Occurrence of *Chaetomidium arxii* on sunn pest in Iran

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Chaetomidium arxii was recovered from dead, overwintering adults of *Eurygaster integriceps* in northern Iran. The species was identified based on morphological characteristics as well as sequence data from LSU and ITS-rDNA regions. This is first report on the occurrence of *C. arxii* on sunn pest and a new record of *C. arxii* for Iran. The fungus is fully illustrated and described.

Key words – Ascomycetes – cephalothecoid – Chaetomiaceae – *Chaetomium* – entomopathogenic

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Introduction

The genus *Chaetomidium* (Zopf) Sacc. (*Chaetomiaceae, Sordariales*), was first described in 1882 with *C. fimeti* (Fuckel) Sacc. as its type species (Crous et al. 2004). The genus is characterized by non-ostiolate, evenly setulose and superficial ascomata, with clavate and evanescent asci (Stchigel et al. 2004). Morphologically related genera such as *Boothiella* Lodhi & Mirza, *Thielavia* Zopf, *Corynascella* Arx & Hodges, *Melanocarpus* Arx and *Corynascus* Arx are distinguishable from *Chaetomidium* based on morphological features including ascospore shape, peridium structure, germ pore characters and anamorphic state (Stchigel et al. 2004). Members of *Chaetomidium* reported in previous studies are considered to be saprobic and they are found predominately on dung (Taparia & Lodha 1974, Benny 1980). Apparently, they play the role of the decomposers in these ecosystems. During a study on the entomopathogenic mycota associated with overwintering population of the sunn pest, *Eurygaster integriceps* Puton, we found a fungal colonist of unknown function. Culturing and molecular techniques were used to identify this organism as a species of *Chaetomidium*. Sunn pest is a serious problem of wheat and barley in Western and Central Asia, and Southeast Europe, causing annual yield loss of 50–90% in wheat and 20–30% in barley.

Methods

Sampling, isolation and morphological study

Dead adults of sunn pest were collected from their overwintering sites in Mianeh, East Azerbaijan Province, Iran. Insects were surface sterilized for 1 min in 70% ethanol, rinsed 2 times with sterilized distilled water, dried, and plated on acidified potato dextrose agar (PDA; Merck, Germany). Pure isolates were obtained by sub-culturing emergent hyphal tips. The cultures were deposited in the living Culture Collection of Tabriz University (CCTU),

Tabriz, Iran. The cultural features were studied on potato carrot agar (PCA; 20 g L^{-1} potato, 20 g L^{-1} carrot, 20 g L^{-1} agar) containing one piece of sterile filter paper, PDA, malt extract agar (MEA; Merck, Germany), and oat meal agar (OA; 30 g L^{-1} oat meal, 15 g L^{-1} agar) after 10 days of incubation at room temperature in the dark. Growth rates were recorded as maximum diameter of colony. Fungal structures were mounted on glass slides with water for microscopic examination. Measurements of all parameters were made at ×1000 magnification, with 30 measurements per structure. All structures except asci were studied in at least 15-day-old colonies, asci were studied in 7-10-day-old cultures. Photographs were captured with a Leica camera system. Line drawings were made using a drawing tube attached to an Olympus BX 41 microscope.

DNA extraction, PCR and sequencing

Genomic DNA extraction was carried out by harvesting of actively growing mycelium using the method of Moller et al. (1992). The internal transcribed spacer region (ITS) of the nuclear ribosomal RNA operon and part of the large subunit 28S rRNA (LSU) gene were amplified from the extracted DNA using universal fungal primer sets ITS1/ITS4 (White et al. 1990) and LROR (Rehner & Samuels 1994)/LR5 (Vilgalys & Hester 1990), respectively. PCR reaction mixture contained 1× PCR buffer, 0.5 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate (dNTPs), 5 pM concentration of each primer, 0.5 U of *Taq* enzyme, 10–15 ng of DNA extract as template. The final volume of the reaction was adjusted to 25 µL by adding sterile deionized water. The thermal-cycling conditions consisted of an initial denaturation step of 96°C for 5 min, which was followed by 40 cycles of 96°C for 30 s, 52°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 7 min. The amplified DNA fragments were sequenced using the BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA) Cycle Sequencing Kits and analyzed on an ABI Prism 3700 (Applied Biosystems, Foster City, CA) as recommended by the vendor. A preliminary search for the identity of isolates was performed in the GenBank database using the NCBI Blast search.

Results

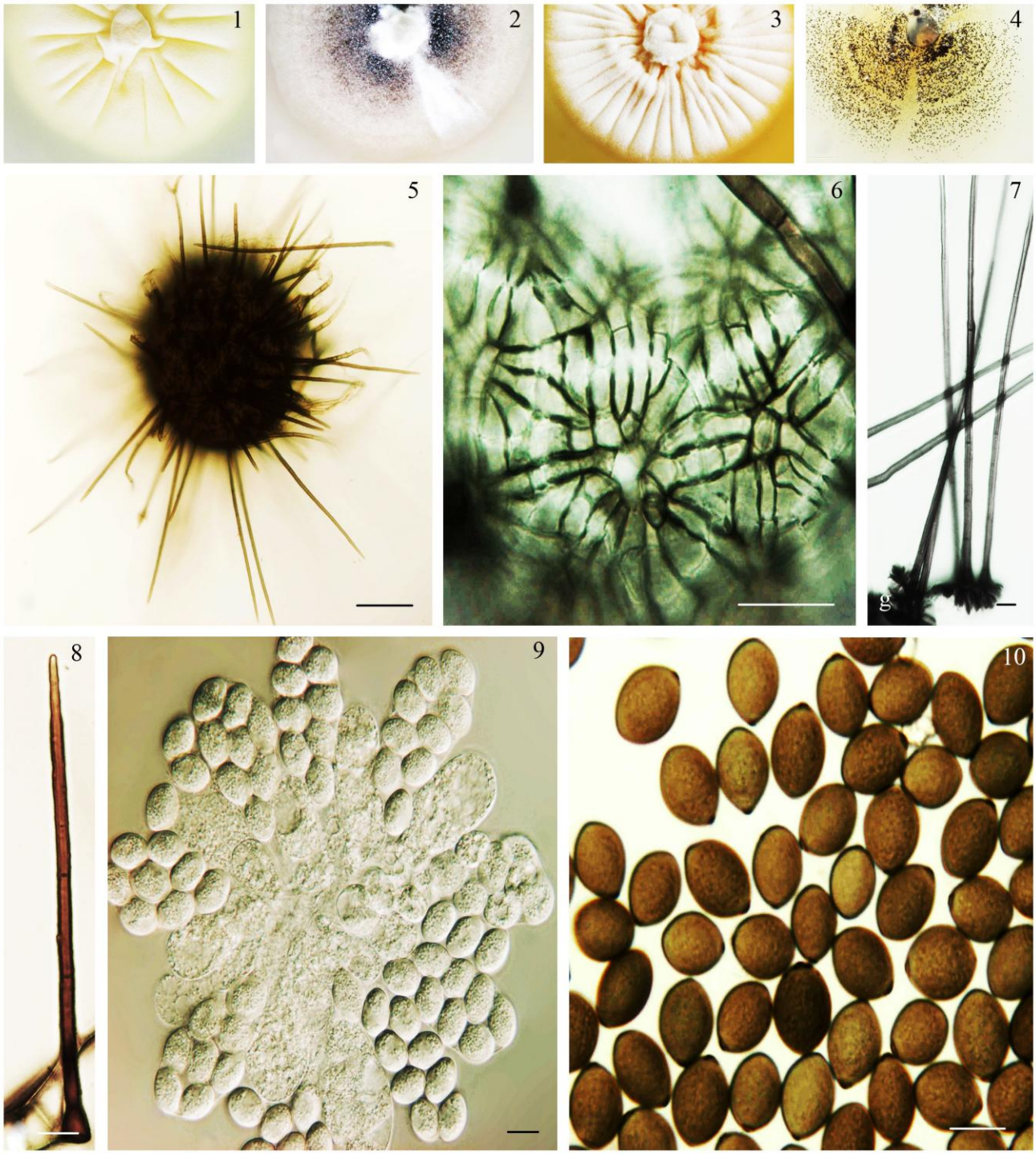
DNA Phylogeny

There are no ITS-rDNA sequences of *Chaetomidium* spp. in GeneBank, and homology searches using NCBI Blast search nested our isolate within the genus *Chaetomium* Kunze. The LSU sequence data were identical to sequences in GeneBank for *Chaetomidium arxii* (accession numbers: FJ666359, FJ666363). The ITS rDNA and nucLSU sequences generated in this study were deposited in GenBank under accession numbers JQ864439 and JQ864438, respect-tively.

Morphology

Chaetomidium arxii Benny, Mycologia. 72: 832, 1980. Figs 1–12

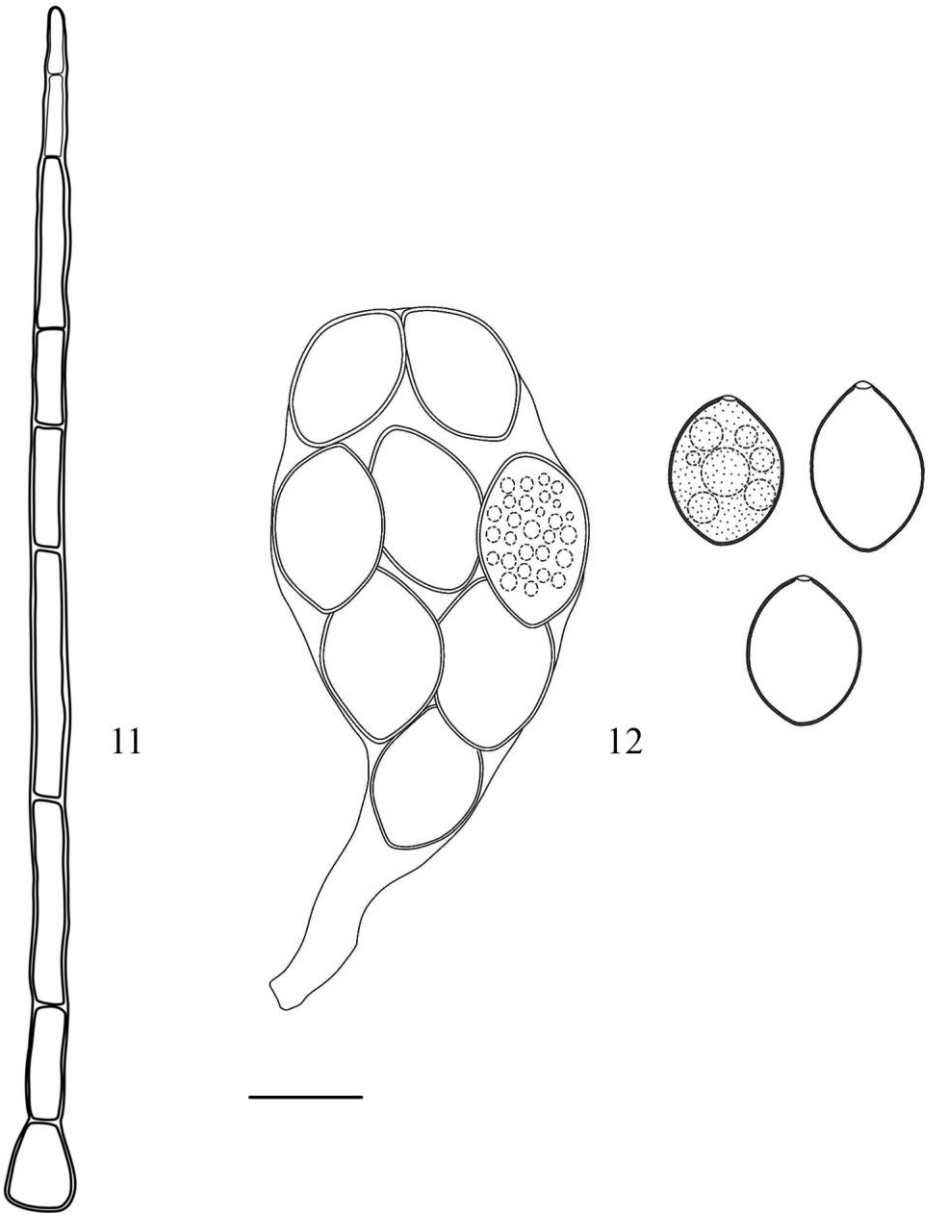
Microscopic characteristics on OA – Aerial mycelium sub-hyaline to dark brown, septate, smooth, branched, thin-walled, 2–4 µm diam, covering the whole colony surface; aerial mycelium around the point of inoculation and in sectors hyaline and appressed, 1–4 µm diam. homothallic. Ascomata cleistothecioid, superficial, densely scattered over the whole surface of the colony, developing at approximately 4 days, maturing rapidly, up to 190 µm in diam, non-ostiolate, globose, arostrate, dull black, opaque, without cirrhi, attached to the substrate by a poorly differentiated, scanty, hyaline to dark brown tuft of hyphae. Peridium cephalothecoid, 1–2-layered, of textura angularis, dark brown, composed of polygonal plates; external cells prismatic. Ascomata clad with hairs; lateral and terminal hairs practically indistinguishable; both unbranched, straight, smooth, thick-walled, regularly septate, up to 290 µm long, 3–4 µm wide basally, 1–2 µm wide apically, blackish brown, gradually tapering and paling upwards, with blunt tips, basal cell swollen, 5–6 µm diam. Asci fasciculate, hyaline, irregularly club-shaped, stalked, 8-spored, deliquescing before spore maturity. Paraphyses absent. Ascospores irregularly disposed in the asci, one-celled, punctuate, broadly ellipsoid, bluntly biapiculate or umbonate, hyaline when immature, darkening to greyish brown at maturity, with numerous guttules, 12–15 × 9–



Figs 1–10 – *Chaetomidium arxii*. **1–4** Colony morphology on PDA, OA, MEA and PCA containing one piece of filter paper, respectively. **5** Cleistothecium. **6** Cephalothecoid peridium. **7**-8 Ascomatal hairs. **9** Asci and ascospores. **10** Mature ascospores. – Scale (5 = 50 µm, 6-10 = 100 µm).

12 µm, mature ascospores thick-walled, with a prominent apical germ pore. Anamorph absent.

Cultural characteristics (Figs 1–4) – Colonies on OA growing moderately, reaching 45 mm in diam after 10 days, without exudates, circular, flat, with smoke grey, cobwebby aerial mycelium, and 1–3 bright white, slightly folded, felty sectors, margins entire, dirty white. Colonies on PDA reaching 40 mm in diam after 10 days, flat, folded, circular, entire, yellowish white, no ascomata. Colonies on MEA reaching 35 mm in diam after 10 days, flat, vigorously folded, bright white, no ascomata. Colonies on PCA reaching 35 mm in diam after 10 days, flat, circular, entire, colorless, forming ascomata in relatively



Figs 11–12 – *Chaetomidium arxii*. **11** Ascomatal hair. **12** Ascus and ascospores. **–** Scale = 10 µm.

concentrically zonate pattern in 3 sectors mostly in center of the colony; the colony and agar reverse, except where ascomata are formed, remain uncolored.

Discussion

Morphological characters of our specimens are in full agreement with the genus *Chaetomidium*. The main features of the present isolates are cephalothecoid ascomatal peridium, straight, smooth and relatively short hairs, with ascospores measuring 12–15 × 9–12 µm. These characters revealed our isolates belonging to *C. arxii* (Benny 1980). Presently, the genus *Chaetomidium* comprises 12 species (Crous et al. 2004, Greif et al. 2009), six of which *viz.*, *C. cephalothecoides* (Malloch & Benny) Arx, *C arxii*, *C. galaicum* Stchigel & Guarro, *C. khodense* Cano, Guarro & El Shafie, *C. megasporum* Doveri, Guarro, Cacialli & Caroti (Stchigel et al. 2004) and *C. leptoderma* (Booth) Greif & Currah (Greif & Currah 2007) have cephalothecoid peridia. Chephalothecoid peridia are considered to be an adaptation for cleistothecial fungi to liberate the ascospores (Greif & Currah 2007). Doveri et al. (1998) provided a dichotomous key for the identification of four species of the genus with cephalothecoid peridia (*C. cephalothecoides*, *C arxii*, *C. megasporum* and *C.*

khodense). Stchigel et al. (2004) provided a key for the identification of 11 accepted morphospecies of *Chetomidium*. Taxonomy and phylogeny of the genus *Chaetomidium*, however, have been subject of some confusion. In recent years, it has been speculated that the current morphological concept of *Chaetomidium* is polyphyletic (Greif et al. 2009). Hence, it appears that optimal markers for species distinction have to be established.

The genus *Chaetomidium* is closely related to *Chaetomium* (*Chaetomiaceae*, *Sordariales*), a genus with ostiolate ascomata, possessing hairs of various shapes (Hanlin 2001). *Chaetomium* is a large genus comprising over 300 species (Crous et al. 2004), and occurs worldwide in a variety of habitats including soil, plant debris, and wood (Badali et al. 2011). Moreover, members of the genus are increasingly recognized as being associated with human disease. In 2011, Badali et al. (2011) described a fatal phaeohyphomycotic infection, and reviewed published human infections caused by *Chaetomiaceae*. They pointed out that the species classified in these genera are able to cause infections in apparently healthy hosts.

C. arxii has been reported to occur mainly on dung (Benny 1980). This is first record of sunn pest colonization by *Chaetomidium* species worldwide. This species seems to cause insect mortality, however, Koch's postulates should be performed to verify that the isolated fungus is the cause of insect death. If there is a pathogenic relationship, it is important to determine the severity of the virulence of the infection, as *C. arxii* would be a promising candidate for control of *E. integriceps*. The problems of insecticide resistance as well as the environmental pollution, and human health hazards associated with agrochemical residues, caused by overuse and misuse of pesticides, have focused attention on alternative methods for the control of *Eurygaster integriceps*, hence the integration of chemical and biological control against this insect is an urgent need (Canhilal et al. 2007).

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