

Taxonomic Study of Sixteen Unrecorded and Five New Species of *Hypocreales* from the Korean Marine Environment

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ABSTRACT

The order *Hypocreales*, which belongs to the *Ascomycota* class *Sordariomycetes*, has a large number of species and occupies a variety of ecological niches, including saprophytic, symbiotic, and parasitic fungi. While much research has focused on terrestrial *Hypocrealean* fungi, there remains a significant gap in our understanding of their diversity and ecological roles in marine environments. In this study, we isolated 47 fungal strains from various marine habitats in South Korea. Through the polyphasic study, including phylogenetic analysis using multi-genetic markers (ITS, LSU, *TEF1*, *RPB2*, *TUB*, and *ACT*) and morphological analysis, we identified 21 species previously undiscovered in Korea, including 5 new and 16 unrecorded species. Our findings illustrate the species diversity of marine *Hypocreales*, highlighting the need for additional research into their ecological functions and potential in biotechnology and medicine.

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1. Introduction

Hypocreales is an order within the class *Sordariomycetes* of *Ascomycota*, comprising 14 families and 303 genera, making it the largest order in *Sordariomycetes* [1–4]. With its high species diversity, *Hypocrealean* fungi occupy a wide range of ecological niches, from saprophytic to symbiotic and parasitic fungi [5–7]. Many parasitic fungi in this order, particularly those affecting insects and plants, have garnered significant attention due to their economic impact. A notable example is the family *Cordycipitaceae*, which includes genera like *Beauveria*, *Cordyceps*, and *Lecanicillium*, known for their entomopathogenic species used as biocontrol agents (such as cyclosporine, pyridones, and fumosorinone) [8–10]. Furthermore, the other *Hypocrealean* fungi also directly affect plants, humans, and animals, which has been the subject of extensive research [7,11–14].

Hypocrealean fungi are distributed globally across terrestrial and aquatic environments, but research has predominantly focused on their diversity and function in terrestrial ecosystems [7,14–17]. In contrast, studies on aquatic *Hypocrealean* fungi, particularly those in marine environments, have been relatively limited, with most attention directed toward

freshwater habitats [15,18–22]. This has left a gap in our understanding of their ecological roles and adaptations in these environments, challenging traditional concepts about their ecological niches [15,23–26]. Marine *Hypocrealean* fungi have been reported in various marine environments, from coastal sediments to deep-sea [27–30], with 168 species documented across 64 genera and 15 families (accessed September 09 2024, via <https://www.marinefungi.org/>) [31]. Although marine fungi are less studied compared to terrestrial fungi, they play vital roles in oceanic ecosystems, including organic matter decomposition, nutrient recycling, and symbiotic interactions with marine flora and fauna [23,32,33]. Exploring marine *Hypocreales* is especially interesting because they can make new bioactive compounds in response to the special physicochemical conditions of marine environments [10,34], which could be used in medicine, agriculture, and industry.

Despite growing interest, taxonomic research on marine *Hypocreales* is still in its early stages. Although *Hypocrealean* fungi are frequently found in various marine environments, such as macroalgae and sediment, sometimes constituting up to 30% of its fungal communities [27–30], they account for only 7% of the total marine fungi listed to date

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families (accessed September 09 2024, via <https://www.marinefungi.org/>) [31]. Morphological identification of marine *Hypocreales* is challenging due to the limited observable traits in cultured isolates, making DNA-based identification essential [4–7]. The nuclear ribosomal internal transcribed spacer (ITS), a universal fungal DNA barcode marker has been used to identify species within the *Hypocreales*. However, due to its low resolution, additional genetic markers, such as the partial actin (*ACT*), the RNA polymerase II subunit (*RPB2*), the translation elongation factor 1- α (*TEF1*), and the beta-tubulin (*TUB*) have been applied in phylogenetic analyses [2,4,35,36]. Nevertheless, of the 168 documented marine *Hypocreales* species, only 88 have been identified based on DNA sequences (accessed September 09 2024, via <https://www.marinefungi.org/>) [31], highlighting the need for further molecular studies to confirm their taxonomic accuracy.

In South Korea, 262 species of *Hypocreales* across 8 families have been documented (National Institute of Biological Resources, accessed 2024.06.13). In comparison, only 131 species have been reported from marine environments (Marine Bio-Resource Information System, updated 2024.03.29). Over the past decade, our research has aimed to bridge the gap in marine fungal diversity, leading to the establishment of the Marine Fungal Resource Bank (MFRB), which isolates fungal strains from various marine environments [37–41]. As part of this effort, we recently applied polyphasic phylogenetic analyses using multi-genetic markers to newly isolated Hypocrealean fungal strains. This resulted in the discovery of 21 species, including 5 new species and 16 unrecorded species. Our findings underscore the need for further research into the diversity, ecological interactions, and adaptation mechanisms of marine *Hypocreales* to understand their roles in marine ecosystems better.

2. Materials and methods

2.1. Sampling and fungal isolation

Fungal strains were isolated from various substrates in marine environments in South Korea between 2015 and 2022 (Table 1). Sampling and isolation methods followed previously described protocols [37,38,41]. Marine organisms such as macroalgae, sandfish eggs, and sponges were collected and washed with sterilized seawater (SSW) at least three times. The samples were then cut into pieces and placed on different media used for fungal isolation. For marine sediment samples (mudflats, sand, and seabed sediment), samples were diluted to 1/10 or

1/100, and 100–200 μ L of the diluted samples were inoculated onto fungal isolation media. Seawater samples were collected from the surface or bottom using Niskin bottles equipped with conductivity, temperature, and depth (CTD) rosettes. The sampled seawater was filtered through a 0.2 μ m pore-sized PETE membrane filter using a vacuum pump, and the filters were placed on DRBC media. All plates were incubated at 25 °C or room temperature for 7–21 d. Each fungal colony grown on the isolation media was transferred to new PDA media supplemented with SSW. Fungal strains were preserved in 20% (v/v) glycerol with SSW at –80 °C and deposited in the Seoul National University Fungus Collection.

2.2. Molecular analysis

Fresh mycelium from each strain was ground using a Bead Ruptor Elite Homogenizer (OMNI International, Kennesaw, GA). Genomic DNA was extracted using either a modified cetyltrimethylammonium bromide (CTAB) method [42] or the AccuPrep Genomic DNA Extraction Kit (Bioneer Co., Daejeon, Korea) following the manufacturer's protocols. The ITS region was amplified for all strains using the primer set ITS1F [43]/ITS4 [44]. To ensure accurate species identification within *Hypocreales*, additional genetic markers were selected based on reference studies Supplementary Table 2, and amplification was performed using the primer sets and conditions listed in Supplementary Table 2. PCR was performed using a C1000 thermal cycler (Bio-Rad, Richmond, CA), and the PCR products were confirmed by gel electrophoresis on a 1% agarose gel. Verified PCR products were purified using the PCR Purification Kit (GeneAll Biotechnology, Seoul, South Korea) or the ExoSAP-IT Express PCR Product Cleanup Kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. The purified PCR products were sequenced with the same primers used for PCR on an ABI Prism 3730xl Genetic Analyzer (Life Technologies, Gaithersburg, MD) at Macrogen (Seoul, South Korea).

Generated sequences were proofread and manually edited using Geneious Prime 2023.1 (Biomatters Ltd., San Diego, CA, <https://www.geneious.com>) [45]. Edited sequences were assembled using the *de novo* assembly function in Geneious Prime 2023.1. All sequences were deposited in GenBank (Table 1). Reference sequences were obtained from published studies and downloaded from GenBank using GenMine software [46] (Supplementary Table 1).

Table 1. Strain information and GenBank accessions used in the study.

Species	Strain	Location (in Republic of Korea)	Substrate	ITS	LSU	TEF1	RPB2	TUB	ACT
<i>Achirostachys aurantispora</i>	SFC20240607-M001	Incheon, Ganghwa-gun	Mud flat	PQ304492	PQ304459	PQ319913			
<i>Acrostalagmus annulatus</i>	SFC20240607-M002	Jeollanam-do, Suncheon-si	Sand	PQ304493	PQ304460	PQ319909	PQ319914		
	SFC20240607-M016	Jeollanam-do, Muan-gun	Mud flat	PQ304494	PQ304461	PQ319910	PQ319915		
<i>Beauveria pseudobassiana</i>	SFC20240607-M003	Jeollanam-do, Suncheon-si	Sand	PQ304495	PQ304462	PQ319886			
<i>Cordyceps fumosorosea</i>	SFC20240607-M004	Jeollanam-do, Suncheon-si	Sand	PQ304496	PQ304463	PQ319887	PQ319916	PQ319882	
	SFC20240607-M018	Gyeongsangbuk-do, Ulleung-gun	Unknown sponge	PQ304497	PQ304464	PQ319888	PQ319917	PQ319883	
<i>Emericellopsis atlantica</i>	SFC20240607-M005	Jeollanam-do, Muan-gun	Mud flat	PQ304498	PQ304465	PQ319908	PQ319918	PQ319884	
<i>Fusarium concentricum</i>	SFC20240607-M006	Jeollanam-do, Suncheon-si	Mud flat	PQ304499	PQ304466	PQ319912	PQ319919		
<i>Lasioneictiella arenuloides</i>	SFC20240607-M007	Jeollanam-do, Suncheon-si	Sand	PQ304500	PQ304467	PQ319892	PQ319920		
<i>Lecanicillium verrucum</i>	SFC20240607-M029 T	Gyeongsangbuk-do, Pohang-si	Sand	PQ355558	PQ355577	PQ355481			
	SFC20240607-M030	Jeju-do, Chuja-myeon	Unknown macroalgae	PQ355559	PQ355578	PQ355482			
	SFC20240607-M031	Jeju-do, Chuja-myeon	Unknown macroalgae	PQ355560	PQ355579	PQ355483			
	SFC20240607-M032	Incheon, Jung-gu	Unknown	PQ355561	PQ355580	PQ355484			
<i>Metapochonia rubescens</i>	SFC20160907-M17	Gangwon-do, Gangneung-si	Egg of <i>Arctoscopus japonicus</i> (Sandfish)	PQ304501		PQ319896	PQ319921		
	SFC20240607-M010	Jeollanam-do, Suncheon-si	Sand	PQ304502			PQ319922	PQ319885	
<i>Neoacremonium distortum</i>	SFC20240607-M011	Incheon, Ganghwa-gun	Mud flat	PQ304503	PQ304468	PQ319897	PQ319923		
	SFC20240607-M034	Incheon, Ganghwa-gun	Sand	PQ304504	PQ304469	PQ319899	PQ319924		
	SFC20240607-M035	Jeollanam-do, Muan-gun	Sand	PQ304505	PQ304470	PQ319902	PQ319925		
	SFC20240607-M036	Incheon, Ganghwa-gun	Mud flat	PQ304506	PQ304471	PQ319900	PQ319926		
	SFC20240607-M037	Incheon, Ganghwa-gun	Mud flat	PQ304507	PQ304472	PQ319898	PQ319927		
	SFC20240607-M038	Incheon, Ganghwa-gun	Mud flat	PQ304508	PQ304473	PQ319901	PQ319928		
<i>Neocosmospora tuberculata</i>	SFC20240607-M039 T	South sea of Korea	Seawater in 40 m bottom	PQ355562	PQ355581	PQ355485	PQ355473		
	SFC20240607-M040	South sea of Korea	Seawater in 40 m bottom	PQ355563	PQ355582	PQ355486	PQ355474		
	SFC20240607-M041	South sea of Korea	Seawater in 40 m bottom	PQ355564	PQ355583	PQ355487	PQ355475		
	SFC20240607-M042	South sea of Korea	Seawater in 40 m bottom	PQ355565	PQ355584	PQ355488	PQ355476		
<i>Niesslia marinisedimenta</i>	SFC20171120-M03	Jeollanam-do, Muan-gun	Sand	PQ355566	PQ355585	PQ355489	PQ355477	PQ355469	
	SFC20240607-M014 T	Jeollanam-do, Muan-gun	Sand	PQ355567	PQ355586	PQ355490	PQ355478	PQ355470	
	SFC20240607-M043	Jeollanam-do, Muan-gun	Sand	PQ355568	PQ355587	PQ355491	PQ355479	PQ355471	
<i>Parasarocladium mabikii</i>	SFC20240607-M044	Incheon, Ganghwa-gun	Mud flat	PQ355569	PQ355588	PQ355492	PQ355480	PQ355472	PQ355463
	SFC20240607-M025	Incheon, Ganghwa-gun	Mud flat	PQ355570	PQ355589	PQ355493			PQ355464
	SFC20240607-M026	Incheon, Ganghwa-gun	Mud flat	PQ355571	PQ355590	PQ355494			PQ355465
<i>Parasarocladium multimorphologicum</i>	SFC20240607-M027 T	Incheon, Ganghwa-gun	Mud flat	PQ355572	PQ355591	PQ355495			PQ355466
	SFC20240607-M022	Jeju-do, Chuja-myeon	Unidentified seaweed	PQ355573	PQ355592	PQ355496			
	SFC20240607-M023	Jeju-do, Chuja-myeon	Unidentified seaweed	PQ355574	PQ355593	PQ355497			PQ355467
<i>Protocreopsis rutila</i>	SFC20240607-M024 T	Jeju-do, Chuja-myeon	<i>Grateloupia</i> sp.	PQ355575	PQ355594	PQ355498			PQ355468
<i>Purpureocillium lavendulum</i>	SFC20170718-M03	Incheon, Ganghwa-gun	Sand	PQ304509	PQ304474	PQ319911			
	SFC20240607-M017	South sea of Korea	Sediment in 40 m	PQ304510		PQ319889	PQ319929		
	SFC20240607-M033	Jeollanam-do, Muan-gun	Sand	PQ304511		PQ319890	PQ319930		
	SFC20240607-M015	Gangwon-do, Goseong-gun	Sand	PQ304512		PQ319891	PQ319931		
<i>Sarocladium bacillisporum</i>	SFC20240607-M028	Gangwon-do, Goseong-gun	Sand	PQ304513		PQ319904			PQ319877
<i>Sarocladium terricola</i>	SFC20240607-M008	Jeju-do, Jeju-si	<i>Gelidium</i> sp.	PQ304514	PQ304476	PQ319905			PQ319878

(Continued)

Table 1. Continued.

Species	Strain	Location (in Republic of Korea)	Substrate	ITS	LSU	TEF1	RPB2	TUB	ACT
<i>Sarocladium zeae</i>	SFC20240607-M009	Jeollanam-do, Muan-gun	Sand	PQ304515	PQ304477	PQ319906			PQ319879
	SFC20240607-M012	Gyeongsangbuk-do, Ulleung-gun	Unknown sponge	PQ304516	PQ304478	PQ319907			PQ319880
	SFC20240607-M013	Jeollanam-do, Gangjin-jun	<i>Fulvia mutica</i>	PQ304517	PQ304479	PQ319903			PQ319881
<i>Verruciconidia infusata</i>	SFC20240607-M019	Jeollanam-do, Suncheon-si	Sand	PQ304518	PQ304480	PQ319893	PQ319932		
<i>Verruciconidia persicina</i>	SFC20240607-M020	Jeju-do, Chuja-myeon	<i>Sargassum thunbergii</i>	PQ304519		PQ319894	PQ319933		
	SFC20240607-M021	Incheon, Ganghwa-gun	Mud flat	PQ304520	PQ304481	PQ319895	PQ319934		

Due to the large dataset and computational requirements, the dataset was divided into four subsets following a previous study [4,7,21,35,36,47,48], and RAxML phylogenetic analysis was conducted for each.

The first dataset included *Bionectriaceae* and *Sarocladiaceae*, with phylogenetic analysis using six genetic markers (ITS, LSU, *TEF1*, *RPB2*, *TUB*, and *ACT*). *ACT* sequences were used for the analysis of only two genera (*Sarocladium* and *Parasarocladium*) in the family *Sarocladiaceae*. The second dataset comprised sequences of four genetic markers (ITS, LSU, *TEF1*, and *RPB2*) from three families (*Nectriaceae*, *Neoacremoniaceae*, and *Stachybotryaceae*). The third and fourth datasets included sequences of five genetic markers (ITS, LSU, *TEF1*, *RPB2*, and *TUB*) from three families (*Clavicipitaceae*, *Hypocreaceae*, and *Ophiocordycipitaceae*) and two families (*Cordycipitaceae* and *Niessliaceae*), respectively. All sequences for each genetic marker were aligned using MAFFT version 7.490 [49], and uninformative ends were trimmed in Geneious Prime 2023.1 (<https://www.geneious.com>) [45]. The alignments of each genetic marker were concatenated, and phylogenetic trees were constructed using the GTR+GAMMA model with 1000 replications through RAxML version 8 on Geneious Prime (<https://www.geneious.com>) [45].

2.3. Morphological observations

Each strain used for morphological observations was sub-cultured on potato dextrose agar (PDA; Difco, Pinellas Park, FL) supplemented with distilled water and inoculated on the following media: PDA, malt extract agar (MEA; Oxoid, Baddhoevedorp, Netherlands), oatmeal agar (OA; Difco), corn meal agar (CMA; Difco), potato carrot agar (PCA; Himedia, Mumbai, India), and synthetic nutrient-poor agar (SNA; KH_2PO_4 1 g, KNO_3 1 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 5 g, KCl 0.5 g, glucose 0.2 g, saccharose 0.2 g, and Bacto agar 20 g/L) [50]. Cultures were incubated for 7 d at 25 °C. Cultures for microscopic observation were incubated at 25 °C for more than 7 d on media appropriate for each genus until the conidia formation was confirmed. The specific media used for each genus are indicated under each taxon's Taxonomy section. Conidial structures were observed using a Nikon 80i (Tokyo, Japan) or Leica DM2500 (Wetzlar, Germany) light microscope. When necessary, lactic acid or lactophenol blue dye was used for observation. More than 30 conidial structures were measured per strain using ImageJ software [51]. Colony color,

including the surface and reverse side, was described using the Methuen Handbook of Colour [52].

3. Results

We isolated 47 strains from various marine substrates environments (macroalgae, animals, seawater, and sediments) and obtained 190 sequences for six genetic markers (ITS, LSU, *TEF1*, *RPB2*, *TUB*, and *ACT*) from these strains (Table 1). Phylogenetic analysis was performed using these genetic markers, along with 886 reference sequences. The dataset was divided into four subsets based on families to improve computational efficiency, and phylogenetic analysis was conducted using RAxML for each subset. The resulting phylogenetic trees are presented in Figures 1–4.

On the basis of the ITS phylogeny, the 47 strains were classified into 21 taxa, which were further assigned to 17 genera across 10 families. Different sets of genetic markers were applied depending on the family. For the six families, *Bionectriaceae*, *Hypocreaceae*, *Clavicipitaceae*, *Cordycipitaceae*, *Niessliaceae*, and *Ophiocordycipitaceae*, five markers (ITS, LSU, *TEF1*, *RPB2*, and *TUB*) were used. In the case of *Sarocladiaceae*, six markers (ITS, LSU, *TEF1*, *RPB2*, *TUB*, and *ACT*) were applied. For *Stachybotryaceae*, *Nectriaceae*, and *Neoacremoniaceae*, four markers (ITS, LSU, *TEF1*, and *RPB2*) were used.

Through the phylogenetic analysis based on these multi-genetic markers, the 47 strains were identified as 21 species within the order *Hypocreales*. Of these, 16 are identified as described but previously unrecorded species in South Korea: *Bionectriaceae* (*Emericellopsis atlantica*, *Lasionectriella arenuloides*, *Protocreopsis rutila*, *Verruciconidia infusata*, *V. persicina*), *Clavicipitaceae* (*Metapochonia rubescens*), *Cordycipitaceae* (*Beauveria pseudobassiana*, *Cordyceps fumosorosea*), *Hypocreaceae* (*Acrostalagmus annulatus*), *Nectriaceae* (*Fusarium concentricum*), *Neoacremoniaceae* (*Neoacremonium distortum*), *Ophiocordycipitaceae* (*Purpureocillium lavendulum*), *Sarocladiaceae* (*Sarocladium bacillisporum*, *S. terricola*, and *S. zae*), and *Stachybotryaceae* (*Achroiostachys aurantispora*). Each species formed robust clades alongside sequences from described species. However, *Lasionectriella arenuloides* and *La. marigotensis* showed high similarity in both molecular and morphological characteristics [35,53]. Our strain was identified as *La. arenuloides*, with further details available in the species note in the Taxonomy section.

The remaining five taxa were confirmed as new species candidates, as they did not match any known

described species and formed distinct clades supported by high bootstrap values. The number of new species candidates identified in each family is as follows: *Cordycipitaceae* (1), *Nectriaceae* (1), *Niessliaceae* (1), and *Sarocladiaceae* (2). Detailed descriptions of these 21 species, including the 5 new (*Lecanicillium verrucum* sp. nov., *Neocosmospora tuberculata* sp. nov., *Niesslia marinisedimenta* sp. nov., *Parasarocladium mabikii* sp. nov., and *Parasarocladium multimorphologicum* sp. nov.) and 16 unrecorded species, are provided in the Taxonomy section.

4. Taxonomy

***Achroiostachys aurantispora* L. Lombard & Crous** (Figure 5(A))

MycoBank: MB815917

Family *Stachybotryaceae*

Materials examined: South Korea. Incheon, Ganghwa-gun (37°36'36.3"N 126°31'13.8"E), July 2021, isolated from mudflat, M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M001, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on MEA *Mycelia* smooth, septate, hyaline; *Conidiophores* mononematous, unbranched, erect, straight, 1–4-septate, hyaline, smooth, occasionally rough with nodules toward apex, 40–101 × 2.5–5.4 µm, bearing a whorl of 5–6 conidiogenous cells. *Conidiogenous cells* terminal, ampulliform to ventricose, hyaline, smooth, 6.2–14 × 2.9–4.6 µm. *Conidia* aseptate, ellipsoidal, smooth, hyaline, 1–2 guttules, 5–8.7 × 3.2–4.6 µm (av. 7.1 × 3.9 µm).

Culture characteristics: Colonies on PDA reaching 56–60 mm diam in 7 d at 25 °C; flat, plain, floccose, sometimes felty, forward yellowish white (1A2), light grey (1C1); margin entire or irregular; reverse yellow (3B8), golden brown (5D7) at center. Colonies on OA reaching 53–58 mm diam in 7 d at 25 °C; flat, plain, floccose, granular, sometimes having patches with scarce aerial mycelia, forward orange white (5A2); margin entire; exudate light orange (5A4); reverse pastel yellow (3A4). Colonies on CMA: reaching 53–62 mm diam in 7 d at 25 °C; flat, plain, forward hyaline to reddish (or pinkish) white (7A2); few pustule-like formation; reverse hyaline. Colonies on SNA reaching 20–25 mm diam in 7 d at 25 °C; vegetative mycelial growth scarce, scattered, forward hyaline to yellowish white (4A2); reverse yellowish white (4A2).

Notes: The SFC20240607-M001 strain, isolated from a mudflat, is nearly identical to the holotype in morphology, with the following exceptions: it has more septa in its conidiophores compared to the

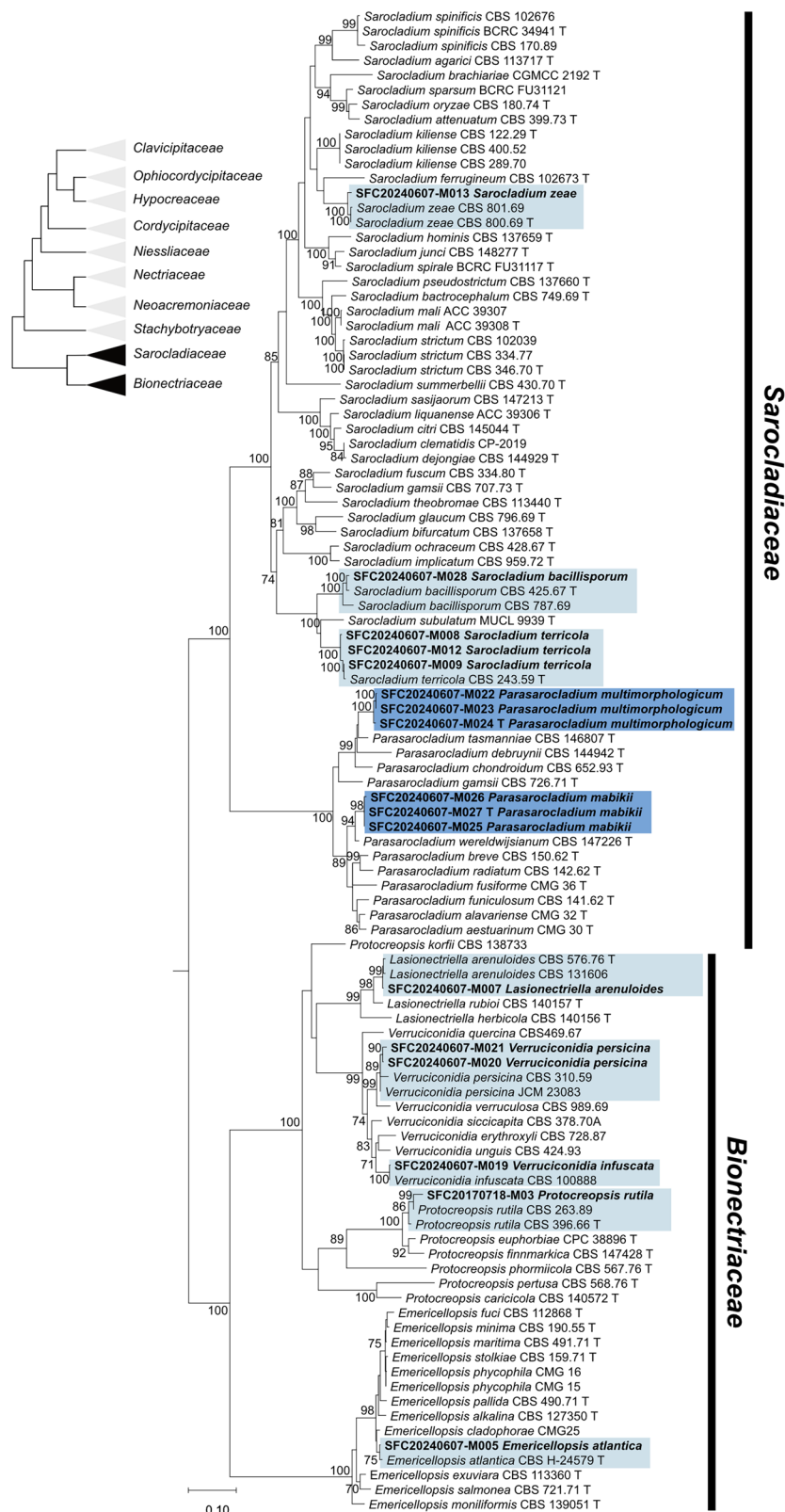


Figure 1. A phylogenetic tree of *Bionectriaceae* and *Sarcocladiaceae* generated by RAXML analysis using ITS, LSU, *TEF1*, *RPB2*, *TUB*, and *ACT*. Bootstrap values over 70% are indicated at the branch nodes. Newly generated sequences from this study are represented in bold. The boxes indicate new species in blue and unrecorded in light blue. The summarized tree is illustrated in reference to Hou et al. [35].

holotype (1–2 septa) [54]. Additionally, its conidiophores are more widely distributed, but its conidiogenous cells and conidia are shorter than those of the holotype DAOM 695772 [54].

Acrostalagmus annulatus (Berk. & M.A. Curtis)
Seifert (Figure 5(B))
Mycobank: MB518663
Family *Hypocreaceae*

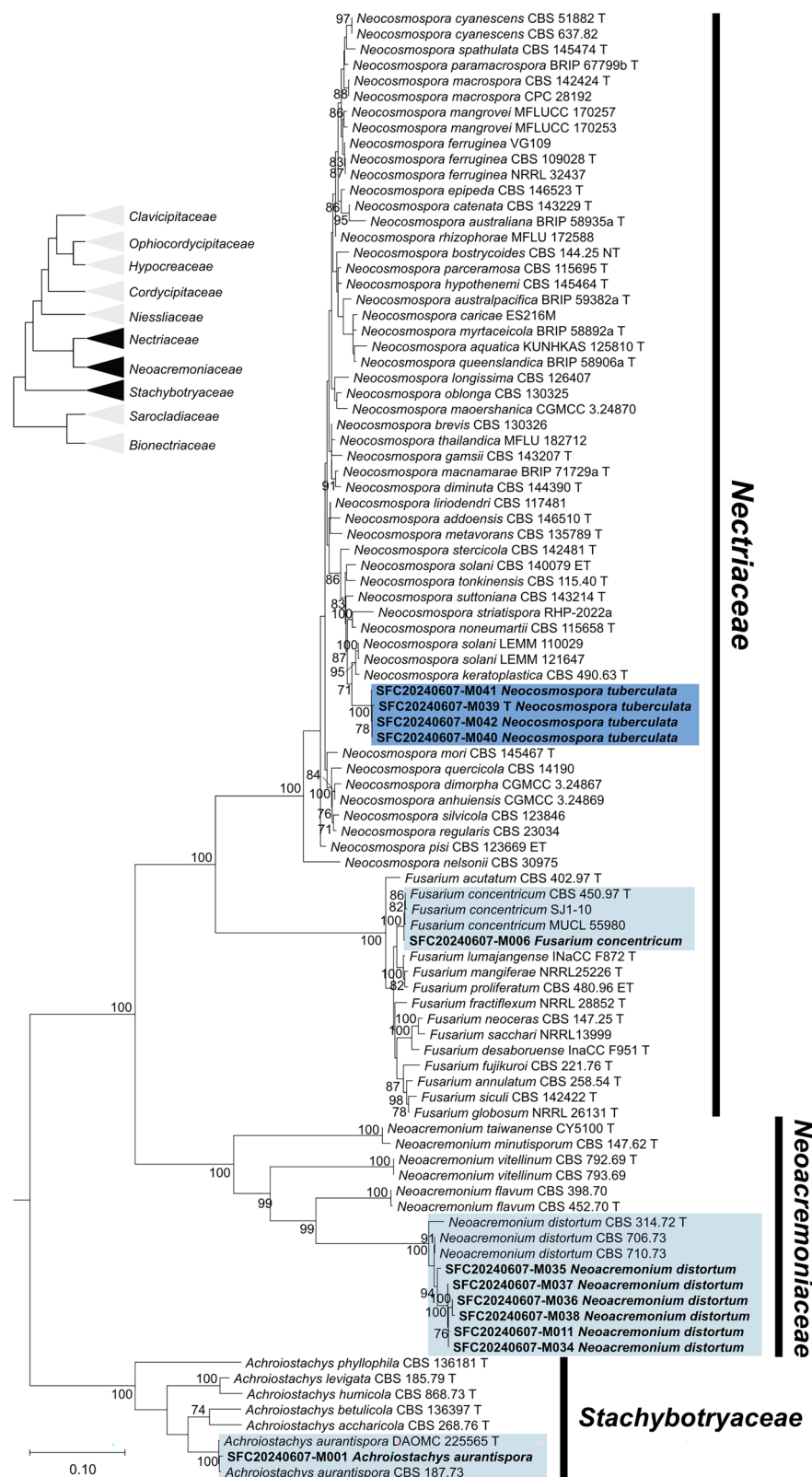


Figure 2. A phylogenetic tree of Nectriaceae, Neoacremoniaceae, and Stachybotryaceae generated by RAxML analysis using ITS, LSU, *TEF1*, and *RPB2*. Bootstrap values over 70% are indicated at the branch nodes. Newly generated sequences from this study are represented in bold. The boxes indicate new species in blue and unrecorded in light blue. The summarized tree is illustrated in reference to Hou et al. [35].

Basionym: *Stilbum annulatum* Berk. & M.A. Curtis, in Berkeley 1874

Materials examined: South Korea. Jeollanam-do, Suncheon-si (34°50'29.7"N 127°29'09.4"E), January

2020, isolated from sea sand, M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M002, stored in a metabolically inactive state); Jeollanam-do, Muan-gun (35°1'38.57"N 126°25'17.06"E), January 2020,

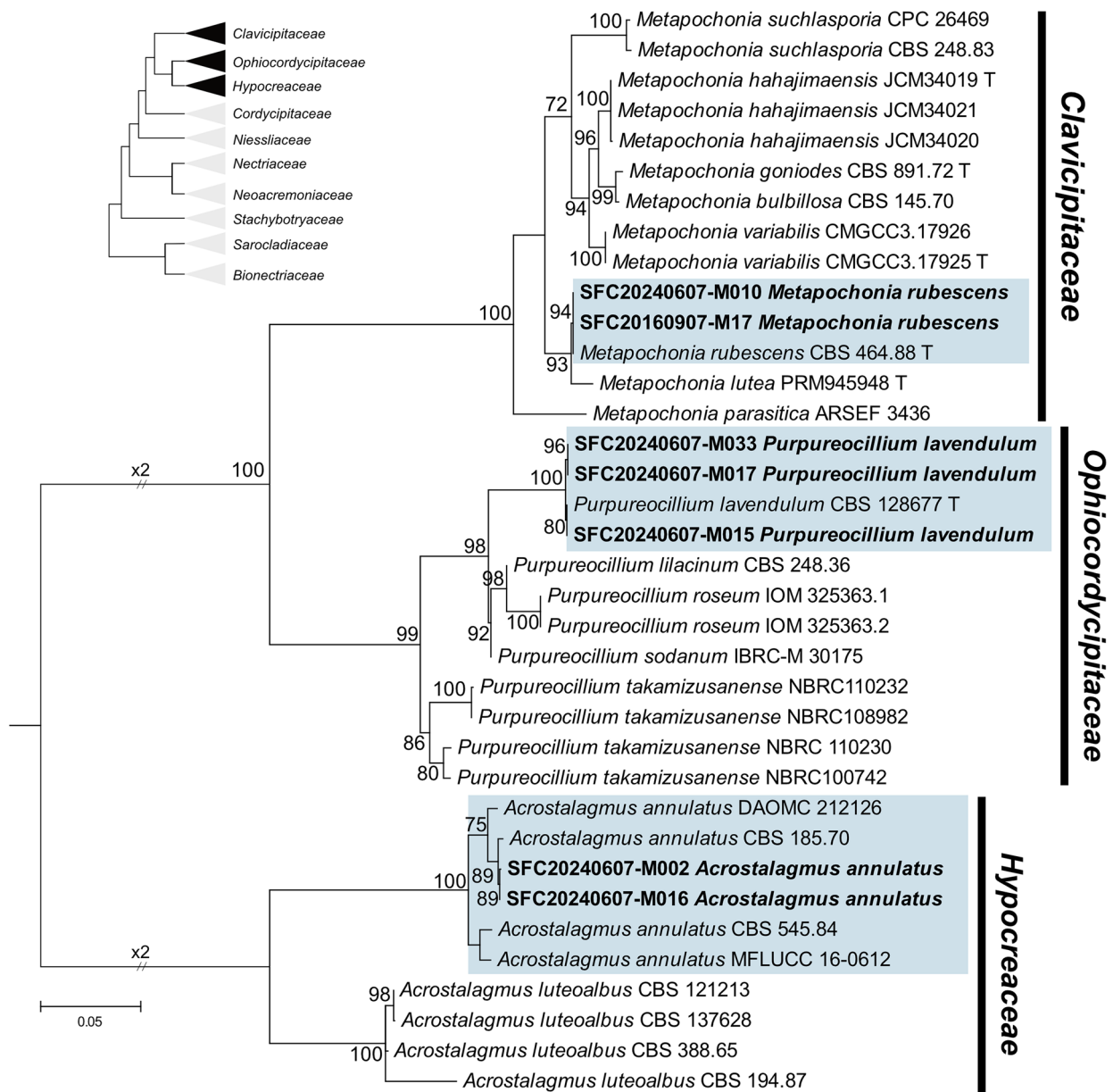


Figure 3. A phylogenetic tree of *Clavicipitaceae*, *Ophiocordycipitaceae*, and *Hypocreaceae* generated by RAXML analysis using ITS, LSU, *TEF1*, *RPB2*, and *TUB*. Bootstrap values over 70% are indicated at the branch nodes. Newly generated sequences from this study are represented in bold. The boxes in light blue indicate unrecorded. The summarized tree is illustrated in reference to Hou et al. [35].

isolated from mudflat, M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M016, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on MEA *Mycelia* smooth, septate, hyaline; *Conidiophores* repeatedly branched, erect, septate, pale reddish brown, smooth, 2–4.2 µm wide. *Conidiogenous cells* phialidic, narrowly flask-shaped in the widest part, collarete, concolorous, smooth, 9–27 × 1.7–3.9 µm. *Conidia* aseptate, ellipsoidal, cylindrical, smooth, concolorous, guttules, 4.9–7.6 × 2.5–3.9 µm (av. 6.1 × 3.1 µm).

Culture characteristics: Colonies on PDA reaching 17–22 mm diam in 7 d at 25°C; raised, plain, floccose, hairy, forward white, pale yellow (4A3); margin fimbriate; reverse pastel yellow (3A4).

Colonies on MEA reaching 19–26(–29) mm diam in 7 d at 25°C; flat, plain, hairy, forward white, yellowish white (3A2); margin fimbriate; reverse grayish orange (5B6). Colonies on OA reaching 18–24 mm diam in 7 d at 25°C; flat, plain, hairy, felty, forward white, orange (5B8); margin fimbriate; reverse pale yellow (4A3), light brown (6D8) at center. Colonies on CMA reaching 18–23 mm diam in 7 d at 25°C; flat, plain, forward yellowish white (1A2), margin entire to irregular; exudate brownish red (8C7); reverse yellowish white (2A2).

Notes: Korean strains, isolated from marine sediments, share similar morphological characteristics with *Acrostalagmus annulatus* (MFLUCC 16-0612) isolated from decaying fruits [55]. However,

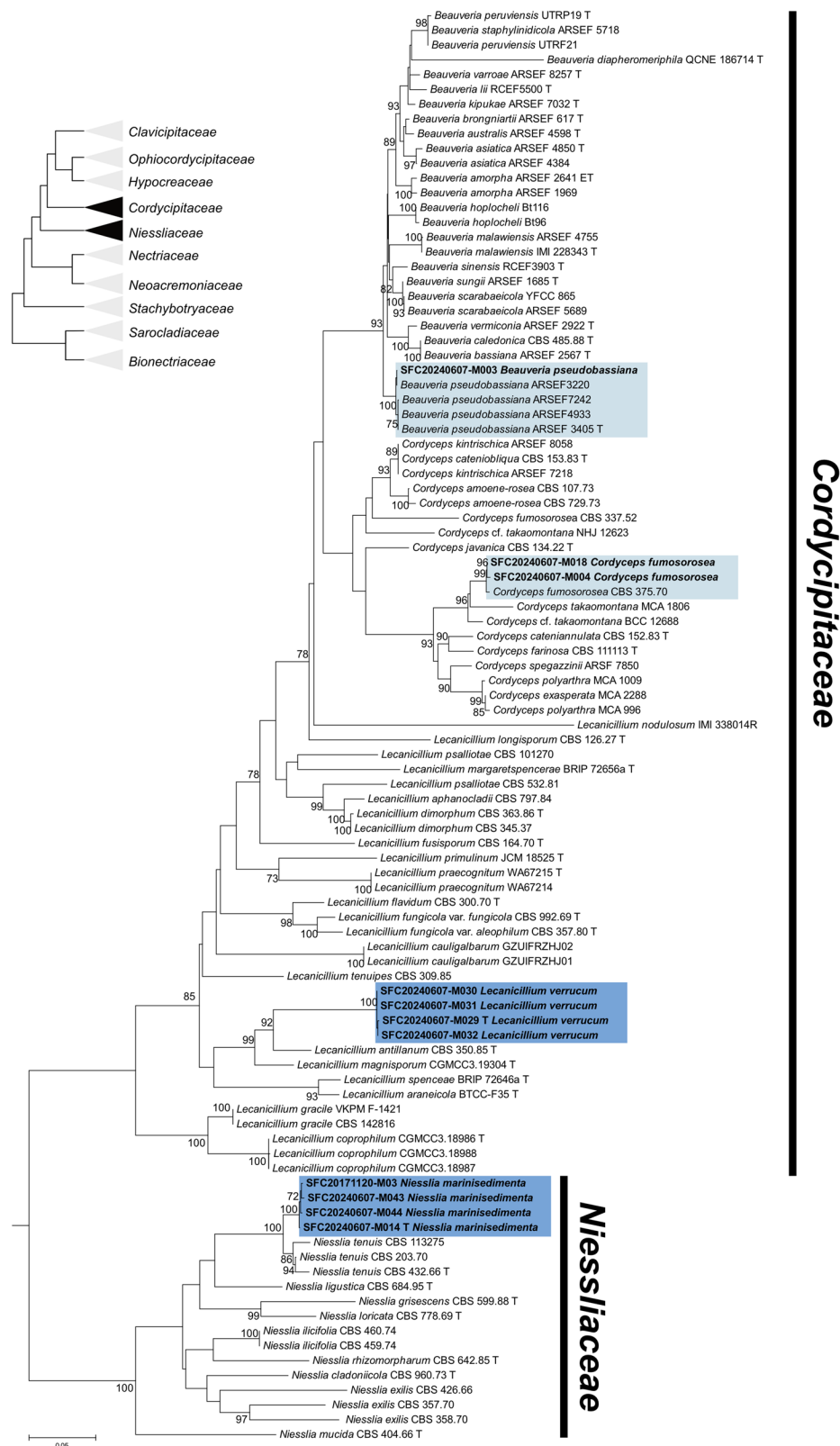


Figure 4. A phylogenetic tree of *Cordycipitaceae* and *Niessliaceae* generated by RAxML analysis using ITS, LSU, *TEF1*, *RPB2*, and *TUB*. Bootstrap values over 70% are indicated at the branch nodes. Newly generated sequences from this study are represented in bold. The boxes indicate new species in blue and unrecorded in light blue. The summarized tree is illustrated in reference to Hou et al. [35].

compared to the holotype [56], the Korean strain has slightly wider conidiophores and conidia.

Beauveria pseudobassiana S.A. Rehner & Humber (Figure 5(C))

MycoBank: MB519125

Family *Cordycipitaceae*

Materials examined: South Korea. Jeollanam-do, Suncheon-si (34°50'29.7"N, 127°29'09.4"E), July 2018, isolated from sea sand, M.S. Park & Y.W. Lim

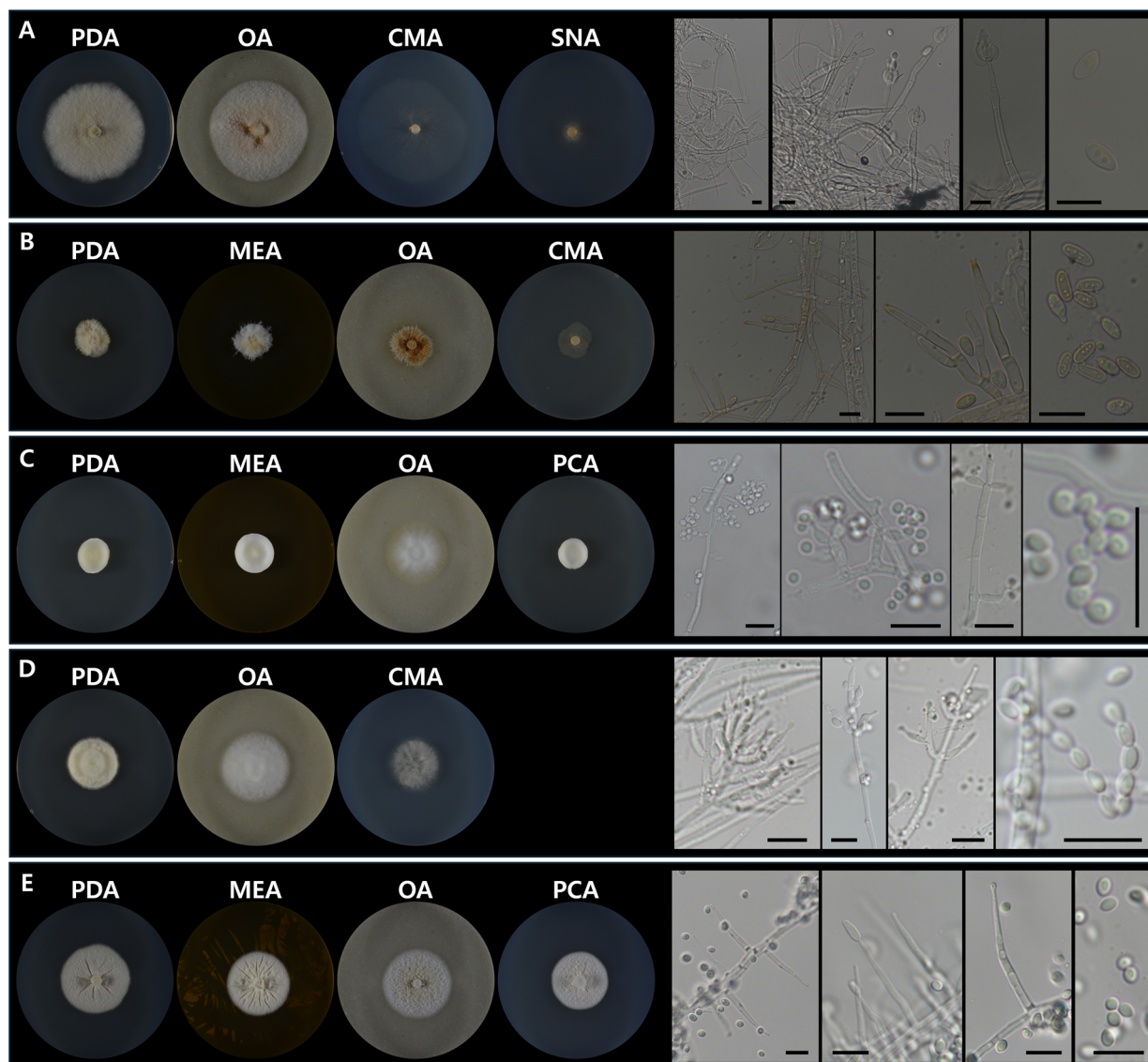


Figure 5. Cultural and conidial morphology. (A) *Achroiostachys aurantisporea*. (B) *Acrostalagmus annulatus*. (C) *Beauveria pseudobassiana*. (D) *Cordyceps fumosorosea*. (E) *Emericellopsis atlantica*. Scale bar: 10 µm.

(SFC20240607-M003, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on PDA *Mycelia* smooth, septate, hyaline; *Conidiophores* branched, erect, septate, hyaline, smooth, 1.3–2.9 µm wide. *Conidiogenous cells* solitary but usually consisting of dense lateral clusters, base subspherical to ampulliform, hyaline, smooth, 3.8–8.1 × 1.5–2.8 µm. *Conidia* aseptate, globose, subglobose, smooth, hyaline, 1.6–2.6 × 1.2–2.4 µm (av. 2.1 × 1.9 µm).

Culture characteristics: Colonies on PDA reaching 17–20 mm diam in 7 d at 25°C; flat to crateriform, plain, cottony, forward white, yellowish white (2A2) at center; margin entire; reverse light yellow (3A5). Colonies on MEA reaching 21–23 mm diam in 7 d at 25°C; crateriform, radially sulcate, cottony, forward white, yellowish white (2A2) to grey (2B1) at center; margin entire; reverse butter yellow (4A5), brownish orange (7C4) at center. Colonies on OA

reaching 33–37 mm diam in 7 d at 25°C; flat, floccose, forward yellowish white (3A2), white at center; margin entire; reverse yellowish white (3A2). Colonies on PCA reaching 15–17 mm diam in 7 d at 25°C; crateriform, plain, cottony, forward white, yellowish white (3A2) at center, margin entire; reverse white, pale yellow (3A3) at center.

Notes: *Beauveria pseudobassiana* shows intraspecific variation in morphology [57,58]. Korean strains isolated from sea sand have long conidiogenous cells compared to other strains within the species [58].

Cordyceps fumosorosea (Wize) Kepler, B. Shrestha & Spatafora (Figure 5(D))

Mycobank: MB 820980

Family Cordycipitaceae

Basionym: *Isaria fumosorosea* Wize 1904

Materials examined: South Korea. Jeollanam-do, Suncheon-si (34°50'29.7"N 127°29'09.4"E), July 2021,

isolated from sea sand, M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M004, stored in a metabolically inactive state); Gyeongsangbuk-do, Ulleung-gun (37°32'2.05"N 130°49'24.87"E), unknown date in 2018, isolated from a sponge, M.S. Park & Y.W. Lim (SFC20240607-M018, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on PDA *Mycelia* smooth, septate, hyaline; *Conidiophores* branched, erect, septate, hyaline, smooth, 1.6–2.8 µm wide. *Phialides* ampulliform, subcylindrical tapering into the apex, subulate in immature, hyaline, smooth, solitary or in whorls of 2–4 on each branch, up to five phialides on a whorl, 6.8–18 × 1.4–3.4 µm. *Conidia* aseptate, ellipsoidal, smooth, hyaline, 1.9–3.6 × 1.2–2.3 µm (av. 2.8 × 1.8 µm).

Culture characteristics: Colonies on PDA reaching 29–30 mm diam in 7 d at 25°C; umbonate, raised plain, floccose, velvety at margin, forward white; margin entire; reverse butter yellow (4A5). Colonies on OA reaching 36–39 mm diam in 7 d at 25°C; raised, floccose, forward white; margin entire; reverse yellowish white (4A2). Colonies on CMA reaching 27–32 mm diam in 7 d at 25°C; flat, plain, floccose, felty, forward white to hyaline; margin filiform; reverse yellowish white (3A2).

Notes: *Cordyceps fumosorosea*, formerly *Isaria fumosorosea* [59], is recognized to infect many pest species [60]. It is very interesting that this species was isolated from sponge in the marine environment.

***Emericellopsis atlantica* L.W. Hou, Crous, Rämä & Hagestad (Figure 5(E))**

MycoBank: MB838493

Family *Bionectriaceae*

Materials examined: South Korea. Jeollanam-do, Muan-gun (35°1'38.57"N 126°25'17.06"E), July 2021, isolated from mudflat, M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M005, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on OA *Mycelia* smooth, septate, hyaline; *Conidiophores* repeatedly branched, erect, septate, hyaline, smooth, 1.3–3.6 µm wide. *Conidiogenous cells* phialidic, narrowly flask-shaped in the widest part, hyaline, smooth, 16–44 × 1.5–3.2 µm. *Conidia* aseptate, broadly ellipsoidal, sometimes obclavate, smooth, hyaline, mostly 1 globose, or subglobose, guttule, 2.7–4.2 × 2–3.6 µm (av. 3.5 × 2.7 µm).

Culture characteristics: Colonies on PDA reaching 36–40 mm diam in 7 d at 25°C; flat, radially sulcate, felty, velvety at center, forward yellowish white (1A2); margin entire; reverse pale yellow (3A3). Colonies on MEA reaching 34–36 mm diam

in 7 d at 25°C; flat, radially sulcate, velvety, felty at margin, forward white, yellowish white (4A2) at center; margin entire; exudate clear; reverse orange (6B7). Colonies on OA reaching 38–39 mm diam in 7 d at 25°C; flat, felty, forward white, yellowish white (4A2) at center; margin entire; exudate clear; reverse pastel yellow (3A4). Colonies on PCA reaching 30–32 mm diam in 7 d at 25°C; flat, slightly radially sulcate, felty; forward yellowish white (4A2), margin entire; reverse white, pale yellow (3A3) at center.

Notes: The Korean strain SFC20240607-M005 matches the holotype of *Emericellopsis atlantica*, a member of the marine clade within the genus. However, there are several differences in their conidial structures: the conidiogenous cells of SFC20240607-M005 are shorter than those of the holotype (vs. 24.5–50(–64) µm) [61]. Additionally, the conidia of SFC20240607-M005 are shorter and wider (vs. 3–6(–9) × 2–2.5 µm). The guttules in the conidia of SFC20240607-M005 are globose or subglobose, while those of the holotype are irregular in shape [61].

***Fusarium concentricum* Nirenberg & O'Donnell (Figure 6(A))**

MycoBank: MB 444884

Family *Nectriaceae*

Materials examined: South Korea. Jeollanam-do, Suncheon-si (34°50'51.8"N 127°29'31.8"E), January 2017, isolated from mudflat, M.S. Park & Y.W. Lim (SFC20240607-M006, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on PCA *Mycelia* septate, smooth, hyaline; *Conidiophores* septate, branched, erect, smooth, hyaline, 1.5–3.3 µm wide. *Phialides* lateral or terminal, subulate to subcylindrical, cylindrical, hyaline, smooth, 6.6–32 × 1.9–3 µm, polyphialides observed. *Microconidia* 0–1 septate, subcylindrical, smooth, hyaline, 5–13 × 1.5–3 µm, solitary or in chain. *Sporodochia* 0–5(–6)-septate, straight to slightly curved, broadest at the half and tapering toward both ends, foot-shaped, abundant guttules, 9.8–59 × 1.9–4.3 µm. *Chlamydospores* not observed.

Culture characteristics: Colonies on PDA reaching 76–80 mm diam in 7 d at 25°C; flat, plain, floccose, forward white; margin fimbriate; reverse grayish yellow (4B3). Colonies on OA reaching 90 mm diam in 7 d at 25°C; flat to raised, plain, floccose, forward white; margin entire; reverse yellowish grey (4B2). Colonies on SNA reaching 67–73 mm diam in 7 d at 25°C; flat, plain, felty, forward yellowish white (4A2); margin entire; reverse pale yellow (4A3).

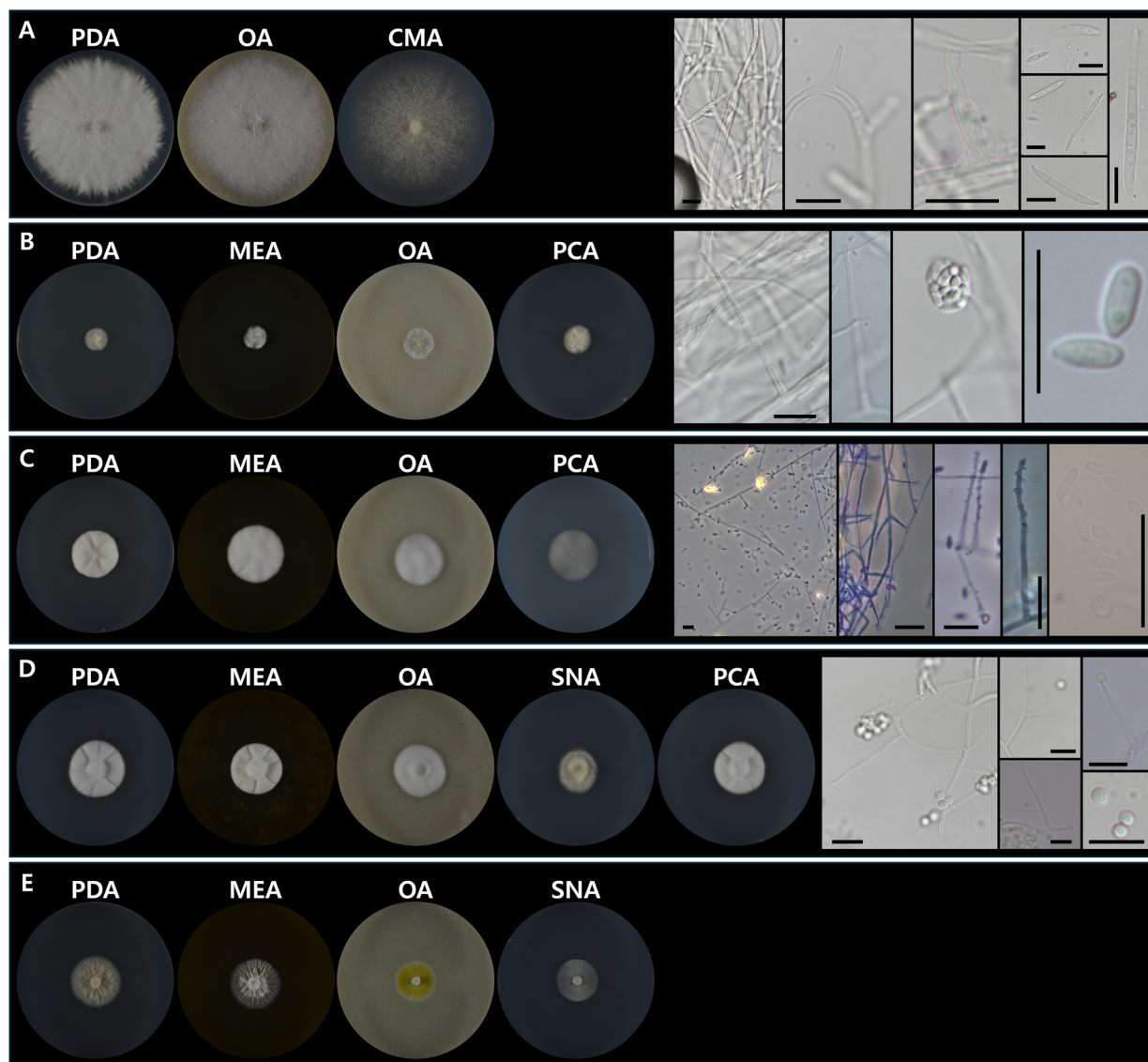


Figure 6. Cultural and conidial morphology. (A) *Fusarium concentricum*. (B) *Lasionectriella arenuloides*. (C) *Lecanicillium verrucum* sp. nov. (D) *Metapochonia rubescens*. (E) *Neoacremonium distortum*. Scale bar: 10 µm.

Notes: False heads are not observed in Korean strain SFC20240607-M006, but are observed in the holotype of this species [62].

***Lasionectriella arenuloides* (Samuels) L.W. Hou, L. Cai & Crous (Figure 6(B))**

MycoBank: MB 845846

Family Bionectriaceae

Basionym: *Nectria arenuloides* Samuels, New Zealand J. Bot. 14: 254. 1976

Synonym: *Hydropisphaera arenuloides* (Samuels) Rossman & Samuels, Stud. Mycol. 42: 30. 1999.

Lasionectriella marigotensis (Lechat & J. Fourn.) L.W. Hou, L. Cai & Crous.

Materials examined: South Korea. Jeollanam-do, Suncheon-si (34°50'29.7"N 127°29'09.4"E), January 2020, isolated from sea sand, M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M007, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on MEA *Mycelia* smooth, septate, hyaline; *Conidiophores* branched, erect, septate, hyaline, smooth, 1.3–2 µm wide. *Phialides*, subulate, hyaline, smooth, 24–50 × 1.8–2.3 µm. *Conidia* aseptate, ellipsoidal to subcylindrical, smooth, hyaline, forming a mucous, rounded head at the tip of phialide, guttules, 3.1–5.8 × 1.6–2.3 µm (av. 4.4 × 1.9 µm).

Culture characteristics: Colonies on PDA reaching 13–15 mm diam in 7 d at 25°C; flat, plain, rugose, membranous, forward yellowish white (2A2); margin filiform; reverse pale yellow (2A3). Colonies on MEA reaching 11–13 mm diam in 7 d at 25°C; raised, plain, felty to floccose, forward white; margin fimbriate; reverse light yellow (4A4). Colonies on OA reaching 17–19 mm diam in 7 d at 25°C; flat, plain, felty, hairy, forward white; margin filiform; reverse yellowish white (2A2). Colonies on PCA reaching 15–17 mm diam in 7 d at 25°C; flat, plain,

rugose, hairy, membranous, forward white; margin entire to filiform; reverse pale yellow (2A3).

Notes: *Lasionectriella arenuloides* and *L. marigotensis* were recently transferred to the genus *Lasionectriella* and exhibited little differences in morphological features and four genetic markers (ITS, LSU, *TEF1*, and *RPB2*) [35]. In this study, we synonymized *Lasionectriella marigotensis* as *Lasionectriella arenuloides* because the polyphasic analysis produced similar results to those of the previous study [35]. The conidia of the Korean strain SFC20240607-M007 are bigger than those of CBS131606 (previous *L. marigotensis*) [53].

***Lecanicillium verrucum* Wonjun Lee & Y.W. Lim, sp. nov.** (Figure 6(C))

MycoBank: MB 855663

Family *Cordycipitaceae*

Etymology: Referring to the phialides that form warts at the upper part.

Typus: South Korea. Gyeongsangbuk-do, Pohang-si (36°15'07"N 129°22'31"E), August 2015, isolated from sea sand, M.S. Park & Y.W. Lim (**holotype** SFC20240607-M029, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on PDA *Mycelia* smooth, septate, hyaline; *Conidiophores* branched, erect, septate, hyaline, 1–2.2 µm wide. *Phialides* narrowly flask-shaped in the widest part, hyaline, rough-walled with warts at the upper part, 11–27 × 0.9–2 µm, polyphialides observed. *Conidia* aseptate, ellipsoidal, oblong-ellipsoidal, smooth, hyaline, 2.9–5.4 × 1.5–2.4 µm (av. 4.1 × 1.9 µm).

Culture characteristics: Colonies on PDA reaching 25–29 mm diam in 7 d at 25°C; raised, radially sulcate, velvety, forward white; margin irregular; exudate clear; reverse pastel yellow (2A4). Colonies on MEA reaching 31–33 mm diam in 7 d at 25°C; raised, radially sulcate, velvety, forward white; margin entire; reverse light orange (5A4). Colonies on OA reaching 27–29 mm diam in 7 d at 25°C; convex, plain, floccose, forward white; margin entire; reverse yellowish white (3A2). Colonies on SNA reaching 27–29 mm diam in 7 d at 25°C; flat, plain, velvety, forward white; margin entire; reverse hyaline to white.

Additional materials examined: South Korea. Jeju-do, Chuja-myeon (33°56'31"N 126°18'50"E), 31 August 2021, isolated from macroalga, M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M030, stored in a metabolically inactive state); *Ibid.* (SFC20240607-M031, stored in a metabolically inactive state); Incheon, Jung-gu (37°21'54.0"N 126°31'40.8"E), January 2019, isolated from unknown substrate, M.S. Park & Y.W.

Lim (SFC20240607-M032, stored in a metabolically inactive state).

Notes: *Lecanicillium verrucum* is phylogenetically related to *L. antillanum*. On PDA, *L. verrucum* colonies grow faster than those of *L. antillanum* CBS 350.85 (vs. 18 mm in 10 d) and have entire, regular margins compared to the irregular margins of *L. antillanum* [63]. *Lecanicillium verrucum* is characterized by rough phialides with warts and does not produce fusiform primary conidia, unlike *L. antillanum*.

***Metapochonia rubescens* (Zare, W. Gams & López-Llorca) Kepler, S.A. Rehner & Humber** (Figure 6(D))

MycoBank: MB 806075

Family *Clavicipitaceae*

Basionym: *Pochonia rubescens* Zare, W. Gams & López-Llorca, Nova Hedwigia 73:69, 2001.

Materials examined: South Korea. Jeollanam-do, Suncheon-si (34°50'29.7"N 127°29'09.4"E), July 2021, isolated from sea sand, M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M010, stored in a metabolically inactive state); Gangwon-do, Gangneung-si (37°51'30.82"N 128°51'15.90"E), January 2015, isolated from sandfish egg, M.S. Park & Y.W. Lim (SFC20160907-M17=SFC102204, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on PCA *Mycelia* smooth, septate, hyaline; *Conidiophores* branched, erect, septate, hyaline, smooth, 1.1–2.3 µm wide. *Phialides* terminal, lateral, narrowly flask-shaped in the widest part, hyaline, smooth, 15–28 × 1.1–2.3 µm, schizophaialides observed. *Conidia* aseptate, globose, smooth, hyaline, 2.2–3.2 × 2–3.1 µm (av. 2.7 × 2.6 µm).

Culture characteristics: Colonies on PDA: reaching 29–32 mm diam in 7 d at 25°C; umbonate, radially sulcate, velvety, forward white; margin entire; reverse pale yellow (2A3). Colonies on MEA reaching 28–29 mm diam in 7 d at 25°C; umbonate, radially sulcate, velvety, forward yellowish white (1A2) to white; margin entire; reverse orange (5B8). Colonies on OA reaching 26–29 mm diam in 7 d at 25°C; umbonate, plain, velvety, forward white; margin entire; reverse yellowish white (2A2). Colonies on SNA reaching 21–25 mm diam in 7 d at 25°C; raised due to abundant aerial mycelium, plain, floccose, felty, forward white, pale yellow (2A3) at center; margin entire; reverse white, pale yellow (2A3). Colonies on PCA reaching 27–29 mm diam in 7 d at 25°C; umbonate, radially sulcate, velvety, forward white; margin entire; reverse pale yellow (2A3).

Notes: The strain SFC102204, isolated from a yellow sandfish egg, was initially identified as

Metapochonia suchlasporia based on *TUB* and was reported to have endoglucanase and gelatinase activities [41]. However, in this study, it was identified as *M. rubescens* through phylogenetic analysis using ITS, *TEF1*, and *RPB2*.

***Neoacremonium distortum* L.W. Hou, L. Cai & Crous (Figure 6(E))**

MycoBank: MB 845811

Family *Neoacremoniaceae*

Materials examined: South Korea. Incheon, Ganghwa-gun (37°36'36.3"N 126°31'13.8"E), January 2017, isolated from mudflat, M.S. Park & Y.W. Lim (SFC20240607-M011, stored in a metabolically inactive state); *Ibid.* July 2018, isolated from mudflat, M.S. Park & Y.W. Lim (SFC20240607-M038, stored in a metabolically inactive state); *Ibid.* April 2017, isolated from mudflat, M.S. Park & Y.W. Lim (SFC20240607-M036, stored in a metabolically inactive state); *Ibid.* (SFC20240607-M037, stored in a metabolically inactive state); Ganghwa-gun (37°35'33.72" 126°27'30.29"), October 2016, isolated from sea sand, M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M034, stored in a metabolically inactive state); Jeollanam-do, Muan-gun (35°3'43.65" 126°20'13.91"), October 2016, isolated from sea sand, M.S. Park & Y.W. Lim (SFC20240607-M035, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph not observed on PDA, MEA, OA, SNA, and PCA.

Culture characteristics: Colonies on PDA reaching 27–29 mm diam in 7 d at 25°C; flat, radially sulcate, rugose, forward white to butter yellow (4A5); margin fimbriate; reverse yellowish white (3A2). Colonies on MEA reaching 25–29 mm diam in 7 d at 25°C flat, radially sulcate, rugose, floccose at center, forward white to hyaline; margin fimbriate; reverse light orange (5A4). Colonies on OA reaching 22–25 mm diam in 7 d at 25°C; flat, plane, sometimes radially sulcate, membranous, moist, forward vivid yellow (2A8), white at margin, sometimes partly vivid yellow (2A8); margin entire; reverse pale yellow (3A3). Colonies on PCA reaching 23–25 mm diam in 7 d at 25°C; flat, plain, hairy, forward white to hyaline; margin filiform; reverse yellowish white (1A2).

Notes: *Neoacremonium distortum* is known for its intraspecific variation [35]. On OA media, Korean strains form vivid yellow colonies, whereas the holotype CBS H-6647 forms white colonies with a buff pigment [35]. Korean strains exhibit fimbriate margins on PDA and MEA, and filiform margins on PCA, while the holotype has entire margins on OA, MEA, and PDA [35]. Korean strains also show

radially sulcate colonies on PDA and MEA, whereas the holotype displays only a lightly radially sulcate trait on PDA as shown in [35]. The conidial structures of Korean strains were not observed on any of the cultured media (PDA, MEA, OA, SNA, and PCA). Korean strains are genetically distinct from the other strains. The variations are 4–7 bp for ITS (515 bp), 13–15 bp for *TEF1* (808 bp), and 7 bp for *RPB2* (750 bp), while LSU does not show significant differences between Korean strains and the others.

***Neocosmospora tuberculata* Wonjun Lee & Y.W. Lim \, (Figure 7(A))**

MycoBank: MB 855664

Family *Nectriaceae*

Etymology: Referring to forming tuberculate chlamydospores.

Typus: South Korea. South Sea of Korea (34°27'42.4"N 128°15'34.7"E), August 16 2022, isolated from seawater in 40 m depth (bottom), Wonjun Lee, J.W. Lee & Y.W. Lim (**holotype** SFC20240607-M039, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on OA *Mycelia* smooth, septate, hyaline; *Conidiophores* branched, erect, septate, hyaline, smooth, 1.6–4.2 µm wide. *Phialides* subulate to subcylindrical, cylindrical, non-flared or flared collar-ette, hyaline, smooth, 38–148 × 1.4–4.3 µm, polyphialides observed. *Conidia* 0–3-septate, subcylindrical, cylindrical, ellipsoidal, smooth, hyaline, gently curved due to forming a false head on phialides, with or without papillate, 5.9–27 × 2–6 µm. *Chlamydospores* abundant, globose, subglobose, mostly tuberculate, terminal or intercalary in hyphae, solitary, in chains, or in clusters, 5.4–12 µm diam. *Sporodochia* not observed.

Culture characteristics: Colonies on PDA reaching 66–71 mm diam in 7 d at 25°C; flat, plain, floccose, hairy, felty forward white to dull blue (23E4), amber yellow (4B6) at margin, sometimes pale red (12A3) at center; margin entire to irregular, filiform; exudate yellow (2A7), sometimes pink (11A5), soluble pigment blond (4D4) when mature; reverse garnet brown (9D8) to buttercup yellow (4A7). Colonies on MEA reaching 42–46 mm diam in 7 d at 25°C; flat to raised, plain, floccose, hairy, sometimes felty, forward grayish green (29B3), having blackish blue (20F7) to grayish green (29C4) patches, sometimes brownish red (10D6) patches; margin entire; soluble pigment some strains pastel red (10A5) when mature; reverse English red (8D8), partly reddish brown (8E8), light orange (5A4) at margin. Colonies on OA reaching 50–55 mm diam in 7 d at 25°C; flat, raised, plain, floccose, hairy, felty, forward yellowish white

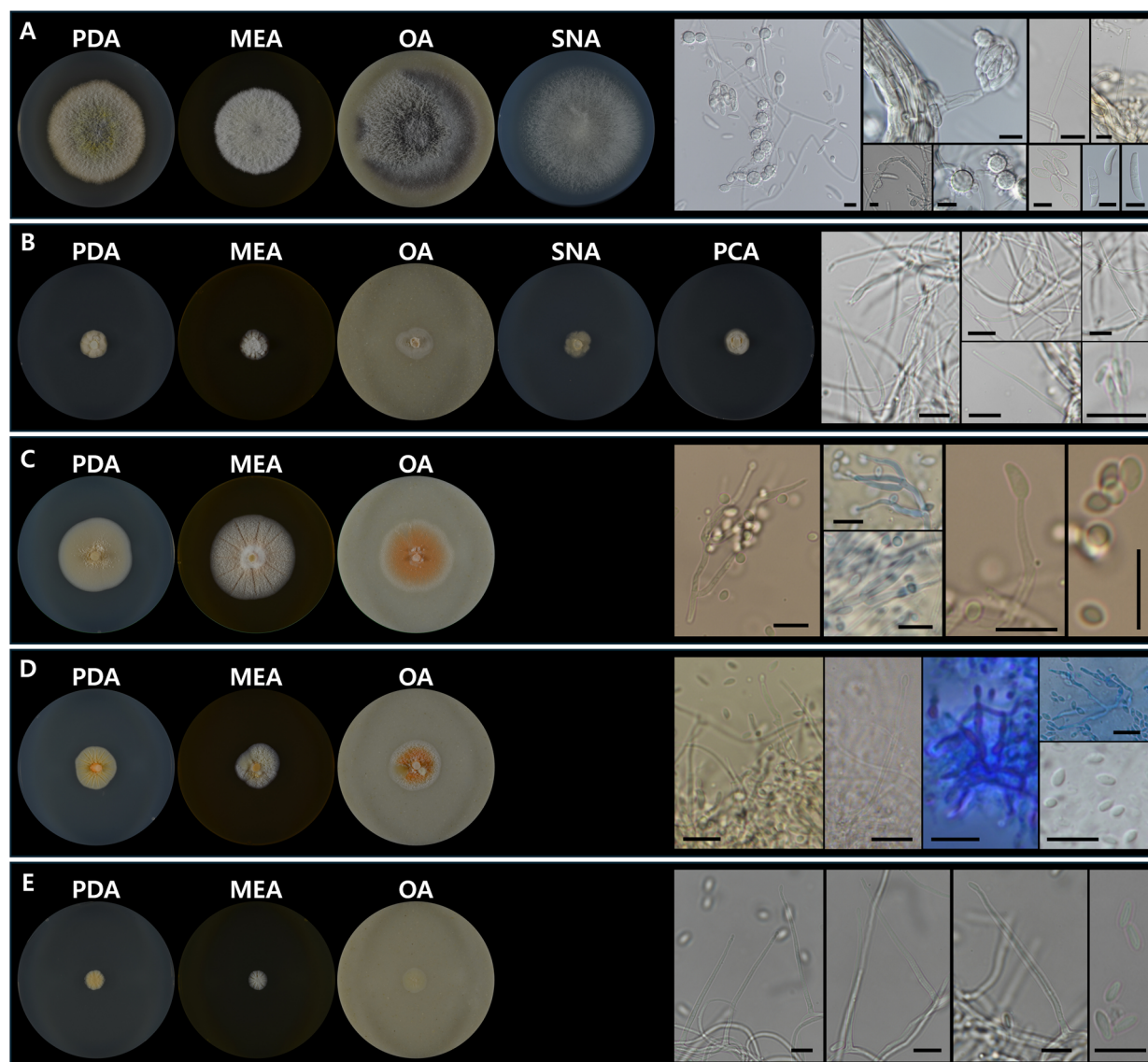


Figure 7. Cultural and conidial morphology. (A) *Neocosmospora tuberculata* sp. nov. (B) *Niesslia marinisedimenta* sp. nov. (C) *Parasarocladium mabikii* sp. nov. (D) *Parasarocladium multimorphologicum* sp. nov. (E) *Protocreopsis rutila*. Scale bar: 10 µm.

(3A2) to dark blue (21F4), pastel grey (15C1) to dull violet (15E4); margin entire; exudate pink (11A5) when mature; reverse golden brown (5D7) to dark brown (8F8), reddish brown (9D4). Colonies on SNA reaching 64–69 mm diam in 7 d at 25 °C; raised due to aerial mycelia, plain, hairy, felty, forward white to hyaline; margin entire; reverse hyaline to yellowish white (1A2).

Additional materials examined: South Korea. South Sea of Korea (34°27'42.4"N 128°15'34.7"E), August 16 2022, isolated from seawater in 40 m depth (bottom), Wonjun Lee, J.W. Lee & Y.W. Lim (SFC20240607-M040, stored in a metabolically inactive state); *Ibid.* (SFC20240607-M040, stored in a metabolically inactive state); *Ibid.* (SFC20240607-M041, stored in a metabolically inactive state); *Ibid.* (SFC20240607-M042, stored in a metabolically inactive state).

Notes: *Neocosmospora tuberculata* is phylogenetically related to *N. solani* and *N. keratoplastica*, but it differs from both species in several characteristics. *N. tuberculata* forms chlamydospores solitarily, in chains, or in clusters, whereas *N. solani* produces chlamydospores that are one or two-celled [64]. Additionally, *N. tuberculata* produces tuberculate chlamydospores, whereas those of *N. keratoplastica* are smooth to rough [65]. The chlamydospores of *N. tuberculata* are also larger, measuring 6.5–8.5 µm compared to 6–8 µm in *N. solani* [64] and *N. keratoplastica* [65].

***Niesslia marinisedimenta* Wonjun Lee & Y.W. Lim, (Figure 7(B))**

MycoBank: MB 855665

Family *Niessliaceae*

Etymology: Referring to the marine sediments (mudflat and sea sand) from which it was isolated.

Typus: South Korea. Jeollanam-do, Muan-gun (35°3'43.65"N 126°20'13.91"E), October 2016, isolated from sea sand, M.S. Park & Y.W. Lim (**holo-type** SFC20240607-M014, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on PCA *Mycelia* smooth, septate, hyaline; *Conidiophores* branched, erect, septate, hyaline, smooth, 1.2–2.6 µm wide. *Phialide* subulate, hyaline, smooth, 27–47 × 1.1–2.4 µm. *Conidia* aseptate, oblong-ellipsoidal, sometimes cylindrical, smooth, hyaline, 2.5–6.6 × 0.9–1.8 µm (av. 3.5 × 1.3 µm).

Culture characteristics: Colonies on PDA reaching 15–17 mm diam in 7 d at 25°C; raised, radially sulcate, velvety, forward white to grayish yellow (4B4); margin lobed; reverse pale yellow (3A3). Colonies on MEA reaching 15–20 mm diam in 7 d at 25°C; raised, radially and concentrically sulcate, felty, downy, forward white, grayish yellow (4B3); margin lobed, filiform; reverse reddish orange (7B8). Colonies on OA reaching 15–20 mm diam in 7 d at 25°C; flat, plain, membranous, granular, forward white; margin irregular; reverse yellowish white (3A2). Colonies on SNA reaching 14–18 mm diam in 7 d at 25°C; flat, plain, membranous, granular, forward white to lemon (3B8); margin entire; reverse pale yellow (3A3). Colonies on PCA reaching 15–17 mm diam in 7 d at 25°C; flat, radially and concentrically sulcate, velvety, membranous, forward white to lemon (3B8); margin entire, fimbriate; reverse pale yellow (3A3).

Additional materials examined: South Korea. Jeollanam-do, Muan-gun (35°3'43.65"N 126°20'13.91"E), October 2016, isolated from sea sand, M.S. Park & Y.W. Lim (SFC20171120-M03, stored in a metabolically inactive state); *Ibid.* (SFC20240607-M043, stored in a metabolically inactive state); *Ibid.* Incheon, Ganghwa-gun (37°36'36.3"N 126°31'13.8"E), January 2019, isolated from mudflat, M.S. Park & Y.W. Lim (SFC20240607-M044, stored in a metabolically inactive state).

Notes: *Niesslia marinisedimenta* is phylogenetically closely related to *N. tenuis* but can be distinguished by the following characteristics. *Niesslia marinisedimenta* does not form white to pinkish colonies on MEA, but *N. tenuis* does [66]. Additionally, *N. marinisedimenta* does not sporulate, *N. tenuis* produces conidia on MEA [66].

***Parasarocladium mabikii* Wonjun Lee & Y.W. Lim, (Figure 7(C))**

Mycobank: MB 855666

Family *Sarocladiaceae*

Etymology: Referring to the name of the National Marine Biodiversity Institute of Korea (MABIK).

MABIK is an institute that investigates Korean marine organisms and strives to unveil a variety of marine organisms.

Typus: South Korea. Incheon, Ganghwa-gun (37°36'36.3"N 126°31'13.8"E), January 2021, isolated from mudflat, M.S. Park, J.S. Kim & Y.W. Lim (**holo-type** SFC20240607-M027, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on PDA *Mycelia* smooth, septate, hyaline, abundant guttules; *Conidiophores* solitary or aggregated, arising directly from aerial or substratal mycelium, branched, erect, straight or irregularly curved, septate, hyaline, smooth, bearing 1–2 levels with 1–3 phialides per node, showing conidiogenous cells as lateral, cylindrical, asymmetrical projections, guttules, 1.1–3 µm wide. *Phialides* lateral or terminal, narrowly flask-shaped in the widest part, subulate to cylindrical, hyaline, smooth, terminal or subterminal proliferation, monophialides, polyphialides with up to three conidiogenous loci, frequently trident-form, guttules, 9.4–48 × 1.4–3 µm. *Conidia* aseptate, ellipsoidal, obclavate, smooth, hyaline, eguttulate, truncated base, 2.8–6.4 × 2–3.5 µm (av. 3.9 × 2.7 µm). *Chlamydoconidia* not observed.

Culture characteristics: Colonies on PDA reaching 38–40 mm diam in 7 d at 25°C; flat, plain, membranous, felty, forward orange white (5A2), white at margin; margin filiform; reverse pale yellow (3A3). Colonies on MEA reaching 44–48 mm diam in 7 d at 25°C; flat, radially sulcate, felty, cottony at center, forward orange white (5A2) to pinkish white (8A2), white at center due to aerial mycelia; margin filiform; reverse pale orange (5A3). Colonies on OA reaching 41–44 mm diam in 7 d at 25°C; flat, plain, granular, moist, forward light orange (5A5), white at margin; margin entire; reverse yellowish white (4A2).

Additional materials examined: South Korea. Incheon, Ganghwa-gun (37°36'36.3"N 126°31'13.8"E), July 2016, isolated from mudflat, M.S. Park & Y.W. Lim (SFC20240607-M025, stored in a metabolically inactive state); *Ibid.* (SFC20240607-M026, stored in a metabolically inactive state).

Notes: *Parasarocladium mabikii* is closely related to *P. wereldwijsianum* but differs in several aspects. The former species grow faster on OA (vs 30–35 mm diam) and MEA (vs. 30 mm diam) compared to *P. wereldwijsianum* [67]. The conidia of *P. mabikii* are shorter than those of *P. wereldwijsianum* (vs. 4–10 µm long) [67]. Additionally, *P. mabikii* contains distinct insertions in *TEF1* genetic marker, which are absent in *P. wereldwijsianum* [67].

***Parasarocladium multimorphologicum* Wonjun Lee & Y.W. Lim, (Figure 7(D))**

MycoBank: MB 855668

Family *Sarocladiaceae*

Etymology: Referring to the morphological characteristics of colonies on MEA media.

Typus: South Korea. Jeju-do, Chuja-myeon (33°57'11.88"N 126°18'07.56"E), August 31 2021, isolated from *Grateloupia* sp. (Macroalga), M.S. Park, J.S. Kim & Y.W. Lim (**holotype** SFC20240607-M024, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on PDA *Mycelia* smooth, septate, hyaline; *Conidiophores* reduced to conidiogenous cells, aggregated, arising directly from aerial or substratal mycelium, branched, erect, septate, hyaline, smooth, 10–34 × 1.1–2.1 µm, polyphialidic structure observed. *Conidia* aseptate, ellipsoidal, oblong-ellipsoidal, smooth, hyaline, 2.3–4.7 × 1.2–2 µm (av. 3.3 × 1.6 µm). *Chlamydospores* not observed.

Culture characteristics: Colonies on PDA reaching 22–25 mm diam in 7 d at 25°C; flat, radially sulcate, membranous, felty, cerebriform at center, sometimes rugose, forward light orange (5A5), yellowish white (3A2) to pale yellow (3A3); margin lobed; reverse pale yellow (4A3). Colonies on MEA reaching 17–24 mm diam in 7 d at 25°C; raised, plain, radially sulcate in some part, rugose, felty, cerebriform, sometimes membranous, felty to floccose in SFC20240607-M023, forward pale yellow (2A3), white at margin, orange white (5A2) to white; margin irregular, lobed filiform; reverse light orange (6A4). Colonies on OA reaching 24–28 mm diam in 7 d at 25°C; flat, plain, sometimes radially sulcate, rugose, felty, sometimes membranous, forward light orange (5A5), white at margin; margin entire, sometimes irregular; reverse orange white (5A2).

Additional materials examined: South Korea. Jeju-do, Chuja-myeon (33°56'31"N 126°18'50"E), August 31 2021, isolated from mudflat, M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M023, stored in a metabolically inactive state); *Ibid.* (SFC20240607-M022, stored in a metabolically inactive state).

Notes: *Parasarocladium multimorphologicum* forms a distinct clade, separate from other species, such as *P. tasmaniae*, *P. debruynii*, *P. chondroidum*, and *P. gamsii* (Figure 1). The colonies of *P. multimorphologicum* grow slowly on OA and MEA compared to those of *P. debruynii* (vs. 45–55 mm and 38–50 mm diam, respectively) [68]. Among closely related species, *P. gamsii* is considered a marine fungus [16,69].

***Protocreopsis rutila* (W. Gams) L.W. Hou, L. Cai & Crous** (Figure 7(E))

MycoBank: MB 845853

Family *Bionectriaceae*

Basionym: *Acremonium rutilum* W. Gams 1971

Materials examined: South Korea. Incheon, Ganghwa-gun (37°35'33.72"N 126°27'30.29"E), July 2016, isolated from sea sand, M.S. Park & Y.W. Lim (SFC20170718-M03, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on OA *Mycelia* smooth, septate, hyaline; *Conidiophores* branched, erect, septate, hyaline, smooth, 1.5–3.6 µm wide, degraded conidiophores not observed. *Phialides* subulate, hyaline, smooth, 39–70 × 2.2–3.8 µm at the base, polyphialides not observed. *Conidia* aseptate, ellipsoidal, oblong-ellipsoidal, smooth, hyaline, 4–6.9 × 1.4–2.6 µm (av. 5.3 × 2 µm).

Culture characteristics: Colonies on PDA reaching 10–11 mm diam in 7 d at 25°C; flat, radially sulcate, rugose, membranous, forward light yellow (4A4); margin lobed; reverse pastel yellow (3A4). Colonies on MEA reaching 10–11 mm diam in 7 d at 25°C; flat, radially sulcate, rugose, membranous, forward yellowish white (3A2); margin entire; reverse light orange (5A4). Colonies on OA reaching 13–15 mm diam in 7 d at 25°C; flat, plain, membranous, forward yellowish white (3A2); margin entire; reverse yellowish white (2A2).

Notes: Korean strain SFC20170718-M03 forms yellowish-white without pigments on PDA media. On the other hand, the holotype CBS 394.70 forms colonies with a sienna center, peach middle, and salmon periphery, producing a yellowish pigment [35]. Additionally, moist slimy heads of conidia and degenerated conidiophores, observed in CBS 394.70 [35], are absent in the Korean strain.

***Purpureocillium lavendulum* Perdomo, Dania García, Gené, Cano & Guarro** (Figure 8(A))

MycoBank: MB 561126

Family *Ophiocordycipitaceae*

Materials examined: South Korea. South Sea of Korea (34°27'42.4"N 128°15'34.7"E), August 16 2022, isolated from seabed sediment 40 m deep, Wonjun Lee, J.W. Lee & Y.W. Lim (SFC20240607-M017, stored in a metabolically inactive state); Jeollanam-do, Muan-gun (35°3'43.65"N 126°20'13.91"E), July 2021, isolated from sea sand, M.S. Park & Y.W. Lim (SFC20240607-M033, stored in a metabolically inactive state); Gangwon-do, Goseong-gun (38°28'53.0"N 128°26'18.1"E), January 2020, isolated from sea sand, M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M015, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on MEA *Mycelia* smooth, septate, hyaline; *Conidiophores* erect, septate, hyaline, smooth

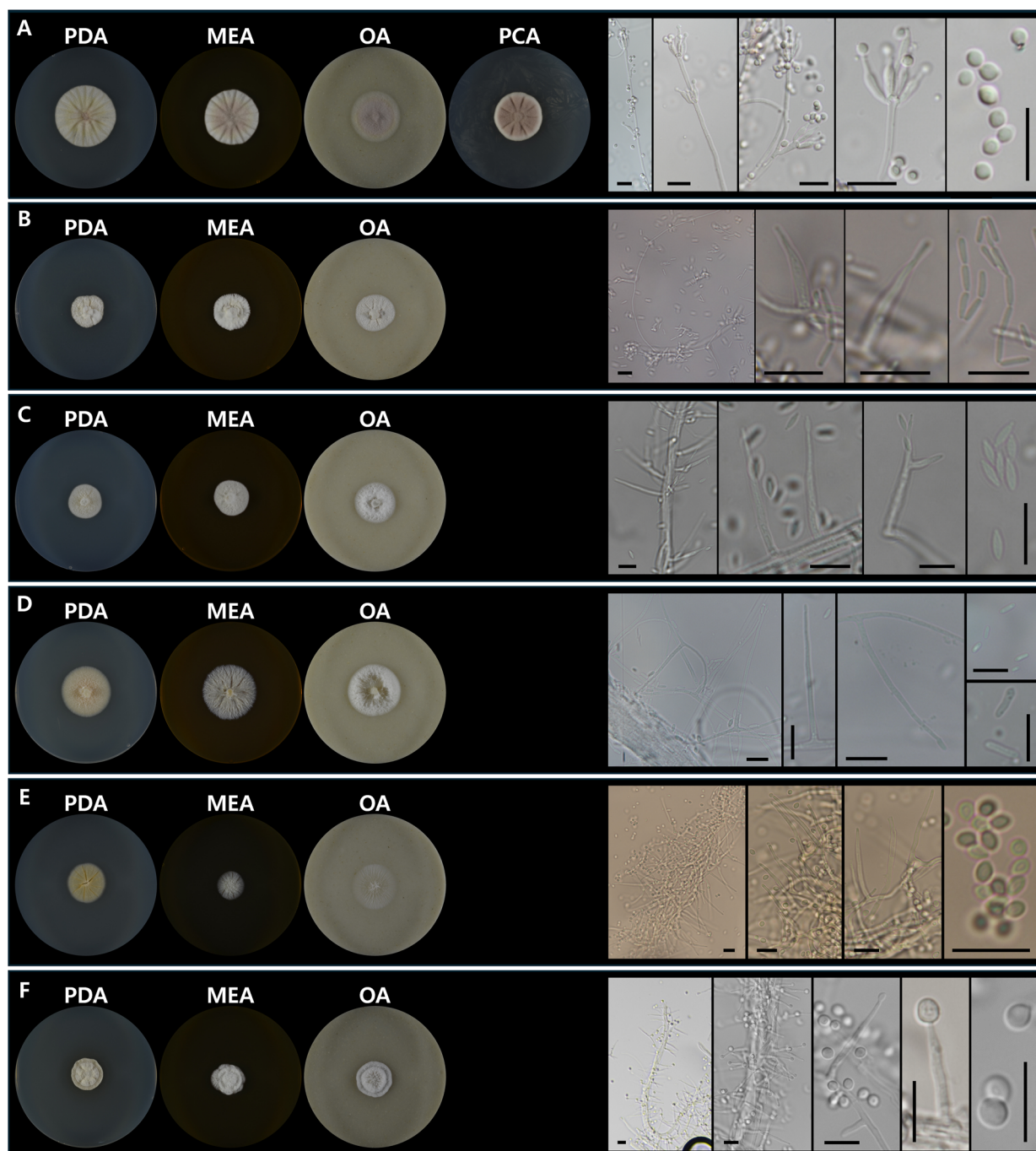


Figure 8. Cultural and conidial morphology. (A) *Purpureocillium lavendulum*. (B) *Sarocladium bacillisporum*. (C) *Sarocladium terricola*. (D) *Sarocladium zeae*. (E) *Verruciconidia infuscatum*. (F) *Verruciconidia persicina*. Scale bar: 10 µm.

or rough-walled stipe, 1–2.2 µm wide, 3–6 phialides per whorl. *Phialides* terminal, lateral, cylindrical, ampulliform, hyaline, smooth, 6.3–14 × 1.8–3 µm, *Acremonium*-like synanamorph observed. *Conidia* aseptate, globose, subglobose, sometimes broadly ellipsoidal, smooth, hyaline, 1.8–2.8 × 1.7–2.5 µm (av. 2.3 × 2.1 µm).

Culture characteristics: Colonies on PDA reaching 37–39 mm diam in 7 d at 25 °C; flat, radially sulcate, velvety, powdery at grayish Magenta center and sulcate, forward pale yellow (2A3), grayish Magenta (13B3) at center; margin entire; reverse light yellow (3A5), yellowish white (3A2) at margin, reddish lilac

(14B4) in age. Colonies on MEA reaching 34–36 mm diam in 7 d at 25 °C; flat, radially sulcate, velvety, forward reddish lilac (14B4), yellowish white (2A2) at middle, white at margin; margin entire; reverse golden yellow (5B7). Colonies on OA reaching 30–32 mm diam in 7 d at 25 °C; flat, plain, velvety, granular, forward purplish pink (14A4), yellowish white (3A2) thinly at middle, white at margin; margin entire; reverse pastel yellow (2A4). Colonies on PCA reaching 27–31 mm diam in 7 d at 25 °C; flat, radially sulcate, velvety, powdery, forward reddish lilac (14B4), white at margin; margin entire; reverse lemon (3B8).

Notes: *Purpureocillium lavendulum* forms acremonium-like phialides and ampuliform phialides with longer necks at the apex compared to *Penicillium* [70]. The stipe ornamentation of *P. lavendulum* (CBS 128677) is well observed under an electron microscope [71], but the Korean strains were not clear visible by a light microscope. However, the overall growth morphology of the Korean strains was similar to CBS 128677 [70].

***Sarocladium bacillisporum* (Onions & G.L. Barron) Summerb. (Figure 8(B))**

MycoBank: MB 519589

Family *Sarocladiaceae*

Basionym: *Paecilomyces bacillisporus* Onions & G.L. Barron 1967

Materials examined: South Korea. Gangwon-do, Goseong-gun (38°28'53.0"N 128°26'18.1"E), July 2018, isolated from sea sand, M.S. Park & Y.W. Lim (SFC20240607-M028, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on OA *Mycelia* smooth, septate, hyaline; *Conidiophores* solitary or aggregated, erect, septate, hyaline, smooth, 1.3–3.3 µm wide. *Phialides* subulate, hyaline, smooth, polyphialides not observed, 11–27 × 1.6–3.3 µm at the base. *Conidia* aseptate, cylindrical, rod-shaped, straight, smooth, hyaline, eguttulate, truncate, arranged in chain, 3.7–5.4 × 0.9–1.6 µm (av. 4.5 × 1.2 µm).

Culture characteristics: Colonies on PDA reaching 19–21 mm diam in 7 d at 25°C; raised, radially sulcate, felty, floccose, forward white to yellowish white (3A2); margin fimbriate; exudate clear; reverse pale yellow (4A3). Colonies on MEA reaching 19–22 mm diam in 7 d at 25°C verrucose, slightly radially sulcate, felty, floccose, forward white; margin fimbriate; exudate clear; reverse light orange (5A4). Colonies on OA reaching 20–23 mm diam in 7 d at 25°C; flat, radially sulcate, granular, felty, forward white; margin entire; reverse yellowish white (4A2).

Notes: The Korean strain SFC20240607-M028 grows faster than the strains (20–24 mm diameter in 14 d) described by Giraldo et al. [36]. SFC20240607-M028 reaches 19–21 mm in diameter in 7 d on PDA media.

***Sarocladium terricola* (J.H. Mill., Giddens & A.A. Foster) A. Giraldo, Gené & Guarro (Figure 8(C))**

MycoBank: MB 807950

Family *Sarocladiaceae*

Basionym: *Fusidium terricola* J.H. Mill., Giddens & A.A. Foster 1958

Materials examined: South Korea. Jeju-do, Jeju-si (33°23'53"N 126°14'24"E), August 15 2021, isolated

from *Gelidium* sp. (Macroalga), M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M008, stored in a metabolically inactive state); Jeollanam-do, Muan-gun (35°3'43.65"N 126°20'13.91"E), July 17 2017, isolated from sea sand, M.S. Park & Y.W. Lim (SFC20240607-M009, stored in a metabolically inactive state); Gyeongsangbuk-do, Ulleung-gun (37°32'2.05"N 30°49'24.87"E), unknown date 2018, isolated from sponge, M.S. Park & Y.W. Lim (SFC20240607-M012, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on OA *Mycelia* smooth, septate, hyaline; *Conidiophores* solitary or aggregated, erect, septate, hyaline, smooth, 1–2.7 µm wide. *Phialides* subulate, hyaline, smooth, schizophtialides observed, adelophtialides observed 11–32 × 1.5–3.1 µm at the base. *Conidia* aseptate, fusiform, smooth, hyaline, eguttulate, truncate, arranged in chain, 3.1–6.3 × 1.3–2.4 µm (av. 4.6 × 1.8 µm).

Culture characteristics: Colonies on PDA reaching 18–21 mm diam in 7 d at 25°C; raised, plain, sometimes radially sulcate, felty, sometimes floccose at center, forward white to pale yellow (4A3); margin entire; reverse yellowish white (4A2). Colonies on MEA reaching 19–22 mm diam in 7 d at 25°C raised, plain, sometimes radially sulcate, felty to floccose, forward white; margin entire; reverse light orange (5A4). Colonies on OA reaching 23–25 mm diam in 7 d at 25°C; raised due to aerial mycelium, plain, felty, hairy to floccose, forward white; margin filiform; reverse yellowish white (3A2).

Notes: The morphological characteristics of the Korean strains resemble those described by Giraldo et al. [36]. While occurrences of the species have been commonly reported in terrestrial environments [36], in this study, the species was isolated from macroalgae, sea sand, and seawater. Notably, it was also discovered at a depth of 5,572 m in the western Pacific [72].

***Sarocladium zeae* (W. Gams & D.R. Sumner) Summerb. (Figure 8(D))**

MycoBank: MB 519595

Family *Sarocladiaceae*

Basionym: *Acremonium zeae* W. Gams & D.R. Sumner, in Gams 1971

Materials examined: South Korea. Jeollanam-do, Gangjin-gun (34°26'49"N 126°49'07"E), 2016, isolated from *Fulvia mutica* (marine bivalve mollusks), M.S. Park & Y.W. Lim (SFC20240607-M013, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on OA Vegetative hyphae septate, hyaline, smooth- and thin-walled, 1.1–2.9 µm wide.

Conidiophores erect, hyaline, smooth-walled. Phialides arising directly from vegetative hyphae and aerial mycelia, acicular, subulate, $34\text{--}56 \times 1.1\text{--}2.7\ \mu\text{m}$ at the base, thin- and smooth-walled, hyaline; adelophialides and schizophialides not observed. Conidia unicellular, cylindrical, $3\text{--}9 \times 0.9\text{--}2.4\ \mu\text{m}$, hyaline to subhyaline, smooth- and thin-walled.

Culture characteristics: Colonies on PDA reaching 28–30 mm diam in 7 d at 25°C; flat, plain, felty, forward orange white (5A2), yellowish white (4A2) at margin; margin filiform; reverse pale yellow (4A3). Colonies on MEA reaching 31–33 mm diam in 7 d at 25°C flat, radially sulcate, rugose, felty, forward yellowish white (3A2), white at margin; margin fimbriate; reverse light orange (5A4). Colonies on OA reaching 29–33 mm diam in 7 d at 25°C; crateriform due to the aerial mycelia at margin and middle, plain, floccose, felty, membranous, forward white to wax white (2B3); margin filiform; reverse yellowish white (4A2).

Notes: The Korean strain SFC20240607-M013, isolated from a marine bivalve (*Fulvia mutica*), clusters with strains of *Sarocladium zae* in the phylogenetic tree. Compared to strains documented by Giraldo et al. [36], SFC20240607-M013 grows faster and produces longer spores on OA media.

***Verruciconidia infusata* L.W. Hou, L. Cai & Crous (Figure 8(E))**

Mycobank: MB845839

Family Bionectriaceae

Materials examined: South Korea. Jeollanam-do, Suncheon-si ($34^{\circ}50'29.7''\text{N}$ $127^{\circ}29'09.4''\text{E}$), July 2021, isolated from sea sand, M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M019, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on OA *Mycelium* branched, septate, hyaline; *Sporulation* abundant; *Conidiophores* solitary or aggregate, unbranched or branched, erect, straight to flexuous, septate, smooth, hyaline, $1.2\text{--}3.3\ \mu\text{m}$ wide, bearing 1–2 phialides per node. *Phialides* cylindrical or subulate, smooth-walled, hyaline, $26\text{--}48 \times 1.4\text{--}3\ \mu\text{m}$ at the base. *Conidia* aseptate, subglobose to broadly ellipsoidal, smooth, hyaline, $2.4\text{--}3.5 \times 1.4\text{--}2.7\ \mu\text{m}$ (av. $2.8 \times 1.9\ \mu\text{m}$), conidia chains not observed. *Chlamydospores* not observed.

Culture characteristics: Colonies on PDA reaching 21–23 mm diam in 7 d at 25°C; flat, radially sulcate, rugose, felty, forward pale yellow (2A3); margin entire; reverse pale yellow (3A3). Colonies on MEA reaching 16–17 mm diam in 7 d at 25°C; flat, radially sulcate, rugose, membranous, forward white to hyaline; margin entire; reverse reddish orange (7B8) to light yellow (4A4). Colonies on OA

reaching 25–27 mm diam in 7 d at 25°C; flat, plain, rugose, hairy, forward white to hyaline; margin filiform; reverse yellowish white (1A2).

Notes: The Korean strain SFC20240607-M019 does not produce pigment, whereas the holotype CBS H-24613 produces pigments on PDA, OA, and MEA media in old cultures [35]. Additionally, SFC20240607-M019 has shorter phialides and conidia compared to the holotypes (38–50.5 and 3–5 μm long, respectively) [35].

***Verruciconidia persicina* (Nicot) L.W. Hou, L. Cai & Crous (Figure 8(F))**

Mycobank: MB845840

Family Bionectriaceae

Basionym: *Paecilomyces persicinus* Nicot 1958

Materials examined: South Korea. Jeju-do, Chuja-myeon ($34^{\circ}0'0.94''\text{N}$ $126^{\circ}20'30.31''\text{E}$), September 12 2017, isolated from *Sargassum thunbergii* (Macroalga), M.S. Park & Y.W. Lim (SFC20240607-M020, stored in a metabolically inactive state); Incheon, Ganghwa-gun ($37^{\circ}36'36.3''\text{N}$ $126^{\circ}31'13.8''\text{E}$), July 2021, isolated from mudflat, M.S. Park, J.S. Kim & Y.W. Lim (SFC20240607-M019, stored in a metabolically inactive state).

Description: Sexual morph undetermined. Asexual morph on OA *Mycelium* branched, septate, hyaline; *Sporulation* abundant; *Conidiophores* solitary or aggregate, unbranched or branched, erect, straight to flexuous, septate, smooth, hyaline, $1.2\text{--}3.3\ \mu\text{m}$ wide, bearing 1–2(–5) phialides per node. *Phialides* subulate, smooth-walled rarely with a nodule, hyaline, $17\text{--}26 \times 1.5\text{--}2.8\ \mu\text{m}$ at the base. *Conidia* aseptate, subglobose to broadly ellipsoidal, smooth, hyaline, $2.6\text{--}4.2 \times 2\text{--}3.2\ \mu\text{m}$ (av. $3.5 \times 2.7\ \mu\text{m}$), conidia chains not observed. *Chlamydospores* not observed.

Culture characteristics: Colonies on PDA reaching 17–19 mm diam in 7 d at 25°C; flat, radially sulcate, velvety, felty, forward white to dull yellow (3B3), pale yellow (3A3); margin entire to slightly irregular; reverse butter yellow (4A5), pale orange (5A3), pale yellow (3A3) at margin. Colonies on MEA reaching 19–20 mm diam in 7 d at 25°C; raised to convex, radially sulcate, floccose, felty, forward white; margin lobed; reverse light orange (5A5), grayish orange (5B5). Colonies on OA reaching 18–21 mm diam in 7 d at 25°C; convex to umbonate due to abundant aerial mycelium, plain, felty, floccose, forward white, sometimes partly pastel yellow (3A4); margin entire to slightly irregular; exudate sometimes wax yellow (3B5); reverse pale orange (5A3) to pale yellow (4A3), sometimes dull red (8B3) at center.

Notes: The Korean strains, isolated from mudflat and green macroalga (*Sargassum thunbergii*), exhibit

textures ranging from velvety to floccose compared to the dusty colonies of the holotype CBS H-6661 [35]. Additionally, the Korean strains have longer conidia, measuring 2.6–4.2 µm, compared to those of the holotype (4.2–6.2 µm) [35].

5. Discussion

This study expanded our understanding of marine *Hypocreales* by identifying 21 species, including 5 new species and 16 unrecorded species in South Korea. Phylogenetic and morphological analyses strongly support that these five species are new to science, as they formed distinct clades separate from closely related species, with apparent morphological differences. Despite being based on a limited number of strains, this is undoubtedly remarkable, especially considering that only 131 species of *Hypocreales* have been previously reported from marine environments in South Korea (Marine Bio-Resource Information System, updated 2024.03.29).

In fungal taxonomy, standardized methods are limited for some groups, with most research focused on economically important genera like *Penicillium* and *Trichoderma* [70,73,74]. The International Code of Nomenclature for Fungi (ICNF) only requires confirmation of description for valid publication of new species, resulting in varying levels of taxonomic detail depending on the classification [75]. Some genera in this study, such as *Purpureocillium* and *Metapochonia*, had also limited taxonomic research, which led to fewer descriptive features being available [11,76]. To address the morphological plasticity of marine fungi, we used various media and multiple genetic markers to establish the starting point of a robust species description framework. Integrating detailed morphological observations with molecular data, this polyphasic approach provided a comprehensive dataset for marine *Hypocreales*. Some species exhibited morphological variability, which is often a response to environmental stressors, such as changes in size, growth rates, and textures [77,78]. Further studies on ecotypes and physiological adaptations are necessary to understand these phenomena better.

Most of the species identified in this study were isolated in sediments (Table 1), and only a few were associated with marine organisms such as marine animals and macroalgae. Ongoing debates question whether fungi found in sediments are “truly marine fungi” – meaning fungi actively functioning in marine environments [25,31,79]. However, recent meta-transcriptome data suggest that fungi, though not dominant, play roles like carbohydrate recycling in marine sediment [80–84]. Microscopy studies

have also provided evidence, such as the presence of branching cells, indicating fungal growth [85]. Although the specific roles of these fungi remain unclear, further research into their diversity, function, and whether they are invaders that have adapted to marine environments is essential.

This study is one of the first to apply a polyphasic approach to reveal the diversity and taxonomic placement of *Hypocrealean* fungi in Korean marine environments. Our findings highlight the high species diversity of *Hypocreales* in these environments and the limitations of relying solely on morphological characteristics or ITS-based single-marker analysis for species-level identification. This study also gives detailed descriptions of *Hypocrealean* fungi that have not been studied much before. These descriptions help us learn more about each species and are meant to be the basis for standardizing taxonomic studies for these groups. Furthermore, this research provides valuable insights for future studies on their ecological interactions, evolutionary relationships, and potential industrial applications.

Disclosure statement

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