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Molecular identification of five species of family Chaetomiaceae (Sordariomycetes, Ascomycota) from Iraqi soil

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Abstract. *Al-Dossary MA, Raheem SS, Almyah MK. 2021. Molecular identification of five species of family Chaetomiaceae (Sordariomycetes, Ascomycota) from Iraqi soil. Biodiversitas 22: 1277-1284.* Five ascomycetes fungi within the family Chaetomiaceae (Sordariomycetes, Ascomycota) were isolated from Iraqi agricultural soil and identified by phenotypic characteristics and molecular analysis. *Chaetomium ascotrichoides* and *C. subaffine* are reported for the first time from the Iraqi mycobiota. *Amesia atrobrunnea*, *Collariella bostrychodes* and *Ovatospora brasiliensis* were previously reported from Iraq as *Chaetomium atrobrunneum*, *C. bostrychodes*, and *C. brasiliense* respectively. All reported species were provided with brief characterizations and photographs

Keywords: Agriculture soils, Ascomycetes, Chaetomiaceae, Iraq

INTRODUCTION

Chaetomiaceae (Sordariomycetes) is one of the largest families of saprotrophic ascomycete fungi with more than 300 species. The type species of this family is *Chaetomium globosum* Kunze. The species within this family are capable of colonizing various substrates and are well-known for their ability to degrade cellulose and to produce a variety of bioactive metabolites. They are present in different microhabitats, including soil, air, decomposed cotton, paper, moist walls, damaged buildings. In the plant rhizoplane, Chaetomiaceae are almost strictly saprophytic and have been shown to be antagonistic against several plant pathogens (Adhikari et al. 2017; Zhang et al.2017; Ruppavalli et al. 2019). *Chaetomium* is well known for its properties for agriculture application. It has a broad spectrum antagonistic ability against wide range of plant pathogens due to production of diverse metabolites (antibiosis) such as chaetomin, cochliodinol, and chaetosin (Moya et al. 2016).

Chaetomium spp. used as fungicide, the fungus has been formulated into bio-pellets and bio powders under the name Ketomium for the biological control of plant diseases such as tomato wilt and basal rot of corn, also used as bioinsecticide for the biological control of sucking insect pests (Soytong et al. 2001; Alsalhi et al. 2018).

Usually, ostiolate ascomata with hairs around the ostiole are one of the common features of this family, also evanescent asci with different shapes that range from clavate to fasciculate with brown to gray-brown ascospores which usually possessing one or two germ pores (von Arex et al.1986). Later, von Arx et al. (1986) established new taxonomic characters for this family, species with ovate or obovate to globose ascomata with textura intricata walls were included. These species exhibit diverse hair morphology that ranges from erect to coiled. Several anamorphic genera have been associated with the genus *Chaetomium*, such as *Acremonium*-like anamorph (Wang et al. 2016a).

At present, the species within this family possesses largely defined morphological variety, many genera in the family have been reevaluated and redefined, and many new genera have been proposed recently. These changes result in a lot of new combinations (Wang et al. 2016a). For this reason, it is necessary to re-understand the diversity of Chaetomiaceae in Iraqi.

The molecular identification of this fungal genus is highly limited, and additional molecular studies on this genus are necessary (Wang et al. 2014). Thus, great effort has been made to classify, identify, and accurately grouping different species of *Chaetomium*, based on DNA sequencing (Sekhar et al. 2018). Due to the lack of genetic studies on the species belonging to this genus in Iraq, this work aimed to investigate the fungal diversity within the family Chaetomiaceae by using morphological characters and molecular sequencing.

MATERIALS AND METHODS

Sample collection

Sixteen soil samples were collected from four different agricultural areas at a depth of 5-10 cm in Basrah Province, Iraq, i.e. Abu-Alkasib, Aljazera, Alkarmah, and Almdinah (Figure1). Approximately 150-200 g was collected from each soil sample. Soil samples were put in clean bags, then transferred to the laboratory, and maintained at 4°C until further use.

Figure 1. Study area in Basrah Province, Iraq

Isolation of fungi

The plate dilution method (Davet, 2000) was used to isolate the ascomycetous fungi within the family Chaetomiaceae. From each soil sample, a total of 10 g was suspended in 100 mL of sterile distilled water and diluted up to $\times 10^3$ after thorough shaking for 10 min. From each dilution, approximately 1 mL was transported to a sterile Petri dish. Then, approximately 15 mL of sterile medium was added. For primary isolation, potato dextrose agar (PDA) and oatmeal agar (OA) media (Hi media, India) supplemented with the antibiotic chloramphenicol (250 mg/L) were used. All the dishes were incubated at 25 ℃ for 7-14 days. All distinct colonies were subjected to additional purification by subculturing on plates containing OA media.

Phenotypic identification

The diameters of each purified 7-14-day-old colony on OA media were measured. Front and reverse colony colors and sporulation grades were also observed. Then, glass slides were prepared from each purified culture and examined under a compound microscope (Samson et al. 2010). Appropriate keys were used for the phenotypic identification of the isolated fungi (Guarro et al. 2012; Wang et al. 2016 a,b).

DNA extraction and phylogenetic identification

Pure cultures of fungi at 7-14-days old were used for DNA extraction. The technique designated by Mirhendi et al. (2006) was used for DNA extraction and PCR amplification. PCR amplification and sequencing were performed by using NL1 and NL4 primers for the large subunit of ribosomal DNA (LSU) with the forward primer NL1 (5′-GCA TAT CAA TAA GCG GAG GAA AAG-3′) and reverse primer NL4 (5′-GGT CCG TGT TTC AAG ACGG-3′) (Friggens et al. 2017). All subsequent operations were performed in accordance with (Mirhendi et al. 2006). The purification and sequencing of the PCR products were done at Macrogen, South Korea. The identification of fungal isolates was done by using BLAST (Altschul et al. 1997) in GenBank (Sayers et al. 2021). The construction of the neighbor-joining phylogentic tree was based on the D1/D2 region of the large subunit rRNA gene and performed in MAFFT (Mirhendi et al. 2006). Bootstrap values were calculated with 500 replicates.

RESULTS AND DISCUSSION

Isolates

On the basis of phenotypic characteristics, 20 isolates were identified as members of the family Chaetomiaceae. Isolates that represented different species were included, whereas identical strains were excluded (Table 1).

Chaetomium species are usually identified according to phenotypic characteristics with emphasis on special features, such as superficial and usually ostiolate ascomata that are surrounded by hairs (Aggarwal et al. 2008), or the presence of stalked, thin-walled, evanescent, eight- spores asci (von Arx et al. 1986).

High morphological diversity has been found for these common features, thus relying on morphological characters alone is insufficient for the identification of this genus (Abdel-Azeem, 2020). Therefore, the accurate identification of the species belonging to this genus should not be based on phenotypic features only; molecular techniques should be also used (Quyet et al. 2018).

When we subjected all isolates of *Chaetomium* to PCR amplification by using primers NL1and NL4, the number of isolates decreased to only 9 species belonging to five genera, among them (*Amesia*, *Collariella*, and *Ovatospora*) were recorded for the first time in Iraq. These genera were

recorded for the first time in Iraq. The NL1and NL4 primers are widely used in rDNA sequencing either alone or with other primers for thorough and accurate phylogenetic investigation of different fungal genera (Zhang et al.2017; Sekhar et al. 2018). The molecular identification of *Chaetomium* species by using the LSU sequence was conducted through GenBank BLAST. The identification percentage was 99 -100%.

Taxonomy

Nine species belonging to the family Chaetomiaceae were recognized through phylogenetic inference and phenotypic characters. Of these species, five belong to the genus *Chaetomium*, and the others belong to other four genera within the Chaetomiaceae family, namely, *Amesia atrobrunnea*, *Collariella bostrychodes*, *Ovatospora brasiliensis*, and *Trichocladium seminis-citrulli*. The use of LSU analysis facilitated the identification of the isolates as clearly reflected by the phylogenetic tree of the species (Figure 2). Several previously reported studies were in agreement with our study Wang et al. (2016a,b). Five of these 9 species were recorded for the first time in Iraq and are described here.

Table 1. Comparison between phenotypic and phylogenetic identification for the fungal isolates

No. of isolates	Fungal species	
	Phenotypic identification	**Phylogenetic identification**
2	*Chaetomium atrobrunneum*	*Amesia atrobrunnea* (Ames) Wang & Samson
1	*C. ascotrichoides*	*Chaetomium ascotrichoides* Calviello
3	*C. elatum*	*C. elatum* Kunze
3	*C. globosum*	*C. globosum* Kunze & Schmidt
3	*C. ascotrichoides*	*C. madrasense* Natarajan
2	*C. seminis citrulli*	*Trichocladium seminis-citrulli* (Sergeeva) X.Wei Wang & Houbraken
2	*C. globosum*	*C. subaffine* Sergeeva
3	*C. bostrychodes*	*Collariella bostrychodes* (Zopf) X. Wei Wang & Samson
1	*C. brasiliense*	*Ovatospora brasiliensis* (Batista & Pontual) Wang & Samson

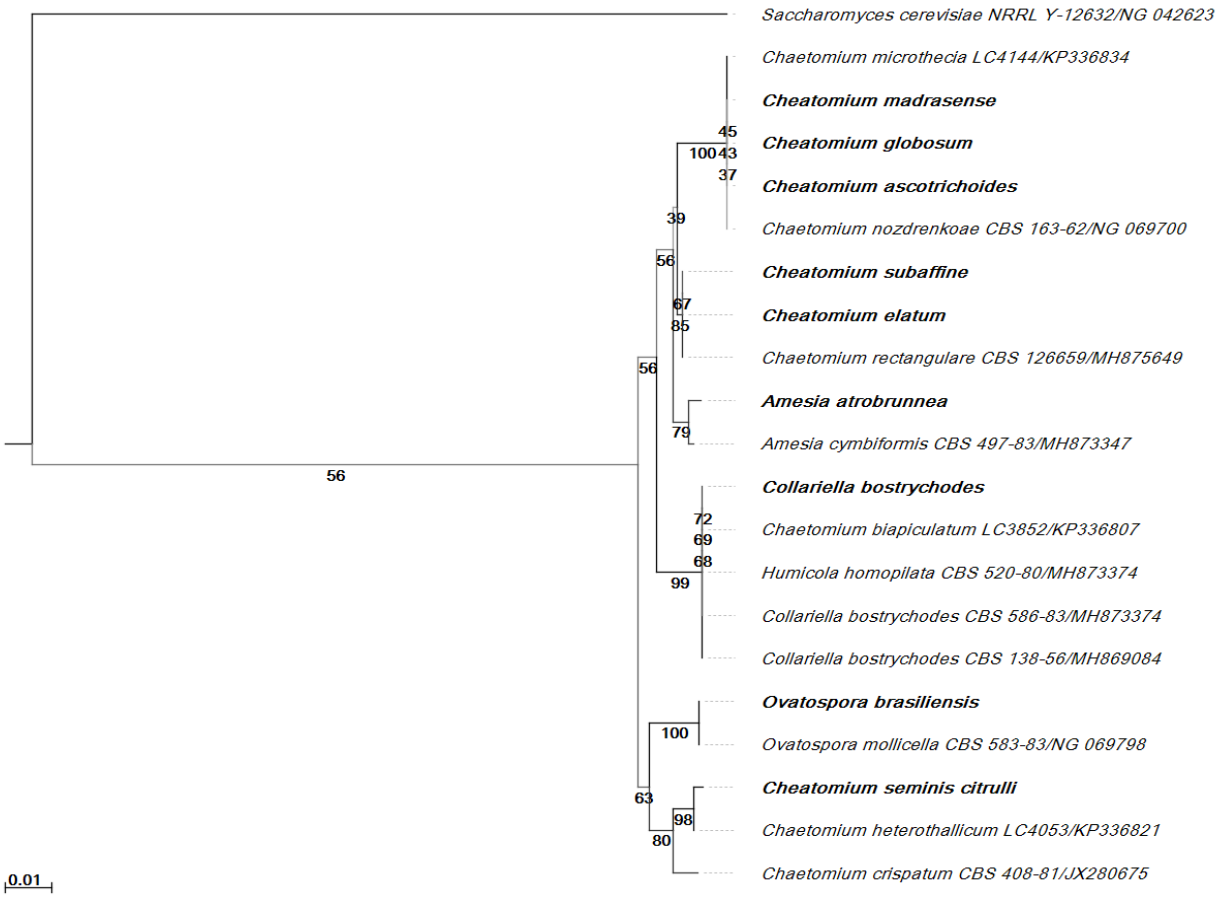

Figure 2. Phylogenetic tree of the family Chaetomiaceae with closely related taxa from GenBank and their accession numbers, derived from neighbor-joining analysis of 28s rDNA D1/D2 domain sequences. Frequencies with which a given branch appeared in 500 bootstrap replications are represented under branches

Amesia atrobrunnea

Amesia atrobrunnea (Ames) Wang & Samson, Stud. Mycol. 84: 158 (2016a). (Figure 3)

Basionym: *Chaetomium atrobrunneum* Ames, Mycologia 41: 641. 1949.

This species was characterized by superficial, ostiolate ovate, or subglobose ascomata with dimensions of 75-165 µm × 70-140 µm , black in reflected light. The wall of the ascomata exhibited textura angularis in surface view and was black to dark brown in color. The hairs were flexuous, smooth, and septate and were 2.3-3.5 µm in diameter near the base. This species possessed eight ascospores and clavate, evanescent, 9-22 µm-long asci. The ascospores were fusiform or elongate and turned dark brown when they matured7.5-10× 4-5.5µm. They possessed an apical germ pore at the more attenuated end. Anamorph stage unknown.

Colony morphology: The colonies exhibited good growth and matured within 7 days on OA medium at 25 ℃. They were nearly 40-50 mm in diameter and appeared black due to the presence of ascomata together with ascospores. They lacked aerial mycelium, and they were grayish to black in color in reverse.

Material examined: this fungus was isolated from two soil samples taken from Abu-Alkasib and Almdinah, Basrah Province. GeneBank accession number LC600695.

This species was previously named *Cheatomium atrobrunneum*, Wang et al. (2016 a) transfer this species to a new genus *Amesia* based on phylogenetic analysis. This genus shows high variety in ascomatal hair and ascospore morphology and is readily isolated from diverse locations (Wang et al. 2016a). Phylogenetic analysis revealed that this species clustered with *A. cymbiformis* in the same clade (Figure 2).

The result was consistent with Wang et al.(2016 a) showing that these two species are associated with each other and can be distinguished on the basis of ascospore size and shape. This species can grow at temperatures reaching 47 ℃, and numerous isolates of this fungus have been proven to cause systemic infections in humans (Li et al. 2012; de Hoog et al. 2013).

Chaetomium ascotrichoides

Chaetomium ascotrichoides Calviello, Revista Mus. Argent. Cien. Nat. B. Aires, Bot. 3: 372. 1972. (Figure 4)

This species is characterized by superficial, olivaceous, ovate, or obovate ascomata with dimensions of 170-290 µm × 130-255 µm, black in reflected light. The ascomatal wall presented textura epidermoidea, brown in color. Hairs are flexuous, finely verrucose, 2.2-3.6 µm in diameter near the base. Around the ostiole, the hairs become shortened and constricted at the septa. It had eight ascospores within clavate, stalked, evanescent, 17-36 µm-long asci. The ascospores were broadly limoniform, occasionally triangle-shaped in side view, and became slightly apiculate at both ends. They turn brown when mature. Their dimensions are 9.5-11 µm × 8.5-9.5 µm × 6.5-7.5 µm. They have an apical germ pore. Anamorph stage unknown.

Colony morphology: The colonies exhibited good growth and matured within 7-10 days on OA medium at 25 ℃. They were nearly 40-50 mm in diameter, olivaceous black in color, and possessed white aerial hyphae. They were black in reverse.

Material examined: this fungus was isolated from agricultural soil collected from Abu-Alkasib, Basrah Province. GeneBank accession number LC600694.

Morphologically, this species is comparable with *Chaetomium madrasense* (von Arx et al. 1986). Our phylogenetic tree clustered these two species together. This result was similar to the result of Wang et al. (2016b). We can distinguish this species from *C. madrasense* due to the shape of ascomatal hairs, which were irregularly branched or flexuous in *C. ascotrichoides* but coiled in *C. madrasense*, and the size of the ascospores, were narrower in this species (6.5-7 µm) than in *C. madrasense* (7.5-8.5 µm).

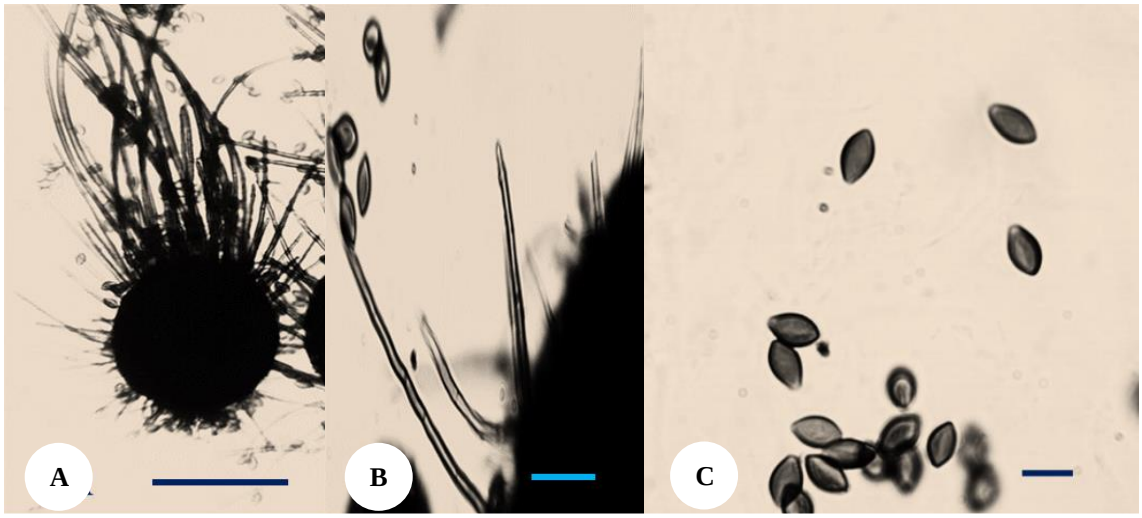

Figure 3. *Amesia atrobrunnea*. A. Ascoma. B. Ascomatal hairs. C. Ascospores. Bars: A = 107 µm; B= 16 µm; C = 10 µm

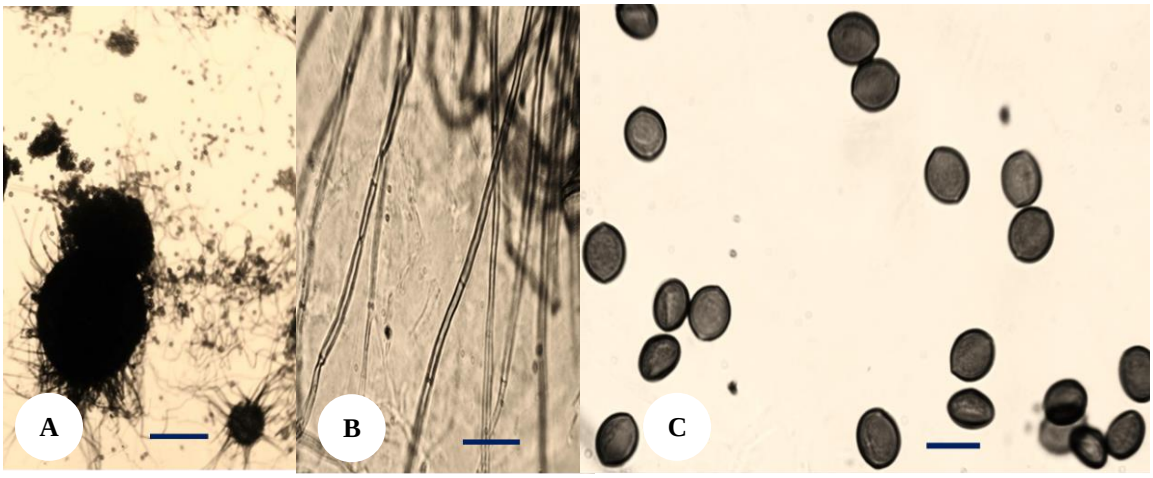

Figure 4. *Chaetomium ascotrichoides*. A. Ascoma. B. Ascomatal hairs. C. Ascospores. Bars: A = 100 µm; B= 30 µm; C = 11 µm.

Chaetomium subaffine

Chaetomium subaffine Sergeeva, Not. Syst. sect. Crypt. Inst. Bot. Acad. Sci. 14: 148. 1961. (Figure 5)

This species was characterized by superficial, dark, obovate, or ovate ascomata with dimensions of 220-410 µm × 180-340 µm, olivaceous or dark in reflected light. The ascomatal wall exhibited textura intricata in surface view and was brown. The hairs were erect to flexuous, septate, verrucose, generally unbranched and tapering towards the tips, and 2.6-4.2 µm in diameter near the base. This species had eight ascospores within clavate and sometimes slightly fusiform evanescent, 17-36 µm-long asci. The ascospores were bilaterally flattened limoniform, and usually biapiculate, when mature become brown, 11.5-13.5 (-14) µm × 8-10 µm × 6-8.2 µm in size, and possessed an apical germ pore. Anamorph not observed.

Colony morphology: Colonies exhibited good growth and matured within 7-10 days on OA medium at 25 °C. They were nearly 45-50 mm in diameter, black, and had abundant white aerial hyphae. They were uncolored in reverse light.

Material examined: this fungus was isolated from two soil samples taken from Alkarmah and Aljazera, Basrah Province. GeneBank accession number LC600693.

Given its large ascospores (11-15 µm × 8-11 µm × 7-8.5 µm), von Arx et al. (1986) maintained that *C. subaffine* is a separate species and distinguished it from *C. globosum*, which has smaller ascospores (9-12 µm × 8-10 µm × 6-8 µm). Recent molecular studies revealed that this fungus is closely related to *Chaetomium spiculipilium*, *Chaetomium cochliodes*, and *Chaetomium pseudocochliodes*. However, we can distinguish it from the other species by its large ascospores and ascomata, which are covered by abundant white aerial mycelia (Wang et al. 2016 b).

Collariella bostrychodes

Collariella bostrychodes (Zopf) X. Wei Wang & Samson, comb. nov. Stud. Mycol. 84: 158 (2016 a). (Figure 6)

Basionym: *Chaetomium bostrychodes* Zopf, Abh. Bot. Ver. Prov. Brandenburg 19:173. 1877.

This species was characterized by superficial, pale greenish-gray, subglobose, or ovate ascomata that were 210-250 µm ×160-240 µm in size, gray in reverse light, and had a black collar around the ostiole. The ascomatal wall exhibited textura angularis and was brown. Around the ostiole the hairs were spirally coiled; obviously rough, septate, dark brown in the upper part, 3.5-6.5 µm in diameter near the base. This species had eight ascospores within clavate or fusiform, evanescent, 20-32 µm-long asci. The ascospores were limoniform, bilaterally flattened, turn pale brown when mature, 6-7.5 µm × 4.5-7 µm × 4-5 µm in size, and possessed apical germ pores. Anamorph stage unknown.

Colony morphology: Colonies exhibited good growth and matured within 7 days on OA medium at 25 °C. They were nearly 30-40 mm in diameter and were black in color and uncolored in reverse.

Material examined: this fungus was isolated from three soil samples collected from Abu-Alkasib, Almdinah, and Aljazera, Basrah Province. GeneBank accession number LC600692.

This genus derives its name from the dark collar that covers the ascomatal ostiole pore. On the basis of morphological and molecular evidence, scientists have classified this genus into two closely related subclades (Wang et al. 2019). The species belonging to this genus possess ascomata with high morphological diversity. The characteristics of our specimen corresponded to that of Wang et al.(2016 a & 2019). Strong evidence shows that this fungus produces a toxic secondary metabolite called chaetochromin (Dosen et al. 2017).

Ovatospora brasiliensis

Ovatospora brasiliensis (Batista & Pontual) Wang & Samson, comb. nov., Stud. Mycol. 84: 207 (2016a). (Figure 7)

Basionym: *Chaetomium brasiliense* Batista & Pontual, Bol. Agr. Com. Pernambuco 15: 70. 1948.

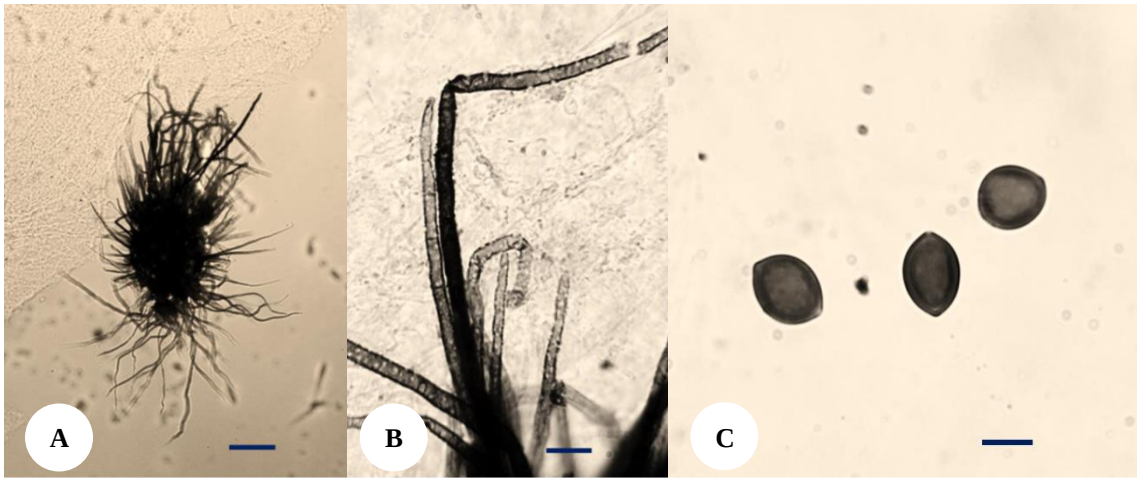

Figure 5. *Chaetomium subaffine*. A. Ascoma. B. Ascomatal hairs. C. Ascospores. Bars: A = 100 µm; B= 14 µm; C = 8 µm.

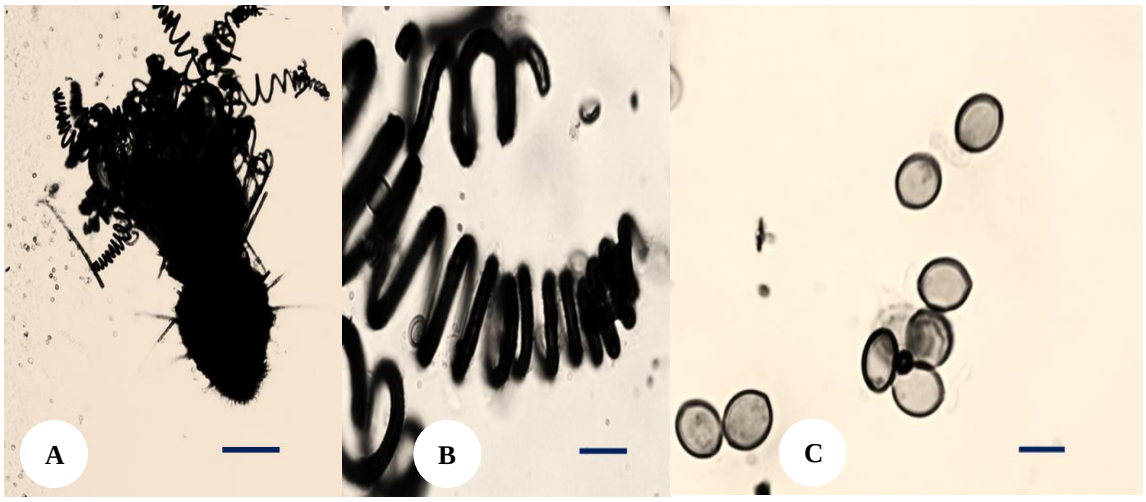

Figure 6. *Collariella bostrychodes*. A. Ascoma. B. Ascomatal hairs. C. Ascospores Bars: A = 100 µm; B= 20 µm; C = 6 µm.

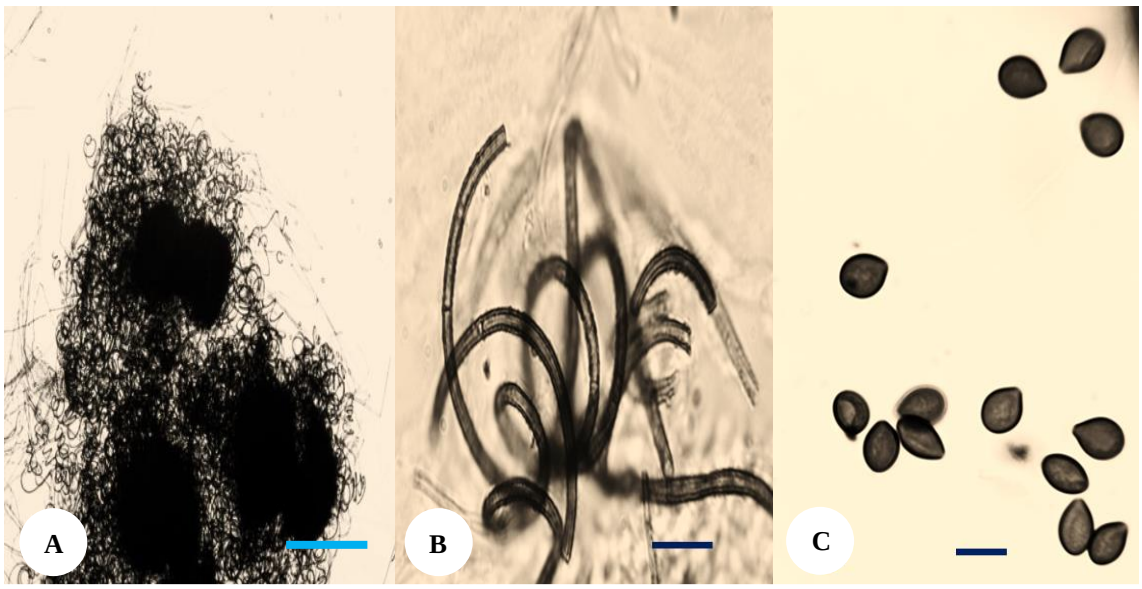

Figure 7. *Ovatospora brasiliensis*. A. Ascoma. B. Ascomatal hairs. C. Ascospores. Scale bars: A = 100 µm; B= 10 µm; C = 9 µm.

This species was characterized by superficial, pale gray, subglobose, or globose ascomata 85-135 µm × 72-115 µm in size, pale olivaceous gray in reverse light. The ascomatal wall presented textura angularis in surface view and was brown. The hairs were loosely coiled or undulate in the upper part and erect in the lower part and obviously rough, septate, grayish to brown in color, and 2-3.7 µm in diameter in the upper part. This species had eight ascospores within cylindrical, evanescent, 34-42 µm-long asci. The ascospores were ovate, bilaterally flattened, turned brown when mature, 6.5-7.7 µm × 5.3-6.2 µm × 5-6.7 µm in size, and had apical germ pores at the attenuated end. Anamorph stage unknown.

Colony morphology: Colonies exhibited good growth and matured within 7 days on OA medium at 25 ℃. They were nearly 40-50 mm in diameter, pale gray to pale olivaceous gray, and black in reverse.

Material examined: the examined materials were isolated from three agricultural soil samples collected from Abu-Alkasib, Basrah Province. GeneBank accession number LC600696.

The name of this genus came from the ovate to broadly ovate ascospores of all of its species (Wang et al. 2016 a). This species was previously isolated from moist jute cloth and from dried freshwater fish, prawns, and shrimps (Wang et al. 2016; Ara et al. 2020). To our knowledge, this is the first record of this species from agricultural soil. This fungus forms a sister lineage to *O. mollicella*. The phylogenetic tree clustered these two species together. This result was similar to that reported by Wang et al.(2016 a) & Ara et al.(2020). We can distinguish *O. mollicella* on the basis of its large ascospores, which have dimensions of 8-9.5 µm × 7-8 µm × 6-7 µm (Wang et al. 2016 a). *O. brasiliensis* is of medical interest considering that it was isolated from a patient with spinocellular carcinoma, which is a case of otitis externa (Hubka et al. 2011).

In conclusion, this study was the first study for the family Chaetomiaceae in Iraq that relied on molecular analysis. The species within this family having diverse morphological characteristics, so the identification of these fungi depending on morphological characters is not enough and must be supported by modern phylogenetic techniques. This study identified five species with three genera *Amesia*, *Collariella*, and *Ovatospora* all of which are recorded for the first time in Iraq.

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