Rostaniha 26(1): 60–76 (2025) – Research Article
رستنیها ۲۶(۱): ۷۶–۶۰ (۱۴۰۴) – مقاله پژوهشی
DOI: 10.22092/bot.j.iran.2025.370380.1429

Molecular and morphological evidence for two *Clonostachys* and one *Sesquicillium* species new to Iran

Received: 16.08.2025 ========== Revised: 10.09.2025 ========== Accepted: 13.09.2025

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Abstract

To identify some fungal species associated with root-knot nematode (*Meloidogyne* species) or inhabiting the rhizosphere of Kiwifruit trees (*Actinidia chinensis*), infected roots and rhizospheric soil samples were collected from Kiwifruit orchards in Guilan Province (North of Iran). For fungal isolation from soil, one g of each soil sample was suspended in 100 mL of sterile distilled water. After making serial dilutions, 100 µL of each dilution was spread onto PDA-Rose Bengal agar supplemented with streptomycin. For isolation from nematodes, egg masses were surface-sterilized in 0.5% sodium hypochlorite, transferred to the same culture medium, and incubated at 25 °C in the dark for 10 days. The isolates were characterized using the morphological assessment and DNA sequence analyses of the internal transcribed spacer (ITS), translation elongation factor 1-α (*tef1*), and beta-tubulin (*tub2*) regions. Some isolates produced monomorphic or dimorphic conidiophores typical of members of the *Clonostachys* and *Sesquicillium* genera. Morphological and molecular data confirmed that the isolates represented three species, including *C. chloroleuca* (from rhizospheric soil), *C. rogersoniana* (from a *Meloidogyne* sp. egg sac), and *Sesquicillium aff. essexcoheniae* (from rhizospheric soil). To the best of the authors' knowledge, this is the first report of the mentioned species from Iran.

Keywords: Biodiversity, Bionectriaceae, Kiwifruit, *Meloidogyne*, phylogeny

شواهد مولکولی و ریخت‌شناسی برای دو گونه *Clonostachys* و یک گونه *Sesquicillium* جدید در ایران

دریافت: ۱۴۰۴/۰۵/۲۵ ========== بازنگری: ۱۴۰۴/۰۶/۱۹ ========== پذیرش: ۱۴۰۴/۰۶/۲۲

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خلاصه

به منظور شناسایی برخی گونه‌های قارچی مرتبط با گونه‌های *Meloidogyne* یا موجود در ریزوسفر درختان کیوی (*Actinidia chinensis*)، نمونه‌هایی از گونه‌های *Meloidogyne* و خاک ریزوسفر از باغ‌های کیوی در استان گیلان جمع‌آوری شد. برای جداسازی قارچ از خاک، یک گرم از هر نمونه خاک در ۱۰۰ میلی‌لیتر آب مقطر سترون معلق گردید. پس از تهیه رقت‌های متوالی، ۱۰۰ میکرولیتر از هر رقت روی محیط کشت PDA-Rose Bengal حاوی استرپتومایسین پخش شد. برای جداسازی از نماتدها، توده‌های تخم با محلول هیپوکلریت سدیم نیم درصد ضدعفونی سطحی شده، به محیط کشت مشابه منتقل و به مدت ۱۰ روز در دمای ۲۵ درجه سلسیوس در تاریکی نگهداری شدند. جدایه‌ها با استفاده از ارزیابی ریخت‌شناختی و تجزیه و تحلیل توالی DNA نواحی فاصله‌دهنده رونویسی‌شونده داخلی (ITS)، فاکتور طویل شدن ترجمه یک آلفا (*tef1*) و بتا-توبولین (*tub2*) شناسایی شدند. برخی از جدایه‌ها کنیدیوفورهای مونومورفیک یا دیمورفیک معمول اعضای جنس‌های *Clonostachys* و *Sesquicillium* تولید کردند. داده‌های ریخت‌شناختی و مولکولی تأیید کردند که جدایه‌ها متعلق به سه گونه *C. chloroleuca* (از ریزوسفر)، *C. rogersoniana* (از کیسه تخم .sp *Meloidogyne*) و *Sesquicillium aff. essexcoheniae* (از ریزوسفر) هستند. براساس اطلاعات نگارندگان، این نخستین گزارش از گونه‌های مذکور از ایران است.

واژه‌های کلیدی: تبارشناسی، تنوع زیستی، کیوی، نماتد مولد غده، Bionectriaceae

Introduction

Members of the Bionectriaceae are herbicolous, corticolous, lichenicolous, fungicolous or coprophilous. They occur mainly in terrestrial or freshwater habitats and are less common in marine environments (Zhao *et al.* 2023, 2025). The family, which currently comprises 58 genera (Hyde *et al.* 2024), is morphologically characterized by perithecial ascomata with light-colored walls (white, pale tan, orange, or brown) that do not change color in 3% KOH or lactic acid (Rossman *et al.* 2001). The genus *Clonostachys* Corda (Hypocreales, Ascomycota) was established in 1839, with *C. araucaria* as the type species. The name *Bionectria* Speg. has been used for the sexual morph, which was segregated into the six subgenera *Astromata*, *Bionectria*, *Epiphloea*, *Myronectria*, *Uniparietina*, and *Zebrinella* (Zhao *et al.* 2023); however, Rossman *et al.* (2013) recommended the use of *Clonostachys* over the *Bionectria* due to priority of publication, thereby resolving nomenclatural conflicts. Morphologically, the sexual morph of *Clonostachys* is characterized by white, yellow to orange or brown color, usually crowded and roughened ascomata, not changing color in 3% KOH or lactic acid. The asexual morph is characterized by penicillate, sporodochial, or dimorphic conidiophores (including primary and secondary conidiophores) and phialidic conidiogenous cells, which produce hyaline conidia (Schroers 2001, Torcato *et al*. 2020, Zhao *et al*. 2023). Species of *Clonostachys* are common as soil-borne fungi, endophytes, epiphytes, saprotrophs, parasites on other fungi, nematodes, or insects, and they are known for their potential to produce secondary metabolites (Schroers 2001, Zheng *et al*. 2006, Abreu *et al.* 2014, Mahmoudi *et al.* 2018, Han *et al*. 2020, Kapeua-Ndacnou 2023, Yao *et al.* 2024). In recent molecular studies, the genus *Sesquicillium*, resurrected to accommodate the former subgenera *Epiphloea* and *Uniparietina* (Zhao *et al.* 2023). This genus is morphologically similar to *Clonostachys* (Zhao *et al*. 2023). Molecular data indicate that they share a common ancestor, and their status as phylogenetic sister groups is strongly supported (Zhao *et al*. 2023). Similar to *Clonostachys*, *Sesquicillium* has simple, branched conidiophores and small, hyaline conidia; however, its conidiophores are predominantly monomorphic (Zhao *et al*. 2023). During recent years, several species previously placed in *Clonostachys* were transferred to *Sesquicillium* following the taxonomic revision of the genus, and some new species have been described, such as *S. cavernum* (Preedanon *et al.* 2023), *S. intermediophialidicum*, *S. neerlandicum*, *S. symmetricum* (Zhao *et al.* 2023), *S. pouteriae*, and *S. thailandense* (Zhao *et al.* 2025). In Iran, most records of *Clonostachys* correspond to *C. rosea*, which has been documented as a nematode-cyst parasite (Abbasi & Afzalinia 2022), an endophyte (Ebrahimi & Fotouhifar 2016, Abdollahi Aghdam & Fotouhifar 2017), and, more recently, as a promising entomopathogen (Mahmoudi *et al*. 2018).

This study aims to identify fungal species associated with the egg sac of *Meloidogyne* species (on roots of Kiwifruit) or inhabiting the rhizosphere of Kiwifruit trees (*Actinidia chinensis* Planch.) in Guilan Province orchards (North of Iran).

Materials and Methods

- Sampling and isolation

Thirty samples were collected from the rhizospheric soil and roots of three Kiwifruit tree orchards infected with root-knot nematodes (*Meloidogyne* spp.) from Astaneh-Ashrafiyeh, Rudsar and Kelachai (Guilan Province, North of Iran) between Aug.–Sept. 2022. Sampling was done selectively from nematode-induced symptomatic trees. Soil and root samples were taken at a depth of 5–30 cm, placed in sterile plastic bags, and stored at 4 °C. To reduce moisture, soil samples were air-dried for 24–48 hrs. For fungal isolation, one g of soil was suspended in 100 mL of sterile distilled water and agitated at 100 rpm for 15–20 min. Serial dilutions (10^{-1} to 10^{-5}) were prepared, and 100 µL of each dilution was spread onto PDA-Rose Bengal agar (Ibresco, Iran) (Onkar & James 1985, Carling & Sumner 1992). Egg masses (both healthy and melanized) were surface-sterilized in 0.5%

sodium hypochlorite (NaClO) for 2 min, then axenically transferred to the same culture medium. Plates were incubated at 25 °C in the dark for 10 days (Singh & Mathur 2010). Pure cultures were obtained by isolating the hyphal tips or the single spore method (Leslie & Summerell 2006). Representatives of the isolates were deposited in the fungal culture collection of the Iranian Research Institute of Plant Protection ("IRAN"), Tehran, Iran.

- Morphology

Pure cultures were grown in triplicate on potato dextrose agar (PDA), oatmeal agar (OA), and synthetic nutrient-poor agar (SNA), following the protocol of Zhao *et al*. (2023). Fungal structures were mounted in sterile distilled water and examined under a Leica DM1000 light microscope coupled with a Canon 600D digital camera. All microscopic measurements (at least 20-50 for each fungal structure) were conducted in sterile water. Key morphological features, including conidiophores, phialides, and conidia, were investigated. Morphological identifications were based on comparative analyses with available descriptions in some published articles (Schroers 2001, Moreira *et al*. 2016, Zeng & Zhuang 2022, Preedanon *et al.* 2023, Zhao *et al.* 2023, 2025, He *et al*. 2025).

- DNA extraction, PCR and sequencing

Whole-genomic DNA was extracted from fresh cultures using the Thermolysis method (Zhang *et al.* 2010). Three genomic loci were amplified, i.e. the internal transcribed spacer region (ITS), translation elongation factor 1-α (*tef1*) and beta-tubulin (*tub2*). The ITS regions were amplified and sequenced using the ITS5 and ITS4 primers (White *et al.* 1990), *tef1* with EF1-688F and EF2-R primers (Alves *et al.* 2008, O'Donnell *et al*. 1998), and *tub2* with T1D and T22D primers (Carbone & Kohn 1999). PCR amplification conditions followed Ghahremani *et al*. (2025). All amplicons were purified and sequenced by Codon Genetic Group (Tehran, Iran).

- Phylogenetic analyses

The raw sequence file was opened in MEGA 7 (Kumar *et al.* 2016). Low-quality sequences were trimmed from the ends, ambiguities were resolved and a clean, reliable FASTA sequence was produced. Initial identification of closely related taxa was performed through separate BLASTn searches for each locus (ITS, *tef1* and *tub2*). Reference sequences were obtained from the National Center for Biotechnology Information (NCBI), with particular attention to type sequences as reported in recent taxonomic studies (Moreira *et al*. 2016, Zhao *et al.* 2023, 2025, He *et al.* 2025). For *Clonostachys*, the phylogenetic dataset comprised 38 *Clonostachys* strains and two outgroup taxa (*Sesquicillium candelabrum* CBS 119045 and CBS 504.67) (Table 1). Sequence alignments were performed using the online server implementation of MAFFT Ver. 7.490 (Katoh *et al*. 2019) and subsequently manually refined in MEGA Ver. 7 (Kumar *et al.* 2016). Individual gene alignments were concatenated using Mesquite Ver. 3.10 (Maddison & Maddison 2015), yielding a final combined alignment of 1,442 characters (ITS: 505 bp, *tef1*: 496 bp, *tub2*: 441 bp).

For *Sesquicillium* species, the phylogenetic analysis was based solely on ITS sequences following the same alignment methodology described for *Clonostachys.* The phylogenetic dataset comprised 45 sequences of *Clonostachys/Sesquicillium* and two outgroup taxa (*Mycocitrus odorus* and *M. coxeniae*).

Maximum likelihood (ML) analyses were performed with RAxML as implemented in raxmlGUI Ver. 2.0 (Edler *et al.* 2021) using the ML + rapid bootstrap setting with 1000 bootstrap replicates and the GTRGAMMA substitution model, followed by a search for the tree with the highest likelihood.

Table 1. *Clonostachys* and *Sesquicillium* sequences used in the phylogenetic analyses. ^{T}: ex-type strain. N/A: Not available or used in this study

Taxon	Strain	Substrate	GenBank accession number			Reference
			ITS	*tef1*	*tub2*	
Clonostachys aquatica	KUNCC 22–12454^{T}	Submerged decaying wood	NR_198353	N/A	N/A	Bao *et al.* (2023)
C. chloroleuca	CML 1941^{T}	Native soil from Cerrado	OQ910549	KX184988	KF871172	Zhao *et al*. (2023; ITS), Moreira *et al*. (2016; *tef1*, *tub2*)
	CML 1922	Native soil from Cerrado	OQ910551	KX184986	KF871170	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*, *tub2*)
	CML 1213	Native soil from Cerrado	OQ910550	KX184978	KF871173	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *TEF1*, *tub2*)
	CML 1927	Soil of soybean field	OQ910552	KX184987	KF871171	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*, *tub2*)
	CML 1919	Native soil from Cerrado	N/A	KX184983	KF871167	Moreira *et al.* (2016; *tef1*, *tub2*)
	CML 1912	Native soil from Cerrado	OQ910554	KX184979	KF871168	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*, *tub2*)
	CML 2537	Bryophyte	OQ910553	KX184989	KX185038	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*, *tub2*)
	IRAN 5412C	**Rhizosphere of *Actinidia chinensis***	**PX285691**	**PX289645**	**PX289647**	**This study**
C. cylindrica	CBS 101113^{T}	Unknown	OQ910569	N/A	N/A	Zhao *et al.* (2023)
C. divergens	CBS 967.73B^{T}	Soil of wheat field	OQ910575	N/A	OQ982612	Zhao *et al.* (2023)
	CBS 102426	Bark	OQ910570	N/A	OQ982608	Zhao *et al.* (2023)
	CBS 532.69	Forest soil under *Thuja occidentalis*	OQ910574	N/A	OQ982611	Zhao *et al.* (2023)
	CBS 381.77	Soil	OQ910573	N/A	OQ982610	Zhao *et al.* (2023)

Table 1 (contd)

C. farinosa	CML 2510	Bark	OQ910614	KX184967	AF358153	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*); Schroers (2001; *tub2*)
	CML 2511	Wood	OQ910615	KX184972	AF358154	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*); Schroers (2001; *tub2*)
	CML 2309	*Fragaria ananassa*	N/A	KX184966	KF871149	Moreira *et al.* (2016)
C. kunmingensis	YFCC 898^{T}	Soil	MW199069	N/A	MW201676	Wang *et al.* (2023)
	CBS 101920	Wood	OQ910635	N/A	OQ982667	Zhao *et al.* (2023)
	YFCC 892	Soil	MW199070	N/A	MW201677	Wang *et al.* (2023)
C. pseudochroleuca	CML 2513^{T}	Base of a decaying palm frond	OQ910665	KX185003	KF871188	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*, *tub2*)
	CML 2562	Bark	OQ910667	KX185016	AF358171	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*); Schroers (2001; *tub2*)
	CML 2406	*Saccharum officinarum*	N/A	KX185007	KF871158	Moreira *et al.* (2016)
C. rhizophaga	CBS 202.37^{T}	Root of *Ulmus americana*	OQ910694	N/A	OQ982723	Zhao *et al.* (2023)
	CML 2514	Culture contaminant	OQ910696	KX184993	AF358158	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*); Schroers (2001; *tub2*)
C. rogersoniana	CML 2557^{T}	Soil under *Araucaria* sp.	OQ910711	KX185022	KX185047	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*, *tub2*)
	CML 2558	Soil from the Amazon forest	OQ910709	KX185023	AF358189	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*); Schroers (2001; *tub2*)
	CML 1944	Soil from secondary forest	N/A	KX185021	KF871181	Moreira *et al.* (2016)
	CML 1216	Native soil from Cerrado	N/A	KX185017	KF871178	Moreira *et al.* (2016)
	CML 2547	Litter	N/A	KX185025	KX185049	Moreira *et al.* (2016)

Table 1 (contd)

	IRAN 5411C	***Meloidogyne* sp. egg sac under *Actinidia chinensis***	**PX285692**	**PX289646**	**PX289648**	**This study**
C. rosea f. *catenulata*	CML 2516^T	Soil	OQ910800	KX184995	AF358160	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*); Schroers (2001; *tub2*)
	CML 2517	Soil	OQ910803	KX184996	AF358166	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*); Schroers (2001; *tub2*)
C. rosea f. *rosea*	CML 2518^T	Soil and sclerotia of *Sclerotinia minor*	OQ910774	KX184999	AF358161	Zhao *et al.* (2023; ITS), Moreira *et al.* (2016; *tef1*); Schroers (2001; *tub2*)
	CML 2549	Litter	N/A	KX185001	KX185040	Moreira *et al.* (2016)
C. samuelsii	CBS 699.97^T	Bark of tree	OQ910812	N/A	OQ982832	Zhao *et al.* (2023)
	CBS 188.94	Bark	OQ910807	N/A	OQ982827	Zhao *et al.* (2023)
	CBS 196.93	Bark of legume	OQ910808	N/A	OQ982828	Zhao *et al.* (2023)
	CBS 198.93	Wood of dead tree	OQ910809	N/A	OQ982829	Zhao *et al.* (2023)
	CBS 701.97	Bark	OQ910814	N/A	OQ982834	Zhao *et al.* (2023)
Mycocitrus coxeniae	BRIP 49599a^T	undetermined insect	OQ629341	N/A	N/A	Tan & Shivas (2023)
M. odorus	CBS 100104^T	Onychomycosis (human)	OQ429717	N/A	N/A	Hou *et al.* (2023)
Sesquicillium candelabrum	CBS 119045^T	Strongly decayed *Fomitopsis pinicola*	OQ911267	N/A	OQ968107	Zhao *et al.* (2023)
	CBS 504.67	Soil	MH859044	KX185029	KF871189	Vu *et al.* (2019; ITS), Moreira *et al.* (2016; *tef1*, *tub2*)
S. cavernum	CBS 180.88^T	Cave environment	NR_190954	N/A	N/A	Preedanon *et al.* (2023)
S. essexcoheniae	BRIP 75170a^T	Soil	OQ629342	N/A	N/A	Tan & Shivas (2023)
S. essexcoheniae	BRIP 75166a	Soil	PV793416	N/A	N/A	Tan & Shivas (2023)
S. essexcoheniae	CBS 918.97	Sphaeriales	OQ911276	N/A	N/A	Zhao *et al.* (2023)
S. aff. essexcoheniae	**IRAN 5413C**	**Rhizosphere of *Actinidia chinensis***	**PX285693**	**N/A**	**N/A**	**This study**

Table 1 (contd)

S. essexcoheniae	CSC22A2125	Unknown	PQ821602	N/A	N/A	NCBI
S. intermediophialidicum	CBS 685.96^{T}	Unknown	OQ911277	N/A	N/A	Zhao *et al.* (2023)
S. lasiacidis	CBS 179.88^{T}	*Lasiacis ligulata* and dead culms	OQ911279	N/A	N/A	Zhao *et al.* (2023)
S. lasiacidis	CBS 147133	Root-associated soil using *Hordeum vulgare* as bait	OQ911278	N/A	N/A	Zhao *et al.* (2023)
S. lasiacidis	NL19-085006	Soil	OQ911284	N/A	N/A	Zhao *et al.* (2023)
S. neerlandicum	CBS 148203^{T}	Soil	OQ911289	N/A	N/A	Zhao *et al.* (2023)
S. neerlandicum	CBS 148201	Soil	OQ911287	N/A	N/A	Zhao *et al.* (2023)
S. phyllophilum	CBS 921.97^{T}	*Viscum album*, leaves, and fallen plant	OQ911297	N/A	N/A	Zhao *et al.* (2023)
S. phyllophilum	CBS 662.83	Decaying sclerophyll leaf	OQ911296	N/A	N/A	Zhao *et al.* (2023)
S. pouteriae	CBS 136497^{T}	Leaf of *Puteria pallida*	PV272803	N/A	N/A	Zhao *et al.* (2025)
S. rossmaniae	CBS 211.93^{T}	Twig of a recently dead tree	OQ911298	N/A	N/A	Zhao *et al.* (2023)
S. rossmaniae	CBS 210.93	Bark of a living liana	AF358227	N/A	N/A	Schroers (2001)
S. saulense	BRFM 2782^{T}	*Bauhinia* sp. and dead bark	MK635054	N/A	N/A	Lechat *et al.* (2019)
S. sesquicillii	CBS 180.88^{T}	Lichen on twig	OQ911300	N/A	N/A	Zhao *et al.* (2023)
S. shanghaiense	CGMCC 3.20773^{T}	Soil	NR_198190	N/A	N/A	Zhang *et al.* (2023)
S. spinulosisporum	CLLG12001^{T}	*Astrocaryum vulgare* and aerial dead palm leaf	MH634702	N/A	N/A	Lechat & Fournier (2018)
S. symmetricum	CBS 124.79^{T}	Agricultural soil	OQ911301	N/A	N/A	Zhao *et al.* (2023)
S. thailandense	CBS 139546^{T}	Leaf litter of bamboo	PV272802	N/A	N/A	Zhao *et al.* (2025)

Results and Discussion

- Molecular phylogeny

For *Clonostachys*, 1442 characters were included in the phylogenetic analyses (ITS-*tef1*-*tub2*), 327 were parsimony informative (70 in ITS, 139 in *tef1*, and 118 in *tub2*). The phylogram of the best ML tree (lnL = − 5,646. 9632) obtained by RAxML is shown in Fig. 1. Estimated base frequencies were as follows: A = 0.181247, C = 0.284574, G = 0.261926, T = 0.272253, with substitution rates AC = 1.260004, AG = 3.312260, AT = 2.216152, CG = 0.772414, CT = 5.373539, and GT = 1.000000.

Clonostachys isolates from the present study fall into two distantly related clades in the phylogenetic tree (Fig. 1). The first clade includes the Iranian sequence of *Clonostachys chloroleuca*, which is almost identical to the sequence of isolates within this clade (such as CML 1919/CBS 141592). These strains formed a well-supported monophyletic group (ML bootstrap = 96%) with the type sequence of *C. chloroleuca*.

The second clade comprised the Iranian isolate of *Clonostachys rogersoniana*, exhibiting 100% sequence identity to some strains (including CBS 582.89). This cluster formed a distinct, strongly supported lineage (ML bootstrap = 98%) with *C. rogersoniana* (CBS 920.97) as type sequence. The phylogenetic reconstruction confirmed *C. rogersoniana* as a sister taxon to *C. divergens* and *C. samuelsii* with maximum support (ML bootstrap = 100%), consistent with previous findings by Zhao *et al*. (2023).

For *Sesquicillium* species, only the ITS sequence was obtained, while *tef1* and *tub2* sequences could not be amplified. Therefore, the present phylogenetic tree is based solely on ITS sequence analysis (Fig. 2). Estimated base frequencies were as follows: A = 0.226131, C = 0.276449, G = 0.254989, T = 0.242431, with substitution rates AC = 1.288814, AG = 1.948917, AT = 1.700820, CG = 0.466945, CT = 4.393817, and GT = 1.000000.

According to figure 2, the isolate from the present study was clustered within a large clade containing several *Sesquicillium* species, which was further divided into two subclades. It fell within a subclade that includes *S. essexcoheniae* (Y.P. Tan, Bishop-Hurley & R.G. Shivas) L. Zhao & Crous, and *Clonostachys aquatica* D.F. Bao, K.D. Hyde & Z.L. Luo. In the present study, the ITS sequence differed from *S. essexcoheniae* (type material, NR185822) by three gaps and was 100% identical to that of *C. aquatica* (type material, NR198353). *S. essexcoheniae* was primarily introduced by Tan *et al*. (2023) as *Clonostachys essexcoheniae* Y.P. Tan, Bishop-Hurley & R.G. Shivas, and later transferred to *Sesquicillium* by Zhao *et al.* (2023). *Clonostachys aquatica* was described by Bao *et al.* (2023) based on rDNA sequences. Protein-coding gene sequences for *C. aquatica* were neither provided in the original description nor deposited in GenBank. In the phylogenetic tree of Bao *et al*. (2023; Fig. 5), *Sesquicillium* species were not included. According to the ITS-based analysis of the present study, *C. aquatica* undoubtedly belongs to *Sesquicillium* and its ITS sequence is almost identical to that of *S. essexcoheniae* (three gaps difference). He *et al*. (2025) recently proposed a new combination for this species as *Sesquicillium aquaticum* (D.F. Bao, K.D. Hyde & Z.L. Luo) S.C. He, K.D. Hyde & Jayaward. However, according to Index Fungorum (https://www.indexfungorum.org/) and MycoBank (https://www.mycobank.org/), this name is invalid (Art. 41.5). Zhao *et al*. (2025) included sequence data from type material of *C. aquatica* in their phylogenetic analysis and, surprisingly, changed its name to *S. essexcoheniae* in their phylogenetic tree. However, they provided no formal synonymization or bibliographic justification for this reclassification. As a result, due to the absence of *tef1* or *tub*2 sequences, it remains unclear whether *C. aquatica* is a distinct species or a synonym of *S. essexcoheniae*. Although the isolate here is morphologically and molecularly (ITS sequence) similar to *S. essexcoheniae*, precise identification requires sequencing of protein-coding genes from both the present specimen and the type material of *C. aquatica*. Therefore, the isolate is conservatively designated as *Sesquicillium aff. essexcoheniae*.

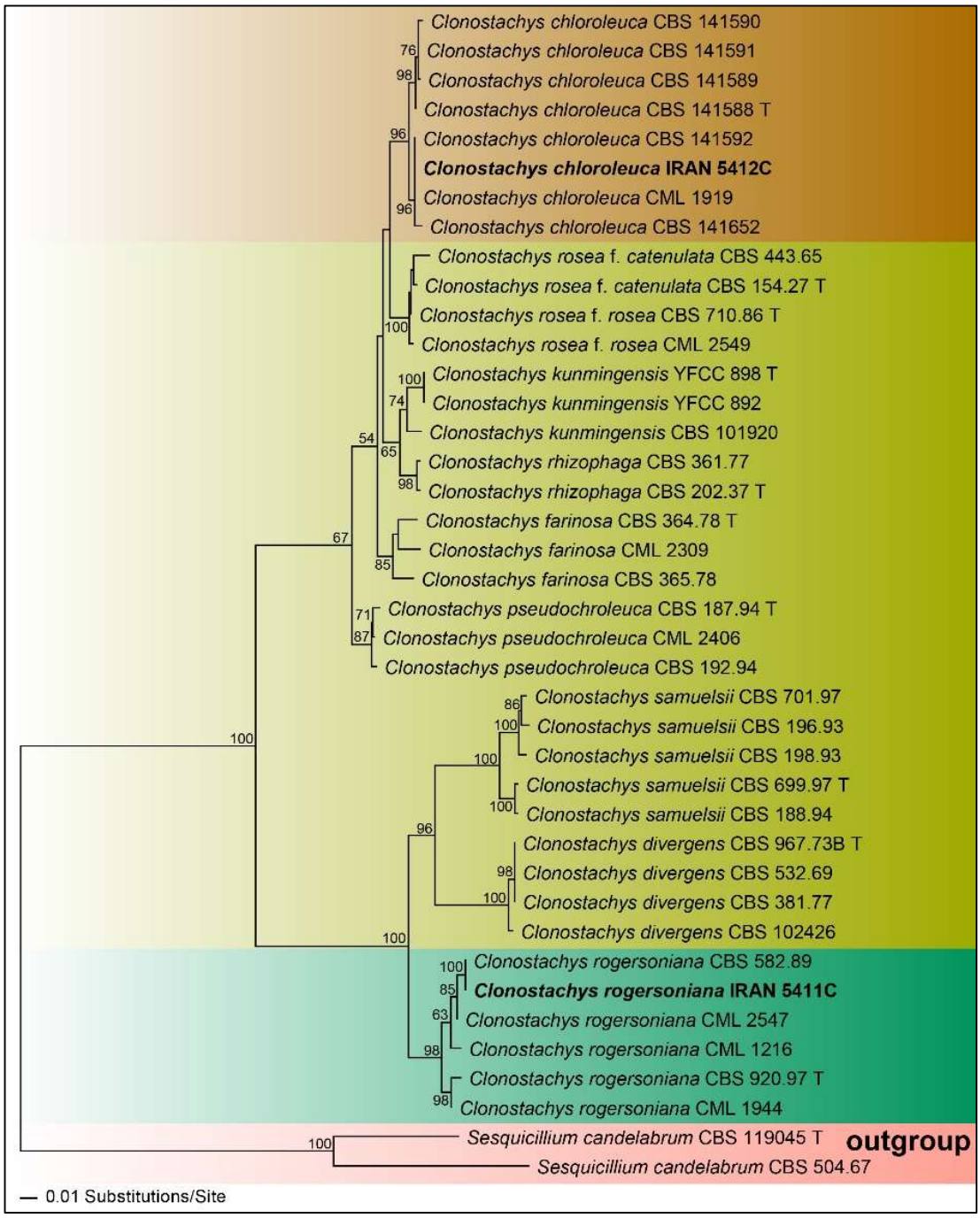

Fig. 1. Phylogenetic tree generated by Maximum likelihood method (lnL = − 5,646. 9632) revealed by RAxML from an analysis of the combined sequences of ITS–*tef1*–*tub2* of selected 38 isolates of *Clonostachys* species. Isolates in bold face were sequenced in the current study. ML bootstrap supports above 50% are given at the positions, above or below the branches. Two sequences of *Sesquicillium candelabrum* were used as outgroup taxa.

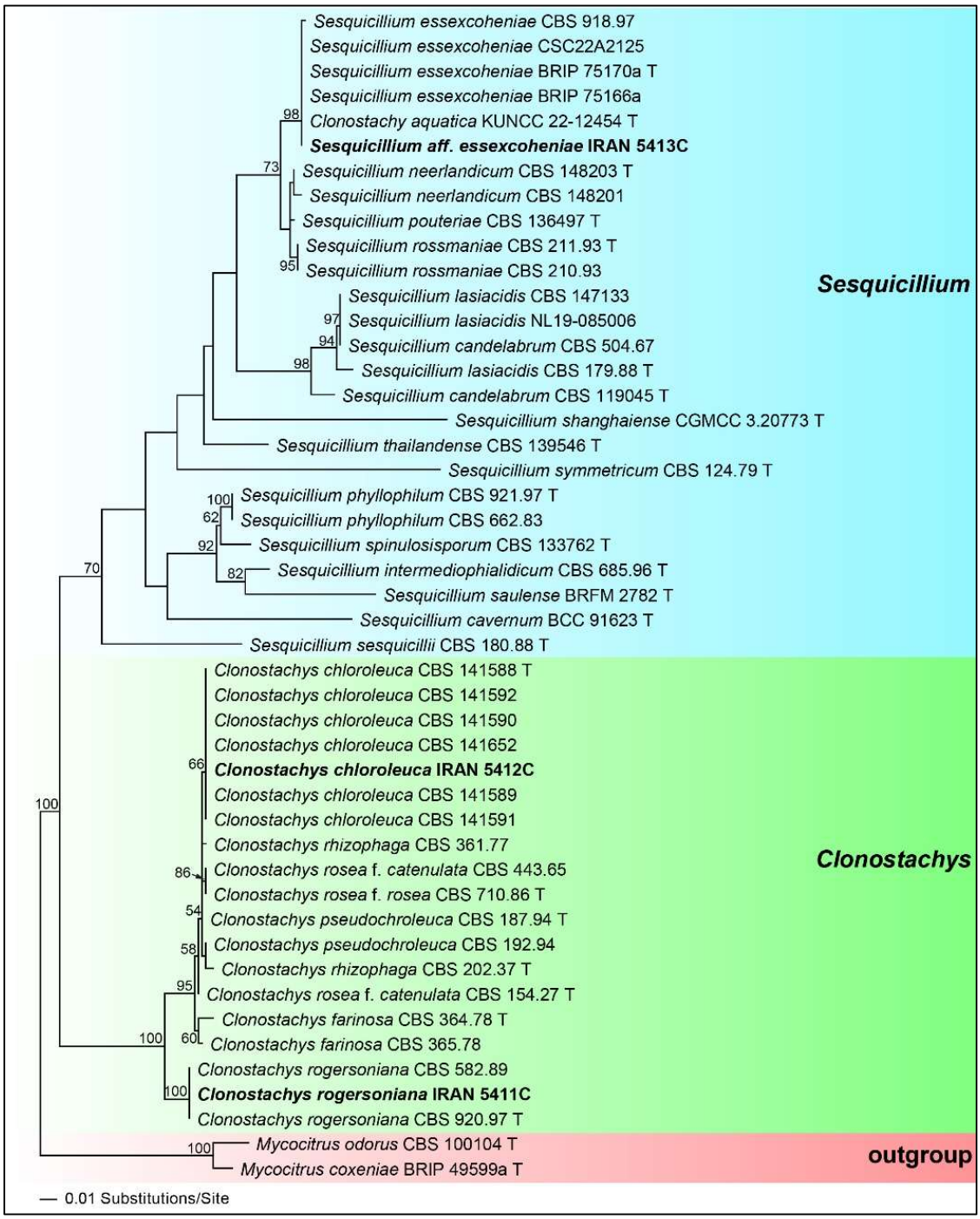

Fig. 2. Phylogenetic tree generated using the best ML method (lnL = − 2,845.8589) revealed by RAxML from an analysis of the ITS sequences of the 45 isolates of the selected *Clonostachys* and *Sesquicillium* species. Isolates in bold face were sequenced in the current study. ML bootstrap supports above 50% are given at the positions, above or below the branches. Two species of *Mycocitrus* were used as outgroup taxa.

- Taxonomy

Clonostachys chloroleuca G. Moreira, L. Abreu, Pfenning & Schroers Mycological Progress 15: 1035 (2016) (Fig. 3)

Description: Conidiophores dimorphic, formed throughout the colony. Primary conidiophores verticillium-like, with monoverticillate, stipes 20–70 µm long, 2.4–3.5 µm wide at base. Phialides generally in whorls of 2–5, 12.7–36.5 µm long, 1.85–2.8 µm wide at base, 0.95–2.2 µm wide near aperture. Secondary conidiophores penicillate, solitary to gregarious, bi- to

quaterverticillate, stipes 25–70 µm long, 3.1–4.6 µm wide at base. Phialides in adpressed whorls of up to 5, 9.9–18.5 µm long, 1.3–3.5 µm wide at base, 0.95–2.3 µm wide near aperture. Conidia aseptate, hyaline, ellipsoidal, slightly curved with one almost straight side, hilum typically laterally displaced, 4.3–6.7 × 2.5–3.9 µm.

Culture characteristics: Colonies on OA reaching 60–65 mm diam. after 7 days at 25 °C in darkness, flat, with entire margin, aerial mycelium moderate, finely to coarsely granular, floccose to felty, whitish, reverse concolorous. Colonies on PDA reaching 30–32 mm, flat, with entire margin, aerial mycelium abundant, felty, reverse concolorous. Colonies on SNA reaching 26–30 mm, whitish, white in the center on the 2nd day, pale green on the 5th day, abundant sporulation, reverse concolorous.

Specimen examined: IRAN: Guilan Province, Astaneh-Ashrafiyeh County, from rhizospheric soil of *Actinidia chinensis*, 1.9.2022, M. Pourshirmohammadi (living culture IRAN 5412C).

Note: Most of the characters of the Iranian isolate are in accordance with the description provided by Moreira *et al.* (2016). *Clonostachys chloroleuca* can be differentiated from *C. rhizophaga* by the fact that the branches in the secondary conidiophores of the latter are even more divergent. In addition, *C. chloroleuca* consistently showed green conidial masses, while conidial masses of *C. rhizophaga* can be greenish, weakly greenish, or unpigmented (Schroers 2001, Moreira *et al.* 2016).

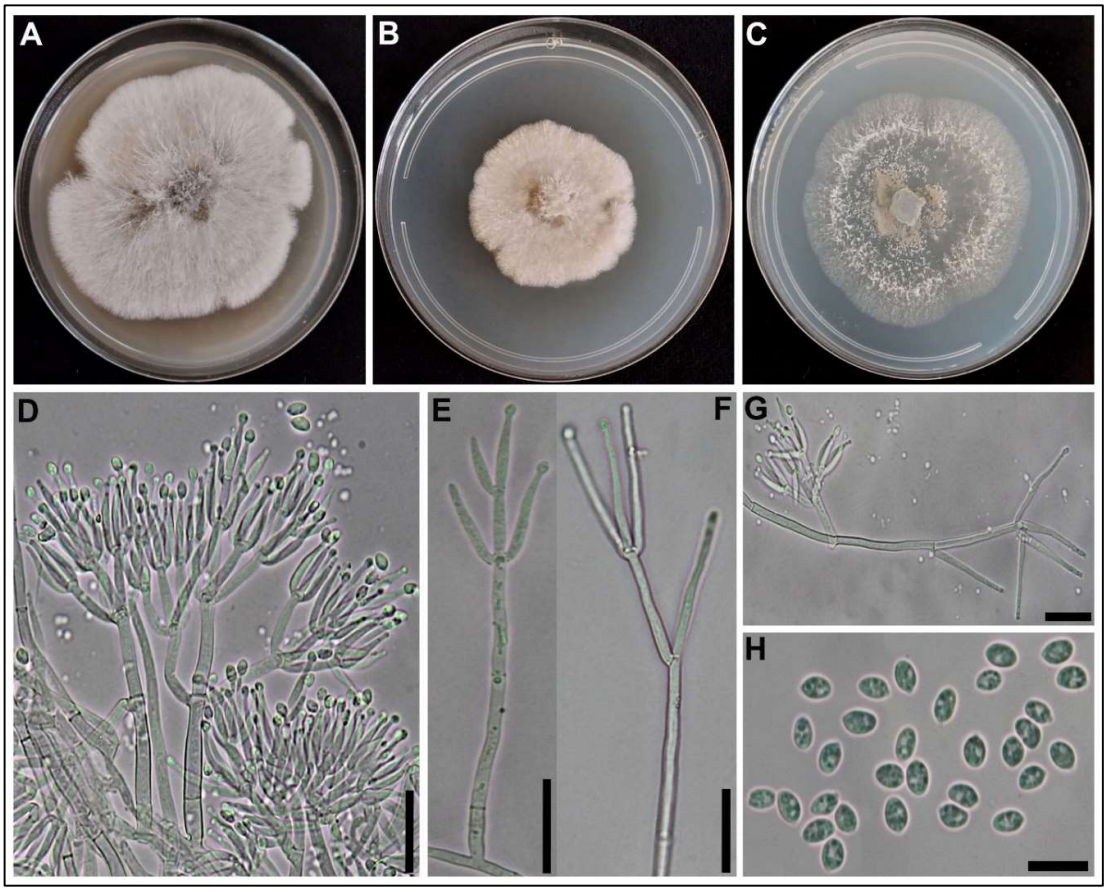

Fig. 3. *Clonostachys chloroleuca* (IRAN 5412C): A-C. Colonies on OA, PDA and SNA after 7 days at 25 °C in dark conditions, D-G. Conidiophores, H. Conidia (Bars: D-G = 20 µm, H = 10 µm).

Clonostachys rogersoniana Schroers, Studies in Mycology 46: 109 (2001) (Fig. 4)

Description: Conidiophores dimorphic, formed throughout the colony. Primary conidiophores verticillium-like, with monoverticillate, stipes 40–250 µm long, 2.3–4.1 µm wide at base. Phialides generally in whorls of 2–4, 12.1–27.5 µm long, 1.4–2.75 µm wide at base, 1–2.1 µm wide near aperture. Secondary conidiophores penicillate, solitary to gregarious, bi-to quaterverticillate, stipes 30–100 µm long, 2.5–5 µm wide at base. Phialides in adpressed whorls of up to 6, 8–18.3 µm long, 1.7–2.6 µm wide at base, 1–2.4 µm wide near aperture. Conidia aseptate, hyaline, broadly ellipsoidal to oval, hilum laterally displaced, 4.2–7.8 × 1.8–3.6 µm.

Culture characteristics: Colonies on OA reaching 45–50 mm diam. after 7 days at 25 °C in darkness, flat, with entire margin, aerial mycelium moderate, finely to coarsely granular, floccose to felty, whitish, reverse concolorous. Colonies on PDA reaching 25–28 mm, flat, with crenate margin, aerial mycelium abundant, finely to coarsely granular, felty to cottony, whitish, reverse concolorous. Colonies on SNA reaching 40–45 mm, whitish, flat, abundant sporulation, margin regular, reverse concolorous.

Specimen examined: IRAN: Guilan Province, Kelachai County, from *Meloidogyne* sp. egg sac under *Actinidia chinensis*, 16.8.2022, M. Pourshirmohammadi (living culture IRAN 5411C).

Note: This species is similar to *C. compactiuscula*, but it can be distinguished from the latter by the frequency of primary conidiophores in culture (in *C. rogersoniana* abundant, in *B. compactiuscula* rare), in shape and size of the conidia [in *C. rogersoniana* broadly ellipsoidal, L/W = (1.5–)1.8–2–2.2(–3.1); in *C. compactiuscula* oblong-ellipsoidal, L/W = (1.8–) 2.9–2.9–3.3(–4.5) (Schroers 2001). *C. rogersoniana* appears closely related to *C. divergens* and *C. samuelsii* based on the phylogenetic tree drawn here, but these species can be differentiated based on greenish pigmented conidial masses and less than 5 µm long conidia in *C. divergens* and sporodochial conidiophores in *C. samuelsii* (Schroers 2001).

Sesquicillium aff. essexcoheniae (Y.P. Tan, Bishop-Hurley & R.G. Shivas) Lin Zhao & Crous, Studies in Mycology 105: 227 (2023) (Fig. 5)

Description: Conidiophores monomorphic, penicillate, up to quaterverticillate, branches typically divergent, phialides divergent or adpressed, stipes 30–60 µm long, 1.9–3.7 µm wide at base. Phialides generally in whorls of up to five, 8–18 µm long, 1.3–2.9 µm wide at base, 2.2–3.4(–4.6) µm at widest point, 0.7–1.6 µm wide near aperture. Conidia aseptate, hyaline, smooth, ellipsoid to subglobose, slightly curved, typically laterally displaced hilum, 3.8–5.9 (–6.1) × 2.1–3.2 µm.

Culture characteristics: Colonies on OA reaching 30–35 mm diam. after 7 days at 25 °C in darkness, flat, white, with entire margin, aerial mycelium, felty, reverse concolorous. Colonies on PDA reaching 27–33 mm, flat, white, cottony, aerial mycelium, crenate margin, reverse concolorous. Colonies on SNA reaching 25–30 mm, whitish, aerial mycelium scanty, sparsely sporulation, reverse concolorous.

Specimen examined: IRAN: Guilan Province, Astaneh-Ashrafiyeh County, from rhizospheric soil of *Actinidia chinensis*, 1.9.2022, M. Pourshirmohammadi (living culture IRAN 5413C).

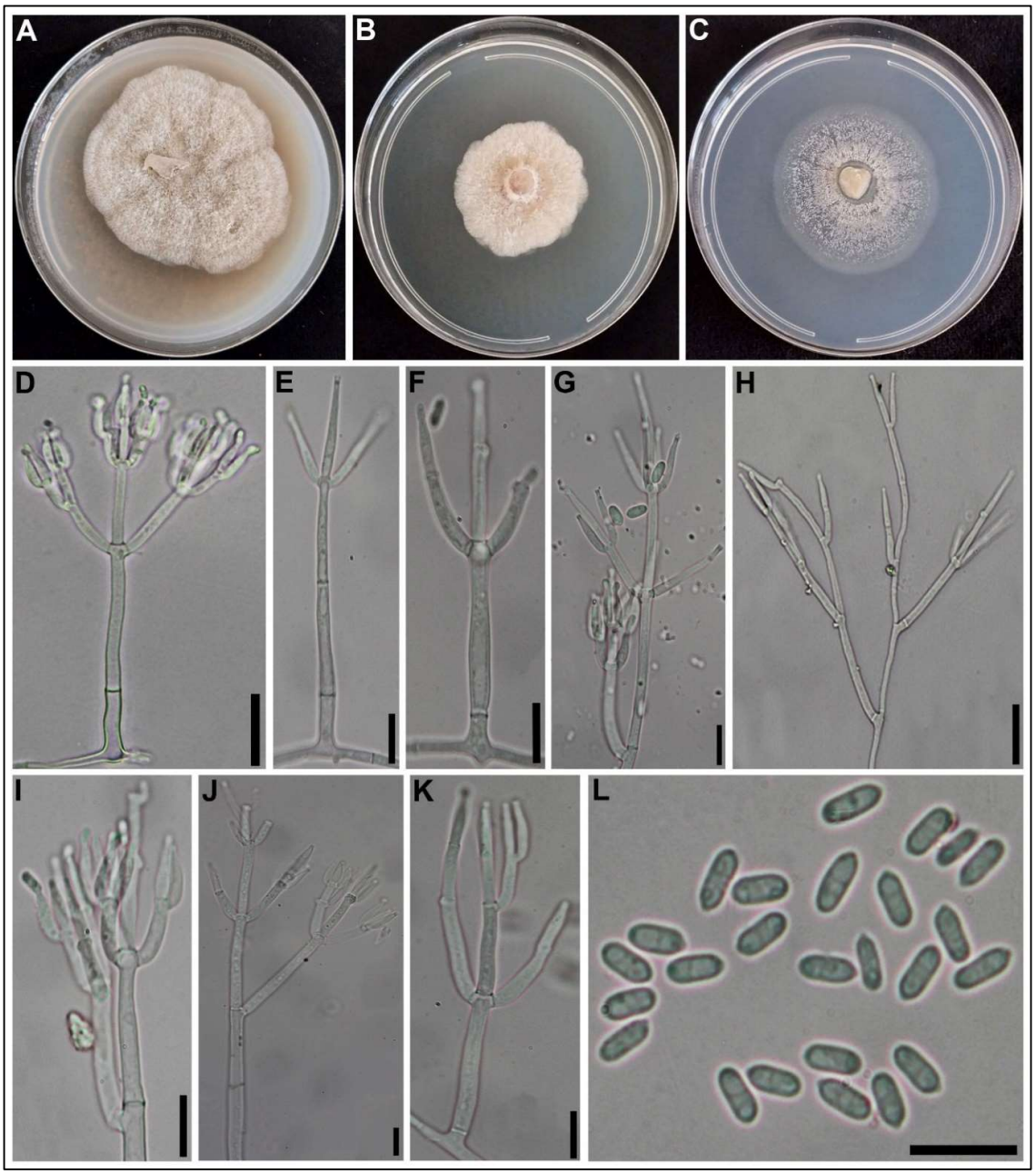

Fig. 4. *Clonostachys rogersoniana* (IRAN 5411C): A-C. Colonies on OA, PDA and SNA after 7 days at 25 °C, D-K. Conidiophores, L. Conidia (Bars: D, H = 20 µm, E-G & I-L = 10 µm).

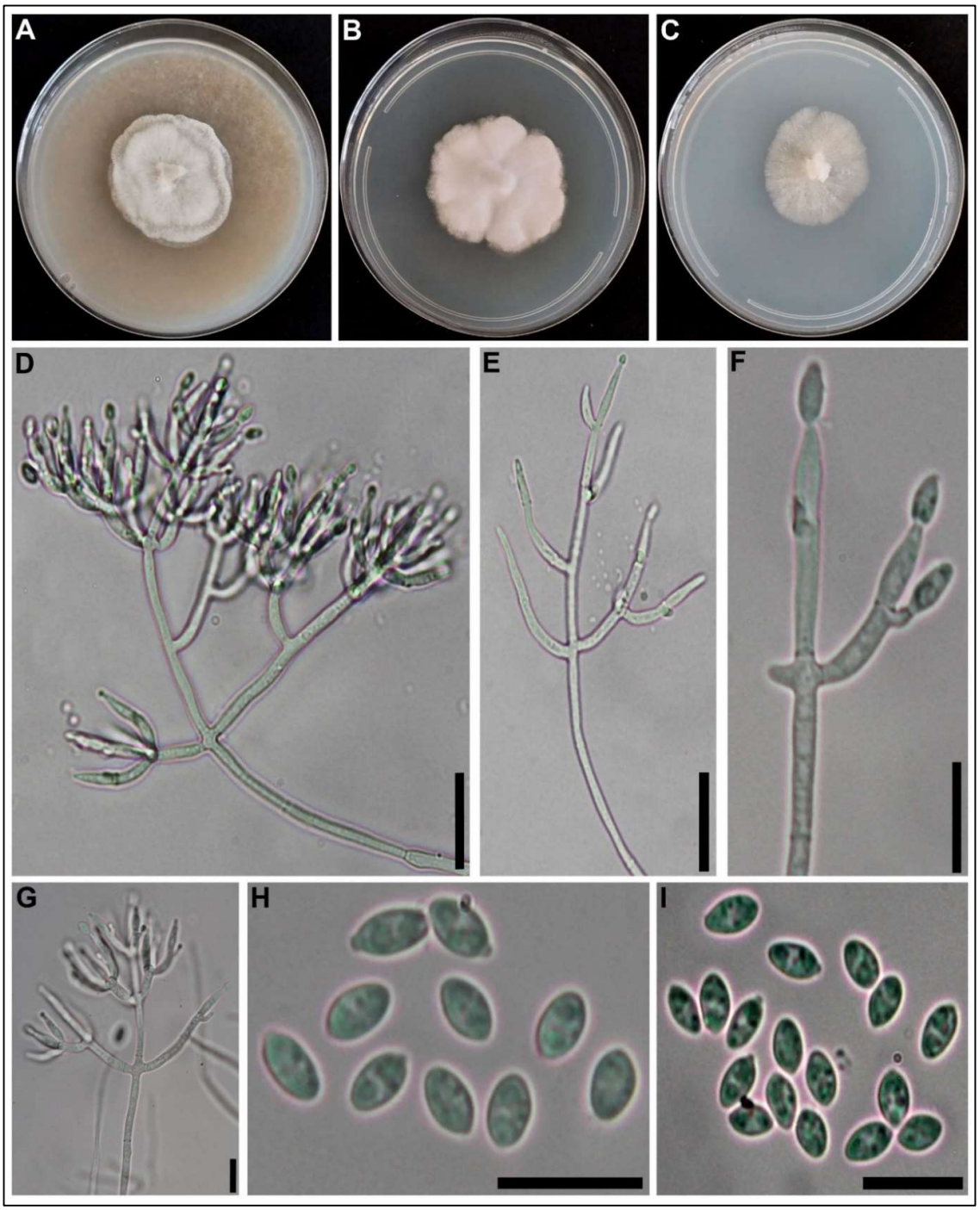

Fig. 5. *Sesquicillium aff. essexcoheniae* (IRAN 5413C): A-C. Colonies on OA, PDA and SNA after 7 days at 25 °C, D-G. Conidiophores, H, I. Conidia (Bars: D, E = 20 µm, F-I = 10 µm).

Acknowledgments

This work was supported by research grants from the Deputy of Research and Technology of the University of Guilan to Dr. Salar Jamali (No. 1756837482) and Dr. S.A. Khodaparast (No. 1756838150). The paper is part of the results derived from the MSc thesis of the first author, supervised by Dr. S. Jamali at the University of Guilan (Rasht, Iran). The authors would like to express their sincere appreciation to PhD student Ms. Mehrnaz Nik for her valuable assistance in this article.

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