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New record of *Cadophora interclivum* from roots of *Ferula ovina* and *F. felabelliloba* in Iran

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Cadophora was described with *C. fastigiata* Lagerb. & Melin as the type species of this dematiaceous hyphomycetous genus that produces solitary phialides with distinct hyaline, flared collarettes (Lagerberg et al. 1927). The generic name *Cadophora* was suggested by Gams (2000) for phialophora-like species with affinities to the Dermateaceae in the Helotiales. *Cadophora* species are primarily isolated from living plants, as pathogens or root colonizers, and produce melanized, septate hyphae that aptly placed them among the fungi labeling dark septate endophytes (DSEs) (Jumpponen & Trappe 1998; Zijlstra et al. 2005). Some samples were collected in May 2016, from *Ferula ovina* and *Ferula felabelliloba* (Apiaceae) in Zoshk highlands of Khorasan Razavi province, Iran (36°26′12.0″ N 59°11′51.6″ E) by Zahra Tazik. Isolation for obtaining endophytic fungi was done according to the method described by Hallmann et al. (2007) with minor modifications. The fresh and disease-free root samples were washed with running tap water and allowed to dry. Then, the obtained samples were cut into pieces of 0.5–1 cm and root pieces were placed in 75% ethanol for 1 min followed by diluted sodium hypochlorite solution 4% for 3 min and finally, in ethanol 75% for 30s. The samples were washed in distilled water after sterilization, placed on filter paper in sterile conditions, and allowed to dry. The root parts were placed on potato dextrose agar (PDA; Merck, Germany) and malt extract agar (MEA; Merck, Germany) containing streptomycin (20 µg/mL) and chloramphenicol (30 µg/mL). The cultures were then incubated at 25-30 °C for 7-28 days. Hyphal tips of fungi emerging out of the root tissues, were picked and grown on PDA, oatmeal agar (OA; 30 g boiled and filtered oat flakes, 15 g agar, 1 L distilled water) and 2% MEA. For sporulation, corn meal agar (CMA; Merck, Germany) and OA media were used, and finally the microscopic slides of fungal isolates were prepared by staining with lactophenol cotton-blue (Vainio et al. 1998) and were examined under a light microscope (Olympus, Tokyo, Japan). The field emission scanning electron microscopy micrograph was taken using FESEM (TESCAN BRNO-Mira3 LMU, 2014, Germany) in the secondary electron imaging (SE) mode. The microscope was operated at 10 kV acceleration voltage, 1.8 kV extraction voltage and a working distance of 5.92 mm. The fungal isolate was deposited in the Fungarium of the Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN) with voucher number: IRAN 3316C.

The fungal genomic DNA was extracted using DenaZist Asia fungal DNA isolation kit according to the manufacturer's instructions. The PCR amplify-cation was performed in a total volume of 25 µL reaction containing 1 ng DNA template, 10 pM of each primer, 10 µL of Taq DNA Polymerase Master Mix RED (Amplicon, Odense, Denmark). Partial sequences of the internal described spacer regions with the 5.8S nuclear ribosomal RNA gene (ITS) were amplified using the ITS5 and ITS4 primers (White et al. 1990). Target regions within the large subunit of the nuclear ribosomal RNA gene cluster (LSU) and translation elongation factor 1-alpha (*TEF1*-α) genes were amplified using primer pairs LRORF/LR5R (Vilgalys & Hester 1990) and 983F/

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EfgrR (Rehner & Buckley 2005), respectively. The PCR products were analyzed in 1.5% agarose gel electrophoresis with 1x Tris-Boric acid-EDTA buffer (TBE) and sent to Macrogen Korea for sequencing. The obtained sequences were subsequently analyzed using the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and closely similar sequences obtained from the National Centre of Biological Information (NCBI) database. The number of sixteen reference strains of *Cadophora* including our isolates and *Cudoniella clavus*, as outgroup, were chosen for phylogenetic analyses. The *TEF1-α* gene was also sequenced (MK512743), but was not used for phylogenetic analyses because comparable sequences of related species were not available. Therefore, we used concatenated data sets of ITS and *LSU* regions to maximize taxon coverage in our phylogenetic analyses. The Bayesian tree was generated with MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003) (Fig. 1). The obtained sequences were deposited in GenBank (NCBI) (accession numbers: MF186880, MK275617 (ITS), MH400226 (LSU), MK512748 (TEF1).

According to BLASTn analysis, our ITS sequences were 98% similar to *Cadophora luteo-olivacea* CBS 141.41 with a 90% similarity to the *LSU* sequence of the same species, indicating that our isolates belong to *Cadophora* genus.

Cadophora interclivum E. Walsh & N. Zhang, Mycologia 110:1, 201-214 (2018); Fig. 2

Colonies on OA and MEA 25 and 46 mm in diameter, respectively, after two weeks of incubation at 25 ºC in darkness. On MEA: Colonies tawny olive, floccose, gibberosus, with light gray aerial hyphae in the middle. Mycelia with septate, dark melanized hyphae. Reverse dark brown olive. On OA: Colonies olive, floccose, gibberosus, with yellow margin. Conidia formed on CMA after 1 month at 25 º C in dark. Collarettes cylindrical, 2–4 × 1.5–3 µm. Conidiogenous cells hyaline, cylindrical, 2.5–15 × 1.5–3 µm. Conidia aggregated, elongated ellipsoidal, a-septate, hyaline, smooth, 5–7 × 1–3 µm. Sexual morph was not observed on culture media.

The result of the morphological identification showed that the species belonged to the genus *Cadophora*. The phylogenetic tree constructed by combined ITS and LSU sequence data sets, showed that it was placed in the same clade with the recently reported species of *Cadophora interclivum* (Fig. 1). To the best of our knowledge, this is the first report of this species for mycobiota of Iran (Ershad et al. 2019).

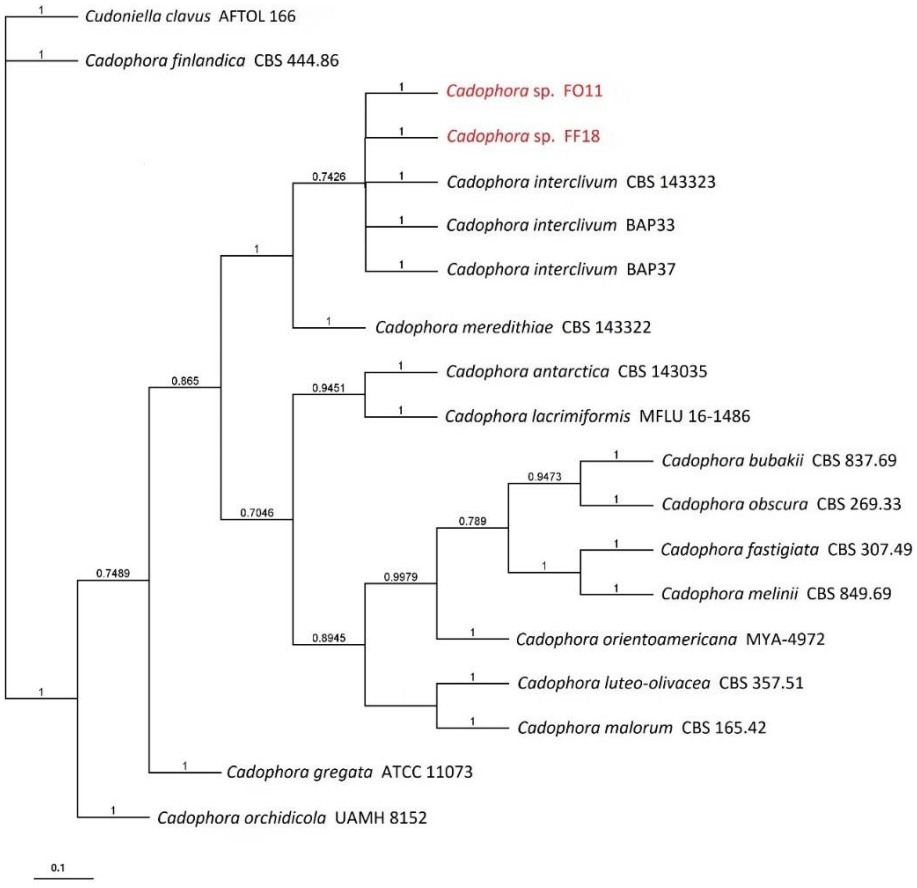

Fig. 1. Bayesian tree from a concatenated ITS and nuLSU data sets showing the phylogenetic position of our isolates among the other *Cadophora* species. Numbers on the branches are bootstrap values for Bayesian posterior probabilities (PP). The tree was rooted with *Cudoniella clavus* as outgroup taxa.

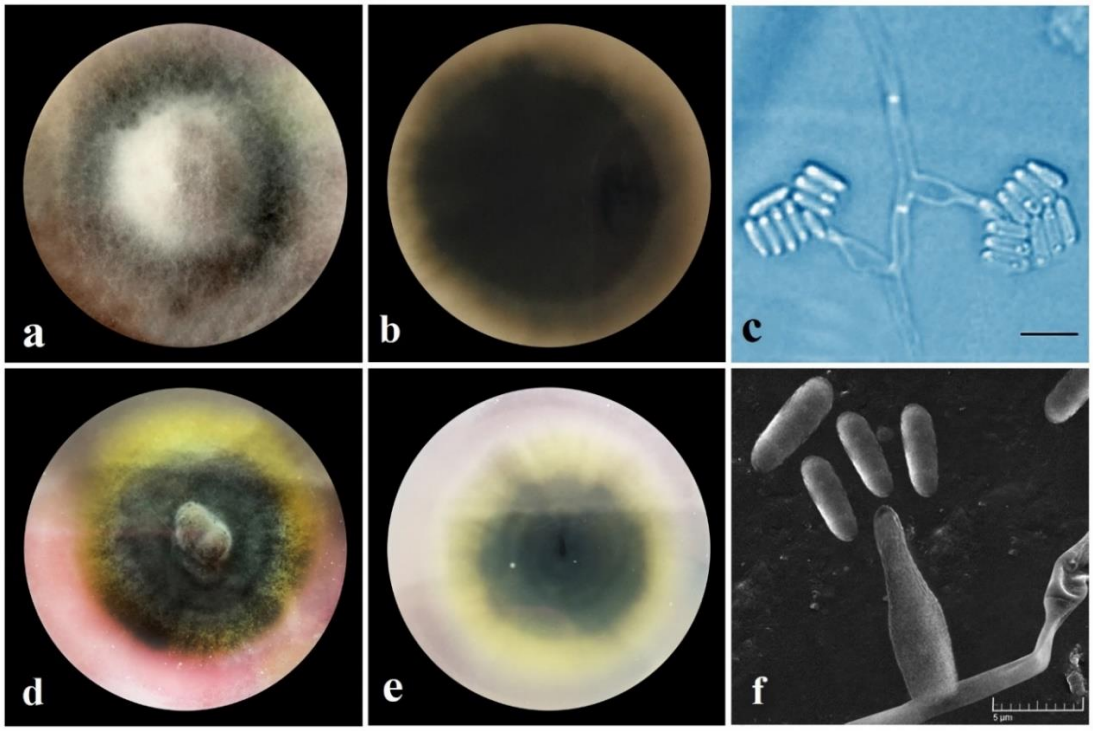

Fig. 2. *Cadophora interclivum*. a, b, d, e. Colony on MEA and OA after 14 d at 25 °C, respectively (surface and reverse), c. conidia and conidiogenous cells. f. field emission scanning electron micrographs (FESEM) of conidia and conidiogenous cells. — Scale bars = c, 10 µm; f, 5 µm.

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