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Comoclathris typhicola, a new species for the funga of Iran

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Abstract: The *Comoclathris* genus belongs to the Pleosporaceae family (*Dothideomycetes*, *Pezizomycotina*, *Ascomycota*), with most species being saprophytes. In the present study, *Comoclathris typhicola* was isolated from *Typha latifolia* (*Typhaceae*, *Poales*) exhibiting leaf spot symptoms. The species was described using PDA, MEA, and OA culture media, and its molecular identification was confirmed through sequencing of the large subunit RNA polymerase II (*RPB2*) gene. This is the first report of this species in Iran.

Keywords: Molecular phylogeny, Morphology, *Pleosporaceae*, Taxonomy.

INTRODUCTION

The genus *Comoclathris* Clem., typified by *Comoclathris lanata* Clem., was described by Clements (1909). It was placed in the family *Pleosporaceae* (*Pleosporales, Dothideomycetes, Pezizomycotina, Ascomycota*) based on *Alternaria*-like asexual morphs (Zhang et al. 2011, Woudenberg et al. 2013). Molecular data is not available for the type species, *C. lanata*, but the two putative strains of *C. compressa* (CBS 157.53 and CBS 156.53) cluster together in a well-supported clade within the family *Pleosporaceae* (Ariyawansa et al. 2014). Subsequent molecular studies confirmed the placement of *Comoclathris* in *Pleosporaceae* (Ariyawansa et al. 2015, Wijayawardene et al. 2017, Boonmee et al. 2021, Crous et al. 2021, Mattoo et al. 2023, Xu et al. 2024). The genus is characterized by ascomata with circular lid-like openings and applanate reddish-brown to dark reddish-brown, muriform ascospores, with single longitudinal septa (Ariyawansa et al. 2015, Wijayawardene et al. 2017, Xu et al. 2024). Presently, the genus consists of 52 registered names in Index Fungorum (https://www.indexfungorum.org; accessed Oct 2024).

In this study, *Comoclathris typhicola* was isolated from *Typha latifolia* (*Typhaceae*, *Poales*), which exhibited leaf spot symptoms. The species was illustrated and described based on morphological characteristics and the sequencing of the large subunit RNA polymerase II (*RPB2*) gene.

MATERIALS AND METHODS

In this study leaf samples from *Typha latifolia* L. (*Typhaceae*, *Poales*) showing necrotic lesions were collected from Miyandoab City, West Azarbaijan province, Iran, in 2021. Leaf samples were surface disinfected using 1% sodium hypochlorite solution for three minutes rinsed in sterile distilled water and incubated in a moist chamber at 25 °C. The incubated leaves were inspected under the stereo microscope (SZ51, Olympus, Japan) and single-spore isolation was done following the method described in Ahmadpour et al. (2021). Germinated spores were transferred to potato dextrose agar (PDA: 39 g/L sterile distilled water, Merck, Darmstadt, Germany) plates and incubated at room temperature for 2–4 weeks. The isolates were grown on PDA, malt extract agar (MEA: 50 g/L, Quelab, Montreal, Canada) and oatmeal agar (OA: 30 g oatmeal and15 g Agar in 1 Liter distilled water) culture media at 25 °C under the near ultraviolet light (NUV)/dark cycle of 12/12 h for 7–14 days to study the morphological characteristics (Mattoo et al. 2023). Measurements and microphotographs were prepared from slide mounts in lactophenol using an Olympus AX70 compound microscope with differential interference contrast (DIC) illumination. Adobe Photoshop 2020 v. 2.10.8 software (Adobe Inc., San Jose, California) was used for manual editing. All the identified isolates were deposited in the fungal culture collections of the Iranian Research Institute of Plant Protection (IRAN) and Urmia University (FCCUU). DNA was extracted from the mycelial mass of each isolate harvested from 10-day-old PDA Petri dishes using chloroform extraction and isopropanol precipitation method (Ahmadpour et al. 2021). Amplification and sequencing of parts of *RPB2* gene was carried out using RPB2-5F2/RPB2-7cr2 primer pairs (Liu et al. 1999, Sung et al. 2007). Maximum likelihood (ML) analysis was conducted in the RAxML–HPC BlackBox v. 8.2.8 (Stamatakis 2014) online server of

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the CIPRES Science gateway portal (https://www.phylo.org/) (Miller et al. 2012) for 1000 bootstrapping iterations, using the general time reversible model (GTR) with a discrete gamma distribution. Sequences of *Neocamarosporium chenopodii* (CBS 206.80) and *N. obiones* (CBS 432.77) served as the outgroup taxa (Mattoo et al. 2023, Xu et al. 2024). The resultant phylogenetic trees were visualized in FigTree v. 1.4.4 (Rambaut 2019), and edited in graphic design software, Adobe Illustrator® CC 2020. The newly generated sequences were submitted to GenBank.

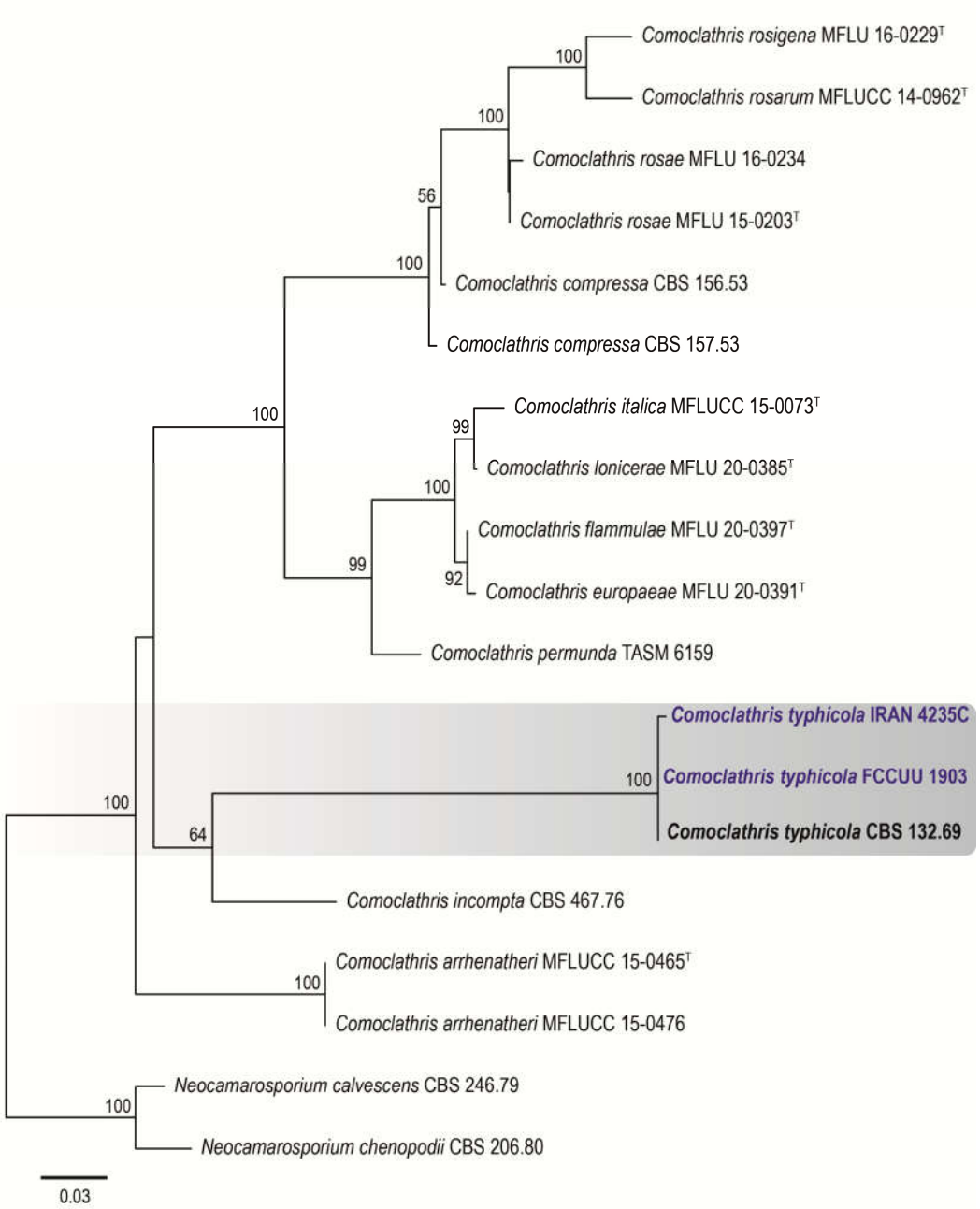

Fig. 1. Maximum Likelihood (ML) analysis based on *RPB2* sequence data of *Comoclathris* species. The Maximum Likelihood bootstrap support (BS) values >50% are given at the nodes. The tree was rooted to *Neocamarosporium chenopodii* (CBS 206.80) and *N. obiones* (CBS 432.77). The scale bar indicates the number of nucleotide substitutions. ^{T} indicates ex-type strains.

RESULTS AND DISCUSSION

The phylogenetic results revealed that our isolates clustered well with *Comoclathris typhicola* (CBS 132.69 strain) in a distinct clade with high bootstrap support values (ML = 100) (Fig. 1). To the best of our knowledge, this is the first report of *C. typhicola* for the funga of Iran.

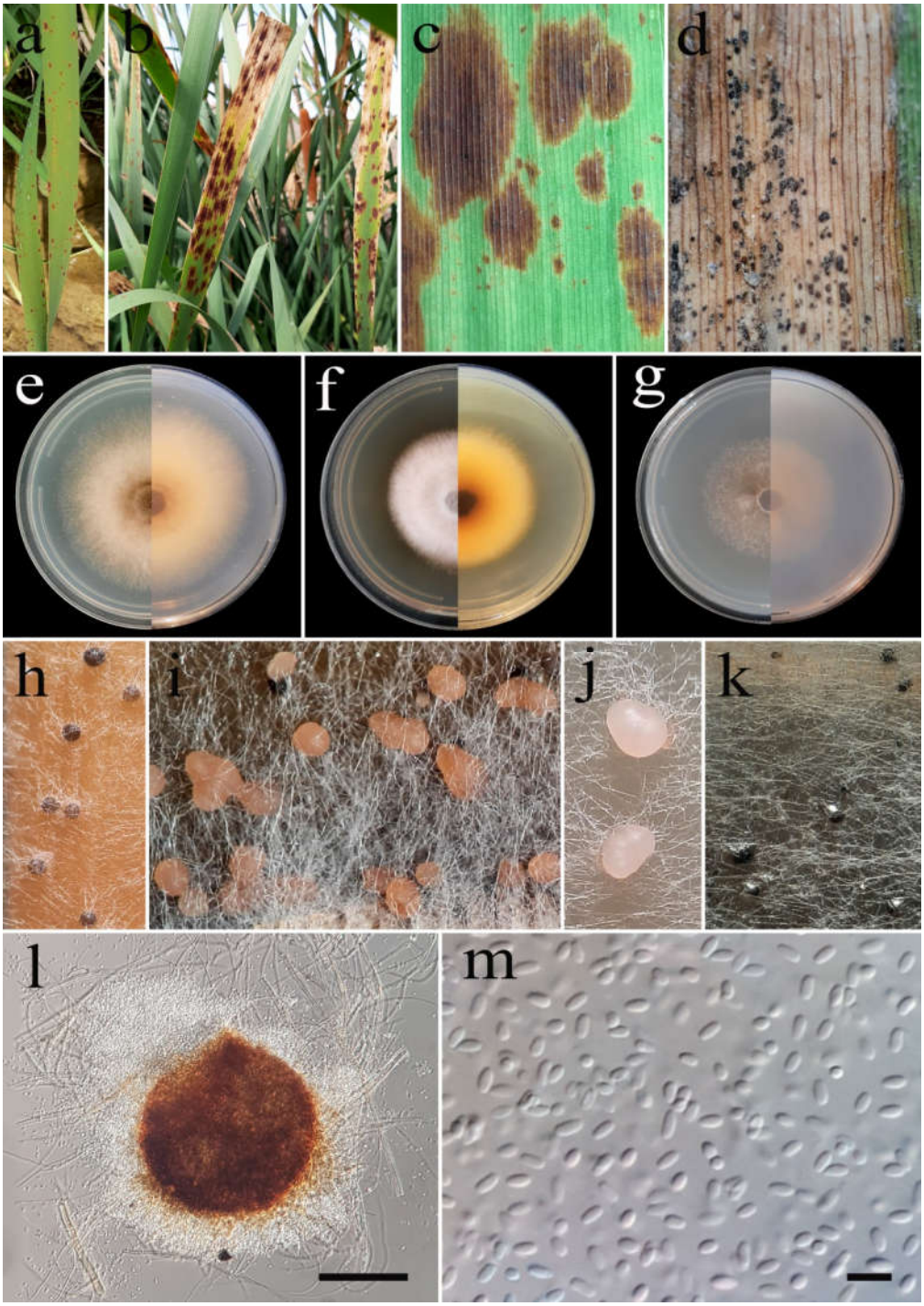

Fig. 2. *Comoclathris typhicola* (IRAN 4235C). **a–d.** Host plant showing necrotic lesions on leaves. **e-g.** Colonies (front and reverse) after seven days on PDA (e), MEA (f) and OA (g). **h**–**k.** Pycnidia with conidial mass on PDA (h-i) and OA (j-k). **l.** Pycnidium. **m.** Conidia. Scale bars: l = 100 µm, m = 10 µm.

Taxonomy and morphology

Comoclathris typhicola (Cooke) Ariyaw. & K.D. Hyde, Fungal Diversity 71: 105 (2015) Fig. 2

Basionym**:** *Sphaeria typhicola* Cooke [as '*typhaecola*'], Grevillea 5(no. 35): 121 (1877).

Synonyms**:**

≡ *Pleospora typhicola* (Cooke) Sacc., Reliq. Libert 2: no. 152 (1875).

≡ *Clathrospora typhicola* (Cooke) Höhn., Annls mycol. 16(1/2): 88 (1918).

≡*Macrospora typhicola* (Cooke) Shoemaker & C.E. Babc., Can. J. Bot. 70(8): 1644 (1992).

≡ *Pyrenophora typhicola* (Cooke) E. Müll., Sydowia 5(3-6): 256 (1951).

≡ *Macrospora typhicola* (Cooke) Shoemaker & C.E. Babc., Canad. J. Bot. 70: 1644 (1992).

= *Phyllosticta typhina* Sacc. & Malbr., Sacc., Michelia 2:88 (1880).

≡ *Phoma typhina* (Sacc. & Malbr.) van der Aa & Vanev, A revision of the species described in *Phyllosticta*: 468 (2002).

= *Phoma typharum* Sacc., Syll. Fung. 3: 163 (1884).

Description: Isolated from the leaves of *Typha latifolia* with necrotic lesions. Lesions up to 5–20 mm diam., spread on the upper surface, scattered, distinct, regular to irregular, pale brown to dark brown, leading to leaf death. Sexual morph: not observed. Asexual morph: Coelomycetous. Conidiomata pycnidial, semi-immersed to immersed, mostly solitary, rarely aggregated, scattered, globose to subglobose, pale brown to brown, thin-walled, glabrous, closed to one inconspicuous pore, with creamy to yellow conidial mass, 180–200 × 190–200 µm. Pycnidial wall pseudoparenchymatous, 3–5 layered, composed of oblong to isodiametric cells, pale brown, 10–25 µm thick. Conidiogenous cells phialidic, hyaline, smooth, globose to ampulliform, 4–6 × 4–7 µm. Conidia ellipsoidal to oblong, occasionally ovoid, with rounded ends, hyaline, smooth and thin-walled, aseptate, with polar guttules, 3–4 × 1–1.2 µm ($\bar{x}$ = 3.5 × 1.1 µm, n = 50).

Culture characteristics: Colonies on PDA reaching 57–59 mm diam. after seven days at 25 °C, smooth margin, with sparse aerial mycelia, grey at the center and white at the margin; reverse white to pale brown. Colonies on MEA reaching 49–50 mm diam. after seven days at 25 °C, floccose, surface white, smooth margin, with sparse aerial mycelia; reverse white and pale brown at the center. Colonies on OA reaching 53–55 mm diam. after seven days at 25 °C, smooth margin, surface white to grey, with sparse aerial mycelia, abundant production of pycnidia, conidial matrix visible; reverse buff and grey near the center.

Specimens examined: IRAN, West Azarbaijan province, Miyandoab city, isolated from infected leaves of *Typha latifolia* (*Typhaceae*, *Poales*), 10 Sept. 2021, *A. Ahmadpour*, IRAN 4235C (*RPB2* = OR611972) and FCCUU 1903 (*RPB2* = OR611973).

Habitat and distribution: From leaf spots and on dead leaves of *Typha latifolia* and *Typha angustifolia* (*Typhaceae*), Austria, Denmark, England, Germany, India, Netherlands, Pakistan, Poland, Portugal, Russia, Switzerland, USA, and Iran (Webster & Lucas 1959, Shoemaker & Babcock 1992, Boerema et al. 2004, Farr et al. 2024, this study).

Notes: *Comoclathris typhicola* was originally described from the leaf lesions on *Typha angustifolia* from Great Britain (Boerema et al. 2004). This species was frequently recorded in association with leaf spots and on dead leaves, leaf sheaths and stems of *Typha latifolia* and *Typha angustifolia* (*Typhaceae*) in European countries (Boerema et al. 2004, Farr et al. 2024). Sexual and asexual morphs of *Comoclathris typhicola* have been recorded *in vitro* and *in vivo* (Webster & Lucas 1959).

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Comoclathris typhicola، گونه جدیدی برای بیوتای قارچی ایران

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چکیده: جنس *Comoclathris* متعلق به خانواده *Pleosporaceae* (*Dothideomycetes*, *Pezizomycotina*, *Ascomycota*) می‌باشد و اغلب گونه‌ها پوده‌رست هستند. در مطالعه حاضر، گونه *Comoclathris typhicola* از گیاه *Typha latifolia* (*Typhaceae*, *Poales*) با علایم لکه‌برگی جداسازی گردید. گونه‌ی *Comoclathris typhicola* روی محیط‌های کشت PDA، MEA و OA توصیف گردید و با توالی‌یابی ژن زیر واحد بزرگ RNA پلیمراز II (*RPB2*) شناسایی مولکولی آن تایید گردید. این اولین گزارش از گونه مذکور در ایران می‌باشد.

کلمات کلیدی: تبارشناسی مولکولی، ریخت‌شناسی، *Pleosporaceae*، آرایه‌بندی.

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