	FN706542	NG_056984
Acremonium hennebertii	CBS 768.69 T	NR_145043	NG_056987
Acremonium inflatum	CBS 212.69		MH877797
Acremonium inflatum	CBS 439.70		MH871550
Acremonium persicinum	CBS 310.59	MH857868	MH869409
Acremonium pinkertoniae	CBS 157.70 T	NR_159611	NG_058554
Acremonium mali	ACCC 39305 T	MF987658	NG_088063
Acremonium nigrosclerotium	CBS 154.72 T	NR_159617	NG_066250
Acremonium roseolum	CBS 289.62 T	MH858153	MH869748
Acremonium roseolum	CBS 267.70	MH859602	MH871365
Acremonium sclerotigenum	CBS 124.42 T	MH856101	MH867594
Acremonium tectonae	CBS 725.87		HQ232144
Acremonium variecolor	CBS 130360 T	HE608647	HE608651
Acremonium verruculosum	CBS 990.69	MH859502	MH871282
Parasarocladium breve	CBS 150.62 T	MH424706	NG_056979
Parasarocladium gamsii	CBS 726.71 T	NR_159615	NG_056985
Parasarocladium radiatum	CBS 142.62 T	NR_161112	MH869704
Pestalotiopsis thailandica	MFLUCC 17-1616 T	NR_164471	NG_070088

ITS: internal transcribed spacer; LSU: large subunit; T: type strain.

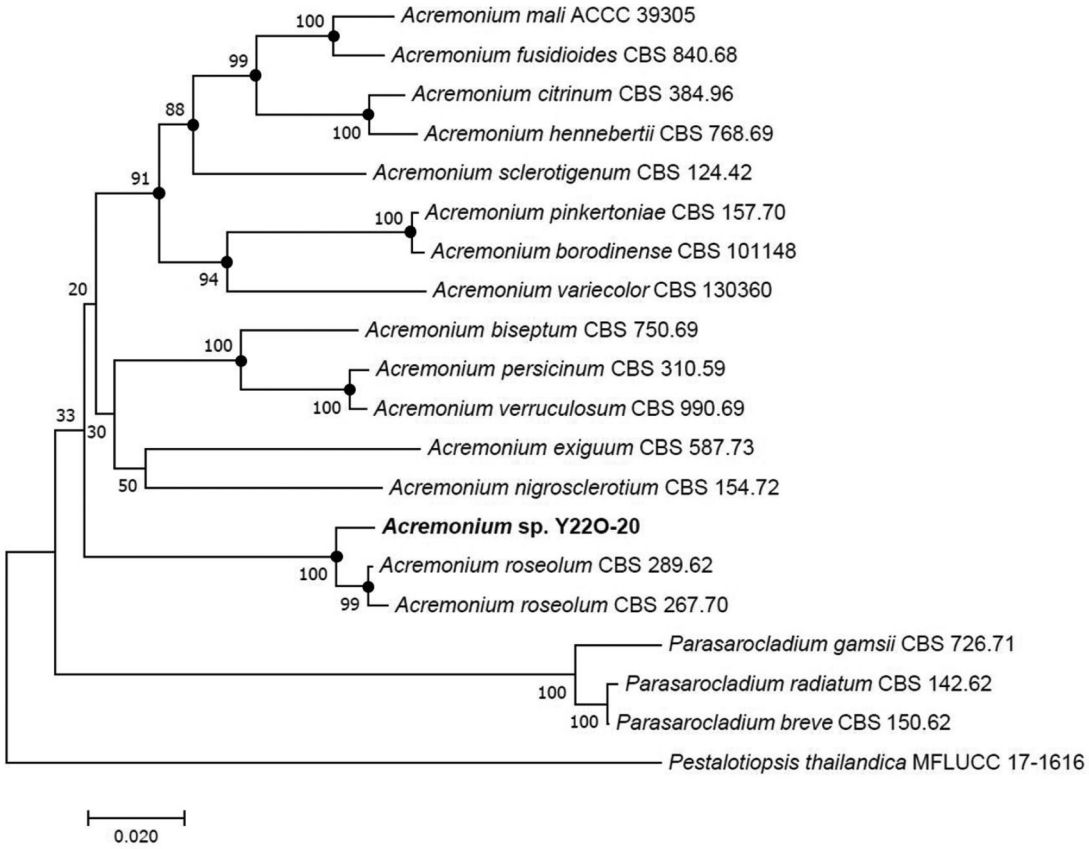

Figure 1. Neighbor-joining phylogenetic tree constructed via the combined internal transcribed spacer and large subunit regions. *Pestalotiopsis thailandica* was included as an outgroup. Bootstrap analysis was performed with 1000 replications. The black circles on the nodes indicate bootstrap support values of ≥75% at both neighbor-joining and maximum-likelihood analyses. The strain Y22O-20 is marked in bold.

color, nearly circular shape, undulate margin, and powdery surface. They were concentric, had a diameter of 20.92–23.75 mm, and possessed an ivory reverse side. On MEA, cultures were characterized with a white color, nearly circular shape, undulated margin, and a velvety surface. Colonies were also concentric, had a diameter of 20.59–23.58 mm, and exhibited a light ivory reverse side. Lastly, colonies grown on OA were white in color, circular in shape, evenly margined, and velvety surfaced. Moreover, they were faintly concentric and had a diameter of 23.68–28.05 mm.

Morphological characteristics: The isolated species exhibited smooth-walled hyaline hyphae of 1.00–1.50 µm width. Conidiophores were curved or erect with smooth walls and a length and width of 13.59–33.91 µm and 2.31–2.69 µm, respectively. The phialides were erect and smooth-walled, measured 10.2–30.5 µm in length and 1.5–2.3 µm in width. Phialides width narrowed near the base to 1.00–1.05 µm. Notably, we observed 1–2 developed phialides per conidiophore. Lastly, the species had conidia that were ellipsoid or bent-ellipsoid in shape, hyaline, and smooth-walled with a diameter of 8.1–13.1 × 2.6–4.8 µm (n = 45, mean = 10.5 × 3.4 µm). Furthermore, we observed that conidia formed in 1–4 clusters at the end of the phialide, along with other 3–4 cluster groups (Figure 3).

Note: Phylogenetic analysis showed that strain Y22O-20 belongs to the same clade as the species *A. roseolum* and *A. inflatum* but forms a separate clade with these two species (Figures 1 and 2). Despite the challenges in classifying *Acremonium* species based on their morphology, the conidial shape of strain Y22O-20 was notably distinct from those of these two species, *A. roseolum* and *A. inflatum* (Table 2). The conidia of *A. roseolum* are lemon-shaped, smooth, with a diameter of 6–7 × 3–3.2 µm (mostly 7 × 3 µm), disposed in very long tangled chains [9]. *A. inflatum* conidia are shaped variably, green in mass, smooth-walled, with 2.8 × 1.9 µm. Moreover, the conidia accumulate at the phialide apex [10]. The conidia of strain Y22O-20, however, were clearly larger than those of the related species, ellipsoid or bent-ellipsoid in shape, and formed in clusters on phialides. The conidiophores of the related species were very short; however, Y22O-20 conidiophores measured up to 13.59–33.91 µm in length. Additionally, the colony growth of the two species was slow, with *A. inflatum* showing a

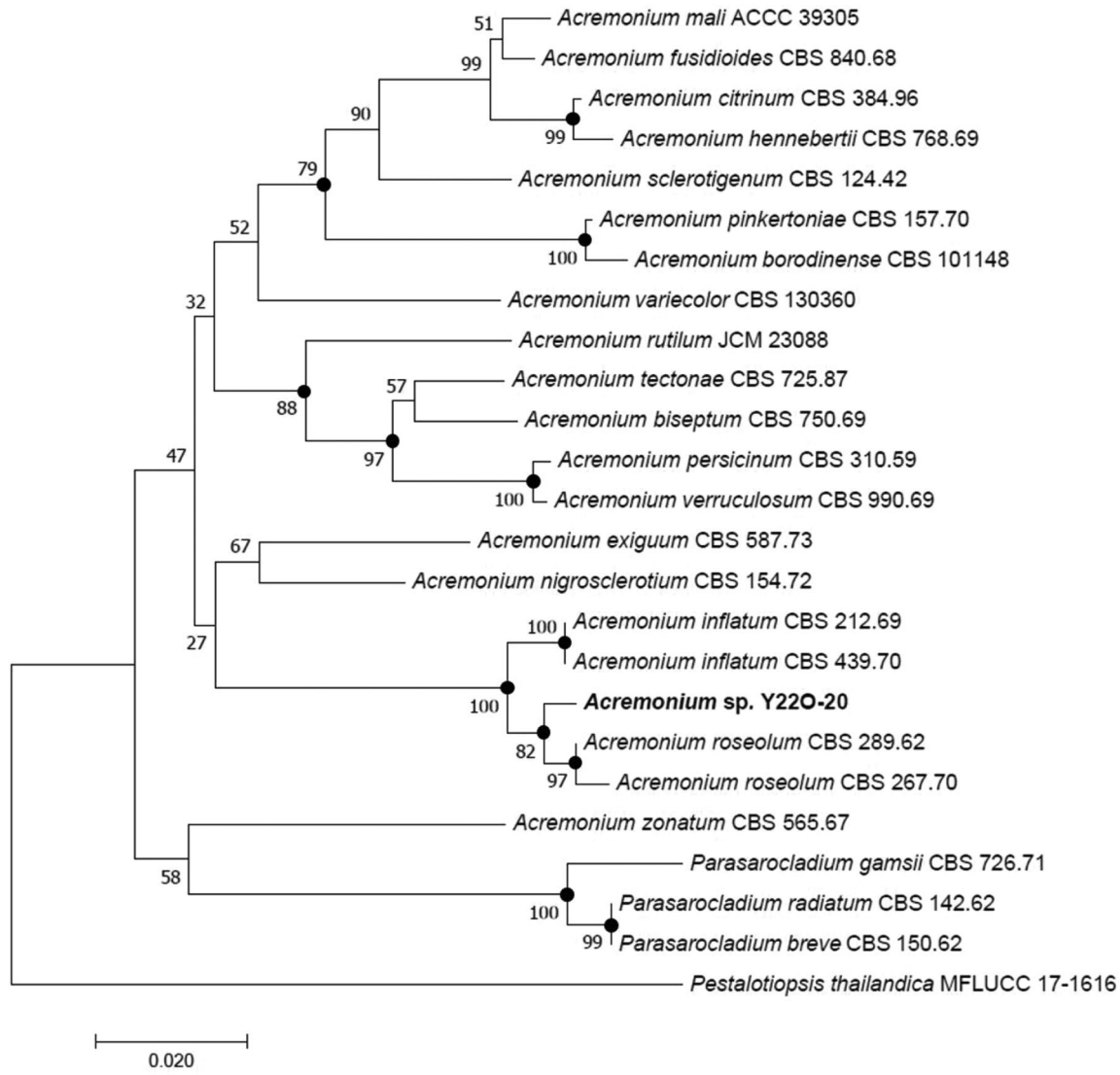

Figure 2. Neighbor-joining phylogenetic tree constructed via the large subunit. *Pestalotiopsis thailandica* was included as an outgroup. Bootstrap analysis was performed with 1000 replications. The black circles on the nodes indicate bootstrap support values of ≥70% at both neighbor-joining and maximum-likelihood analyses. The strain Y22O-20 is marked in bold.

diameter of 8–9 mm on malt agar after a total of 14 days [9,10], whereas strain Y22O-20 showed a diameter of 20.59–23.58 mm after only seven days of growth.

4. Discussion

Acremonium spp. are commonly found in soil and plants [11] and have been shown to be infectious to both plants and humans. Their morphological similarities and low antifungal susceptibility make them difficult to diagnose and treat [4]. In China, they have been identified as the cause of brown spots on bagged apples [12]. Acremonamide, which exhibits wound healing effects, was recently isolated from *Acremonium* [13]. The dual nature of infectivity and the production and extraction of beneficial substances in plants and humans highlights the importance of studying *Acremonium* spp.

Efforts to define *Acremonium* spp. through phylogenetic analyses are ongoing, but the species are not yet fully understood [1]. Strain Y22O-20 formed a distinct single clade with closely related species in phylogenetic analysis, and clear morphological differences were observed in its microstructure. The discovery of this new species is important because it shows major morphological differences from known *Acremonium* spp. Furthermore, it is necessary to continuously evaluate the characteristics and determine the role of *Acremonium* in human ecosystems.

The present study aimed to characterize a novel *Acremonium* sp. isolated from air samples in Korea, using molecular phylogenetic analysis of the ITS and LSU regions. Phylogenetic analysis showed that the isolated strain Y22O-20 was closely related to *A.*

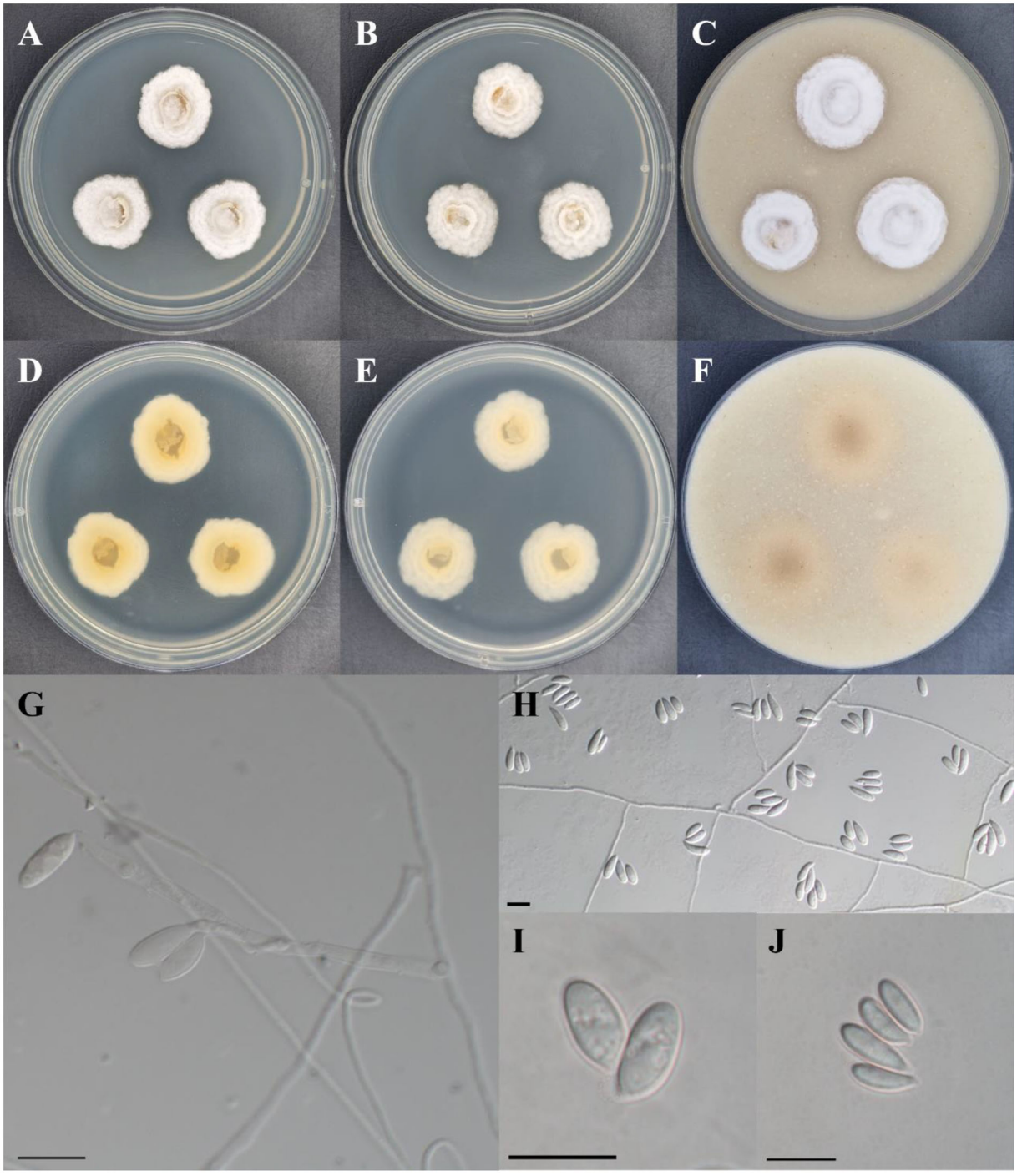

Figure 3. Morphological characteristics of *Acremonium* sp. Y22O-20 colonies on potato dextrose agar (A, D), malt extract agar (B, E), and oatmeal agar (C, F) at 25 °C for 7 days; conidiophore, phialides, and conidia (G); conidia (H–J). Bars = 10 μm.

roseolum and *A. inflatum* but formed a separate clade together with them, indicating a distinct species. Morphological observations further supported the distinction between the new species, *A. conglutinatum*, and the related species, *A. roseolum* and *A. inflatum*. *A. conglutinatum* exhibited differences in conidial shape, size, and cluster formation on phialides compared to *A. roseolum* and *Acremonium exiguum*.

In conclusion, it is essential to consider all aspects of this new species, including its classification, pathogenicity, and ecology. Further investigation is needed to uncover its potential. Based on morphological characteristics and phylogenetic analysis, strain Y22O-20 was distinct from previously recorded species within the genus *Acremonium*. Therefore, *A. conglutinatum* has been proposed as a novel species. Our findings contribute to the understanding of *Acremonium* taxonomy and diversity, and the description of this novel species will serve as a valuable reference for future studies on *Acremonium* spp. and related genera.

Table 2. Morphologic characteristics description of *Acremonium* sp., *Acremonium roseolum*, and *Acremonium inflatum*.

Strain name	Cultural characteristics	Conidiophores and phialides	Conidia
Acremonium sp. Y22O-20	Cultures grown on PDA at 25 °C for 7 days: almost circular, white, undulate margin, powdery texture, concentric, and 20.92–23.75 mm diameter. Cultures grown on MEA at 25 °C for 7 days: almost circular, white, undulate margin, velvety texture, concentric, and 20.59–23.58 mm diameter.	Conidiophores: erect or curved, smooth-walled, 13.59–33.91 μm length, and 2.31–2.69 μm wide. Phialides: erect, smooth-walled, 10.23–30.54 μm length, 1.54–2.26 μm diameter, tapering toward 1.00–1.05 μm. 1–2 phialides were developed per conidiophore.	Ellipse or bend-ellipsoid shape, hyaline, smooth-walled, 8.12–13.09 × 2.63–4.85 μm, formed 1–4 conidia within cluster.
Acremonium roseolum CBS 289.62 T	Cultures grown on PDA and MEA at 24 °C: slow-growing, compact, zonate, almost velvety in appearance but seen to be funiculose when examined under the microscope. White at first then pale rose-pink, with reverse brownish and sometimes with a slight purplish tone.	Absent, or consisting at most of a single very short cell, bearing a single phialide. Phialides: solitary, 20–32 μm long and 2–2.8 μm in diameter at the base tapering from about half the length to a slender tip of 0.5–1 μm diameter.	Lemon-shaped smooth, 6–7 × 3–3.2 μm, mostly 7 × 3 μm, disposed in very long tangled chains.
Acremonium inflatum IMI 100877	Cultures grown on malt agar at 25 °C for 14 days: 8–9 mm diameter, restricted, velvety, with little aerial mycelium, narrow, entire margin, and heavily sporing. Hyaline colony becoming dark green, reverse pale green.	Phialophores: not readily distinguished but frequently vegetative cells that increase in size and produce 2–3 erect, sessile phialides. Phialides: straight or sinuous, hyaline, smooth-walled, 11–22 μm long, often swollen below to 1.9–3.3 μm wide then tapering to a narrow tube of 0.6–1.0 μm wide at the apex, which is usually surmounted by a small, hyaline, and tubular collarette.	Non-septate, variable in shape, globose, ovoid, cylindrical, or allantoid, with thin smooth walls, green in mass, dilute green when viewed singly, 2–4.3 μm long, 1.6–2.3 μm wide, mostly 2.8 × 1.9 μm.

Disclosure statement

No potential competing interest was reported by the authors.

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