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Two new records of *Fusarium incarnatum-equiseti* species complex in Iran

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Abstract

Members of the *Fusarium incarnatum-equiseti* species complex (FIESC), a species-rich and taxonomically challenging lineage within *Fusarium*, are cosmopolitan in various ecological niches. In an investigation on biodiversity of *Fusarium* species in rhizosphere of sugarcane in Khuzestan Province (Iran), four isolates of *Fusarium* belonging to FIESC were subjected to a phylogenetic study. The multilocus phylogeny of translation elongation factor 1-alpha (*tef1*) and RNA polymerase II second largest subunit (*RPB2*) genes, supplemented with morphological characters, enabled the recognition of two new species to the Iranian funga, i.e., *F. fasciculatum* (one isolate) and *F. nothincarnatum* (three isolates). Both species are described and illustrated herein based on Iranian material and compared with closely related species. *Fusarium fasciculatum* is close to *F. citri* and *F. humuli*, and *F. nothincarnatum* is a close relative of *F. incarnatum*. The present study, is the first report of polyphialides and mesoconidia formation on the aerial mycelium by isolates of *F. nothincarnatum*.

Keywords: Biodiversity, multigene phylogeny, rhizosphere, single-copy genes, taxonomy

دو گزارش جدید از کمپلکس گونه‌ای *Fusarium incarnatum-equiseti* در ایران

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خلاصه

اعضای کمپلکس گونه‌ای *Fusarium incarnatum-equiseti* (FIESC) که یک تبار حاوی گونه‌های فراوان و چالش‌برانگیز از نظر تاکسونومی در جنس *Fusarium* است، در محدوده‌های بوم‌شناختی متعدد حضور دارند. در بررسی تنوع‌زیستی گونه‌های *Fusarium* موجود در ریزوسفر نیشکر در استان خوزستان، چهار جدایه *Fusarium* متعلق به کمپلکس گونه‌ای FIESC از نظر تبارزایی مورد مطالعه قرار گرفتند. براساس توالی‌یابی ژن‌های *TEF1* (Translation elongation factor 1-alpha) و *RPB2* (RNA polymerase II second largest subunit) و مطالعات ریخت‌شناختی، دو گونه جدید برای قارچ‌های ایران شامل *F. fasciculatum* (یک جدایه) و *F. nothincarnatum* (سه جدایه) شناسایی شد. در این مقاله، تصاویر و توصیف هر دو گونه شناسایی شده براساس بررسی جدایه‌های ایرانی و نتایج مقایسه آن‌ها با گونه‌های نزدیک ارایه می‌شود. گونه *F. fasciculatum* به دو گونه *F. citri* و *F. humuli* نزدیک بوده و گونه *F. nothincarnatum* هم خویشاوند نزدیک *F. incarnatum* است. در مطالعه حاضر، تشکیل پلی‌فیالیدها و مزوکنیدیوم‌ها روی میسلیوم‌های هوایی توسط جدایه‌های متعلق به گونه *F. nothincarnatum* برای نخستین بار گزارش می‌شود.

واژه‌های کلیدی: تاکسونومی، تبارزایی چندژنی، تنوع زیستی، ریزوسفر، ژن‌های تک‌نسخه‌ای

Introduction

The genus *Fusarium* Link (1809) is one of the most species-rich and economically important genera of filamentous fungi, encompassing plant pathogens, saprobes, and mycotoxigenic species with a global distribution (Leslie & Summerell 2006, Crous *et al.* 2021). Currently, species delimitation in *Fusarium* relies heavily on multilocus phylogenetic approaches, in which the translation elongation factor 1-α (*TEF1*) and RNA polymerase II second largest subunit (*RPB2*) loci are among the most informative and widely used markers for species-level identification (O'Donnell *et al.* 2013, Lombard *et al*. 2019, Crous *et al*. 2021).

The *Fusarium incarnatum-equiseti* species complex (FIESC) represents a species-rich and taxonomically challenging lineage within *Fusarium* (Leslie & Summerell 2006, Xia *et al*. 2019, Han *et al.* 2023). Traditionally, species in this complex were commonly identified as *F. incarnatum* (Roberge ex Desm.) Sacc. or *F. equiseti* (Corda) Sacc., based on the macroconidial morphology and colony characters, and ITS/*tef1* sequences (Khoa *et al*. 2004, Leslie & Summerell 2006, Marín *et al*. 2012). However, the application of genealogical concordance phylogenetic species recognition (GCPSR) enabled the recognition of more than 30 cryptic phylogenetic species (phylo-species) in the complex that were separated in two major clades, i.e., the *Incarnatum* and *Equiseti* clades (O'Donnell *et al.* 2009, Villani *et al.* 2016, Xia *et al.* 2019). Subsequent integrative taxonomic work, by employing morphological characters and multilocus phylogenetic analyses, have resolved the species within the FIESC, and linked the majority of phylo-species to Latin binomials (Wang *et al.* 2019, Xia *et al.* 2019, Han *et al.* 2023). Furthermore, by applying the phylogenomic analyses, Han *et al.* (2023) included the *Fusarium camptoceras* species complex (FCAMSC) in FIESC as the *Camptoceras* clade. Morphologically, members of the FIESC are mainly characterized by dorsiventrally curved, often falcate macroconidia, with abundant chlamydospores, and mostly lacking microconidia (Wang *et al.* 2019).

Members of FIESC have been isolated from soil, air, plants and insects around the world (Summerell *et al.* 2011, Xia *et al.* 2019). However, half of the species in the complex have been reported from cereals (Han *et al.* 2023). The FIESC species may act as saprobes, endophytes, entomopathoges, fungicolous or phyto-pathogens (Summerell *et al.* 2011, Xia *et al.* 2019, da Silva Santos *et al.* 2020, Torbati *et al.* 2021, Mello *et al.* 2024). As phytopathogens, FIESC species have been reported as weak pathogens or secondary invaders (Summerell *et al.* 2003, Leslie & Summerell 2011). To date, diseases such as leaf spot, leaf blight, head blight, wilt , and mainly rot on a various crops have been associated with FIESC species (see da Silva Santos *et al.* 2025). Furthermore, some isolates of FIESC have been reported to produce a range of different mycotoxins (Jedidi *et al.* 2021, da Silva Santos *et al.* 2025), particularly trichothecenes, potent inhibitors of protein synthesis, and zearalenones, causing estrogenic disorders in human and animals (Desjardins 2006).

Sugarcane (*Saccharum officinarum* L.) is one of the important agricultural crops of Iran, that is mainly cultivated in Khuzestan Province (Iran) in an area of 11,245 ha, with annual production of 8 MT and productivity of 72.5 t/ha (Annual agricultural statistics of Iran 2023–24). Members of *Fusarium* are among the most common fungi isolated from sugarcane rhizosphere (Romão-Dumaresq *et al.* 2016, Asgari *et al.* 2019, Liu *et al.* 2023, Ren *et al.* 2024). In Iran, like other worldwide studies (see Tavakol Noorabadi *et al.* 2021), *Fusarium* strains have been mostly isolated from infected and symptomatic plant tissues of sugarcane rather than the rhizosphere soil, and commonly examined by their pathogenicity and mycotoxin profile. Most frequently, the species reported belonged to *F. fujikuroi* species complex (FFSC) (Taherkhani 1995, Alizadeh *et al.* 2010, Mohammadi *et al.* 2012, Tavakol Noorabadi *et al.* 2021), whereas members of other species complexes including *F. sambucinum* species complex (FSAMSC) and *F. oxysporum* species complex (FOSC) (Dashtipoor & Zafari 2024), and FIESC (Taherkhani *et al.* 1998) were

observed at very low frequencies. However, Asgari *et al*. (2019) isolated 1802 strains of fungi from the rhizosphere of sugarcane in Khuzestan Province, and morphologically assigned 253 isolates (14%) to the genus *Fusarium*, the second most frequent genus.

In an investigation on biodiversity of *Fusarium* strains in rhizosphere of sugarcane in Khuzestan Province (2019–25), obtained in the study of Asgari *et al*. (2019), two *Fusarium* species belonging to FIESC were identified as new for Iranian funga that are characterized here using morphological and molecular data.

Materials and Methods

- Strains, media and morphological observations

The soil dilution plating technique modified by Johnson *et al*. (1960) and Warcup (1960) was used to isolate *Fusarium* species from the rhizosphere soil (see Tavakol Noorabadi *et al.* 2020). Pure cultures were obtained by single spore isolations. Subcultures of all strains are preserved at the Fungal Culture Collection (IRAN) of the Iranian Research Institute of Plant Protection (Tehran, Iran).

Morphological characterization followed the standardized protocols described by Crous *et al.* (2021). For macromorphological studies, isolates were cultured on PDA (Merck, Germany), and incubated for 7 d at 25 °C in the dark. Colony morphology, pigmentation and growth rates were recorded. Micromorphological characteristics, including sporodochia, conidiophores, phialides, conidia (sporodochial and aerial conidia), and chlamydospores, were recorded on carnation leaf agar (CLA; Fisher *et al*. 1982, Leslie & Summerell 2006) and synthetic nutrient-poor agar (SNA; Gams *et al*. 1998), incubated for 7–14 days at 25 °C under a 12/12 h near-ultraviolet light/dark cycle. All microscopic observations and measurements were made from samples mounted in water. Photographs were taken using a Dino Capture 2.0 image software installed on an Olympus BH-2 compound microscope (Tokyo, Japan) with differential interference contrast (DIC) optics. Average and standard deviations were calculated manually and with BioloMICS 1.0.2 software (provided by Dr. V. Robert, BioAware, S.A. 2003).

- DNA extraction, sequencing and phylogenetic analysis

DNA extraction was performed using a slightly modified method of Cenis (1992), according to Ansari *et al*. (2023). DNA samples were amplified for the translation elongation factor 1-alpha (*tef1*) and RNA polymerase II second largest subunit (*RPB2*) genes. Primer pairs used for amplification and sequencing included EF1-EF2 (O'Donnell *et al*. 1998) for the *tef1*, and 5F2-7CR (Liu *et al*.1999, O'Donnell *et al*. 2010) for the *RPB2*. The PCR reaction (25 ml) contained 1 µL (10 pmol µl−1) of each primer (CinnaGen), 1 µl DNA template (30 ng µl−1) and 12 µl of Taq DNA Polymerase 2× Master Mix RED (1.5 mM MgCl2 final concentration; Ampliqon). PCR amplification of all regions was carried out using an MWG Thermal Cycler (AG Biotech). PCR conditions for the *tef1* were set as follows: initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 50 s, annealing at 59 °C for 50 s and extension at 72 °C for 7 min, and a final extension step at 72 °C for 10 min. For *RPB2* amplification, the PCR program was initial denaturation at 90 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 60 °C for 2 min and extension at 72 °C for 2 min, and a final extension step at 72 °C for 10 min. The PCR products were purified by Microsynth AG (Switzerland), and were sequenced using a capillary sequencing machine (ABI 3730XL, Applied Biosystem), at the same company.

Sequence chromatograms were checked for quality using FinchTV v. 4.0 (Geospiza Inc., Seattle, WA, USA). The sequences obtained in this study were compared with those of the NCBI's GenBank nucleotide database using the Megablast searches. Datasets were aligned using the G-INS-i strategy implemented in MAFFT v. 7 (Katoh *et al*. 2019), and manually corrected with MEGA 12 (Kumar *et al*. 2024) where necessary. Sequences of the *tef1* and

RPB2 were analysed individually (not shown) and in combination using the maximum-likelihood (ML) and Bayesian inference (BI) methods. *Fusarium concolor* (NRRL 13459) selected as outgroup. The ML analyses were run with IQ-TREE v. 2.1.2 (Minh *et al*. 2020) with ultrafast bootstrapping (UFBoot2) for estimation of branch support. The most suitable evolutionary model for each partition was estimated using ModelFinder (Kalyaanamoorthy *et al*. 2017, Minh *et al*. 2020), as implemented in IQ-TREE. The BI analyses were carried out using MrBayes v. 3.2.7 (Ronquist *et al*. 2012) with a Markov chain Monte Carlo (MCMC) algorithm, and Bayesian posterior probabilities (BI-PP) were estimated. Four MCMC chains were run from random trees for 1 000,000 generations. The sample frequency was set to 100 and the first 25% of trees were removed as burn-in. The best evolutionary model for each dataset or partition was determined by comparing the Akaike information criterion in JModeltest v. 2.1.10 (Darriba *et al*. 2012). Trees were visualized in FigTree v. 1.4.4 (Rambaut 2012). The sequences generated in this study were deposited in GenBank.

Results and Discussion

- Phylogeny

In order to elucidate the relationship of the present study isolates (IRAN 4507C, IRAN 4508C, IRAN 4509C, and IRAN 5650C) within the FIESC, the two-locus (*tef1* and *RPB2*) phylogenetic analysis using ML and BI approaches were conducted. The sequences generated in this study were aligned against sequences of ex-type cultures of all previously described species in the FIESC, mostly from Xia *et al*. (2019), Crous *et al*. (2021), Han *et al*. (2023, 2025), Zhang *et al*. (2023) and da Silva Santos *et al*. (2025). Based on the phylogeny derived from the present study (not shown), all the Iranian isolates clustered in the *Incarnatum* clade of FIESC. Then, the phylogeny (ML & BI) of individual *Incarnatum* clade within the FIESC was studied. In these analyses, the sequences of the present study isolates and all the species phylogenetically assigned to the *Incarnatum* clade were included (Table 1). An overview of the alignment length and composition, best-fit evolutionary models, and tree statistics for all the datasets included in this study is presented in table 2. Analysis of the combined dataset of *tef1* and *RPB2* provided higher support than the individual datasets and resolved the relationships among *Fusarium* species within the FIESC. Furthermore, analyses using ML and BI resulted in phylogenies with congruent topologies. Therefore, the best scoring IQ-TREE-ML tree of the combined dataset is presented in figure 1, with UFboot2-BS and BI-PP shown for supported branches.

In this study, three *Fusarium* strains isolated from sugarcane rhizosphere in the Khuzestan Province (Iran), i.e., IRAN 4508C, IRAN 4509C, and IRAN 5650C were placed in a separate clade (Fig. 1), close to the type strain of *F. nothincarnatum* (CGMCC 3.24286) (100% BS, 1 PP). The other *Fusarium* strain examined in the present study, IRAN 4507C, was placed in a clade containing the type strain of *F. fasciculatum* (CBS 131382) with high bootstrap support (100% BS, 1 PP).

Taxonomy

1. *Fusarium fasciculatum* J.W. Xia, L. Lombard, Sand.-Den., X.G. Zhang & Crous, Persoonia 43: 203 (2019)

Conidiophores and aerial conidia borne on aerial mycelium not observed. Sporodochia formed abundantly on CLA, saffron. Sporodochial conidiophores densely and irregularly branched; sporodochial phialides monophialidic, subcylindrical, smooth, thin-walled, with a short apical collarette, 9–16 × 3–4.5 µm. Sporodochial conidia falcate, slender, curved dorsiventrally, hyaline, thin- and smooth-walled, with a curved, blunt or slightly hooked apical cell and a papillate or notched to poorly developed foot-shaped basal cell, (2–)3–5(–6)-septate, 25–45(–55) × 3–5 µm overall. Chlamydospores not observed (Fig. 2).

Colonies on PDA incubated at 25 °C in the dark occupying an entire 90 mm Petri dish in 7 d; surface beige, flat, radiate, aerial mycelium absent, margin entire; reverse pale beige. Diffusible pigments absent (Fig. 2).

Table 1. *Fusarium* strains included in the phylogenetic analyses

Taxon	Strain	Source	Origin	GenBank accession number	
				TEF-1a	***RPB2***
Fusarium aberrans	CBS 131385^{T}	Stem of *Oryza australiensis*	Australia: Northern Territory, Roper River area	MN170445	MN170378
F. bryceae	BRIP 74865c^{T}	*Spinifex sericeus*	Australia: Queensland, Peregian Beach	PP209369	PP209368
F. caatingaense	MUM 1859^{T} = URM 6779	*Dactylopius opuntiae*	Brazil: Pernambuco, Ibimirim	LS398466	LS398495
F. caulendophyticum	CGMCC 3.25474^{T} = GUCC 191050.1	*Rosa roxburghii*	China: Guiyang	OR043881	OR043826
F. citri	CGMCC 3.19467^{T} = LC6896	Leaf of *Citrus reticulata*	China: Hunan Province	MK289617	MK289771
F. citrullicola	SDBR-CMU422^{T}	Fruit rot lesion of *Citrullus lanatus*	Thailand: Chiang Mai Province	OP020920	OP020928
F. coffeatum	CBS 635.76^{T} = BBA 62053 = NRRL 20841	*Cynodon lemfuensis*	New Zealand: Palmerston North	MN120755	MN120736
F. concolor	NRRL 13459 = CBS 961.87 (ex-type of *F. polyphialidicum*) = ATCC 60096 = FRC M-2405 = IMI 296456	*Hordeum vulgare*	Uruguay: Montevideo	GQ505674	GQ505852
F. fasciculatum	CBS 131382^{T}	Stems of *Oryza australiensis*	Australia: Northern Territories, Roper River area	MN170473	MN170406
″	IRAN 4507C = F30 = S14-15	Rhizosphere of *Saccharum officinarum*	Iran: Ahvaz	**PZ713419**	**PZ713422**
F. fici	CGMCC 3.27796^{T} = SAUCC 3249C-3	*Ficus fistulosa*	China: Baoting Li and Miao Autonomous County	PQ309133	PQ309123
F. guilinense	CGMCC 3.19495^{T} = LC12160	Leaf of *Musa nana*	China: Guilin	MK289594	MK289747
″	CBS 161.25 = NRRL 26857 = NRRL 26918 (ex-type of *F. bubalinum*)	Unknown	Australia	MN170448	MN170381
F. hainanense	CGMCC 3.19478^{T} = LC11638	Stem of *Oryza* sp.	China: Hainan Province	MK289581	MK289735
F. humuli	CGMCC 3.19374^{T} = CQ1039	Leaf of *Humulus scandens*	China, Jiangsu Province	MK289570	MK289724
F. incarnatum	CBS 132.73^{ET} = NRRL 25478 = ATCC 24387 = IMI 128222	*Trichosanthes dioica*	Malawi	MN170476	MN170409
F. irregulare	CGMCC 3.19489^{T} = LC7188	Bamboo	China: Guangdong Province	MK289629	MK289783
F. luffae	CGMCC 3.19497^{T} = LC12167	*Luffa aegyptiaca*	China: Fujian	MK289601	MK289754
F. mariecurieae	CBS 152079^{T} = CMW 58673 = CN072A3	Pasture samples	South Africa: Eastern Cape Province	OR671063	PP158192
F. mianyangense	CGMCC 3.23520^{T} = LC15879 = HSL859	Symptomatic tissues (spikelet rot) of *Oryza sativa*	China: Mianyang	OQ125232	OQ125510
F. monophialidicum	NRRL 54973^{T} = UTHSC 06-1473	Eye of *Rhinoceros unicornis*	USA: Ohio	MN170483	KC808362
F. mourae	URM 4872^{T}	Industrial waste	Brazil: Pernambuco	MZ701762	OM146569
F. multiceps	CBS 130386^{T} = NRRL 43639 = UTHSC 04-135	*Trichechus* sp.	USA: Florida	GQ505666	GQ505844
F. nanum	CGMCC 3.19498^{T} = LC12168	Leaf of *Musa nana*	China: Guilin	MK289602	MK289755

Table 1 (contd)

F. nothincarnatum	CGMCC 3.24286^{T} = LC18436 = HSL221	Symptomatic tissues (spikelet rot) of *Oryza sativa*	China: Daqing	OQ125147	OQ125509
″	IRAN 5650C = F170 = S13-32	Rhizosphere of *Saccharum officinarum*	Iran: Ahvaz	**PZ713417**	–
″	IRAN 4508C = F171 = S30-37	Rhizosphere of *Saccharum officinarum*	Iran: Ahvaz	**PZ713418**	**PZ713421**
″	IRAN 4509C = F228 = S18-4	Rhizosphere of *Saccharum officinarum*	Iran: Ahvaz	**PZ713416**	**PZ713420**
F. pernambucanum	URM 7559^{T} = MUM 1862	*Aleurocanthus woglumi*	Brazil: Pernambuco, Paudalho	LS398489	LS398519
F. persicinum	CBS 479.83^{T}	Unknown	Unknown	MN170495	MN170428
F. peseshetiae	BRIP 53790a^{T}	Leaf blotch of *Aspidistra* sp.	Australia: Queensland	PP712791	PP712790
F. primavesiae	URM 8399^{T}	Rhizosphere soil	Brazil: Pernambuco	OM146561	OM146571
F. ruthhalliae	BRIP 72406h^{T}	Inflorescence of *Sorghum plumosum*	Australia: Queensland, Petford	OP627085	OP627084
F. sulawesiense	InaCC F940^{T}	Infected pseudostem of *Musa acuminata* var. Pisang Cere	Indonesia: South Sulawesi	LS479443	LS479855
F. sylviaearleae	BRIP 72816a^{T}	Leaf lesion of *Sporobolus natalensis*	Australia: Queensland, Murgon	OR269444	OR269438
F. tanahbumbuense	InaCC F965^{T}	Pseudostem of *Musa* var. Pisang Hawa	Indonesia: South Kalimantan	LS479448	LS479863
F. tongrenense	CGMCC 3.28877^{T} = LC21064 = HSL3387	*Nicotiana tabacum*	China: Tongren	PQ823416	PQ823084
F. weifangense	CGMCC 3.24285^{T} = LC18333 = HSL1800	Symptomatic tissues (scab) of *Triticum aestivum*	China: Weifang	OQ125107	OQ125515
F. woodroofeae	BRIP 52905a^{T}	*Vitis vinifera*	Australia: New South Wales, Gulgong	PV459754	PV459753
F. xylosmatis	CGMCC 3.27794^{T} = SAUCC 2416-1	Leaf of *Xylosma congesta*	China: Yunan Province	PQ309131	PQ309119

Sequences generated in this study are shown in bold. ^{T} ex-type strain, ^{ET} ex-epitype strain ATCC, American Type Culture Collection, Manassas, VA, USA; BBA, Collection of the Julius Kühn Institute – Federal Research Centre for Cultivated Plants (former Biologische Bundesanstalt für Land- und Forstwirtschaft) housed at the Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany; BRIP, Queensland Plant Pathology Herbarium, Queensland Department of Agriculture and Fisheries, Dutton Park Queensland, Australia; CBS, Westerdijk Fungal Biodiversity Institute (WI), Utrecht, The Netherlands; CGMCC, China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Science, China; CMW, Culture Collection of Innovation Africa at the University of Pretoria, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa; IMI, CABI Bioscience, Egham, UK; InaCC, National Research and Innovation Agency, Republic of Indonesia (BRIN), Indonesia; IRAN, Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran; MUM, Micoteca da Universidade do Minho, Braga, Portugal; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, USDA, Peoria, IL, USA; URM, Universidade Federal de Pernambuco, Micoteca do Departmento de Micologia, Recife Pernambuco, Brazil. Other abbreviations are not registered.

Table 2. Summary of phylogenetic information generated in this study for isolates of *Fusarium incarnatum-equiseti* species complex

Nuclear region	No. of isolate	Length + gap	PI	Var.	BI unique site pattern	Model (AIC)	Model (BIC)	ML-InL (IQ)
tef1	40	646	129	244	280	GTR+G	TNe+G4	-3321.154
RPB2	39	1751	113	335	222	GTR+I+G	TNe+G4	-4765.942
Combined		2397	242	579	502	n/d	n/d	-8350.740

PI = Parsimony informative characters; Var. = Variable characters; BI = Bayesian inference; Model (AIC) = Evolutionary model selected by JModeltest using the Akaike information criterion; Model (BIC) = Evolutionary model selected by ModelFinder in IQ-TREE; ML-InL (IQ) = Best tree score determined in IQ-TREE; G = Rate of discrete Gamma categories; GTR = General time reversible model; I, Proportion of invariable sites; TNe = Unequal transition/transversion rates with unequal purine/pyrimidine rates and equal base frequencies.

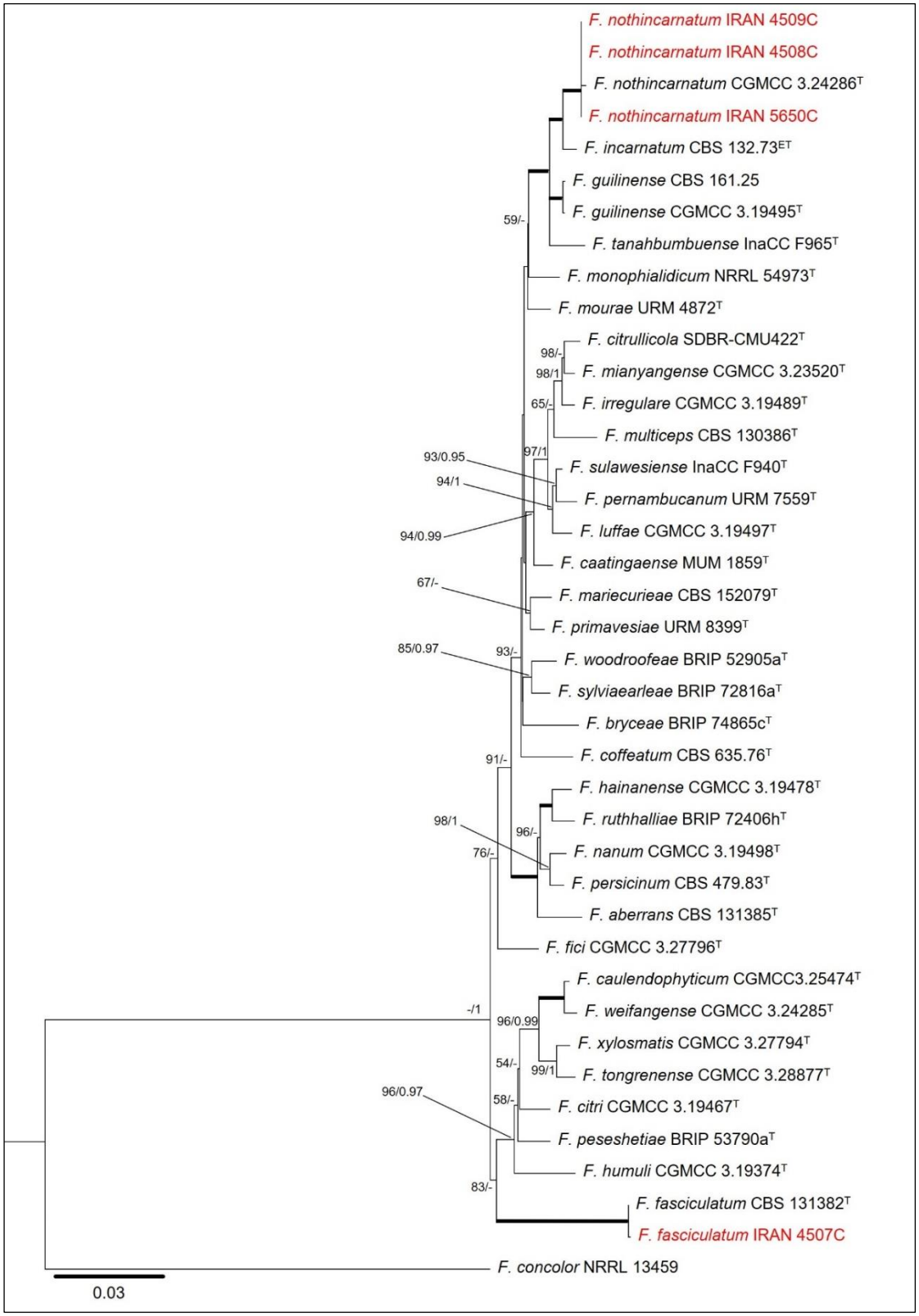

Fig. 1. Maximum-Likelihood (IQ-TREE) tree inferred from the combined *tef1* and *RPB2* gene regions of the *Fusarium incarnatum-equiseti* species complex (FIESC). *Fusarium concolor* (NRRL 13459) was used as an outgroup. Numbers at the branches indicate support values (UFboot2-BS/BI-PP) above 50%/0.95 with thickened branches indicating full support (UFboot2-BS, 100%; BI-PP, 1). Dashes replace non-significant values. The scale bar indicates expected changes per site. ^{T} = ex-type strain, ^{ET} = ex-epitype strain.

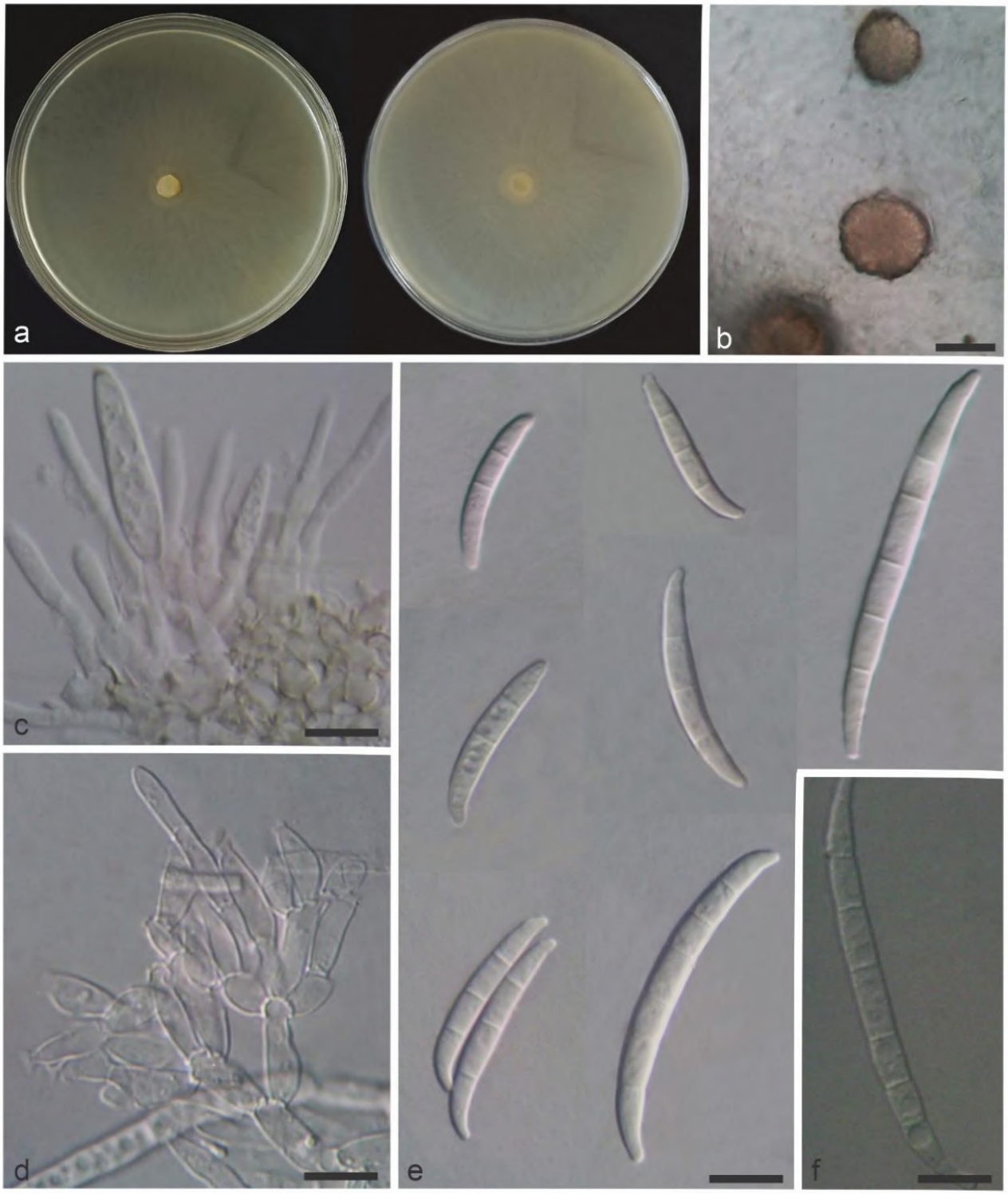

Fig. 2. *Fusarium fasciculatum* (IRAN 4507C): a. Colonies surface (left) and reverse (right) on PDA after 7 d at 25 °C, b. Sporodochial formation on the surface of CLA, c, d. Sporodochial conidiophores and phialides, e, f. Sporodochial conidia (Bars: b = 20 µm, c–f = 10 µm).

Specimen examined: IRAN: Khuzestan Province, Ahvaz, Imam Khomeini sugarcane agro-industry plantation, rhizosphere of *Saccharum officinarum*, 22.02.2014, coll. H. Moazen and K. Taherkhani, isol. B. Asgari, IRAN 4507C (=F30=S14-15).

Morphological characteristics of isolate of the present study (IRAN 4507C), were similar to description of *F. fasciculatum* (Xia *et al*. 2019), but produced beige colonies, compared to the pale orange to orange colonies of *F. fasciculatum*. *Fusarium fasciculatum* was originally described from stems of a native Australian wild rice species (*Oryza australiensis* Domin) (Xia *et al*. 2019), based on the ex-type strain (CBS 131382) and two additional strains (CBS 131383 and CBS 131384). Since then, this species has been reported from seeds of *Triticum aestivum* L. in Türkiye (Saracoglu *et al*. 2024). *Fusarium fasciculatum* is morphologically close to *F. citri* M.M. Wang, Qian Chen & L. Cai (Wang *et al*. 2019) and *F. humuli* M.M. Wang, Qian Chen & L. Cai (Wang *et al*. 2019). All these species generally produce 3–5-septate sporodochial conidia, however, *F. citri* and *F. humuli* do not produce 2- and 6-spetate conidia, and their 3–5-septate conidia are shorter than those of *F. fasciculatum* (25.5–40.5 × 3–5 µm in *F. citri*, and 21–35 × 3–5 µm in *F. humuli*).

Based on a Megablast search of NCBI's GenBank nucleotide database, the closest hit with the strain of *F. fasciculatum* (IRAN 4507C) using the *tef1* sequence are *F. fasciculatum* (strain CBS 131382, GenBank MN170473.1; identities=559/560 (99%); no gaps) and *F. fasciculatum* (strain MFG 59061, GenBank PV667619.1; identities=556/561 (99%); no gaps); and using the *RPB2* sequence are *F. fasciculatum* (strain CBS 131382, GenBank MN170406.1; identities=837/837 (100%), no gaps) and *F. fasciculatum* (strain MFG 91201, GenBank PQ459037.1; identities=836/837 (99%); no gaps). Based on the multigene phylogenies (Fig. 1), the strain IRAN 4507C was grouped with the type strain of *F. fasciculatum* (CBS 131382) in a fully supported clade (100% BS, 1 PP), distinct from other members of the FIESC. The present study represents the first record of *F. fasciculatum* from Iran.

2. *Fusarium nothincarnatum* S.L. Han, M.M. Wang & L. Cai, Stud. Mycol. 104: 133 (2023)

Sporodochia formed abundantly on carnation leaves, cream. Sporodochial conidiophores densely and irregularly branched; sporodochial phialides monophialidic, subcylindrical to subulate, smooth, thin-walled, with a short apical collarette, 8–13 × 3–4 µm. Sporodochial conidia falcate, slender, curved dorsiventrally, the dorsal side more curved than the ventral, hyaline, thin- and smooth-walled, with a curved apical cell and poorly developed foot-shaped basal cell, 3–5-septate, (23–)28–50 × (3–)4–5 overall (Fig. 3).

Aerial conidiophores borne on aerial mycelium, straight or flexuous, smooth- and thin-walled, unbranched or sympodial to irregularly branched; aerial phialides mono- and polyhialidic, subcylindrical to subulate, smooth- and thin-walled, sometimes proliferating percurrently, 14–24 × 3–4 µm. Aerial conidia abundant, of two types: (a) slender with no significant curvature (mesoconidia), ellipsoidal to fusiform, with a pointed to blunt apical cell and obtuse to flattened basal cell, (0–)1–3-septate, 14–30(–35) × 3.5–4.5 µm; (b) falcate, curved (macroconidia), tapering towards both ends, with a curved, pointed or blunt apical cell and blunt to rarely notched basal cell, 3(–4)-septate, 27–36.5 × 3–4.5 µm. Chlamydospores abundant, globose, subglobose to ovoid, smooth-walled, intercalary, solitary, in pairs or forming chains, 6–11.5 µm diam (Fig. 3).

Colonies on PDA incubated at 25 °C in the dark reaching 79–80 mm diam in 7 d; surface white, flat, floccose to felty, with abundant aerial mycelium, margin entire; reverse yellowish white. Diffusible pigments absent (Fig. 3).

Specimens examined: IRAN: Khuzestan Province, Ahvaz, Imam Khomeini sugarcane agro-industry plantation, rhizosphere of *S. officinarum*, 22.02.2014, IRAN 5650C

(=F170=S13-32); Mirza Kuchak Khan sugarcane agro-industry plantation, rhizosphere of *S. officinarum*, 22.05.2013, IRAN 4508C (=F171=S30-37); Debel Khazaei sugarcane agro-industry plantation, rhizosphere of *Saccharum officinarum*, 04.03.2014, IRAN 4509C (=F228=S18-4). All collected by H. Moazen and K. Taherkhani, and isolated by B. Asgari.

Morphological characteristics of the present study isolates (IRAN 4508C, IRAN 4509C and IRAN 5650C) are largely consistent with the original description of *F. nothincarnatum* (Han *et al*. 2023). However, they are slightly different by producing wider, mono- and polyphialides in the aerial mycelium (vs. only monophialides, 1.4–3.5 µm wide in *F. nothincarnatum*), and forming mesoconidia (absent in *F. nothincarnatum*). *Fusarium nothincarnatum* was originally described from symptomatic tissues (spikelet rot) of rice (*Oryza sativa* L.) in China (Han *et al*. 2023), based on the ex-type strain (CGMCC3.24286) and two additional strains (HSL280 and HSL281). *Fusarium nothincarnatum* is morphologically and phylogenetically close to *F. incarnatum*. The controversy on the taxonomy of *F. incarnatum* was recently summarised and resolved by Xia *et al.* (2019) who designated an epitype, CBS 132.73, for this species. *Fusarium nothincarnatum* can be differentiated from *F. incarnatum* by having 0–5-septate sporodochial conidia (vs. 1–6-septate in *F. incarnatum*) with different morphology (the dorsal side more curved than the ventral, with more curved apical cell), and falcate aerial macroconidia with 3–5 septa (vs. 1–7 septa in *F. incarnatum*) (see Xia *et al*. 2019). However, formation of mono- and polyphialides in the aerial mycelium and mesoconidia by Iranian strains of *F. nothincarnatum*, characters shared with *F. incarnatum*, suggests the need for collecting more strains, with a broad geographical coverage to resolve the morphology of *F. nothincarnatum*.

Based on a Megablast search of NCBI's GenBank nucleotide database, the closest hit with the representative strain of *F. nothincarnatum* (IRAN 4509C) using the *tef1* sequence are *Fusarium* sp. (strain NRRL 25081, GenBank JF740712.1; identities=627/627 (100%); no gaps) and *F. incarnatum* (strain YLTZRR607-1-1P, GenBank PX849488.1; identities=627/627 (100%); no gaps); and using the *RPB2* sequence are *F. incarnatum* (strain YLTZRR607-2-2wuP, GenBank PX849489.1; identities=877/877 (100%), no gaps) and *F. incarnatum* (strain XJ-187, GenBank PQ606100.1; identities=877/877 (100%); no gaps). Based on the multigene phylogenies (Fig. 1), all the strains examined in the present study clustered together in the fully supported clade (100% BS, 1 PP) containing the type strain of *F. nothincarnatum* (CGMCC 3.24286), distinct from other members of FIESC, particularly *F. incarnatum*. This is the first report of *F. nothincarnatum* from Iran.

The recovery of *F. fasciculatum* and *F. nothincarnatum* from sugarcane rhizosphere in the present study does not permit conclusions regarding their role in relation to sugarcane; they may function as saprobes, endophytes or phytopathogens, consistent with the broad ecological range documented for FIESC members (Summerell *et al*. 2011, Crous *et al*. 2021). Future studies incorporating pathogenicity assays on sugarcane and other Poaceae, together with broader geographic and host-range surveys, are needed to clarify the ecological roles and potential phytopathological significance of these species in sugarcane agroecosystems.

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Fig. 3. *Fusarium nothincarnatum* (IRAN 4509C): a. Colonies surface (left) and reverse (right) on PDA after 7 d at 25 °C, b–d. Aerial conidiophores and phialides, e. Aerial mesoconidia, f. Aerial macroconidia, g. Sporodochial conidiophore and phialides, h. Sporodochial conidia, i, j. Chlamydospores (Bars: b = 20 µm, c–j = 10 µm).

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