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Occurrence of *Cladosporium Colombiae* as a First Record in Iraq

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Abstract. *Cladosporium* species are the most common fungal component of air, it is cosmopolitan and found on all kinds of plants, soil, food, and organic substrate, this genus belongs to dematiaceous fungi which represent a large and varied group of fungi. *Cladosporium colombiae* first isolate from *Cortaderia* leaves collected in Colombia, and in this study, *Cladosporium colombiae* was isolated from air in Basrah, Iraq, and depending on its morphology and molecular analysis, it was identified.

Keywords: *Cladosporium*, Morphological Characteristics, Sequence analysis, Taxonomy

INTRODUCTION

The genus *Cladosporium* was discovered by Link 1816 and a historical review of this genus was conducted by several studies [1,2,3]. *Cladosporium* represents an important genus of hyphomycetes (Dothideomycetes, *Cladosporiaceae*), and it includes quite 772 names [4], a reason for this massive number returns to strong morphological variability between species [5]. Most species are saprobes and isolated from the different substrate of plant, food, animals, air, and soil [6,7], in certain cases it can become a pathogen and cause various diseases to many plants with different names due to infected plants and type of symptoms or found as endophytic fungi [8,9,10,11] *Cladosporium* species associated with allergic reaction and many species isolated from human and animal clinical samples [12,13,14]. *Cladosporium* is usually found in the conidial stage anamorph and sexual stage teleomorph found rarely in the culture [8], this genus is distinguished by a distinct conidiogenous cell its shape resembles a crown, therefore, it is called coronate. Currently, PCR technology is always used in diagnosing *Cladosporium* [1,15]. *Cladosporium colombiae* first isolate from *Cortaderia* leaves collected in Colombia [16]. During a survey of airborne fungi in Basrah, Iraq we isolate *Cladosporium colombiae* and determine its morphological and molecular characteristics. Previous works on genus *Cladosporium* in Basrah city revealed six species of this genus: *C. cladosporioides*, *C. herbarum*, *C. sphaerospermum*, *C. spongiosum*, *C. oxysporum* and *C. uredinicola* [11,17,18,19].

MATERIAL AND METHODES

Fungal Isolation

Samples were collected from air by exposure of Petri-dishes that contains Potato Dextrose Agar medium to air, plates were located at a high place and on-ground for 20 min. The dishes were incubated and examined regularly. Colonies believed to be *Cladosporium* is being purified. Slide cultures were prepared and morphological characteristics were recorded.

Diagnosis

The monoconidial culture was grown on PDA media for 10 days at 25 °C mycelium mass which was harvested by scraping the culture. DNA was isolated from mycelium using the Preps Mini package of Ez-10 Spin Column Fungal Genomic DNA (Biobasic, Canada). PCR amplifications were done in a 50 µl total volume containing 10 µl DNA template, 25 µl master mix, 11 µl nuclease-free water, and 2 µl of ITS 1 and ITS4 primer [20]. PCR was done in a thermocycler (Eppendorf); the program applied for amplification was a (1 cycle of 3 min denaturation at 94 °C and followed by 35 cycles of 40 sec at 94 °C, 60 sec at 55 °C, 60 sec at 72 °C and 10 min final extension at 72 °C. Sequencing was conducted by Macrogen Company.

Data Analysis

1. DNA Dragon (<http://www.sequentix.de/dnadragon>)
2. BLAST" program (<http://blast.ncbi.nlm.nih.gov>)

RESULTS

The result of morphological study of *Cladosporium Colombiae* can be seen in Table 1. and Figure 1. The followings:

TABLE 1. Morphological characteristics of *Cladosporium colombiae*

Structures	Description
Colony	Effuse, olivaceous grey, hairy, curly, and reaching 45 mm diam after 10 days on PDA
Mycelium	Immersed and superficial, 1–4 µm wide, hyaline to olivaceous brown, septate, with swellings up to 7µm diameter, soft to small verruculose.
Conidiophores	Macronematous, mononematous, terminally or laterally formed from hyphae, smooth, thick, straight or a little flexuous, light brown, velvet to closely granular, length reaches higher than 150 µm long 2.5–3.5 µm wide. Conidiogenous cells incorporated, elongated, like thread, rarely intercalary, 12-32 µm long with 2-3 loci.
Conidia	Catenate in long chains, obovoid, limoniform to ellipsoid-ovoid, smooth to minutely granular 2.5–7.5 × 2.5–3 µm, intercalary conidia limon shape to ovoid, 7–10 × 3–3.5 µm, aseptate. Ramoconidia ellipsoid to subcylindrical 0–2 septa, 10–25 × 2.5–5 µm not constricted at the median septa, with 2-3 hila

According to morphological characteristics, the isolate belonged to *C. colombiae*, we decided to confirm this identification by PCR molecular method by using ITS1 and ITS4 primers. The sequence analysis revealed the congruence of this isolate with *C. colombiae* strain, and it registered in GenBank with accession numbers for nucleotide sequence (MN959978). The phylogenetic tree of ITS sequence of *C. colombiae* isolate in this study was performed by maximum parsimony tree for 19 strains (18 references and 1 isolate) these strains were chosen from BLAST search appeared maximum percentage of homology in sequence. *Fusarium incarnatum* was used as an outgroup for comparison, and *C. colombiae* isolates in this study forming monoclade with reference strain *C. colombiae*. Figure 2.

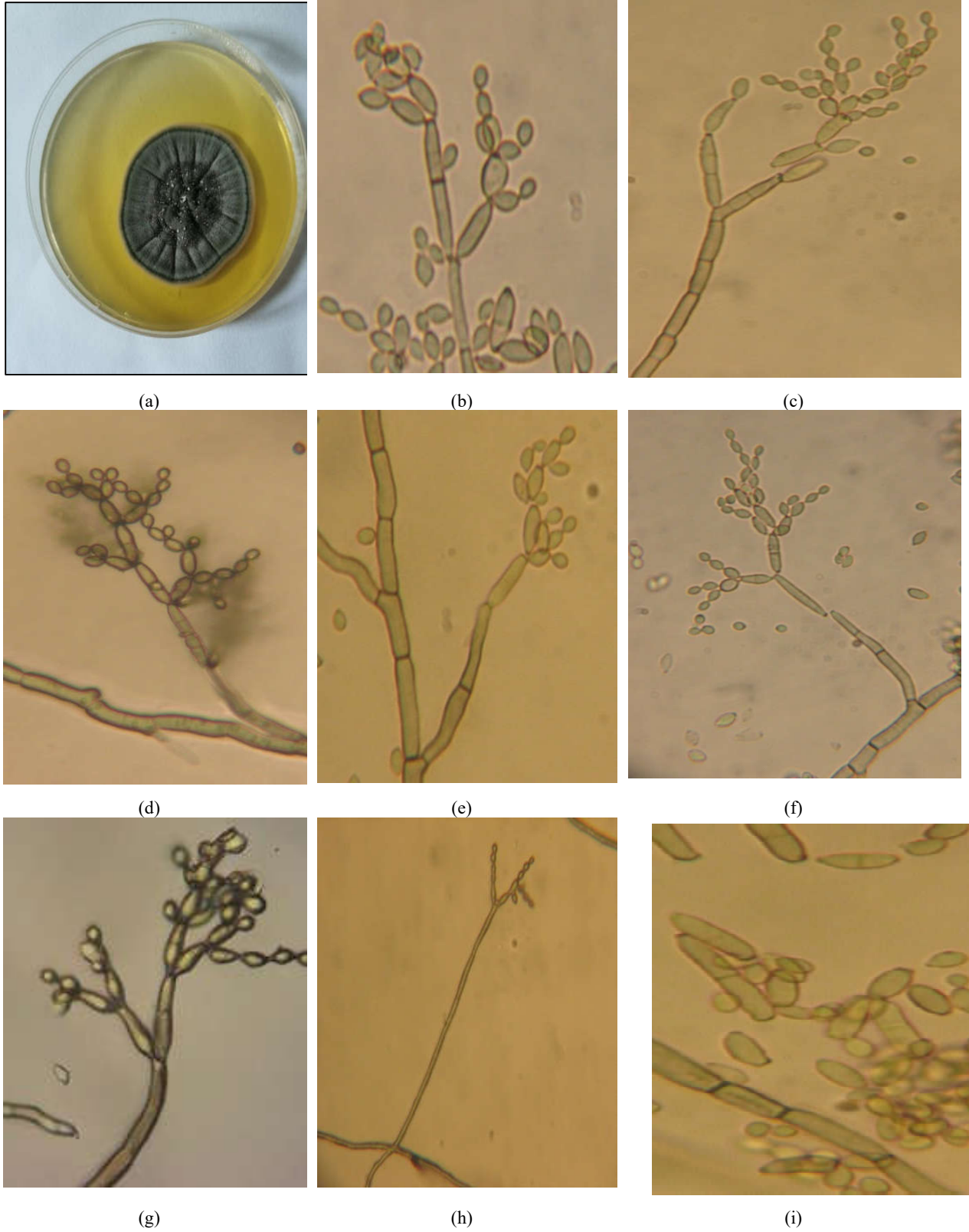


FIGURE 1. *Cladosporium colombiae*. a. Colony on PDA medium; b-g. Conidiophores development appearing ramoconidia and chain of conidia; h. Macronematous conidiophores; i. Conidia. Scale bars a= 10 mm, b-i= 10 μ m.

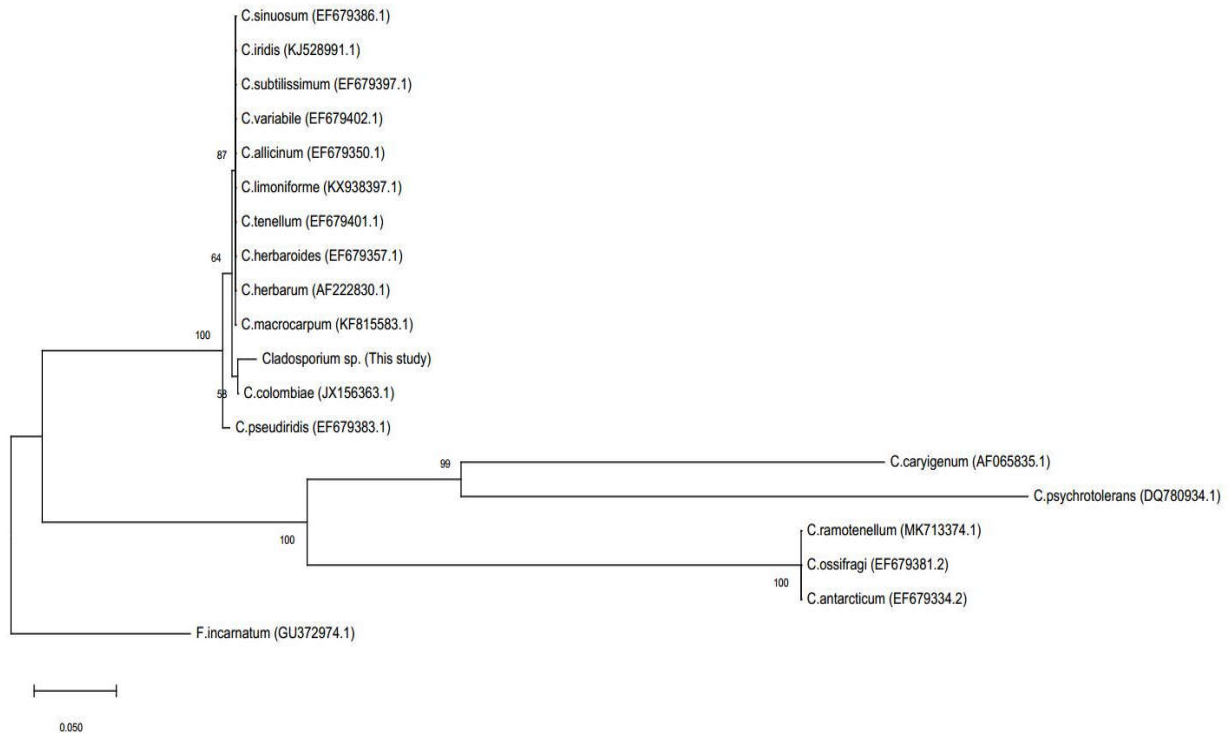


FIGURE 2. Phylogenetic relationships of *Cladosporium colombiae* with other species of *Cladosporium* were obtained from ITS sequencing data.

The comparison group divided the tree into two main clades, the first clade (upper) includes genetically close species and the second clade includes genetically divergent species. *Cladosporium colombiae* in this study clustered with the first clade.

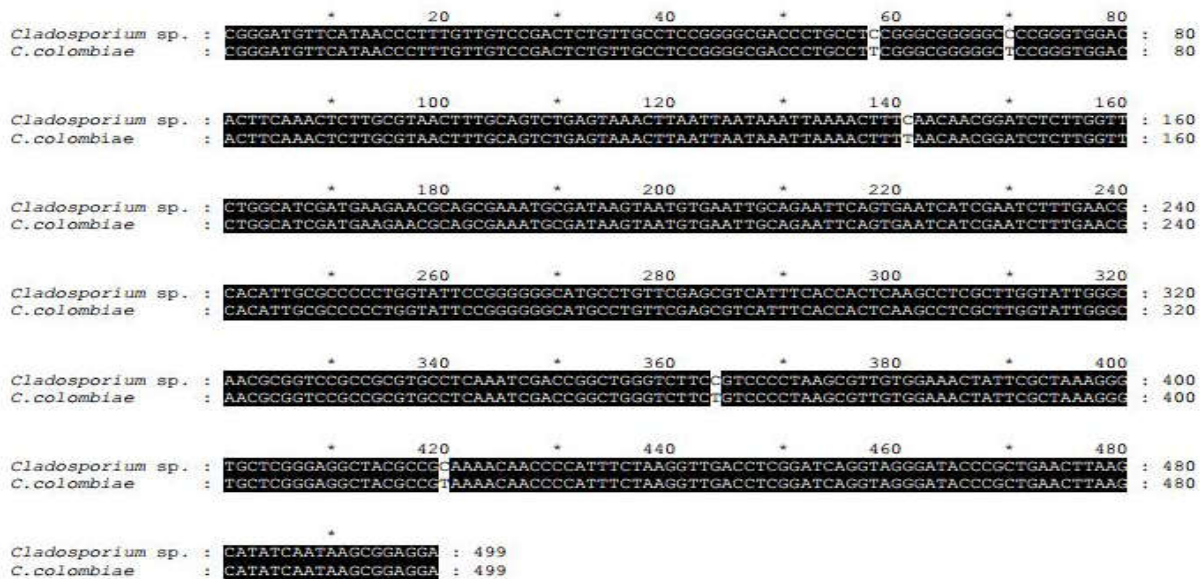


FIGURE 3. ITS Nucleotide Sequence Comparison of *C. colombiae*

Figure 3 shows an alignment of ITS nucleotide sequences from the *C. colombiae* isolate (JX156363.1) obtained from NCBI and the *C. colombiae* isolate (MN959978) obtained in the present study. The current study isolate was highly similar to the reference isolate. However, there were 5 polymorphism sites found in the ITS sequences obtained in the current study. At positions 58, 70, and 141, the nucleotide thymine (T) found in the reference isolate was replaced with cytosine (C) in the present isolate. Similarly, at positions 364 and 420, the nucleotide thymine (T) found in the reference isolate was substituted with cytosine (C) in the present isolate. Thus, the present *C. colombiae* isolate was highly similar to the reference isolate.

DISCUSSION

Cladosporium is a diverse genus of Ascomycetes it contains species difficult to distinguished morphologically and in recent years there are many studies try to clarify the taxonomic relationship between its species [3]. Mainly, in the beginning, *C. colombiae* was isolated in 1980 and deposited as *C. tenuissimum* by W. Gams then [16] isolated it from *Cortaderia* leaves collected in Colombia and described it as *C. colombiae*, in this time *Cladosporium tenuissimum* distinguish by produce very long conidiophores up to 500µm which became easy to characterized it from *C. colombiae*.

C. colombiae is more linked phylogenetically with *C. chubutense* but morphology is distinct, *C. chubutense* forming long conidiophore with conidiogenous loci (up to six), and short chains of conidia while those in *C. colombiae* forming extended branched chains (more than 10 conidia), and differ in the size of intercalary and ramoconidia [16].

The morphological similarity between close species of *Cladosporium* making recent studies move towards molecular methods and sequencing analysis to obtaining data on genetic structure and variation between species to confirm or supports the morphological classification.

To our knowledge, this is the first record of *Cladosporium colombiae* in Iraq isolated from the air. The morphological characters matched those of *C. colombiae*, and the use of ITS sequence confirmed the traditional identification.

CONCLUSION

The morphological identification of the genus *Cladosporium* is difficult because of the close relationship between species. In this study, *C. colombiae* isolated from air and identified depending on morphology and DNA sequence which is the best method to diagnose this species. We recommended researchers studies about this species and isolate it from another place.

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