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Paracremonium moubasheri, a new species from an alkaline sediment of Lake Hamra in Wadi-El-Natron, Egypt with a key to the accepted species

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Abstract

During surveys of extremophilic fungi in hypersaline, alkaline lakes of Wadi-El-Natron, Egypt, an interesting fungus was recovered from a mud sample collected from Lake Hamra in Wadi-El-Natron, Egypt. Maximum likelihood analysis of Internal Transcribed Spacer (ITS) gene along with morphological comparisons of related taxa revealed a novel taxon, *Paracremonium moubasheri* which is described and illustrated in the current study. *Paracremonium moubasheri* can be distinguished from the known species of the genus by its large conidia, in addition to the presence of chlamydospores.

Key words – Acremonium-like – Hypersaline – Hypocreales – new taxon – Phylogeny

Introduction

Paracremonium is acremonium-like genus related to order Hypocreales which includes approximately 2700 species belonging to 240 genera (Crous et al. 2014). The genus *Paracremonium* was introduced by (Lombard et al. 2015), and since this date 5 species appertaining to that genus were described namely *P. binnewijzendii* Houbraken, van der Klij & L. Lombard, *P. contagium* L. Lombard & Crous, *P. inflatum* L. Lombard & Crous, *P. pembeum* S.C. Lynch & Eskalen, and *P. variiforme* Z.F. Zhang, F. Liu & L. Cai. The genus *Paracremonium* is characterized by hyaline, septate, branched hyphae which sometimes forming sterile coils from which conidiophores arising. These morphological characters can distinguish the genus *Paracremonium* from other acremonium-like genera. Some species of *Paracremonium* are associated with human infections as *P. inflatum* which isolated from a granulomatous lesion of a male in India and *P. contagium* from a subcutaneous lesion of a male in Canada (Lombard et al. 2015). However some others as *P. binnewijzendii* was isolated from stream embankments in the Netherlands (Crous et al. 2017), *P. pembeum* from *Acer negundo* L., *Persea americana* Mill., *Platanus racemosa* Nutt., *Ricinus communis* L. trees and heads of *Euwallacea* sp. in California, USA (Lynch et al. 2016), and *P. variiforme* from water sample of karst cave in China (Zhang et al. 2017). Reports of fungi from extremely hypersaline environments are very limited compared with other habitats like soil. However, lack of representation in the literature may reflect the little effort that has been payed to recover them. Therefore, the present study was conducted for

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identification of new taxa in such extreme habitats like saline lakes of Wadi-El-Natron in Egypt.

Materials and methods

Sampling and strain isolation

Mud samples were collected from Lake Hamra. The dilution plate technique (Harris & Sommers 1968) was employed for isolation of the fungi from mud samples using 1 % glucose-Cz medium (Ismail et al. 2017). After incubation at 25 °C for 15 days, the developed colonies were purified and maintained on Cz slants at 4 °C for further investigation. It was preserved and deposited as pure culture in the culture collection of the Assiut University Mycological Centre as AUMC 11030 and the sequence of ITS region was uploaded to GenBank as KX384655.

Morphological studies

Cultural morphological characteristics and growth rates were studied on malt extract agar (MEA, (Samson 2010), Czapek's agar (CZ, (Raper & Fennell 1965), and potato dextrose agar (PDA, (Smith & Onions 1994) at 30 °C in addition to on Cz at 5 °C, 25 °C and 37 °C. Inoculations were made from spore suspension prepared in a 0.2 % agar and 0.05 % Tween 80 solution (Samson et al. 2014). Plates were inoculated in three-point pattern using a micropipette and inoculum size of 1 µl per spot. Unwrapped cultures were incubated in the dark reverse side. Microscopic features on Cz were examined in lacto-phenol cotton blue.

Molecular studies

DNA extraction

Prior to DNA extraction, small piece of fungal mycelia of *Paracremonium moubasheri* cultured on Cz agar plates at 30 °C for 7 days were collected and transferred to 2 ml-Eppendorf tube. DNA extraction was performed following the method of (Moubasher et al. 2019).

PCR amplification

The PCR reaction was performed using SolGent EF-Taq. The universal primers ITS1 and ITS4 (White et al. 1990) were used for DNA amplification. In the PCR tubes 1µl of DNA template, 1 µl 2.5 mM dNTP mix, 0.2 unit of Taq polymerase, 5 µl of 10x complete buffer and 40 µl of sterile ddH2O, 10 pmol of ITS1 (5´ TCC GTA GGT GAA CCT TGC GG 3´) and ITS4 (5´ TCC TCC GCT TAT TGA TAT GC 3´) were added. Amplification was conducted using the following PCR reaction conditions: one round of amplification consisting of denaturation at 95 °C for 15 min followed by 30 cycles of denaturation at 95 °C for 20 sec, annealing at 50 °C for 40 sec and extension at 72 °C for 1 min, with a final extension step of 72 C for 5 min. The PCR products were then purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) prior to sequencing. 1 % agarose gel was used for confirmation of the purified PCR products by electrophoreses. Then these bands were eluted and sequenced in the forward and reverse directions.

Phylogenetic analysis

Sequence data of all published *Paracremonium* species including sequences of the available type materials were downloaded from GenBank. The phylogenetic analysis was carried out using the Maximum Likelihood method and General Time Reversible model (Nei & Kumar 2000). The bootstrap consensus tree inferred from 100 replicates was taken to represent the phylogenetic analysis of the taxa analyzed (Felsenstein 1985). Branches corresponding to partitions reproduced in less than 50 % bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches (Felsenstein 1985). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log

likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 1.4098)). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 25.53% sites). This analysis involved 13 nucleotide sequences. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). There was a total of 380 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018)

Results

The new taxon was isolated from mud sample characterized by alkaline pH value of 9.74, high total dissolved solids (TDS) of 12.6 %, high sodium (185.7 g/kg dry mud) and total chlorides (59.3 g/kg dry mud) contents (Ismail et al. 2017) revealing the extremophilic nature of the new species since it was isolated from a typical hypersaline, alkaline habitat.

Phylogenetic analysis

The ITS dataset comprised 13 sequences, of which 10 are *Paracremonium* and 3 sequences of *Cosmospora* (Fig. 1). The ML analysis of ITS data set yielded a best scoring RAxML tree (Fig. 1). The phylogenetic analysis revealed that *P. moubasheri* is grouped with other species of *Paracremonium* but in a single branch in the phylogenetic tree indicating that this is a new species. It has a homology percentage ranged from 92.68 % with *P. contagium* to 96.95 % with *P. inflatum*.

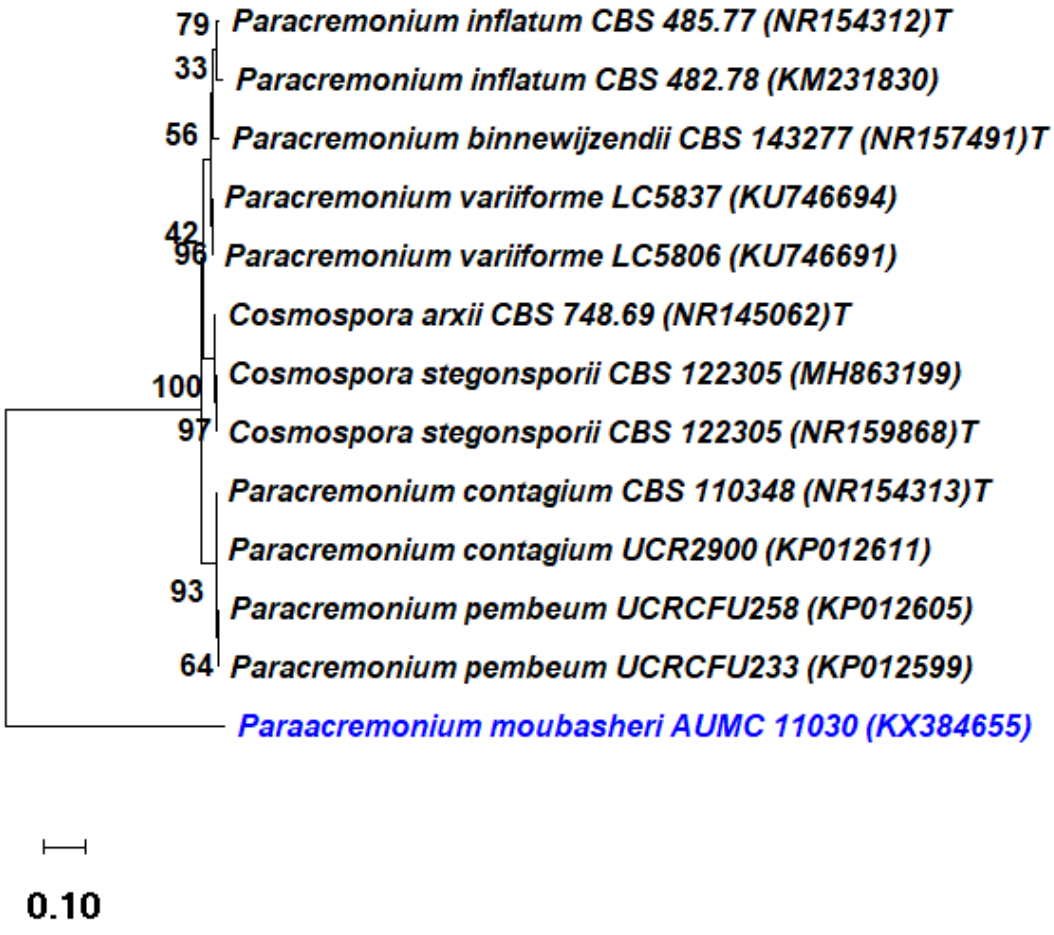

Fig. 1 – Maximum Likelihood tree of *Paracremonium moubasheri* (AUMC 11030) and other *Paracremonium* species based on the data of the ITS gene sequences (GenBank accession numbers in parentheses; type species are signed with (T); sequence of the new species in blue).

Taxonomy

Paracremonium moubasheri Al-Bedak OA & Ismail MA, sp. nov. Fig. 3

GenBank number: KX384655; Mycobank number: MB831989;

Facesoffungi number: FoF 06585

Etymology – Named after Moubasher AH, Founder of the Assiut University Mycological Centre (AUMC), Assiut University, Assiut Governorate, Egypt.

Typification: EGYPT. Wadi-El-Natron: Lake Hamra, from alkaline mud sample, 2 Feb 2012, Osama A. Al-Bedak (holotype AUMC 11030).

Cultural characteristics – Colonies on Cz agar attaining a diameter of 40–50 mm after 14 days at 30 °C, greyish-orange (5B-3), raised in the center with aerial mycelial tufts; margin entire, flat, paler than the colony center, narrow; reverse clay (5D-5). On PDA at 30 °C colonies reaching 35–42 mm in diameter after 14 days, orange white (5B-2), flat to slightly raised in the center, margin entire, flat, wide; reverse champagne (4B-4). On MEA colonies attaining 43–50 mm in diameter after 14 days at 30 °C, brownish-orange (5C-3), flat to slightly raised colony center, margin entire, flat, paler than the colony center; reverse yellowish brown (5D-8). On Cz at 5 °C after 14 days colonies restricted, attaining 6-8 mm diameter. Colonies on Cz after 14 days attaining a diameter of 35 mm at 25 °C and a diameter of 35–38 mm at 37 °C, greyish-orange (5B-3), raised in the center with aerial mycelial tufts; margin entire, flat, paler than the colony center; reverse nougat (5D-3) (Fig. 2).

Asexual morph: *Vegetative hyphae* 1–2 µm width, hyaline, smooth- and thin-walled, septate, branched. Sterile coiled hyphae absent. *Conidiophores* consisting of single, hyaline, smooth-walled, erect, tapered, unbranched or rarely branched, 0–1 septate phialides, commonly (15-) 30–50 (–100) × 2–3 µm (n=50) or 2 phialides may be borne on a stipe arising from vegetative and aerial hyphae. *Phialides* 1–1.5 µm width, hyaline, smooth, elongate-ampulliform, tapering towards apex. *Conidia* (7-) 10–12 (-16) × 2–3 µm (n=100), hyaline, smooth-walled, aseptate, fusiform, straight to slightly curved, commonly formed in slimy heads. *Chlamydospores* abundant, 10–17 × 7–15 µm (n=100), globose to subglobose. Sexual morph: *Ascomata* not observed (Fig. 3).

Key to known species of Paracremonium

1. Chlamydospores present ……2
- Chlamydospores absent ……3
2. Conidia 10–12 (–16) × 2–3 µm ……***P. moubasheri***
- Conidia 4.5–7.0 µm ……*P. pembeum*
3. Conidiophores bearing whorls of 2–4 phialides, conidia 9–14.5 × 4–6 µm ……*P. variiforme*
- Conidiophores simple ……...4
4. Sterile coils from which conidiophores radiating outwards present, conidia 5–6 × 1–2 µm ……..*P. inflatum*
- Sterile coils absent ……5
5. Conidia 7–11 (–13) × (1.5–)2.5–3.5(–4.5) µm ……..*P. binnewijzendii*
- Conidia 4–6 (–7) × 2–3 µm ……...*P. contagium*

Discussion

The genus *Paracremonium* was recently established for different fungal strains previously treated as *Acremonium recifei* (Lombard et al. 2015). According to the available literatures, five species were described in the genus *Paracremonium* namely *P. binnewijzendii*, *P. contagium*, *P. inflatum*, *P. pembeum* and *P. variiforme*. *Paracremonium binnewijzendii* was isolated from stream embankments in The Netherlands (Crous et al. 2017), while *P. contagium* (Canada) and *P. inflatum* (India and Colombia) are associated with human infections (Lombard et al. 2015), *P. pembeum* with trees of *Acer negundo*, *Persea americana*, *Platanus racemose* and *Ricinus communis* in addition to heads of *Euwallacea* sp. in California, USA (Lynch et al. 2016) and *P. variiforme* from water sample of karst cave in China (Zhang et al. 2017), in addition to the new species *P.*

moubasheri (described here) which was isolated from an alkaline sediment from Lake Hamra in Wadi-El-Natron, Egypt.

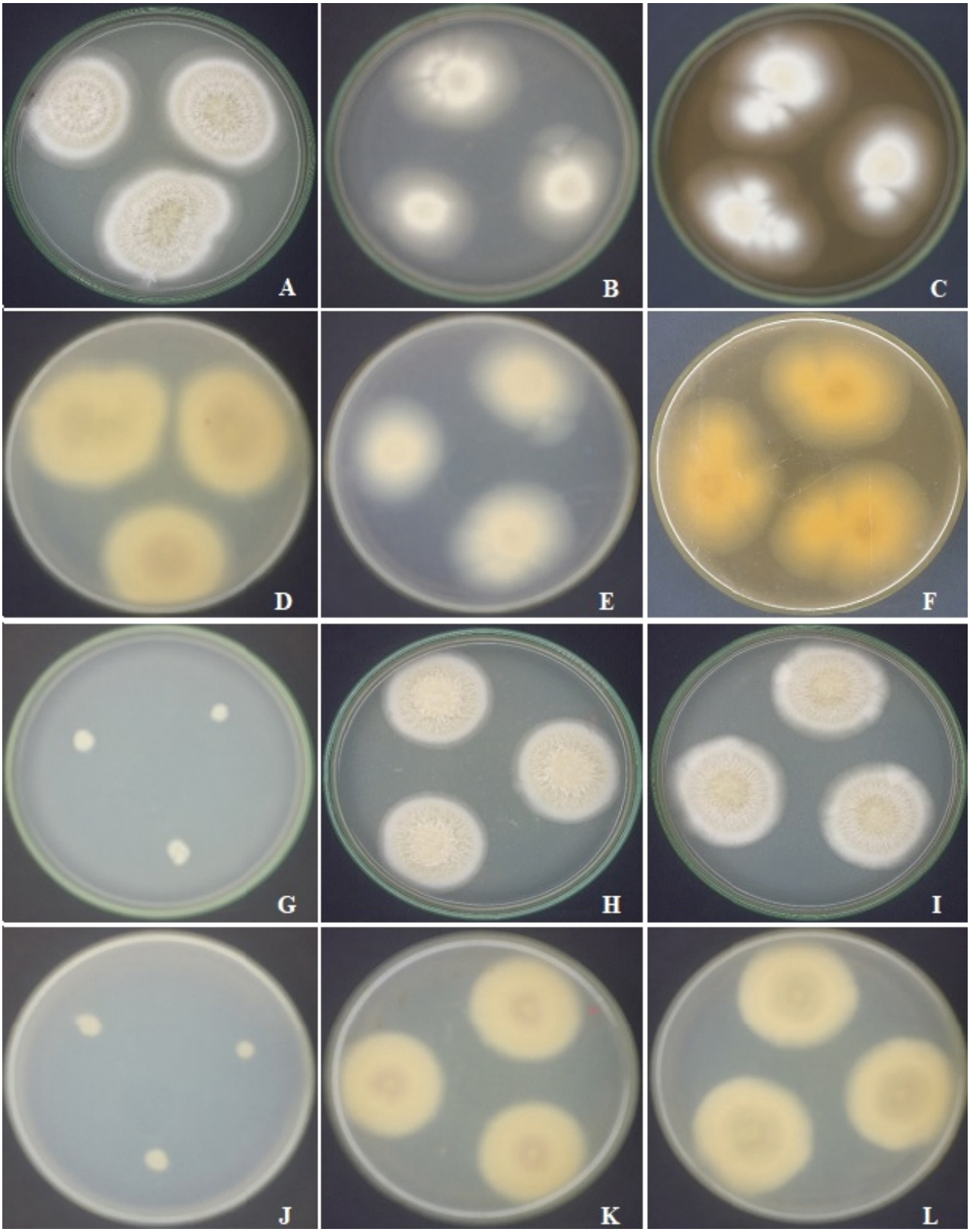

Fig. 2 – *Paracremonium moubasheri* (AUMC 11030). A–C colonies on Cz, PDA and MEA after 14 days at 30 °C. D–F reverse on Cz, PDA and MEA. G–I colonies on Cz after 14 days at 5 °C 25 °C and 37 °C. J–L reverse on Cz at 5 °C, 25 °C and 37 °C.

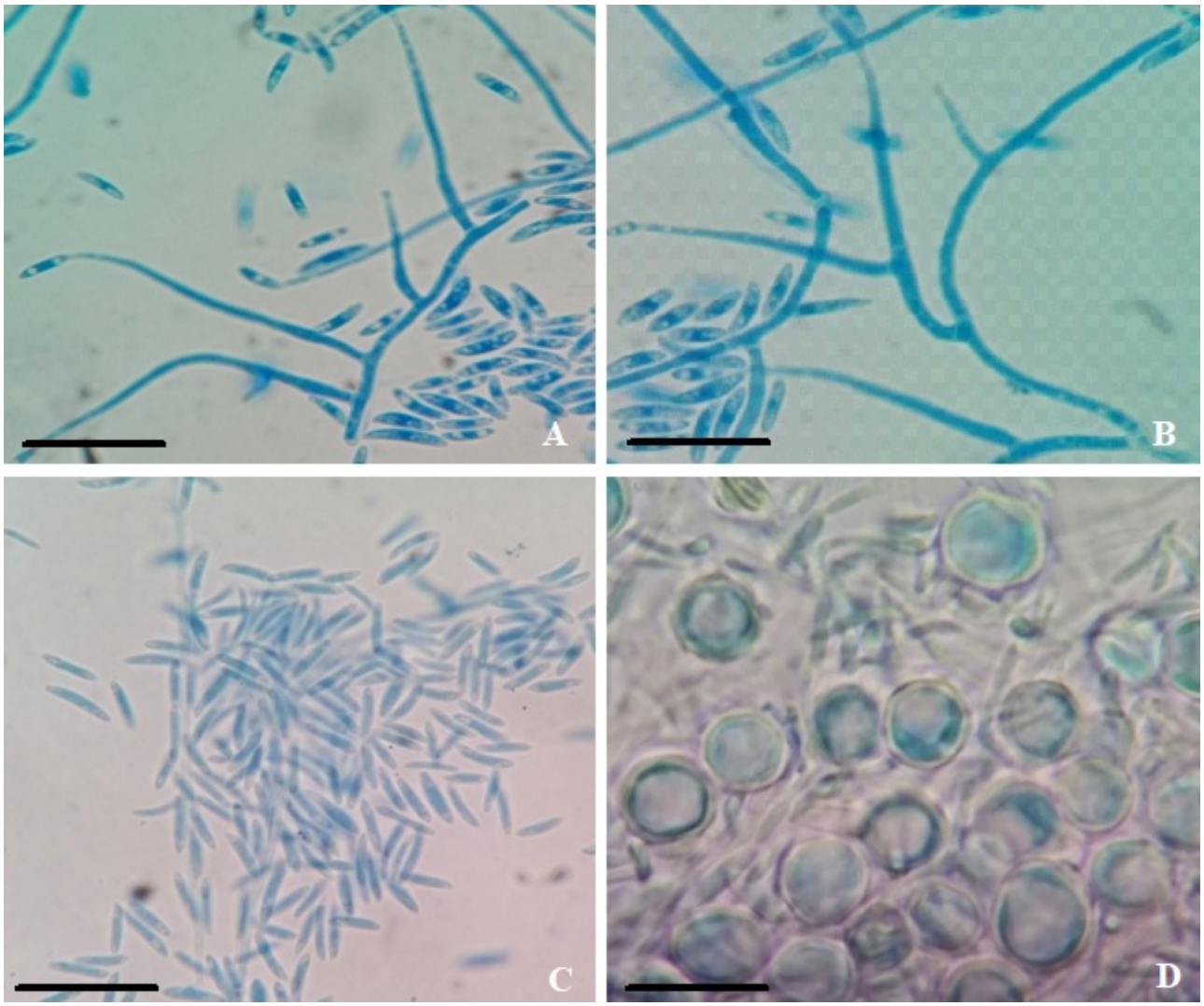

Fig. 3 – *Paracremonium moubasheri* (AUMC 11030). A unbranched conidiophores. B branched conidiophores. C fusiform, straight to slightly curved conidia. D globose to subglobose chlamydospores (Scale bar = 20 µm).

Based on megablast search using the ITS sequence of the new species, the closest matches in GenBank nucleotide database were *P. inflatum* CBS 485.77 [(GenBank NR154312 and KM231829; Identities 540/557 (96.95 %), 2 gaps (0 %)] with interspecific difference of 17 nucleotides, and *P. binnewijzendii* CBS 143277 [(GenBank NR157491; Identities 537/562 (95.55 %), 7 gaps (1 %)] with interspecific difference of 25 nucleotides.

Paracremonium moubasheri can be distinguished from the other species in the genus by its large conidial size and presence of chlamydospores. The conidia of *P. moubasheri* are commonly 10–12 (-16) µm in length, and the conidia of the other species in the genus are usually less than 7 µm long except *P. binnewijzendii* and *P. variiforme* which have large conidia but no chlamydospores are absent. *Paracremonium pembeum* produces globose to ellipsoidal, hyaline, thick-walled chlamydospores but differs from the new species by its small conidia (4.5–7.0) µm. *P. moubasheri* can be distinguished from *Paracremonium inflatum* by the absence of sterile coils from which conidiophores radiate and presence of chlamydospores which not produced in *P. inflatum*. Also, *P. moubasheri* can be distinguished from *P. contagium* by its large size conidia and the presence of chlamydospores.

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