



Toxicocladosporium irritans, a new species for funga of Iran

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The genus *Toxicocladosporium* (*Cladosporiaceae*, *Cladosporiales*, and *Dothideomycetes*) was first introduced by Crous et al. (2007) based on *Toxicocladosporium irritans* Crous & U. Braun. To date, 20 species namely *T. aquimarinum* R. Fotedar, Sand.-Den., Kolecka & Boekhout, *T. banksiae* Crous, R.G. Shivas & McTaggart, *T. cacti* J.D.P. Bezerra, Souza-Motta & Crous, *T. crousianum* M. Bakhshi, *T. chlamyosporum* Crous & M.J. Wingf., *T. eucalyptorum* M. Bakhshi, Zare & Jafary, *T. ficiniae* Crous & A.R. Wood, *T. glendoranum* Crous & Jurjević, *T. hominis* Sand.-Den., Gené & Deanna A. Sutton, *T. immaculatum* J.D.P. Bezerra, Souza-Motta & Crous, *T. irritans*, *T. losalamitosense* Crous & Jurjević, *T. pini* Crous & Y. Zhang, *T. posoqueriae* Crous & R.G. Shivas, *T. protearum* Crous & Roets, *T. pseudovelox* Crous, *T. pterocarpi* Crous, *T. qatarse* R. Fotedar, Sand.-Den., Kolecka & Boekhout, *T. rubrigenum* Crous & M.J. Wingf., and *T. strelitziae* Crous have been described in this genus based on a combination of morphological and molecular data (Crous et al. 2007, 2021a, 2021b; Bezerra et al. 2017; Fotedar et al. 2019; Bakhshi et al. 2022b). Species of this genus are cosmopolitan and occur in a wide range of host plants, different ecosystems, and extreme habitats (Bezerra et al. 2017; Fotedar et al. 2019). In addition, they display diverse lifestyles, from plant pathogens to epiphytes, endophytes, and saprobes (Bezerra et al. 2017; Bakhshi et al. 2022b).

Loquat or Japanese medlar (*Eriobotrya japonica* Lindl.) belongs to the family *Rosaceae*, is predominantly adapted to tropical and subtropical climates worldwide, and is a highly nutritious fruit tree of economic relevance (Wang & Paterson 2021). In an investigation aimed at revealing the diversity of

follicolous fungi associated with fruit orchards in northern Iran, one isolate of *Toxicocladosporium* was isolated from the leaf surface of a loquat tree in Golestan Province. The living culture was deposited in the culture collection of the Iranian Research Institute of Plant Protection (IRAN) in Tehran, Iran. To extract genomic DNA using the protocol described by Möller et al. (1992), the fungal isolate was transferred onto a plate containing malt-extract agar (MEA; Merck, Darmstadt, Germany) and incubated at 25 °C for 10 days. Amplification and sequencing of two nuclear loci, DNA-directed RNA polymerase II second largest subunit (RPB2), and internal transcribed spacer regions and intervening 5.8S nrRNA gene of the nrDNA operon (ITS), were carried out with primers RPB2-5F2 + fRPB2-7cR and V9G + ITS4, respectively, as described by Bakhshi et al. (2021). The new sequences generated in this study were further edited and concatenated using the MEGA v. X (Kumar et al. 2018) software in the sense and antisense directions, submitted to GenBank, and accession numbers were obtained. The Bayesian method was performed using MrBayes v. 3.2.6 (Ronquist et al. 2012) for the two-locus DNA phylogenetic analysis, as elucidated by Bakhshi et al. (2021). *Neocladosporium leucadendri* (Crous) J.D.P. Bezerra, Sand.-Den., Souza-Motta & Crous (CBS 131317, *Cladosporiaceae*) was used as the outgroup taxon. According to phylogenetic analyses (Fig. 1), the Iranian strain obtained from Japanese medlar clustered together with the *T. irritans* reference culture (CBS 185.58). To the best of our knowledge, this is the first report of *T. irritans* for funga of Iran (Bakhshi et al. 2022a). The fungus exhibited the following morphology:

Toxicocladosporium irritans Crous & U. Braun, Stud. Mycol. 58: 39 (2007)

Description *in vitro*: on SNA: Mycelium consisting of subhyaline to brown, smooth to finely verruculose branched, septate, 2–3.5 µm wide hyphae. Conidiophores solitary, brown, smooth to finely verruculose, dimorphic. Macronematous conidiophores arising from hyphae, erect, unbranched or branched, subcylindrical, straight to geniculous-sinuous, or irregularly curved, walls thick, septa dark brown, 20–75 × 3–5.5 µm, 1–6-septate.

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Micronematous conidiophores aseptate, reduced to conidiogenous cells on hyphae, erect, subcylindrical, $8\text{--}25 \times 2.5\text{--}4 \mu\text{m}$. Conidiogenous cells integrated, lateral or terminal, smooth to slightly verruculose, brown, subcylindrical with slight taper towards apex, $6\text{--}11 \times 2.5\text{--}4 \mu\text{m}$, proliferating sympodially; loci thickened, darkened and refractive, $1\text{--}2 \mu\text{m}$ wide. Conidia in general catenulate in branched or unbranched chains, medium brown to brown, thick-walled, with dark, thick septa, smooth to finely verruculose. Ramoconidia, subcylindrical to broadly ellipsoid, straight or slightly flexuous, polyblastic, with sympodial proliferation, 0–3-septate, $8\text{--}18 \times 3\text{--}4\text{--}(5) \mu\text{m}$. Conidia ellipsoid to ovoid, 0-1-septate, 6--

$10 \times 3\text{--}5 \mu\text{m}$; hila darkened and thickened, $1\text{--}1.5 \mu\text{m}$ wide.

Culture characteristics: (in the dark, at $25 \text{ }^\circ\text{C}$ after 20 days): Colonies on MEA, surface folded, velvety, olivaceous grey to olivaceous, with an even to irregularly undulating margin, with moderate aerial mycelium, reverse dark grey, slow-growing, reaching 12 mm. On SNA, surface flat, grey-olivaceous, with sparse aerial mycelium, with even, smooth margins, reaching 11 mm.

Specimens examined: Iran: Golestan province, Fazel Abad, on leaves of *Eriobotrya japonica* (*Rosaceae*), 27 April 2019, M. Bakhshi, IRAN 4788C (ITS = OQ625992, RPB2 = OQ632795).

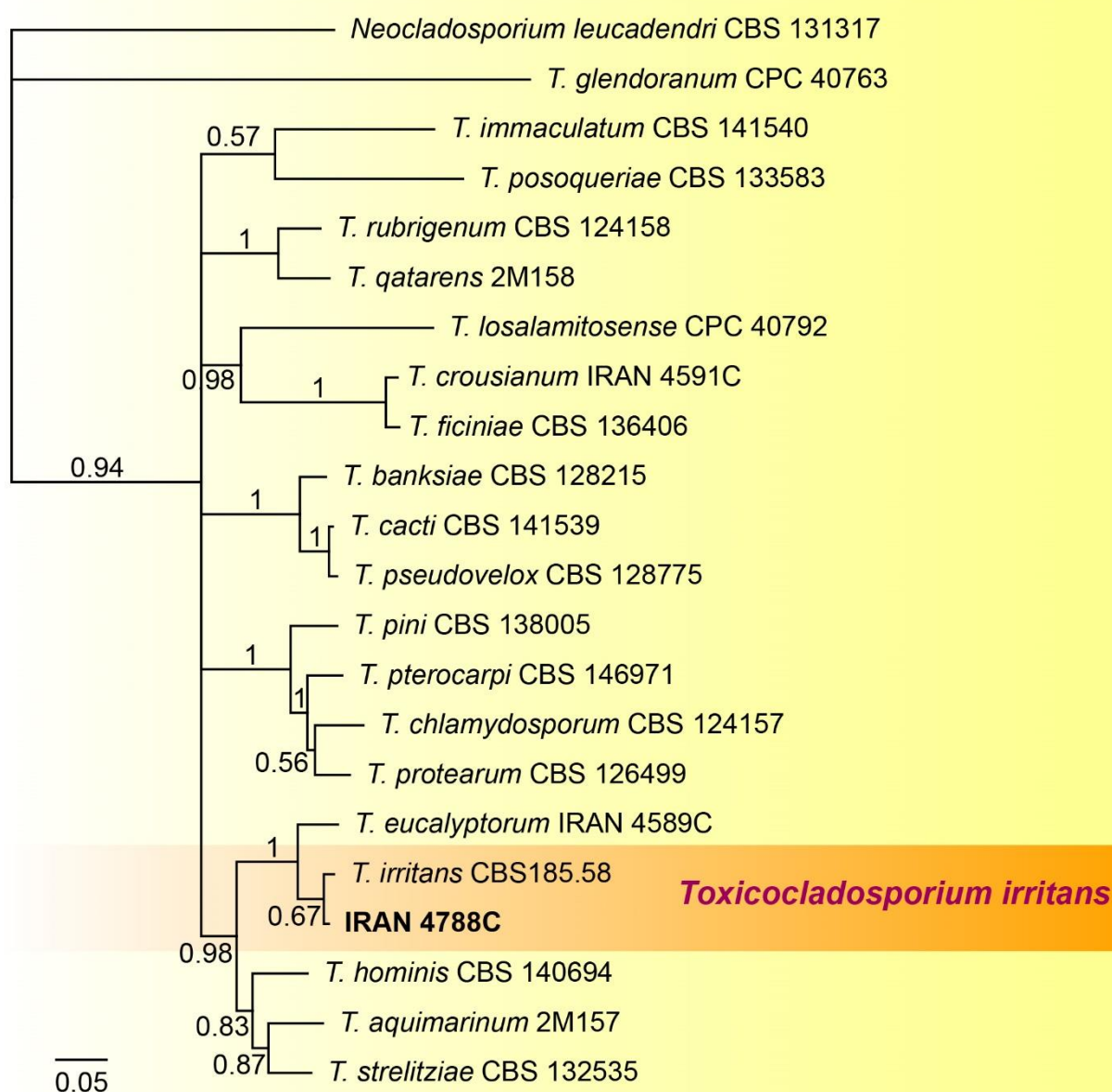


Fig. 1. Phylogenetic tree inferred by Bayesian analysis of the combined two-locus (ITS and RPB2) sequence alignment using MrBayes v.3.2.6 for *Toxicocladosporium* species. The scale bar indicates 0.05 expected changes per site. The isolate of this study is in bold. The tree was rooted to *Neocladosporium leucadendri* (CBS 131317).

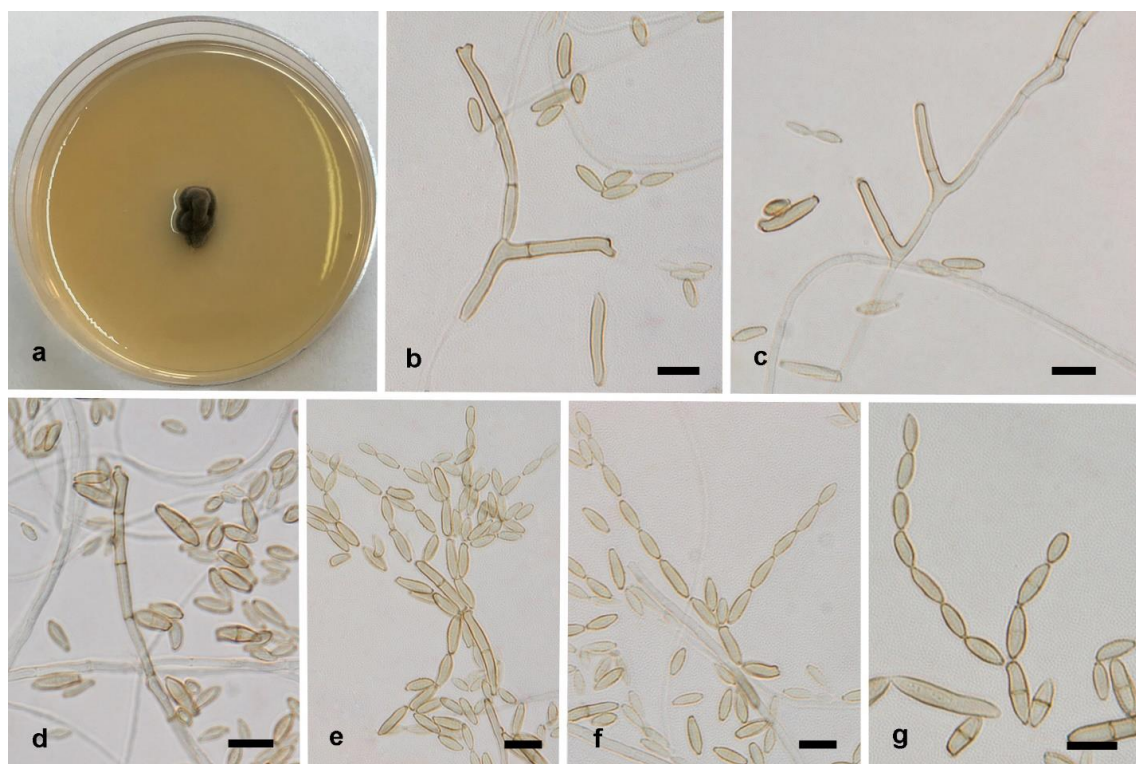


Fig. 2. *Toxicocladosporium irritans* (IRAN 4788C): a, 20-day-old colony on MEA at 25 °C, b–g Conidiophores, conidiogenous cells, ramoconidia and conidia (Bars = 10 μm).

REFERENCES

- Borde M, Kshirsagar Y, Jadhav R, Baghela A. 2021. Bakhshi M, Braun U. 2022. *Acericercospora hyrcanica* gen. et sp. nov. (*Mycosphaerellaceae*) and *Paramycocentrospora acericola* gen. et sp. nov. (*Dothidotthiaceae*) on maple trees in Hyrcanian forests. *Mycological Progress* 21(8): 1–15.
- Bakhshi M, Zare R, Braun U, Taheri H. 2021. Polyphasic taxonomy of four passalora-like taxa occurring on fruit and forest trees. *Mycological Progress* 20(9): 1157–1173.
- Bakhshi M, Zare R, Ershad D. 2022a. A detailed account on the statistics of the Fungi and fungus-like taxa of Iran. *Mycologia Iranica* 9 (2): 1–96.
- Bakhshi M, Zare R, Jafary H. 2022b. *Toxicocladosporium crousianum* and *T. eucalyptorum*, two new foliar fungi associated with Eucalyptus trees. *Mycological Progress* 21 (9): 1–10.
- Bezerra JD, Sandoval-Denis M, Paiva LM, Silva GA, Groenewald JZ, Souza-Motta CM, Crous PW. 2017. New endophytic *Toxicocladosporium* species from cacti in Brazil, and description of *Neocladosporium* gen. nov. *IMA Fungus* 8: 77–97.
- Crous PW, Braun U, Schubert K, Groenewald JZ. 2007. Delimiting *Cladosporium* from morphologically similar genera. *Studies in Mycology* 58: 33–56.
- Crous PW, Cowan DA, Maggs-Kölling G et al. 2021a. Fungal planet description sheets: 1182–1283. *Persoonia* 46: 313–528.
- Crous PW, Osieck ER, Jurjevi Ž et al. 2021b. Fungal planet description sheets: 1284–1382. *Persoonia* 47: 178–374.
- Fotadar R, Sandoval-Denis M, Kolecka A, Zeyara A, Al Malki A, Al Shammari H, Al Marri M, Kaul R, Boekhout T. 2019. *Toxicocladosporium aquimarinum* sp. nov., *Toxicocladosporium qatarse* sp. nov., isolated from marine waters of the Arabian Gulf surrounding Qatar. *International Journal of Systematic and Evolutionary Microbiology* 69(10): 2992–3000.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6): 1547–1549.
- Möller E, Bahnweg G, Sandermann H, Geiger HH. 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Research* 20: 6115–6116.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- Wang Y, Paterson AH. 2021. Loquat (*Eriobotrya japonica* (Thunb.) Lindl) population genomics suggests a two-staged domestication and identifies genes showing convergence/parallel selective sweeps with apple or peach. *The Plant Journal* 106(4): 942–952.