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MORPHOLOGICAL DESCRIPTION AND A NEW RECORD OF GENUS *CUNNINGHAMELLA* (MUCORALES) FROM PAKISTAN

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Abstract

In this study, two species of industrially important and pathogenic zygomycete genus *Cunninghamella, C. bertholletiae* and *C. echinulata*, have been described, illustrated, identified on morphological details. One species, *C. bertholletiae* is a common opportunistic human pathogen of mucormycosis, is reported here as a new record for Pakistan. *C. echinulata* has an industrial relevance by producing large quantities of lipids. Both fungi have been isolated from rhizosphere of rice and wheat plants. Identification of these taxa was confirmed by morphological and microscopic characterization. A comprehensive key to identify *Cunninghamella* species from Pakistan is also provided in this paper.

Key words:

Colony morphology, Filamentous fungi, Micrographs, Punjab, Taxonomy

INTRODUCTION

The genus *Cunninghamella* is the most common member of the order Mucorales described by Matruchot (1903). Species of this genus are commonly found in soil, dung, grains, and other organic substrates as saprobes and play important role as decomposers in natural ecosystems (Baijal and Meheotba, 1980; Voigt *et al.,* 1999). Regarding distribution, members have been reported mainly from mediterranean, subtropical or warmer zones, however, can also be found in temperate regions (Walther *et al.,* 2019). Most are saprobes and some taxa are human pathogens. Species of this genus have traditionally been characterized on morphological and anatomical basis (Liu *et al.,* 2005). Genus is characterized by a having branched/unbranched, septate or aseptate sporangiophore, globose to oval sporangial vesicle, and smooth to echinulate sporangiospores which can be globose to oval (Liu *et al*., 2005; Alves *et al.*, 2017). Although filamentous fungi play very important ecological roles such as nutrient recycling and maintenance of soil fertility, but the available knowledge is limited in Pakistan. This research aims to expand the knowledge of diversity, distribution and morphology of filamentous fungi associated with the rhizosphere of agriculturally important crops such as wheat and rice.

MATERIALS AND METHODS

Cunnighamella species described in this study have been isolated from rhizosphere of wheat (*Triticum aestivum*) and rice (*Oryza sativa*) crops from two agricultural sites of Lahore (31.5204° N, 74.3587° E) and Hafizabad (32.0712° N, 73.6895° E) districts of Punjab province, Pakistan. Soil samples were collected near the root zones at approximately 10-15 cm depth. Soil samples were brought to the

laboratory in sterilized and labeled zip-lock bags for further analysis. Isolation of filamentous fungi was performed using culture dependant methods following the protocol of Warcup (1950). For this purpose, 1 gram of soil sample was used to make serial dilution and poured on PDA medium containing streptomycin (50 mg/L).

Plates were incubated in the dark at 28 ℃ for a week. Distinct colonies of *Cunnighamella* were observed and selected for obtaining pure culture. Pure cultures were established following Nguyen *et al*. (2018). Pure colonies were obtained after incubation at the same conditions for 7 days. These taxa have been characterized based on colony and microscopic morphology. Colony morphology included colour, appearance, form, margins and elevations were recorded following Domsch *et al.* (2007). Microscopic morphology included shape and size of the sporangiophore, sporangia and sporangiospores. Measurements have been done using ocular micrometre and illustrations were made using Camera Lucida.

RESULTS

1. *Cunninghamella bertholletiae* Stadel, Über neuen Pilz, Cunn. bertholletiae, (Diss., Kiel): 1-35 (1911) (Fig. 1)

Colony morphology: Greyish brown to dark brown, rapidly growing, floccose, raised, pattern central, margins irregular.

Microscopic characterization:

Sporangiophore brown, branched, septate, lack cytoplasmic aggregations, thick-walled, bear globose vesicle, 6–10 × 2–3 µm. Sporangium brown, globose, contains metullae. Collumella brown, terminal, globose, uniseriate, bear broad phialides at the apex on which spores is attached, 30-40 µm in diam. Spores brown, globose, thick-walled, smooth, 7–9 µm in diam.

Material examined – Pakistan, Punjab, Hafizabad, in soil of rice fields, 15th November 2019, (collector) S. Ali, (SFP1).

2. *Cunninghamella echinulata* (Thaxt.) Thaxt., Rhodora 5: 98 (1903) (Fig. 2)

Colony morphology: White, rapidly growing, raised, pattern irregular, margins not well-defined, white spots appear on the colonies.

Microscopic characterization: Sporangiophore hyaline, erect, diversely branched, irregular and verticillate, cytoplasmic aggregations present, septum is present above the origin of branching, also bears short lateral branches, each end into a small vesicle, 6.5–12 µm in length. Sporangium hyaline, globose, 8.5–12 × 20–25.5 µm. Vesicles hyaline, terminal, globose to sub-globose, bears spine like sterigmata, up to 1.5 µm. Sporangiola hyaline to light brown, globose to ovoid, echinulate, up to 13 µm in diam., echines up to 4 µm. Spores brown, globose, thick-walled, echinulate, with thick spines, 5-22 µm

KEY TO PAKISTANI SPECIES OF GENUS *CUNNINGHAMELLA*

1a. Spores smooth……... 2

1b. Spores ornamented…… 3

2a. Sporangiophore brown, septate, branched, thick-walled, having globose vesicle. Sporangium brown, globose, Spores brown, globose, thick walled, 7–9 µm……. ***C. bertholletiae***

2b. Sporangiophore hyaline, aseptate, branched, branching arising solitary or in verticils, thick walled, having spherical to ellipsoidal vesicle. Sporangium hyaline, globose to ellipsoidal. Spore hyaline, globose, thick-walled, 7–10 µm…….…… …. ***C. blakesleeana***

3a. Spores hyaline……...4

3b. Spores brown……....5

4a. Sporangiophore hyaline, aseptate, verticillately brachned, thick-walled, having sub globose to oval vesicle. Sporangium hyaline, spherical to ellipsoidal. Spores hyaline to pale brown, punctate to spinulose, 6–10

µm.....

..... ***C. elegans***

4b. Sporangiophore hyaline, aseptate, branched, branching arises singly, thick-walled, having globose to sub-globose vesicle. Sporangium hyaline, globose. Spores hyaline, globose, conspicously echinulate 6–10 µm.

……

…… ***C. phaespora***

5a. Sporangiophore hyaline, aseptate, verticillate to pseudo-verticillate branching, thick-walled, having globose vesicle. Sporangium brown, globose. Spores brown, globose, shortly echinulate, 9–15 µm……

… ***C. bainieri***

5b. Sporangiophore hyaline, aseptate, irregular to verticillate branching, thick-walled, ends with small vesicle. Sporangium hyaline, globose. Spores brown, globose, thick-walled, echinulate, with thick spines, 5–22

µm……

… ***C. echinulata***

Discussion:

In this study two species of genus *Cunninghamella* are reported, viz. *C. bertholletiae* and *C. echinulata*. *C. bertholletiae* is characterized by branched, septate sporangiophore which is 6–10 × 2–3 µm bearing round vesicle at the ends, and smooth sporangiospores of up to 9 µm. *C. bertholletiae* is also similar to *C. blakesleeana* because both have smooth to echinulate spores but in *C. bertholletiae* sporangiophore is septate which is aseptate in *C. blakesleeana.* It is presented here as a new record for Pakistan.

Morphologically *C. echinulata* is identified by diversely branched, erect, sporangiophore up to 12 µm, with a septum present above the origin of branching. Vesicles are characteristically sterigmate bearing globose to ovoid, echinulate spores of up to 6 µm (Milko and Beljakova, 1967). *C. echinulata* closely resembles with *C. homothallica* due to the presence of septate sporangiophore and echinulated spores in both but both species were distinguished based on size of spores that were 5–22.5 µm vs 9–17 µm and size of spines that were 4 µm in *C. echinulata* and 3.3 µm in *C. homothallica* (Baijal and Meheotba, 1980).

It also resembles *C. baineri* due to presence of brown and echinulated spores in both but former lacks globose sporangium which was present in *C. baineri*. *C. echinulata* is also close to *C. clavata* because both have clavate vesicle but *C. echinulata* lacks globose sporangium which were present in *C. clavata* (Alves *et al*., 2017). It *is* also related to *C. elegans* and *C. phaespora* having similar features of echinulate spores but former has septa present above the branching in sporangiophore while this character is absent in *C. elegans* and *C. phaespora.* This taxon has previously been isolated from soil in Punjab (Lahore, Faisalabad), Sindh (Karachi), KP and Baluchistan, on *Sonchus roxburghii*, on goat dung in Lahore (Ahmad, 1956; Rizvi, 1966; Hussain *et al*., 1966; Qureshi, 1966; Ghaffar *et al*., 1971; Mirza *et al*., 1979).

CONCLUSION:

During efforts to investigate the micro-flora from rhizosphere fields of rice and wheat fields, two

interesting species of genus *Cunninghamella* were reported and among these, one species *C. bertholletiae* is found new for Pakistani fungal flora. This study also highlights the occurrence of two important *Cunnighamella* species in soils of Pakistan. One taxon (*C. echinulata*) is famous for its relevance in lipid production. While microbial lipids are gaining popularity amongst researchers, it can be exploited in future for lipid extraction for further use in commercial fats, oils or in food products. Another reported taxon is *C. bertholletiae,* which is a reported opportunistic pathogen of mucromycosis causing disease in humans. Identifying the taxa using the key provided in the article will be helpful to clinicians and mycologists of the country for the accurate diagnosis and subsequent treatment.

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AUTHOR'S CONTRIBUTION

Naila Younas and Shanzay Ali: Methodology, Formal analysis; Naila Younas: Writing the original draft; Nousheen Yousaf and Umer Farooq Awan Supervision of lab work and fungal identification, Manuscript review and editing.

CONFLICT OF INTEREST:

Authors of this manuscript agree that there is no conflict of interest.

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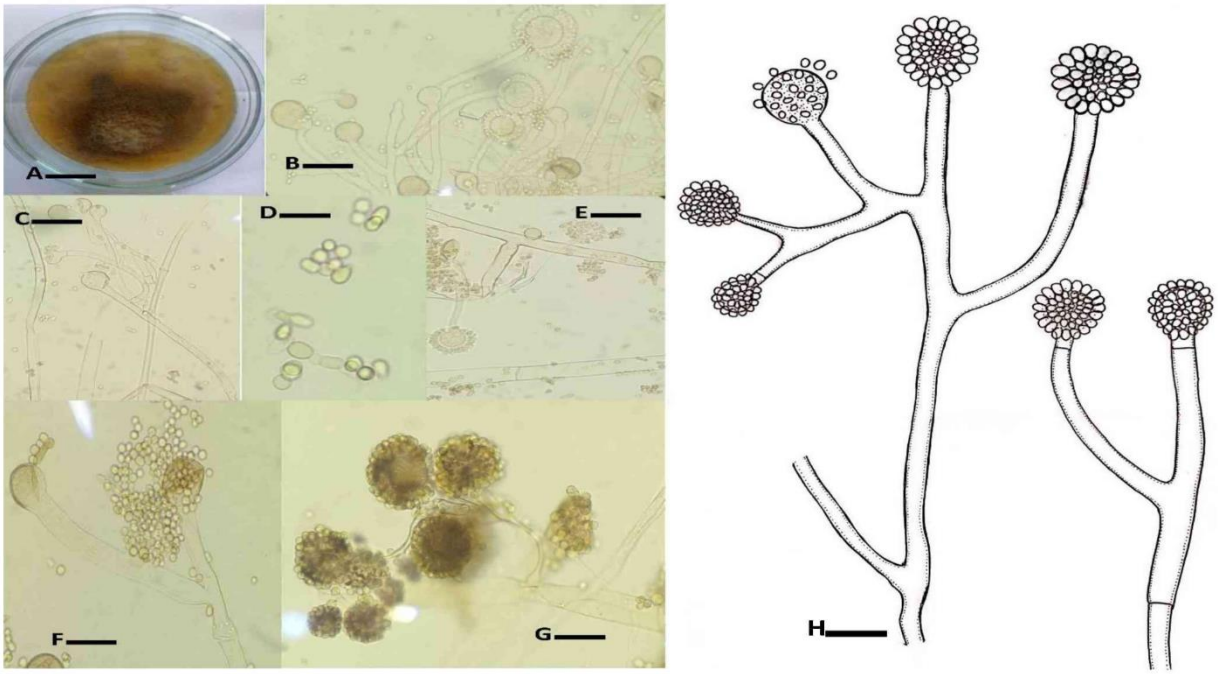

Fig. 1. A-E. Morphology of *Cunninghamella bertholletiae*. **A** Pure culture. **B, E-G** Light micrographs of sporangia. **C** Light micrographs of sporangiophore. **D** Light micrograph of sporangiospores**. H** Illustration of sporangiophores having sporangia. Scale bars: **A** = 1.5 cm, **B and C** = 35 µm, **D** = 6 µm, **E** = 23 µm, **F** = 11.5 µm**, G =** 15 µm.

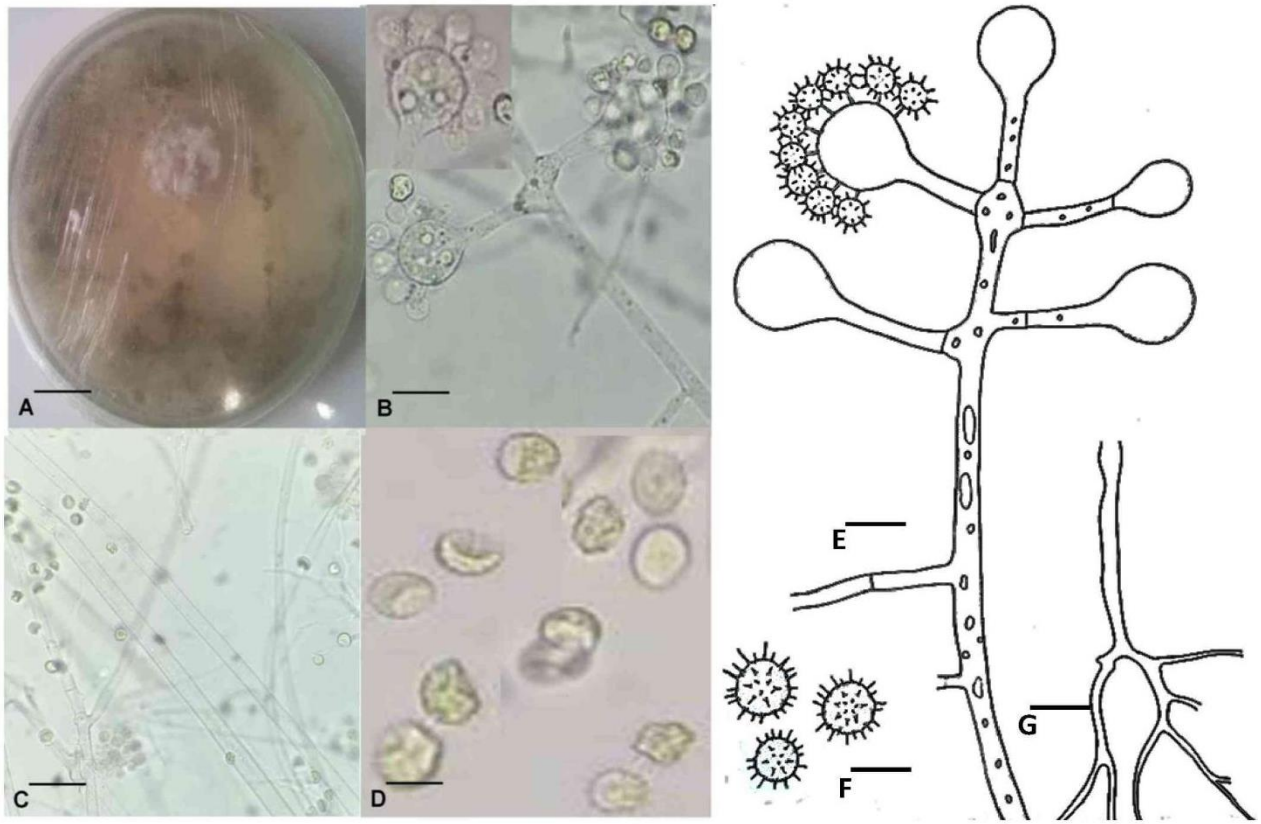

Fig. 2. A-G – Morphology and illustration of *Cunninghamella echinulata*. **A** pure culture**. B** Light micrograph of sporangiola. **C** Light micrograph of sporangiophore. **D** Light micrograph of spores. **E** Illustration of branched sporangiophores with terminal vesicle. **F** Illustration of spores. **G** Illustration of branched rhizoids**. Scale bars: A** = 1.6 cm, **B** = 22.5 µm, **C and F** = 24 µm, **D and E** = 6 µm**.**

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