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Colletotrichum species associated with some wild grasses in Iran; Two new records for the Funga of Iran

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Abstract: In this study, twenty-one isolates of the genus *Colletotrichum* with straight conidia were isolated from leaf spots on wild *Poaceae* plants in three provinces of Iran, including East Azarbaijan, Golestan, and Guilan. Based on morphology, the isolates belonged to three morphotypes. Representative isolates were studied by a polyphasic approach, which included morphology, and phylogeny inferred from multi-locus sequences including the nuc rDNA ITS1-5.8S-ITS2 (ITS), partial sequences of the *β-tubulin* (TUB2), and *actin* (ACT) genomic regions. Three distinct species were identified namely *C. chrysophilum*, *C. destructivum* and *C. orientalis*. *Colletotrichum chrysophilum* and *C. orientalis* are new records for the Funga of Iran. This study provides new insights into the host range and geographical distribution of these identified species.

Keywords: Anthracnose, Ascomycota, Morphology, Phylogeny, Poaceae, Systematics.

INTRODUCTION

The genus *Colletotrichum* Corda (1831) is known to infect a wide range of plant species, including both monocots and dicots, and many of its species are considered to be important plant pathogens (Crouch et al. 2009a, Damm et al. 2012a, Alizadeh et al. 2022). The host range of *Colletotrichum* is vast and encompasses over 1,000 different plant species belonging to more than 100 different families, making it one of the most diverse genera of plant pathogenic fungi (Farr & Rossman 2023). Some of the most common crops affected by *Colletotrichum* include fruits such as apples, mango, and strawberry, as well as vegetables like beans, peppers, and tomatoes. *Colletotrichum* also infects ornamental plants, the most important of which are roses, carnations, and chrysanthemums (Farr & Rossman 2023).

Colletotrichum species are known to exhibit high levels of morphological and genetic diversity, making their identification and classification challenging (Crouch et al. 2009c). Traditional morphological methods are often insufficient to accurately identify *Colletotrichum* species, and molecular techniques are required for accurate identification and classification (Hyde et al. 2009, Jayawardena et al. 2020, Liu et al. 2022). In recent years, the evolution of classification schemes for the genus *Colletotrichum* has been shaped by a polyphasic approach using morphology and multigene phylogeny (Cai et al. 2009, Jayawardena et al. 2020, Liu et al. 2022). Recent taxonomic revisions have resulted in the discovery of new species and the reclassification of existing ones, which provide a better understanding of the diversity and complexity of this economically important genus (Cannon et al. 2012, Marin-Felix et al. 2017, Jayawardena et al. 2020, Liu et al. 2022). In the light of using the modern taxonomy and subsequently advancing the systematics of *Colletotrichum*, approximately 290 species have been accepted within the genus (Liu et al. 2022, Viera et al. 2023).

The majority of these species belong to large species complexes, including the Acutatum, Agaves, Bambusicola, Boninense, Dematium, Destructivum, Dracaenophilum, Gigasporum, Gloeosporioides, Graminicola, Magnum, Orbiculare, Orchidearum, Spaethianum, and Truncatum species complexes (Crouch et al. 2009c, Damm et al. 2009, 2012a, 2012b, 2013, 2014, 2019, Liu et al. 2014, 2022, Weir et al. 2012, De Silva et al. 2021, Bhunjun et al. 2021, Jayawardena et al. 2020, Guo et al. 2022, Guevara-Suarez et al. 2022, Tan et al. 2022, Chen et al. 2022, Li et al. 2022, Zheng et al. 2022;).

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Colletotrichum species associated with more than 40 genera of *Poaceae* as well as with *Bletilla ochracea* (*Orchidaceae*) belong to the Graminicola complex of the genus (Crouch et al. 2009a, 2009b, 2009c, Crouch & Tomaso-Peterson 2012, Moriwaki & Tsukiboshi 2009, Tao et al. 2013, Crouch 2014, Alizadeh et al. 2022). To date, the Graminicola complex comprises 23 species, all of which are characterized by falcate or curved spores (Liu et al. 2022). Although, there have been reports of *Colletotrichum* species with straight conidia on *Poaceae* plants (Farr & Rossman 2023), no comprehensive study has yet been conducted to target these species using multigene phylogenetic analyses. With the continued evolution of the taxonomy of this genus, there is a growing need for a comprehensive investigation of *Colletotrichum* species associated with *Poaceae* forming straight conidia.

Iran, located in Western Asia, boasts a diverse range of environments, from the cool, humid climates of the northwest mountains to the soaring deserts of the eastern central plains. As part of the Irano-Turanian region, Iran is renowned for its high biodiversity, particularly in *Poaceae* plants (Noroozi et al. 2016). Despite this, knowledge of *Colletotrichum* species with straight conidia associated with *Poaceae* plants in Iran has been limited to a few outdated reports listed in the book "Fungi and fungal analogues of Iran" (Ershad 2022). However, these reports are based on old species concepts that are now known to be inadequate for reliable species delimitation. Complicating this issue, there are no large collections available of the reported fungi to practice modern systematics for these fungi in Iran, in the light of recent molecular revisions of *Colletotrichum*. Despite recent comprehensive studies on the diversity of *Colletotrichum* species with falcate/curved spores obtained from Poaceae (Crouch et al. 2009a, 2009b, 2009c, Crouch & Beirn 2009, Crouch 2014, Alizadeh et al. 2022), the knowledge of species within the genus that form straight conidia still remains poorly understood. In this study, we aimed to shed light on this knowledge gap by identifying and characterizing *Colletotrichum* isolates with straight conidia obtained from several *Poaceae* plants in three provinces of Iran including East Azarbaijan, Golestan and Guilan using a combination of morphology, and phylogenetic analyses.

MATERIALS AND METHODS

Sample collection and fungal isolation

Samples were collected from several wild *Poaceae* plants showing anthracnose symptoms or leaf spots in the East Azarbaijan, Golestan and Guilan provinces of Iran during the years 2018 and 2019. To isolate the fungi, the leaf samples (approximately 1 cm in diameter) were washed in tap water, surface-disinfected in a 2% sodium hypochlorite solution for one minute, and rinsed in sterile distilled water. The samples were then incubated in glass Petri dishes on autoclaved paper towels soaked with sterile tap water and kept in the dark at a temperature of 25 °C. Conidial masses or mycelia indicative of *Colletotrichum* were transferred to water agar (WA, 2%) plates supplemented with chloramphenicol (50 mg/L). Single spore or single hyphae isolates were obtained on potato dextrose agar (PDA, Merck, Darmstadt, Germany) (Goh 1999). The isolates were stored in Fungal Culture Collection of Azarbaijan Shahid Madani University, Tabriz, Iran (AZFC), and the Iranian Fungal Culture Collection (IRAN...C) (Table 1).

Morphological analysis

Cultural and microscopic features were studied on synthetic nutrient agar (SNA) Nirenberg (1976) and oatmeal agar (OA) Crous et al. (2019) following the methodology of Alizadeh et al. (2015). Cultures were inoculated with 5 mm diameter plugs from cultures that were 4-7 days old. To promote sporulation, isolates were transferred to SNA containing autoclaved filter paper and double-autoclaved stems of *Anthriscus sylvestris*. Incubation of SNA and OA cultures took place at 20 °C under near-UV light with a 12-hour photoperiod for a duration of 10 days. Fungal structures such as conidia, conidiophores, conidiomata, setae, and appressoria were measured and photographed following the method described by Damm et al. (2007). Appressoria were observed on the underside of SNA plates, while conidia were collected from acervuli. Microscopic preparations were made using clear lactic acid or methylene blue, with a minimum of 40 measurements per structure. The observations were conducted using a Nikon SMZ1000 dissecting microscope (DM) or an Olympus BX53 microscope with differential interference contrast illumination (DIC). Colony characteristics and pigment production on SNA and OA were recorded after 10 days, while growth rates were measured after seven and ten days.

Phylogenetic analysis

Fungal isolates were cultured on PDA medium at 25 °C for 7–10 days. Genomic DNA was extracted using a standard phenol-chloroform extraction protocol (Sambrook 2001). The following loci were amplified: the 5.8S nuclear ribosomal RNA gene with the two flanking internal transcribed spacers (ITS), partial sequences of the *β-tubulin* (*TUB2*) and actin (*ACT*) genes. The primer pairs used were ITS–1 + ITS–4 (White et al. 1990), T1 O'Donnell & Cigelnik (1997) + Bt–2b (Glass & Donaldson 1995), and Act–512F + Act–783R (Carbone and Kohn 1999), respectively, following the protocols explained in the respective references. The PCR products were sequenced by a commercial sequencing service provider (Biomagic Gene Company, BMG, China) using amplifying primers.

The viewing, editing, and assembly of sequences were carried out using Geneious version 5.6. To compare the sequences with other DNA sequences, particularly those from ex-type and reference strains in previous studies (Table S1), the BLAST tool from

GenBank (http://ncbi.nlm.nih.gov/genbank) was utilized (https:/ /blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences showing similarity were included in the alignment as reference strains (Fig. 1). Alignments were performed on the MAFFT web server Katoh et al. (2019) using the Q-INS-i algorithm (Katoh et al. 2013), which is the latest available version. Bayesian inference was employed for phylogenetic analysis on the TrEase web server (http://thines-lab.senckenberg.de/trease/), also using the latest available version. The GTR model was chosen for the Bayesian analysis, and random trees were generated for 1,000,000 generations. The first 30% of trees were discarded as burn-in steps, and posterior probabilities were determined from the remaining trees. The sequences obtained in this study have been deposited in GenBank, and their accession numbers are provided in Table 1.

RESULTS

Phylogeny

In the multi-locus analysis of the *Colletotrichum acutatum* complex, 31 isolates were examined along with the outgroup and 1249 characters, including alignment gaps were processed. The gene boundaries of ITS, *TUB2*, and *ACT* were 1–519, 520–1019 and 1020–1249, respectively. Of these characters, 85 were parsimony-informative, 314 were variable, and 904 were constant. The examined isolate AZFC R61N180 from *Echinochloa crus-galli* was placed in a well-supported clade (BP: 0.96) with the type strain of *C. orientalis* (F10PGBYS08) (Fig. 1). Many other species were well-separated from closely related species.

In the multi-locus analysis of the *Colletotrichum destructivum* complex, 45 isolates were examined along with the outgroup and 778 characters, including alignment gaps were processed. The gene boundaries of *TUB2* and *ACT* were 1–532, and 533–778, respectively. Of these characters, 150 were parsimony-informative, 282 were variable, and 472 were constant. The single isolate AZFC R84N255 from an unknown *Poaceae* plant investigated in this study was placed in a well-supported clade (posterior probability 1) with the type strain of *C. destructivum* (CBS 136228) (Fig. 2). Many other species were well-separated from closely related species.

In the multi-locus analyses of the *Colletotrichum gloeosporioides* complex (gene boundaries of ITS: 1–556, *TUB2*: 557–1198, *ACT*: 1199–1462), 88 isolates as well as the outgroup, and 1249 characters including the alignment gaps were processed, of which 207 characters were parsimony-informative, 492 characters were variable and 889 constants. The single isolate AZFC R40N103 from *Setaria viridis* investigated in this study in the tree was placed in a well-supported clade (BP: 1) with the type strain of *C. chrysophilum* (URM 7368) (Fig. 3). Many other species were well-separated from closely related species**.**

Taxonomy

Based on the DNA sequence data and morphology, the three isolates (Table 1) from three wild *Poaceae* species belonged to three *Colletotrichum* species, namely *C. chrysophilium*, *C. destructivum* and *C. orientalis.* All species studied in culture are characterized below.

Colletotrichum chrysophilum W.A.S. Vieira, W.G. Lima, M.P.S. Câmara & V.P. Doyle, Mycologia 109(6): 927 (2017). Fig. 4.

Cultural characteristics: Colonies on PDA initially pale gray to dark gray, aerial mycelium dark gray, with visible orange conidial masses in the center, Sclerotia absent, reverse pale gray to dark gray, >60 mm diam in 7 days at 25 °C (>60mm diam in 10 d).

On *Anthriscus* stem: Conidiomata acervular, Conidiophores mostly formed directly on hyphae. Setae not observed. Conidiogenous cells are hyaline, cylindrical to ampulliform and smooth-walled. Conidia, hyaline, smooth-walled, cylindrical with rounded ends, sometimes oblong, 10–17 × 5– 7.5 µm, mean ± SD = 13.6 ± 2× 5.3 ± 0.9 µm, L/W ratio = 2.57.

Sexual morph: Ascomata perithecia, solitary, globose to ovoid, dark brown, 51.5–196 × 51.5–169 µm, mean ± SD = 116.7 ± 35 × 107.5 ± 31.1 µm, L/W ratio = 1.08. Asci 8-spored, cylindrical to sub-fusoid, 37–66× 9.5–10 µm, mean ± SD = 51.7 ± 6.8 × 9.8 ± 5.4 µm, L/W ratio = 5.28. Ascospores often are cylindrical, ends broadly rounded, not tapering to the ends often slightly curved, 12–17 × 5 µm, mean ± SD = 13.8 ± 1.3 × 4.9 ± 2.7 µm, L/W ratio = 2.83.

On SNA: Hyphal appressoria solitary, aseptate, smooth-walled, medium brown, irregularly shaped, edges entire or more or less lobed, 5.5–12.5 × 4.5–12.5 µm, mean ± SD =8.3 ± 1.7 ×6.9 ± 1.5 µm, L/W ratio = 1.20.

Specimens examined. IRAN, Golestan province, Gorgan, Tuskestan, from leaves of *Setaria viridis*, Sep 2019, Alireza Alizadeh & Fatemeh Salimi. culture AZFC R40N103.

Geographical distribution and host range

This species has been identified previously in several Brazilian states, including São Paulo, Minas Gerais, Pernambuco, and Santa Catarina, where it causes anthracnose symptoms on *Musa* sp. fruits. It was first discovered as an endophyte in Panama, where it was found in *Theobroma* and *Genipa* plants (Rojas et al. 2010). Additionally, it was also found in Puerto Rico in *Mycopteris* plants under the name "Terpsichore" (Doyle et al. 2013).

Genetic identification

According to Vieira et al. (2017), *C. chrysophilum* is most closely related to *C. fructicola* and *C. nupharicola* in the multi-locus trees. However, the species can be differentiated from these two species based on most individual gene trees including *ACT*, *APN2*, *APN2/M-IGS*, *CAL*, *GAP2-IGS*, *GAPDH*, *GS*, and *TUB2* sequences. The results of the study showed that the *TUB2* and *ACT* sequences are informative in differentiating this species from others. Although ITS

sequencing was not effective in distinguishing between them.

Notes: According to Vieira et al. (2017), *C. chrysophilum* can be distinguished from *C. fructicola* by producing larger and wider conidia, and from *C. nupharicola* by producing smaller conidia. While *C. chrysophilum* and *C. fructicola* have been found to infect a wide range of hosts (Prihastuti et al. in 2009, Rojas et al. in 2010, Weir et al. in 2012, Doyle et al. in 2013, and Lima et al. in 2013), *C. nupharola* has been reported to be specific to aquatic plants in the Nymphaeaceae family (Johnson et al. 1997, Weir et al. 2012, Doyle et al. 2013). Based on our knowledge, *C. chrysophilum* is a new record for the Funga of Iran.

To date, only two species of *Colletotrichum*, *C. caudatum* Crouch & Beirn (2009) and *C. truncatum* Yu et al. (2023) which both have curved conidia and belong to the Graminicola and Truncatum complexes, respectively, have been reported from *Setaria viridis* worldwide. As a result, *Setaria viridis* is a novel host for *Colletotrichum* in Iran, and this research marks the first report of a *Colletotrichum* species with straight conidia from *Setaria viridis* globally.

Colletotrichum destructivum O'Gara, Mycologia 7(1): 38 (1915). Fig. 5.

Cultural characteristics: Colonies on PDA initially dark olive green to black, reverse same colors, 55 mm diam in 7 days at 25 °C (>60mm diam in 10 d). Colonies on MEA flat with entire margin, pale gray to dark gray, aerial mycelium grayish white, reverse brickish brown, 33–37 mm diam in 4 days at 25 °C (>60 mm diam in 10 d). Colonies on OA flat with entire margin, dark olive green to black, aerial mycelium whitish to gray, reverse dark gray, 30–35 mm diam in 4 days at 25 °C (>60 mm diam in 10 d).

On OA: Sexual morph not observed. Conidiomata acervular, conidiophores and setae formed directly on hyphae or a cushion of pale to medium brown cells. Setae medium brown, smooth-walled to finely verruculose, sometimes verrucose, 1–3-septate, 44–152 µm long, smooth-walled, tapering to an acute to rounded apex, base cylindrical to conical sometimes inflated. Conidiogenous cells pale to medium brown, smooth-walled, elongate-ampulliform to cylindrical, 24.5–61× 5 µm. Conidia enteroblastic, aseptate, hyaline, smooth-walled, cylindrical, straight to slightly curved, with both ends rounded, 12–17.5 × 5 µm, mean ± SD = 14.7± 1.4 × 4.9 ± 2.7 µm, L/W ratio = 3.

On SNA: Conidiomata acervular. Conidiophores mostly formed directly on hyphae. Hyphal appressoria solitary, aseptate, smooth-walled, medium brown, fusiform to ellipsoidal outline, clavate, with a lobate, undulate or crenate margin, 5.5–22.5 × 4.5–10 µm, mean ± SD =10.3 ± 3.2 × 7.1 ± 1.3 µm, L/W ratio = 1.45.

Specimens examined. Iran, East Azarbijan Province, Marand, Kouhkamar, from leaves of an unknown *Poaceae* plant, Oct 2019, Alireza Alizadeh. culture AZFC R84N255.

Geographical distribution and host range

Previously, this species has been identified in various hosts and countries across all continents (Farr & Rossman 2023). It has been found in *Medicago sativa* in Canada, *Rumex* sp. in Korea, *Trifolium* in the Netherlands, *Medicago* in Serbia, *Antirrhinum* and *Robinia* in Japan, *Bletilla* in China, house dust, *Phragmites* sp. and *Trifolium hybridum* in the USA, and *Crupina* in Greece (Damm et al. in 2014), indicating its broad host range and global distribution.

Genetic identification

According to Damm et al. (2014), *C. destructivum* can be identified by its ITS, *HIS3*, *ACT* and *TUB2* sequences, however, the *GAPDH* sequence is identical to that of *C. ocimi*. The results of the current study also showed that the *TUB2* and *ACT* sequences are efficient loci in discriminating this species from all described species within the Destructivum complex.

Notes: *Colletotrichum destructivum* was described by O'Gara (1915) from stems and petioles of red clover (*Trifolium pratense*) and alsike clover (*T. hybridum*) in clover fields in the Salt Lake Valley, Utah, USA. This species has been reported for the first time from alfalfa and clover in Iran (Zafari & Tarah Hamadani 2009). According to previous research, this species appears to have a broader range of hosts and a more geographical distribution in Iran. To our knowledge, this is the first report of *C. destructivum* from a *Poaceae* plant in Iran**.**

Colletotrichum orientalis Dandan Fu & G.Y. Sun, Journal of Fungi 8(7): 10 (2022). Fig. 6.

Cultural characteristics: Colonies on PDA flat with entire margin, compacted cottony to felty, orange towards the center and pale gray towards the edge, aerial mycelium white to pale gray, with masses of orange conidia, reverse pale brackish orange, 45 mm diam in 7 days at 25 °C (>60 mm diam in 10 d). Colonies on MEA flat with entire margin, compacted cottony to felty, orange towards the center and pale gray towards the edge, aerial mycelium white to pale gray, reverse pale brickish orange, 20–25 mm diam in 7 days at 25 °C (45 < 60 mm diam in 10 d). Colonies on OA flat with entire margin, aerial mycelium sparse, white to pale gray, masses of orange conidia scattered in circles; reverse pale buff, growth rate 20 mm diam in 7 days at 25 °C (>60 mm diam in 10 d).

On *Anthriscus* stem**:** Conidiomata acervular, Conidiophores mostly formed directly on hyphae. Setae not observed. Conidia enteroblastic, aseptate, hyaline, smooth-walled, straight and fusiform or cylindrical with both ends acute, 10–17 × 5–7.5 µm, mean ± SD = 15.1± 1.9 × 5.4 ± 1 µm, L/W ratio = 2.77.

On SNA: Conidiomata acervular. Conidiophores mostly formed directly on hyphae. Hyphal appressoria solitary or in loose groups, smooth-walled, medium brown, irregularly shaped, ovoid and ellipsoidal, 6.5–11.5 × 4.5–9 µm, mean ± SD = 8.7 ± 1.2 × 6.7 ± 0.9 µm, L/W ratio = 1.29.

Table 1. Isolates of *Colletotrichum* spp. studied, with collection details and GenBank accession numbers.

Species	Isolate[1]	Host	Province/Region	GenBank number[2]		
				ITS	TUB2	ACT
C. chrysophilum	AZFC R40N103	*Setaria viridis*	Golestan/Gorgan	OR755829	OR762651	OR762647
C. destructivum	AZFC R84N255	*Poaceae* sp.	East Azarbijan/Marand	—	OR762650	OR762648
C. orientalis	AZFC R61N180	*Echinochloa crus-galli*	Guilan/Lahijan	OR755828	OR762652	OR762649

[1] AZFC: Fungal Culture Collection, Mycology Laboratory of the Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz, Iran.

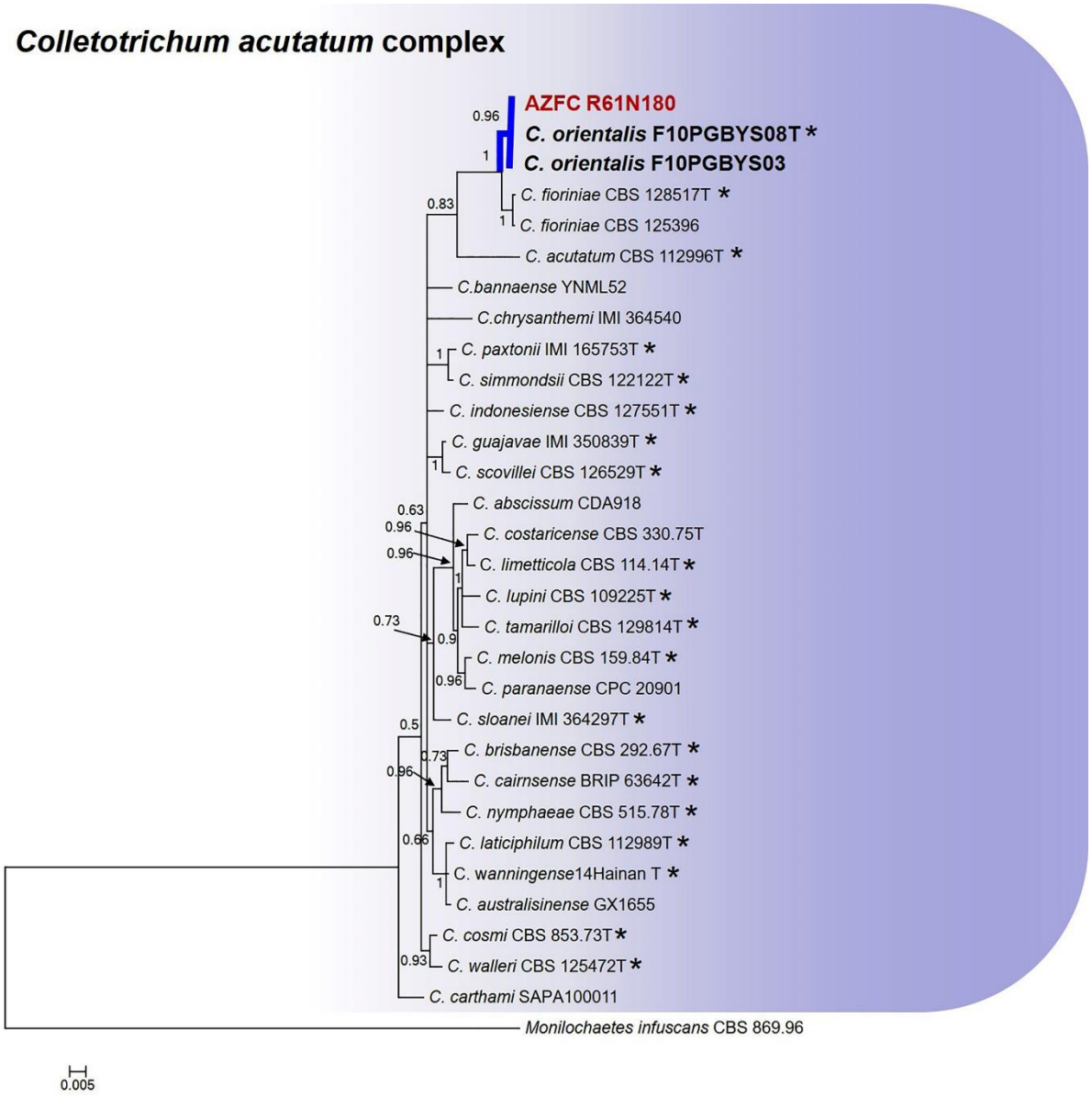

Fig 1. Phylogram generated from Bayesian analysis of the combined ITS, *TUB2*, and *ACT* sequence alignment of the Acutatum complex. Bayesian posterior probability values above 0.50 are shown at the nodes. *Monilochaetes infuscans* strain CBS 869.96 is used as outgroup. The newly generated isolate in this study is in red and numbers of types or ex-type strains are emphasized with an asterisk (*).

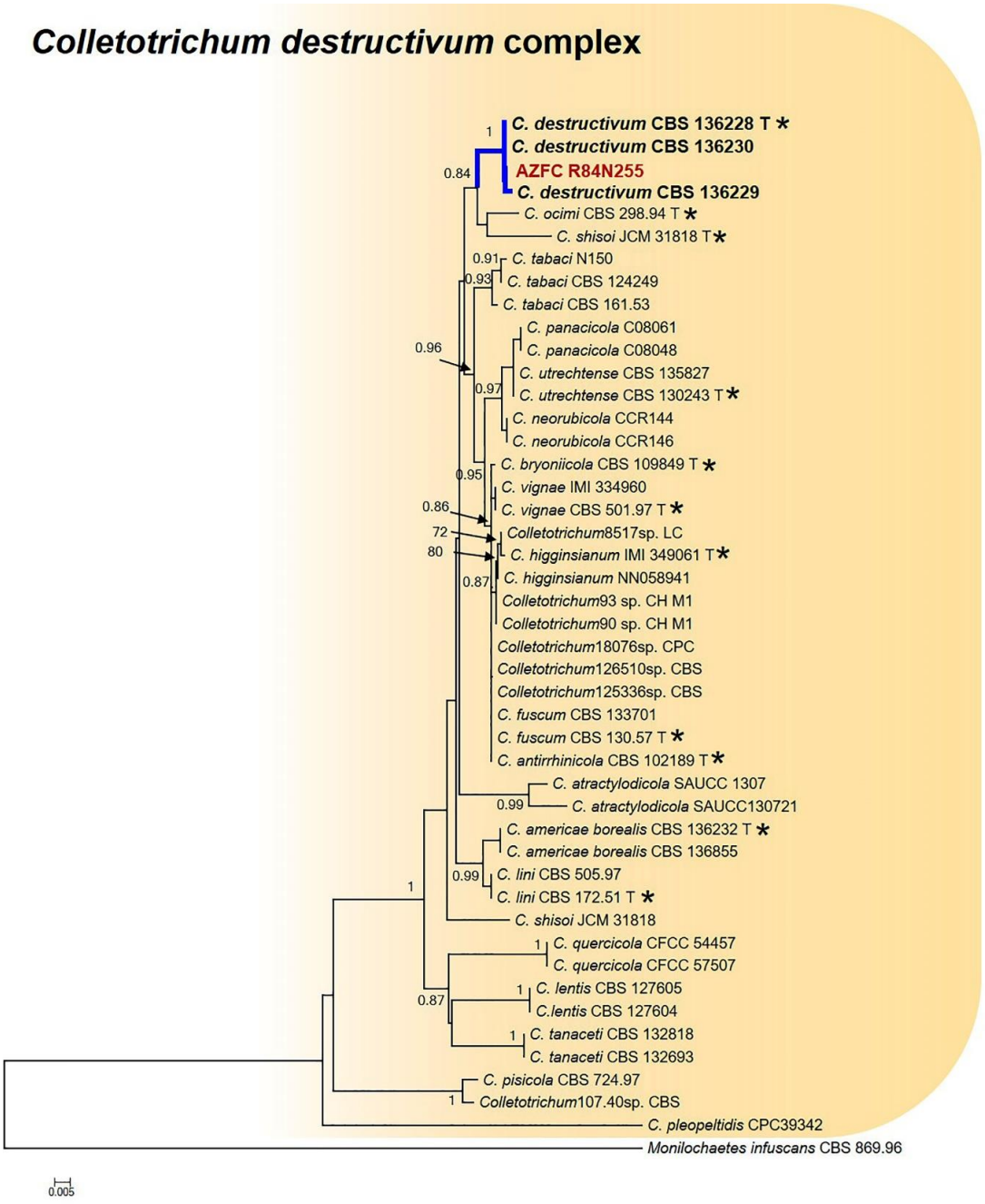

Fig 2. Phylogram generated from Bayesian analysis of the combined *TUB2* and *ACT* sequence alignment of the Destructivum complex. Bayesian posterior probability values above 0.70 are shown at the nodes. *Monilochaetes infuscans* strain CBS 869.96 is used as outgroup. The newly generated isolate in this study is in red and numbers of types or ex-type strains are emphasized with an asterisk (*).

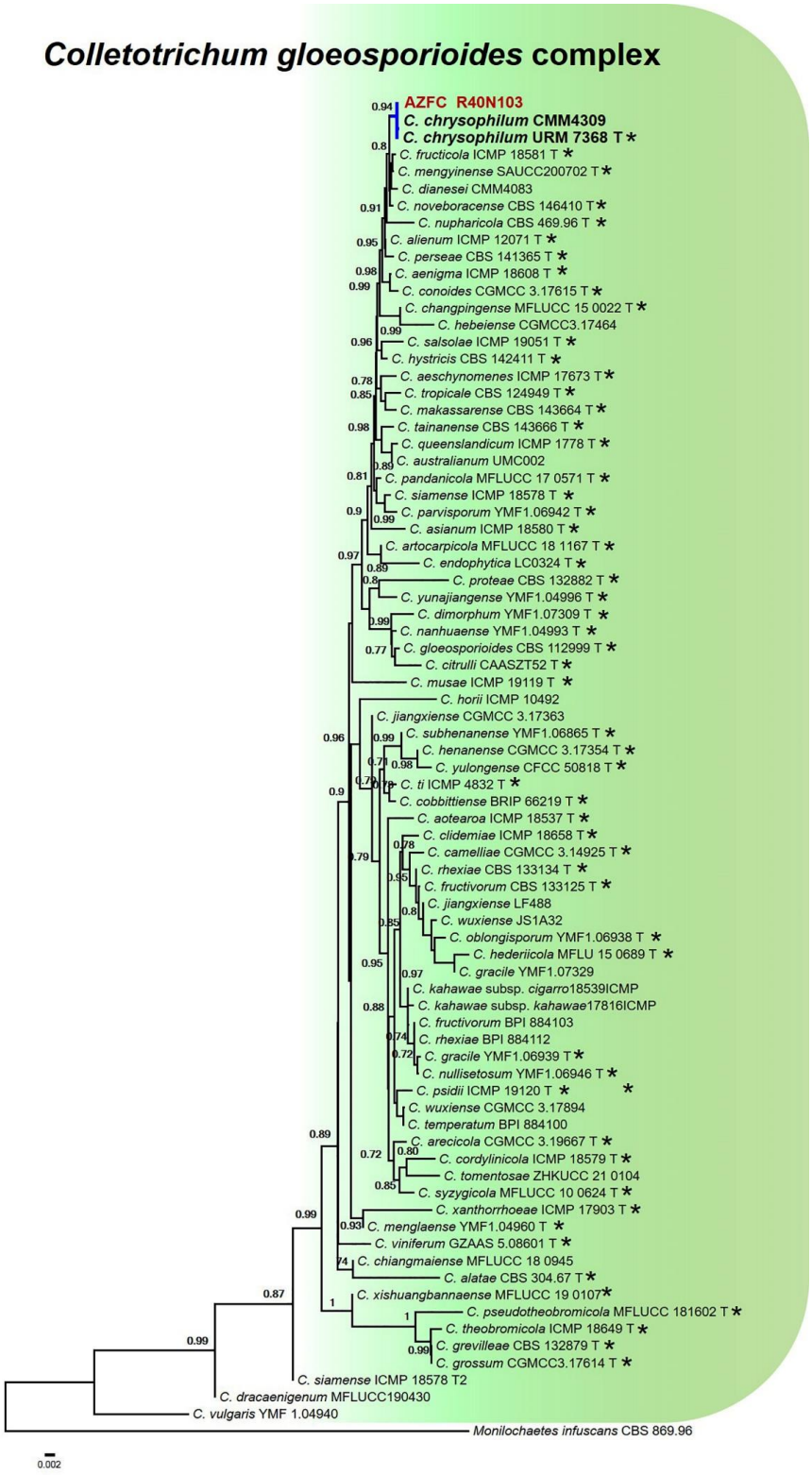

Fig 3. Phylogram generated from Bayesian analysis of the combined ITS, *TUB2* and *ACT* sequence alignment of the Gloeosporioides complex. Bayesian posterior probability values above 0.70 are shown at the nodes. *Monilochaetes infuscans* strain CBS 869.96 is used as outgroup. The newly generated isolate in this study is in red and numbers of types or ex-type strains are emphasized with an asterisk (*).

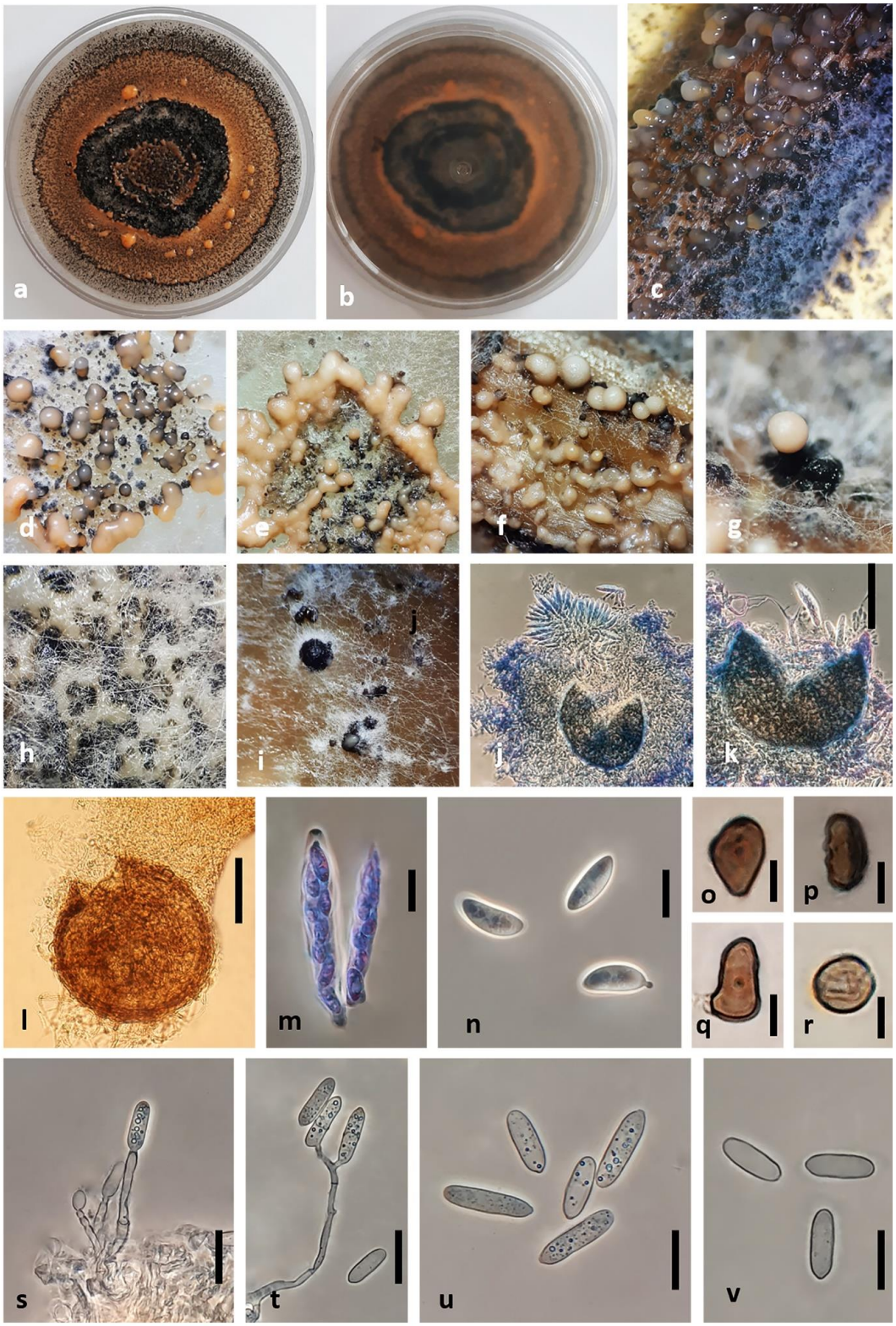

Fig 4. *Colletotrichum chrysophilum* (AZFC R40N103). a**, b.** Colony on PDA after 10 days at 25 °C, **a.** upper and **b.** reverse side. **c, f, g.** Ascomata and Conidial mass on *Anthriscus sylvestris* stem. **d, e.** Ascomata on filter paper. **h, i.** Ascomata on SNA. **j, k, l.** Perithecia**. m.** Asci. **n.** Ascospores. **o-r.** Appressoria. **s, t.** Conidiogenous cells. **u, v.** Conidia. Scale bars: **k,l** = 100 µm, **m** = 30 µm, **n, s-v** = 10 µm, **o-r** = 5 µm.

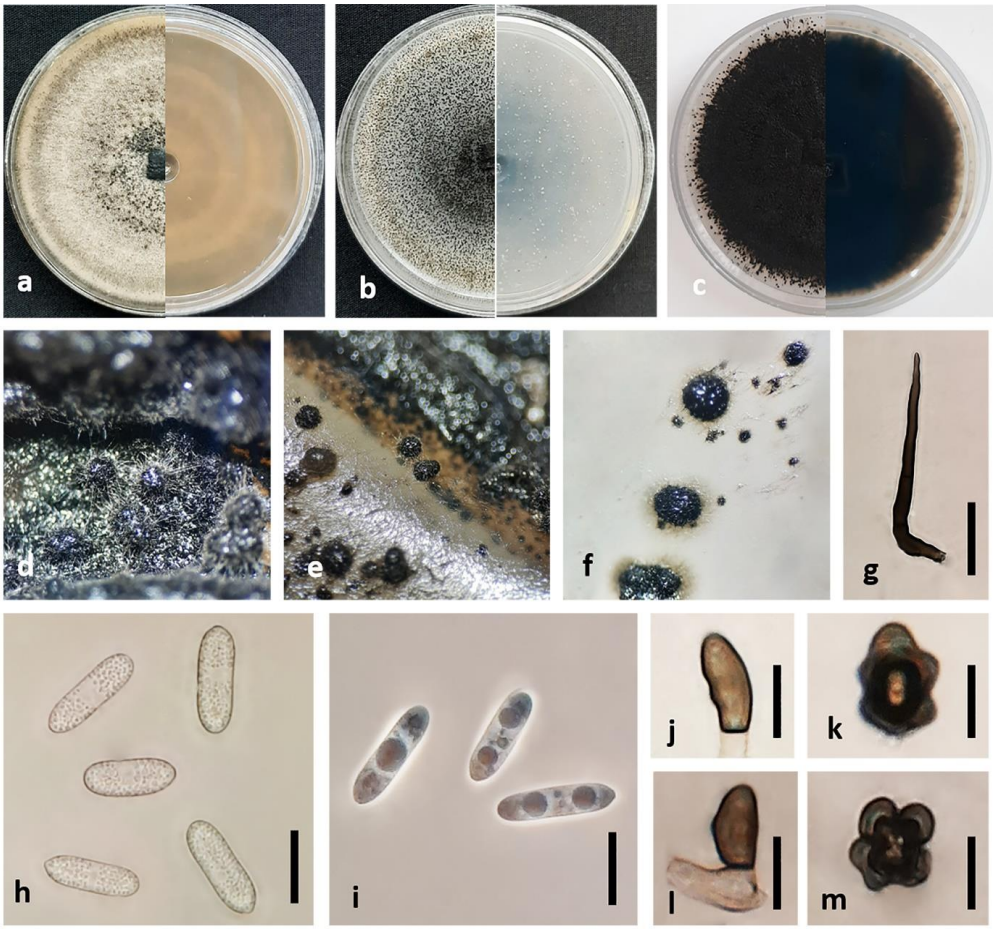

Figure 5. *Colletotrichum destructivum* (AZFC R84N255). **a–c** Colonies on media above and below after 10 days at 25 °C (**a** MEA, **b** OA, **c** PDA). **d, e, f.** Conidiomata on OA. **g.** Setae. **h, i.** Conidia**. j-m.** Appressoria on SNA. Scale bars: **g** = 50 µm, **h, i** = 10 µm, **j-m** = 5 µm.

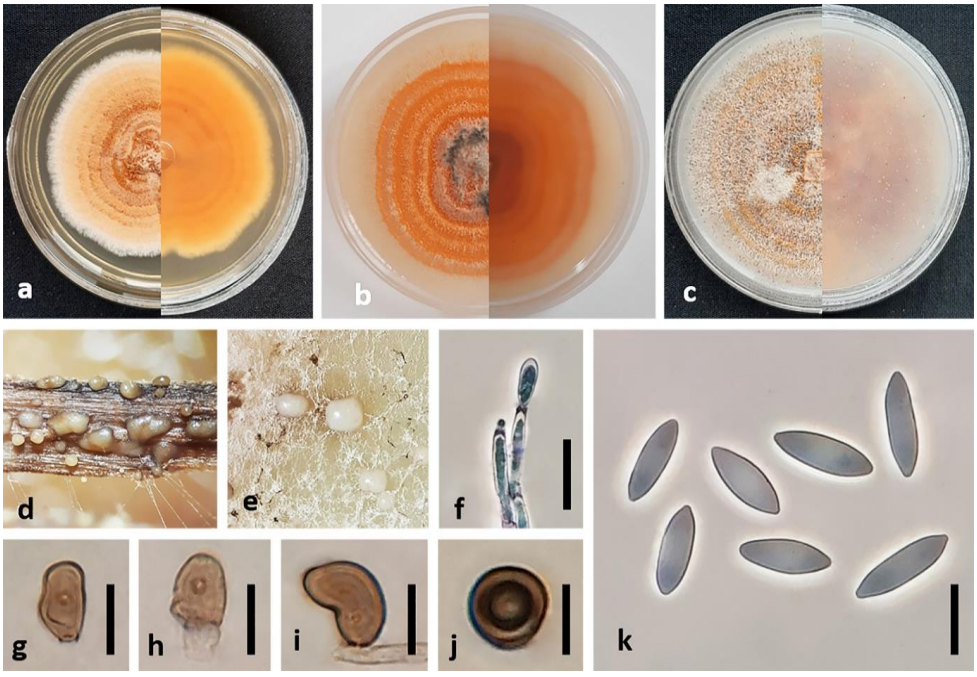

Fig 6. *Colletotrichum orientalis* (AZFC R61N180). **a–c** Colonies on media above and below after 10 days at 25 °C (**a:** MEA, **b:** PDA, **c:** OA). **d.** Conidial masses on *Anthriscus sylvestris* stem. **e.** Conidial masses on SNA. **f.** Conidiogenous cells. **g-j.** Appressoria. **k.** Conidia. Scale bars: **f, k**= 10 µm, **g-j** = 5 µm.

Specimens examined. IRAN, Guilan province, Lahijan, from leaves *of Echinochloa crus-galli*, Sep 2018, Alireza Alizadeh & Fatemeh Salimi. culture AZFC R61N180.

Geographical distribution and host range Previously, this fungus has been identified in various hosts and countries across different continents (Farr & Rossman 2023). It has been found in apple bitter rot in China; *Solanum lycopersicum*, *Vacinum corymbosum* (blueberry) and *Malus domestica* in New Zealand and from *Malus domestica* and *Rubus* sp. in the USA.

Genetic identification: According to the findings of the present study, the *TUB2* and *ACT* loci have proven to be effective in distinguishing this species from other species within the Gloeosporioides complex. However, ITS sequencing was not successful in differentiating this species from several closely related species.

Notes: *Colletotrichum orientalis*, was recently introduced based on a multigenic analysis of six isolates obtained from apple bitter rot in Liaoning province, China along with the six isolates (CBS 129938, CBS200.35, ATCC 28992, CBS 119293, CBS 128555, and CBS 490.92) that were previously classified as *C. fioriniae* in a study by Damm et al. (2012a). The *C. fioriniae* isolates were divided into two clades in the Damm et al. (2012a) study, with one clade including the six isolates mentioned above and the other group containing the *C. fioriniae* holotype CBS 128517. Chen et al. (2022) used five gene regions (ITS, *ACT*, *GAPDH*, *CHS-1*, and *TUB2*) to classify the clade containing the six isolates from apple bitter rot in China and the six isolates from the Damm et al. (2012a) study as a new species, *C. orientalis*.

According to our current knowledge, *C. orientalis* is a new record for the Funga of Iran. To date, only one species of *Colletotrichum*, *C. echinochloae* (Moriwaki & Tsukiboshi, 2009), which has curved conidia and belongs to the Graminicola complex, has been reported from *Echinochloa crus-galli* (Barnyard grasses) worldwide. As a result, *E. crus-galli* is a new host plant for *Colletotrichum* in Iran, and this research marks the first report of a *Colletotrichum* species with straight conidia from Barnyard grasses globally.

DISCUSSION

This study utilized a polyphasic approach to examine three representative *Colletotrichum* isolates with straight conidia obtained from wild Poaceae. The approach combined multi-locus phylogeny, colony characteristics, morphology and host data and three *Colletotrichum* species namely *C. chrysophylum*, *C. destructivum* and *C. orientalis* were identified. Previous studies had used different sets of loci to discriminate species among the different species complexes in the genus *Colletotrichum* (Crouch et al. 2009c, Damm et al. 2009, 2012a, 2012b, 2013, 2014, 2019, Liu et al. 2014, Weir et al. 2012, De Silva et al. 2021, Bhunjun et al. 2021, Chen et al. 2022, Guo et al. 2022, Guevara-Suarez et al. 2022, Tan et al. 2022, Li et al. 2022, Zheng et al. 2022). However, ITS sequences were the only common locus available for all species in these studies, and their resolution was too low to resolve all species of the genus *Colletotrichum*. The current study opted to utilize ITS, *TUB2*, and *ACT* sequences for phylogenetic analysis, as they have been previously shown to offer improved discrimination of species boundaries within the *Colletotrichum* complexes. Our analyses showed that *TUB2* and *ACT* sequences are informative in differentiating *C. chrysophylum*, *C. destructivum*, and *C. orientalis* within the Gloeosporioides, Destructivum, and Acutatum complexes, respectively. However, ITS sequencing was not useful in distinguishing the identified species with close taxa within the corresponding complexes (Crouch et al. 2009c).

The results of this study are valuable for understanding the host-pathogen relationships in the Acutatum, Destructivum, and Gloeosporioides complexes. Additionally, this study comprises the first records of *C. chrysophylum* and *C. orientalis* for the Funga of Iran. It is also the first report of *Colletotrichum* spp. having straight conidia from *Echinochloa crus-galli* and *Setaria viridis*. Previously, *C. echinochloa* was only known to occur on *E. crus-galli*, and only *C. caudatum* with falcate conidia had been identified from *Setaria viridis*. Based on this knowledge, *E. crus-galli* and *Setaria viridis* are reported as new hosts for *C. chrysophylum* and *C. orientalis*, respectively.

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گونه‌های *Colletotrichum* همراه با برخی از گرامینه‌های وحشی در ایران – دو گزارش جدید برای فهرست قارچ‌های ایران

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چکیده: در این پژوهش، ۲۱ جدایه از جنس *Colletotrichum* با کنیدیوم‌های مستقیم از علایم لکه‌برگی برخی گیاهان گرامینه وحشی در سه استان ایران شامل آذربایجان شرقی، گلستان و گیلان جداسازی شد. بر اساس ریخت‌شناسی، جدایه‌ها به سه مورفوتیپ تعلق داشتند. از راهبرد شناسایی چند فازی شامل ریخت‌شناسی و تجزیه و تحلیل تبارزایی چندژنی بر پایه توالی نوکلئوتیدی نواحی ژنومی nuc rDNA ITS1-5.8S-ITS2 (ITS)، بتاتوبولین (*TUB2*) و اکتین (*ACT*) برای مطالعه جدایه‌های نماینده استفاده شد. سه گونه به نام‌های *C. chrysophilum*، *C. destructivum* و *C. orientalis* شناسایی شدند. بر اساس اطلاعات موجود، گونه‌های *C. chrysophilum* و *C. orientalis* گزارش‌های جدیدی برای فهرست قارچ‌های ایران محسوب می‌شوند. این مطالعه اطلاعات جدیدی در رابطه با دامنه میزبانی و پراکنش جغرافیایی گونه‌های شناسایی شده را ارائه می‌دهد.

کلمات کلیدی: آسکومیکوتا، آنتراکنوز، پوآسه، تبارزایی، ریخت‌شناسی، سیستماتیک.

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