A revision of the family *Cucurbitariaceae* with additional new taxa from forest trees in Iran

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Abstract

The family *Cucurbitariaceae* is rich in species diversity and has a wide host range and geographic distribution. In this study, we identified 12 *Cucurbitariaceae* isolates which were obtained from disease symptoms in two forest trees in Khuzestan province, Iran. In addition, this family is reassessed using phylogenetic analyses based on DNA sequences from five nuclear regions (ITS, LSU, *TUB2*, *TEF1α*, and *RPB2*). The phylogenetic analyses showed that the present isolates represent one new genus, *Nothocucurbitaria*, and three new species, *Allocucurbitaria galinsogisoli*, *Nothocucurbitaria izehica*, and *Parafenestella quercicola*, which are described and illustrated. Furthermore, the genus *Allocucurbitaria* is emended to accommodate *Seltsamia ulmi* that grouped with the type species of *Allocucurbitaria*. *Parafenestella pittospori* and *A. prunicola* are recombined into the genera *Neocucurbitaria* and *Nothocucurbitaria*, respectively. Comparative analysis of single-locus trees revealed that the *TUB2* and *TEF1α* can distinguish most genera and species in *Cucurbitariaceae*, while the ITS and LSU phylogenies show low resolution at both generic and species level. The best single-locus marker, *RPB2*, was able to distinguish all generic and most species lineages in *Cucurbitariaceae*.

Keywords: *Crataegus* ; New taxa; Pycnidia; Phylogenetic analyses; *Quercus*

Introduction

Cucurbitariaceae represent an important family in *Pleosporales* (*Dothideomycetes*) (Wijayawardene et al. 2020). Most members of *Cucurbitariaceae* are saprobic, mainly found on the wood, bark, and leaves of shrubs and trees, or in the soil at the base of woody plants (Wanasinghe et al. 2017; Jaklitsch et al. 2018). Some members of *Cucurbitariaceae* are reported to be endophytes (Jaklitsch et al. 2018; Hill et al. 2023), or plant pathogens, including *Rhytidiella moriformis* Zalasky causing perennial rough-bark disease of *Populus balsamifera* L. (Doilom et al. 2013) and *Parafenestella pittospori* Crous causing leaf spot disease of *Pittosporum tenuifolium* Variegata (Crous et al. 2019b). Others are fungicolous, parasitising

genera such as *Cytospora* Ehrenb. (Jaklitsch and Voglmayr 2020), or opportunistic pathogens of animals and humans, mostly colonising skin and nails (Wanasinghe et al. 2017). Members of this family are mostly widespread in temperate and Mediterranean regions and are rarely found in the tropical climates (Jaklitsch et al. 2018; Wijayawardene et al. 2020).

The *Cucurbitariaceae* was established by Winter (1885) to accommodate some genera of pyrenomycetes which produce non-stromatic ascomata in erumpent clusters beneath the host periderm or superficially on wood (Barr 1990), including genera such as *Cucurbitaria* Gray et al., *Nitschkia* G.H. Otth ex P. Karst., and *Otthia* Nitschke ex Fuckel. This family is typified by the genus *Cucurbitaria* and the type species *C. berberidis* (Pers.) Gray. *Cucurbitariaceae* are commonly recognised by having immersed or superficial clustered ascomata with scattered ostioles and hyphal outgrowths, and having cylindrical to oblong bitunicate asci containing ellipsoid, fusoid, or oblong muriformly septate ascospores (Jaklitsch et al. 2018, 2020). The taxonomy of *Cucurbitariaceae* has received considerable attention (Arx and Müller 1950; Barr 1987; Aveskamp et al. 2010; de Gruyter et al. 2010; de Gruyter 2012; Doilom et al. 2013; Wanasinghe et al. 2017; Jaklitsch et al. 2018; Valenzuela-Lopez et al. 2018; Jaklitsch and Voglmayr 2020; Su et al. 2022). Arx and Müller (1950) reduced the family *Cucurbitariaceae* to the genus *Cucurbitaria* and placed it in the *Pleosporaceae*. Barr (1987); however, recognised several genera in the family, including *Cucurbitaria*, *Cucurbidothis* Petr., *Otthia*, *Rhytidiella* Zalasky, and *Syncarpella* Theiss. & Syd. However, these classifications were based on morphology only, which led to different interpretations and an incorrect taxonomic placement of taxa within this family (Doilom et al. 2013; Jaklitsch et al. 2018; Jaklitsch and Voglmayr 2020). The taxonomic concept of *Cucurbitariaceae* was subsequently re-defined based on molecular phylogenetic studies combined with morphology (Aveskamp et al. 2010; de Gruyter et al. 2010; de Gruyter 2012; Doilom et al. 2013). Accordingly, the genera *Cucurbidothis*, *Cucurbitaria*, *Pyrenochaeta* De Not., *Pyrenochaetopsis* Gruyter et al., *Rhytidiella*, and *Syncarpella* were accommodated in the family (Doilom et al. 2013). In further studies, species of the genera *Cucurbidothis*, *Pyrenochaeta*, and *Pyrenochaetopsis* were excluded from *Cucurbitariaceae* and transferred to other families, including *Didymosphaeriaceae*, *Pyrenochaetopsidaceae*, and *Teichosporaceae* in *Pleosporales* (Jaklitsch et al. 2016; Valenzuela-Lopez et al. 2018; Wijayawardene et al. 2020). Recently, several new genera were assigned to the *Cucurbitariaceae* based on molecular phylogenetic studies, i.e. *Allocucurbitaria* Valenz.-Lopez et al., *Astragalicola* Jaklitsch & Voglmayr, *Cucitella* Jaklitsch & Voglmayr, *Fenestella* Tul. & C. Tul., *Neocucurbitaria* Wanas. et al., *Paracucurbitaria* Valenz.-Lopez et al., *Parafenestella* Jaklitsch & Voglmayr, *Protofenestella* Jaklitsch & Voglmayr, *Seltsamia* Jaklitsch & Voglmayr, and *Synfenestella* Jaklitsch & Voglmayr (Wanasinghe et al. 2017; Jaklitsch et al. 2018; Valenzuela-Lopez et al. 2018; Wijayawardene et al. 2020).

The aims of the present study were thus to (1) identify *Cucurbitariaceae* fungi associated with disease symptoms in *Crataegus* and *Quercus* plantations using a polyphasic approach based on morphological characters and molecular data and (2) delimit the phylogenetic lineages within *Cucurbitariaceae* and improve the taxonomic position of the genera and species in this family.

Material and methods

Sample collection and fungal isolation

The specimens were collected from various forest areas in Izeh (Khuzestan Province, Iran) during 2021–2022. Symptomatic trees included *Crataegus* sp. (stem cankers) and *Quercus*

brantii Lindl. (leaf spots). Diseased samples were washed with sterile distilled water, and small pieces from the border of infected and healthy tissues were excised. Plant pieces were superficially disinfected in 1–2% sodium hypochlorite for 1–3 min and then washed in sterile water, followed by drying on sterile filter paper and placing on semi-selective potato dextrose agar medium (PDA, potato extract 200–400 gL^{-1}, sucrose 10 gL^{-1}, agar 12 gL^{-1}, and streptomycin sulphate 30 mgL^{-1}) in Petri plates. Plates were incubated at 25 °C in darkness for 5–10 days and examined daily to observe the emerging colonies. Small agar plugs from the edge of dissimilar fungal colonies were transferred to fresh PDA plates for further isolation and purification. A hyphal-tip isolation technique was performed to obtain pure cultures.

Fungarium materials, including a dried culture of each new species, were deposited at the Fungus Reference Collection (IRAN…F) of Herbarium Ministerii Iranici Agriculturae "IRAN", Iranian Research Institute of Plant Protection (Tehran). Living cultures (Table 1) are preserved at the Iranian Fungal Culture Collection (IRAN…C) of "IRAN" Herbarium and the Collection of Fungal Cultures, Department of Plant Protection, Shahid Chamran University of Ahvaz, Iran (SCUA). Nomenclatural and taxonomic data of new taxa were deposited in MycoBank.

Morphological study

Colony diameter and morphology were determined on PDA up to 20 days of incubation at 25 °C and 30 °C in the dark. Colony colours (obverse and reverse) were assessed according to the Methuen handbook of colour (Kornerup and Wanscher 1967). Morphological characters were studied by observing cultures sporulating on PDA after 8–20 days of incubation at 25 °C under a 12-h cycle of near-ultraviolet/dark conditions. Micro-morphological characteristics were obtained by mounting fungal structures (pycnidia) in a drop of lactophenol or lactophenol cotton blue on microscopic slides. Microscopic sections of pycnidia (approx. 3 μm thickness) were prepared using a Leica RM 2235 microtome (Germany) and mounted in a drop of hematoxylin and eosin mix on microscopic slides (Ahmadpour et al. 2022), to study the anatomy of pycnidial walls and morphology of conidiogenous cells. Observation and measurement of fungal structures were done using a Leitz Wetzlar (SM-LUX) Basic Biological Light Microscope (Germany) at × 100, × 400, and × 1000 magnification. At least 50 observations for each fungal structure were measured and reported as minimum–maximum values, 95% confidence intervals and average values with standard deviations. Fungal structures were photographed using an OLYMPUS BX-50 compound microscope (Japan) fixed with a TUCSEN GT 12 digital camera (China).

DNA extraction and amplification

Mycelia were scraped from the surface of 10-day-old colonies grown on PDA at 25 °C in darkness. Genomic DNA was extracted from mycelia powdered in liquid nitrogen according to the procedure described by Mehrabi-Koushki et al. (2018). The internal transcribed spacer regions 1 and 2 including the intervening 5.8S nuclear ribosomal DNA (ITS) and partial nuclear 28S ribosomal DNA (LSU) were amplified using the primer pair ITS1 and NL4 (White et al. 1990; O'Donnell 1993). The primers Btub2Fd and T2 (O'Donnell & Cigelnik 1997; Woudenberg et al. 2009) were used for amplification and sequencing of the partial region of the β-tubulin gene (*TUB2*). Part of the translation elongation factor 1 alpha (*TEF1α*) gene was amplified and sequenced using the primer pair EF1-688F (Alves et al. 2008) and EF2 (O'Donnell et al. 1998) and partial RNA polymerase II second largest subunit (*RPB2*) with primer pair RPB2-5F2 and fRPB2-7cR (Liu et al. 1999; Sung et al. 2007). PCR amplification

Table 1 Strains used in this study and their GenBank accession numbers. The new taxa and combinations are designated in bold

Taxon	Strain[a]	Source	Origin	GenBank accession numbers				
				ITS	LSU	*TUB2*	*TEF1α*	*RPB2*
Allocucurbitaria botulispora	CBS 142452 [T]	Human	USA	LT592932	LN907416	LT593001	-	LT593070
A. galinsogisoli	CBS 140956 [E]	*Galinsoga parviflora*	China	KU759584	KU759581	-	-	-
A. mori	HMJAU 60183 [T]	*Morus alba*	China	OL996120	OL897171	OL898725	-	OL944505
A. ulmi	CBS 143002 [T]	*Ulmus glabra*	Norway	MF795794	MF795794	MF795918	MF795882	MF795836
Astragalicola amorpha	CBS 142999 [T]	*Astragalus angustifolius*	Greece	MF795753	MF79753	MF795883	MF795842	MF795795
Cucitella opali	CBS 142405 [T]	*Acer opalus*	France	MF795754	MF795754	MF795884	MF795843	MF795796
Cucurbitaria berberidis	C241	*Berberis* sp.	Austria	MF795756	MF795756	MF795886	MF795845	MF795798
C. berberidis	CB	*Berberis vulgaris*	Austria	MF795757	MF795757	MF795887	MF795846	MF795799
C. oromediterranea	C229 [T]	*Berberis cretica*	Greece	MF795761	MF795761	MF795890	MF795849	MF795803
C. oromediterranea	CB2	*Berberis cretica*	Greece	MF795763	MF795763	MF795892	MF795851	MF795805
Fenestella crataegi	C287	*Crataegus monogyna*	Austria	MK356281	MK356281	MK357598	MK357554	-
F. crataegi	CBS 144857 [T]	*Crataegus monogyna*	Austria	MK356282	MK356282	MK357599	MK357555	MK357512
F. fenestrata	FP9 [T]	*Alnus glutinosa*	Austria	MF795765	MF795765	MF795893	MF794843	MF795807
F. gardiennetii	FM [T]	*Acer saccharum*	France	MK356283	MK356283	MK357600	MK357556	MK357513
F. granatensis	C279 [T]	*Acer granatense*	Spain	MK356284	MK356284	MK357601	MK357557	MK357514
F. media	FP [T]	*Corylus avellana*	Austria	MK356285	MK356285	MK357602	MK357558	MK357515
F. media	FP1	*Corylus avellana*	Austria	MK356287	MK356287	MK357603	MK357560	MK357517
F. parafenestrata	C306 [T]	*Quercus robur*	Austria	MK356291	MK356291	MK357607	MK357564	MK357521
F. parafenestrata	C317	*Salix* sp.	Austria	MK356292	MK356292	MK357608	MK357565	MK357522
F. subsymmetrica	FP4	*Corylus avellana*	Austria	MK356296	MK356296	MK357609	MK357568	MK357524
F. subsymmetrica	FP6 [T]	*Acer campestre*	Austria	MK356297	MK356297	MK357610	MK357569	MK357525
F. viburni	FP2	*Viburnum lantana*	France	MK356299	MK356299	MK357612	MK357571	MK357527
F. viburni	FVL [T]	*Viburnum lantana*	Austria	MK356300	MK356300	MK357613	MK357572	MK357528
Neocucurbitaria acanthocladae	C225 [T]	*Genista acanthoclada*	Greece	MF795766	MF795766	MF795894	MF795854	MF795808
N. acerina	C255	*Acer pseudoplatanus*	Austria	MF795768	MF795768	MF795896	MF795856	MF795810
N. acerina	C26a	*Acer pseudoplatanus*	Austria	MF795767	MF795767	MF795895	MF795855	MF795809
N. aetnensis	C261 [T]	*Genista aetnensis*	Italy	MF795769	MF795769	MF795897	MF795857	MF795811
N. aetnensis	C270	*Genista aetnensis*	Italy	MF795770	MF795770	MF795898	MF795858	MF795812
N. aquadulcis	FMR 17840 [T]	Plant debris in freshwater	Spain	LR897770	-	LR897794	-	LR897793
N. aquatica	CBS 297.74 [T]	Sea water	Serbia and Montenegro	LT623221	EU754177	LT623238	-	LT623278
N. cava	CBS 115979	Unknown	The Netherlands	AY853248	EU754198	LT623234	-	LT623273
N. cava	CBS 257.68 [T]	Wheat-field soil	Germany	JF740260	EU754199	KT389844	-	LT717681
N. chlamydospora	KNUF-22-1-8-B [T]	*Hygia lativentris*	Sout Korea	OQ060587	OQ060588	OQ148365	-	OQ148364
N. cinereae	KU9 [T]	*Genista cinerea*	Spain	MF795771	MF795771	MF795899	MF795859	MF795813
N. cisticola	C244 [T]	*Cistus monspeliensis*	Spain	MF795772	MF795772	MF795900	MF795860	MF795814

Table 1 (continued)

Taxon	Strain[a]	Source	Origin	GenBank accession numbers				
				ITS	LSU	*TUB2*	*TEF1α*	*RPB2*
N. hakeae	CPC 28920 [T]	*Hakea* sp.	Australia	KY173436	KY173526	KY173613	-	KY173593
N. irregularis	CBS 142791 [T]	Human arm	USA	LT592916	LN907372	LT592985	-	LT593054
N. juglandicola	BW6 [T]	*Juglans regia*	Austria	NR_156358	MF795773	MF795901	MF795861	MF795815
N. juglandicola	C316	*Quercus rubra*	Austria	MK356301	MK356301	MK357614	MK357573	MK357529
N. keratinophila	CBS 121759 [T]	Human	Spain	NR_137017	NG_070610	LT623236	-	LT623275
N. pittospori	CPC 34462 [T]	*Pittosporum tenuifolium*	New Zealand	MN562098	MN567606	-	-	-
N. populi	C28 [T]	*Populus* sp.	Sweden	MF795774	MF795774	MF795902	MF795862	MF795816
N. quercina	CBS 115095 [T]	*Quercus robur*	Italy	LT623220	GQ387619	LT623237	-	LT623277
N. rhamni	C1 [T]	*Rhamnus frangula*	Austria	MF795775	MF795775	-	MF795863	MF795817
N. rhamni	C112	*Rhamnus frangula*	Austria	MF795776	MF795776	MF795903	MF795864	MF795818
N. rhamnicola	C185 [T]	*Rhamnus lycioides*	Spain	MF795780	MF795780	MF795906	MF795868	MF795822
N. rhamnicola	KRx	*Rhamnus alaternus*	Spain	MF795781	MF795781	MF795907	MF795869	MF795823
N. rhamnioides	C118 [T]	*Rhamnus myrtifolius*	Spain	MF795782	MF795782	MF795908	MF795870	MF795824
N. rhamnioides	C223	*Rhamnus saxatilis* subsp. *Prunifolius*	Greece	MF795784	MF795784	MF795910	MF795872	MF795826
N. ribicola	C155	*Ribes rubrum*	Austria	MF795786	MF795786	MF795912	MF795874	MF795828
N. ribicola	C55 [T]	*Ribes rubrum*	Austria	MF795785	MF795785	MF795911	MF795873	MF795827
N. salicis-albae	CBS 144611 [T]	*Salix alba*	Germany	NR_163365	MK442535	MK442738	-	MK442669
N. unguis-hominis	CBS 111112	*Agapornis* sp.	The Netherlands	LT623222	GQ387623	LT623239	-	LT623279
N. vachelliae	C192 [T]	*Vachellia gummifera*	Morocco	MF795787	MF795787	MF795913	MF795875	MF795829
N. variabilis	FMR 17552 [T]	Plant debris in freshwater	Spain	LR897768	-	LR897792	-	LR897791
N. variabilis	VMD-2020	Plant debris in freshwater	Spain	LR897784	-	LR897807	-	LR897806
Nothocucurbitaria prunicola	CBS 145033 [T]	*Prunus padus*	Ukraine	MK442594	MK442534	MK442737	-	MK442668
Nothocucurbitaria izehica	**IRAN 4856C; SCUA-IS-A10**	***Crataegus* sp.**	**Iran**	**OR440607**	**OR440612**	**OR450816**	**-**	**OR450808**
Nothocucurbitaria izehica	**SCUA-IS-A10-2**	***Crataegus* sp.**	**Iran**	**OR440608**	**OR440613**	**OR450817**	**-**	**OR450809**
Paracucurbitaria corni	CBS 248.79	*Fraxinus excelsior*	The Netherlands	LT903672	GQ387608	LT900365	-	LT903673
P. italica	CBS 234.92 [T]	*Olea europaea*	Italy	LT623219	EU754176	LT623235	-	LT623274
Parafenestella alpina	C198 [T]	*Cotoneaster integerrimus*	Austria	MK356302	MK356302	MK357615	MK357574	MK357530
P. alpina	C249	*Salix appendiculata*	Austria	MK356303	MK356303	MK357616	MK357575	MK357531
P. austriaca	C152 [T]	*Rosa canina*	Austria	MK356304	MK356304	MK357617	MK357576	MK357532
P. changchunensis	HMJAU 60182 [T]	*Populus*	China	OL996119	OL897170	OL898719	-	-
P. faberi	MFLUCC 16-1451	*Rosa canina*	Germany	KY563071	KY563074	-	-	-
P. germanica	C307 [T]	*Corylus avellana*	Germany	MK356305	MK356305	MK357618	MK357577	MK357533

Table 1 (continued)

Taxon	Strain[a]	Source	Origin	GenBank accession numbers				
				ITS	LSU	*TUB2*	*TEF1α*	*RPB2*
P. ontariensis	EI-6	*Acer negundo*	Canada	OM286882	OM286884	-	-	-
P. ostryae	MFLU 16-0184 [T]	*Ostrya carpinifolia*	Italy	KY563072	KY563075	-	-	-
P. parasalicum	C318 [T]	*Salix cinerea*	Austria	MK356306	MK356306	MK357619	MK357578	MK357534
P. pseudoplatani	C26 [T]	*Acer pseudoplatanus*	Austria	MF795788	MF795788	MF795914	MF795876	MF795830
P. pseudosalicis	C301 [T]	*Salix* cf. *alba*	Ukraine	MK356307	MK356307	MK357620	MK357579	MK357535
P. rosacearum	C203	*Pyrus communis*	Austria	MK356308	MK356308	MK357621	MK357580	MK357536
P. rosacearum	C309 [T]	*Pyracantha coccinea*	Austria	MK356311	MK356311	MK357624	MK357583	MK357539
P. salicis	C303	*Salix alba*	Austria	MK356316	MK356316	MK357628	MK357588	MK357544
P. salicis	C313 [T]	*Salix alba*	Austria	MK356317	MK356317	MK357629	MK357589	MK357545
P. salicum	C311 [T]	*Salix alba*	Austria	MK356318	MK356318	MK357630	MK357590	MK357546
Parafenestella quercicola	**IRAN 4857C; SCUA-IS-B1**	***Quercus brantii***	**Iran**	**OR440609**	**-**	**OR450818**	**OR450812**	**-**
Parafenestella quercicola	**SCUA-IS-B1-2**	***Quercus brantii***	**Iran**	**OR440610**	**-**	**OR450819**	**OR450813**	**-**
Parafenestella tetratrupha	C304 T	*Alnus glutinosa*	Austria	MK356319	MK356319	MK357631	MK357591	MK357547
P. ulmi	HMJAU 60179	*Ulmus pumila*	China	OL996116	OL897167	OL898717	-	OL944502
P. ulmi	HMJAU 60178 [T]	*Ulmus pumila*	China	OL996115	OL897166	OL898723	-	OL944501
P. ulmicola	HMJAU 60180 [T]	*Ulmus pumila*	China	OL996117	OL897168	OL898724	-	OL944503
P. ulmicola	HMJAU 60181	*Ulmus pumila*	China	OL996118	OL897169	OL898718	-	OL944504
P. vindobonensis	C302 [T]	*Salix babylonica*	Austria	MK356320	MK356320	MK357632	MK357592	MK357548
Protofenestella ulmi	FP5 [T]	*Ulmus minor*	Austria	MF795791	MF795791	MF795915	MF795879	MF795833
Synfenestella pyri	C297 [T]	*Pyrus communis*	Austria	MK356321	MK356321	MK357633	MK357593	MK357549
S. sorbi	C196	*Sorbus aucuparia*	Austria	MK356324	MK356324	MK357635	MK357596	MK357552
S. sorbi	FR [T]	*Sorbus aucuparia*	Austria	MK356322	MK356322	MK357634	MK357594	MK357550
Pyrenochaetopsis leptospora	CBS 101635 [T]	*Secale cereale*	Unknown	MF795793	MF795793	MF795917	MF795881	MF795835
Pyrenochaeta nobilis	CBS 407.76 [T]	*Laurus nobilis*	Italy	MF795792	MF795792	MF795916	MF795880	MF795834
Pseudopyrenochaeta terrestris	CBS 282.72 [T]	Soil	The Netherlands	LT623228	LT623216	LT623246	-	LT623287
Neopyrenochaeta acicola	CBS 812.95 [T]	Waterpipe	The Netherlands	LT623218L	GQ387602	LT623232	-	T623271
Neopyrenochaetopsis hominis	UTHSC: DI16-238[T]	Human	USA	LT592923	LN907381	LT592992	-	LT593061
Leptosphaeria doliolum	CBS 505.75	*Urtica dioica*	The Netherlands	JF740205	GU301827	JF740144	-	KT389640

[a]Abbreviation of culture collections: CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; IRAN, Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran; MFLUCC, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; others are not registered abbreviations. [T]Ex-type strains

was carried out in a 30 µL reaction mixture containing 3 µL of × 10 PCR Buffer (GenetBio, South Korea), 100–200 ng of genomic DNA, 1.2 µL of each primer (10 µM), 1.5 µL of dNTP mix (2.5 mM of each dNTP), 1.8 µL of $MgCl_2$ (25 mM), 3 units of Prime Taq DNA polymerase (GenetBio, South Korea), and nuclease-free Milli-Q water up to final volume (30 µL). PCR was performed in a SimpliAmp ™ Thermal Cycler and consisted of pre-denaturation for 3 min at 95 °C, followed by 35 cycles of 30-s denaturation at 95 °C, 30 s-annealing at 56 °C (ITS-LSU), 57 °C (*TUB2*), 57 °C (*TEF1α*), or 58 °C (*RPB2*), and 30-s elongation at 72 °C, and a final elongation at 72 °C for 5 min. PCR products were analysed and sequenced as described by Mehrabi-Koushki et al. (2021) and Artand et al. (2022). PCR products were sequenced at the CardiGenetics Research Center or Codon Genetics Group (Tehran Province, Iran).

Phylogenetic analyses

Phylogenetic analyses were performed as described in Safi et al. (2021). Forward and reverse sequences of each sample were assembled using DNA Baser Sequence Assembler v.4 (2013, Heracle BioSoft, http:// www. dnaba ser.com) and subjected to BLASTn search algorithm against the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest matching ex-type strains, which were added to the sequence alignment. Accordingly, the sequences of representative taxa were downloaded and used for phylogenetic analyses (Table 1). All sequences of each individual gene were aligned with ClustalW in BioEdit Alignment Editor v.7.2.5 software (Hall 1999) and trimmed based on the longest start and end gap of the alignment. Alignments were checked and edited manually where necessary. Phylogenetic analyses were performed first for each genomic region (not shown) and then for a combined matrix (ITS + LSU + *TUB2* + *TEF1α* + *RPB2*). This combined matrix was made by concatenation of all single-region alignments using raxmlGUI 2.0 beta software (Edler et al. 2021). The unavailable sequences of each genomic region for each strain in the combined matrix were treated as missing data. Six outgroup taxa from the families *Leptosphaeriaceae*, *Neopyrenochaetaceae*, *Pseudopyrenochaetaceae*, *Pyrenochaetopsidaceae*, or *incertae sedis* in *Pleosporales* were used to root phylogenetic trees, according the results of previous studies (Jaklitsch et al. 2018; Valenzuela-Lopez et al. 2018; Jaklitsch and Voglmayr 2020; Su et al. 2022). The maximum likelihood (ML) analysis was performed using raxmlGUI 2.0 beta (Edler et al. 2021) with parameters adjusted for 1000 bootstrap replications (MLBS) and the general time-reversible (GTR) substitution model with a gamma-distributed rate variation and invariant site (G + I). Maximum parsimony (MP) analyses were performed in MEGA v.7 (Tamura et al. 2013) with selecting the heuristic search for tree inference with 1000 bootstrap replicates (MPBS). Bayesian inference analyses (BI) were performed in MrBayes v.3.2.6 software (Ronquist et al. 2012) using Markov chain Monte Carlo sampling to determine Bayesian posterior probabilities values (BPP). The best model of evolution for BI analysis was estimated for each genomic region using jModelTest 2 (Darriba et al. 2012). Accordingly, the following substitution models were used: GTR + I + G for ITS, LSU, *TEF1α*, and *RPB2* regions and HKY + I + G for *TUB2*. Four simultaneous chains were run for 3,000,000 generations, with trees sampled every 300 generations and discarding the first 25% of sampled trees to calculate BPP values. The newly generated sequences were deposited in the GenBank nucleotide database (Table 1).

Results

In this study, a total of 12 isolates belonging to the family *Cucurbitariaceae* were obtained. Following a comprehensive morphological evaluation, these isolates were grouped in two morphologically identical types, respectively, isolated from symptomatic tissues of *Crataegus*

sp. (seven isolates) and *Quercus brantii* (five isolates). Among them, two isolates of each morphologically identical types are represented in the phylogenetic reassessment of *Cucurbitariaceae*. Accordingly, one new genus, three new species, and three new combinations are proposed.

DNA analyses and phylogeny

Phylogenetic analysis of combined ITS, LSU, *TUB2*, *TEF1α*, and *RPB2* dataset included 420 sequences from 95 strains, including the six outgroup taxa. The combined DNA dataset consisted of a total length of 2772 nucleotide sites, including gaps (ITS: 452 bp, LSU: 820 bp, *TUB2*: 341 bp, *TEF1α*: 459 bp, *RPB2*: 700 bp). Of those sites, 1721 were constant (ITS: 274 bp, LSU: 711 bp, *TUB2*: 171 bp, *TEF1α*: 197 bp, *RPB2*: 368 bp), 203 were parsimony uninformative (ITS: 40 bp, LSU: 40 bp, *TUB2*: 18 bp, *TEF1α*: 51 bp, *RPB2*: 54 bp), and 808 were parsimony informative (ITS: 133 bp, LSU: 67 bp, *TUB2*: 147 bp, *TEF1α*: 183 bp, *RPB2*: 278 bp). The ITS, LSU, *TUB2*, *TEF1α*, and *RPB2* sequence data showed congruency in the tree topologies for the 70% reciprocal bootstrap trees, which allowed to combine the sequences to a concatenated DNA dataset. The ML tree had a similar topology with those obtained from MP and BI methods in major clades. Accordingly, the ML tree is here depicted to show the phylogenetic relationships of the representative taxa in *Cucurbitariaceae* (Fig. 1). In the phylogenetic tree (Fig. 1), 11 major lineages are supported by bootstrap and posterior probability values, of which 10 represent described genera, and the remaining lineage is described as new genus.


85/79/1.0
Neocucurbitaria aquadulcis FMR 17840 T
-/53/0.86
Neocucurbitaria chlamydospora KNUF-22-1-8-B T
-/61/1.0
Neocucurbitaria irregularis CBS 142791 T
-/55/1.0
Neocucurbitaria keratinophila CBS 121759 T
100/100/1.0
Neocucurbitaria acerina C255
Neocucurbitaria acerina C26a
100/100/1.0
90/99/1.0
Neocucurbitaria variabilis VMD-2020
Neocucurbitaria variabilis FMR 17552
Neocucurbitaria unguis-hominis CBS 111112
100/100/1.0
Neocucurbitaria aquatica CBS 297.74 T
Neocucurbitaria vachelliae C192 T
100/100/1.0
Neocucurbitaria rhamnicola C185 T
100/100/1.0
Neocucurbitaria rhamnicola KRx
Neocucurbitaria rhamnioides C223
-/-/0.74
83/84/1.0
Neocucurbitaria rhamnioides C118 T
100/100/1.0
Neocucurbitaria rhamni C112
Neocucurbitaria rhamni C1 T
-/-/0.74
Neocucurbitaria pittospori CPC 34462 T comb. nov.
Neocucurbitaria ribicola C55 T
100/100/1.0
Neocucurbitaria ribicola C155
100/100/1.0
Neocucurbitaria aetnensis C261 T
100/99/1.0
Neocucurbitaria aetnensis C270
100/99/1.0
Neocucurbitaria cinereae KU9 T
85/69/1.0
Neocucurbitaria acanthocladae C225 T
79/77/0.89
Neocucurbitaria salicis-albae CBS 144611 T
99/91/1.0
Neocucurbitaria quercina CBS 115095 T
100/100/1.0
Neocucurbitaria juglandicola C316
76/66/0.99
Neocucurbitaria juglandicola BW6 T
100/100/1.0
Neocucurbitaria populi C28 T
100/100/1.0
Neocucurbitaria cava CBS 115979
100/98/1.0
Neocucurbitaria cava CBS 257.68 T
99/97/1.0
Neocucurbitaria cisticola C244 T
Neocucurbitaria hakeae CPC 28920 T
L1
89/95/0.96
Parafenestella germanica C307 T
Parafenestella pseudoplatani C26 T
-/-/0.66
Parafenestella austriaca C152 T
99/99/1.0
Parafenestella rosacearum C203
52/-/0.65
Parafenestella rosacearum C309 T
97/99/1.0
Parafenestella parasalicum C318 T
-/76/0.60
Parafenestella salicum C311
70/76/0.92
Parafenestella tetratrupha C304 T
100/100/1.0
Parafenestella quercicola SCUA-IS-B1-2 sp. nov.
Parafenestella quercicola IRAN 4857C T sp. nov.
54/-/-
Parafenestella ostryae MFLU 16-0184 T
54/78/0.98
Parafenestella faberi MFLUCC 16-1451
100/100/1.0
Parafenestella ulmicola HMJAU 60180 T
Parafenestella ulmicola HMJAU 60181
100/99/1.0
Parafenestella ulmi HMJAU 60178 T
81/92/1.0
Parafenestella ulmi HMJAU 60179
L2

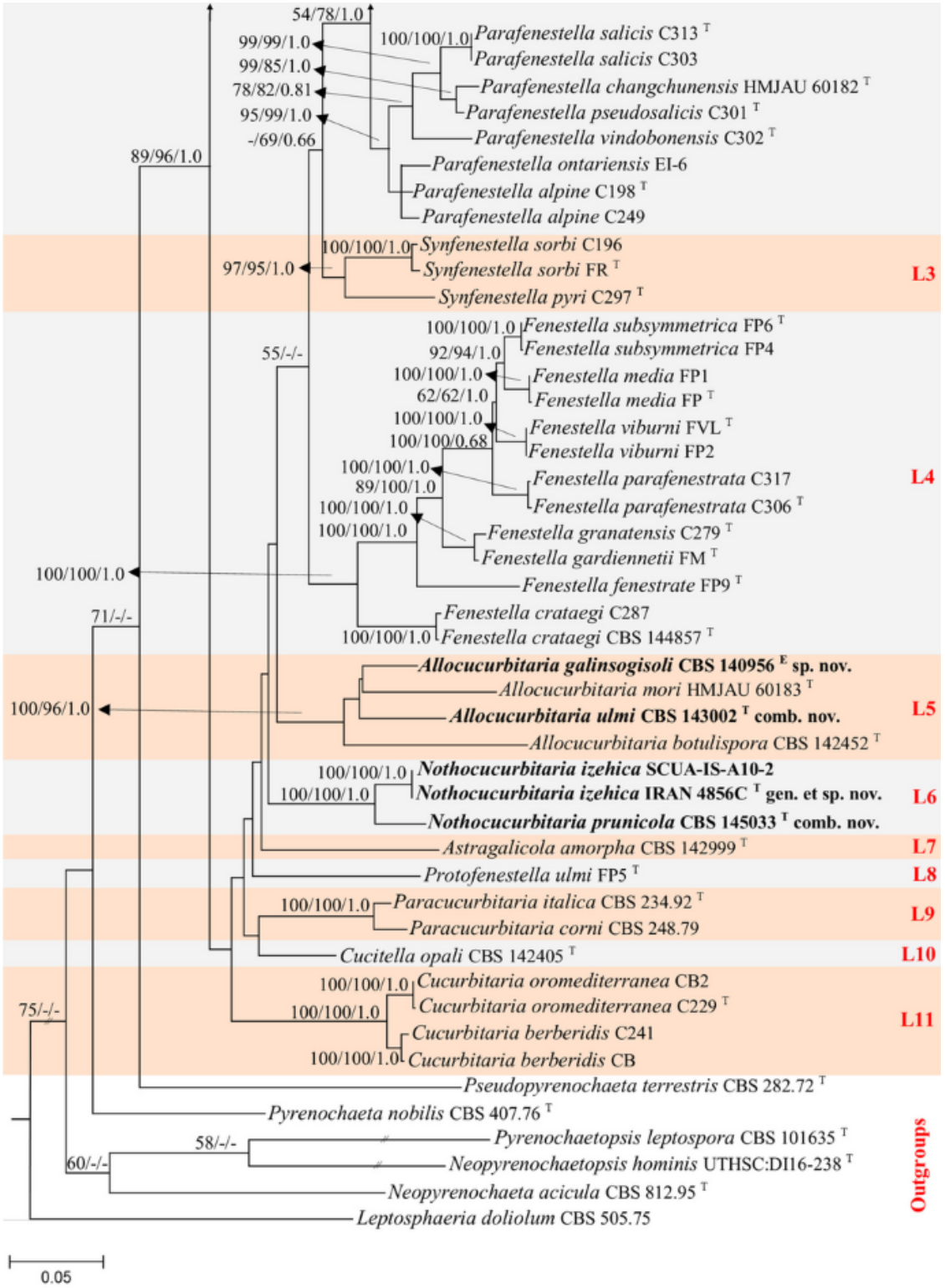

Fig. 1. Phylogenetic tree constructed from a maximum likelihood analysis based on a combined matrix of ITS, LSU, *TUB2*, *TEF1α*, and *RPB2* sequences. Bootstrap values obtained in maximum likelihood (MLBS) and maximum parsimony (MPBS) analyses ≥ 50% and Bayesian posterior probability values (BYPP) ≥ 0.5 are shown at the nodes, respectively. The scale bar displays the expected number of

changes per site. The tree is rooted with six taxa from related families. Letter T indicates the ex-type strains

Lineage 2, a moderately supported clade (MLBS 54%, MPBS 78%, BPP 1.0), accommodated all the known species of the genus *Parafenestella*, with the exception of *P. pittospori* Crous (CPC 34462) which grouped with lineage 1 and is placed in *Neocucurbitaria*. In addition, two strains representing the new species *Parafenestella quercicola* (IRAN 4857C and SCUA-IS-B1-2) clustered in a well-supported clade (MLBS 100%, MPBS 100%, BPP 1.0) distinct from other previously known species of *Parafenestella* in the lineage 2. Lineage 5, a well-supported clade (MLBS 100%, MPBS 96%, BPP 1.0), included two species of the genus *Allocucurbitaria*, *A. botulispora* Valenz.-Lopez et al. and *A. mori* W.X. Su et al., one new species *Allocucurbitaria galinsogisoli* (syn. *Seltsamia galinsogisoli* T.Y. Zhang & Y.X. Zhang), and the recombined species *A. ulmi* (syn. *Seltsamia ulmi* Jaklitsch & Voglmayr). Lineage 6, a well-supported clade (MLBS 100%, MPBS 100%, BPP 1.0), comprised two isolates obtained in this study (IRAN 4856C and SCUA-Is-A10) and the ex-type strain of *Allocucurbitaria prunicola* (Crous & Akulov) Magaña-Dueñas et al., which represents a novel genus *Nothocucurbitaria* to accommodate *N. izehica* sp. nov. and *N. prunicola*. The remaining lineages represent other known genera in *Cucurbitariaceae*, including *Astragalicola*, *Cucitella*, *Cucurbitaria* (MLBS 100%, MPBS 100%, BPP 1.0), *Fenestella* (MLBS 100%, MPBS 100%, BPP 1.0), *Neocucurbitaria* (MLBS 79%, MPBS 77%, BPP 0.89), *Paracucurbitaria* (MLBS 100%, MPBS 100%, BPP 1.0), *Protofenestella*, and *Synfenestella* (MLBS 97%, MPBS 95%, BPP 1.0).

Taxonomy

Allocucurbitaria Valenz.-Lopez *et al.*, Stud. Mycol. 90: 51. 2017. ***emend.*** M. Mehrabi-Koushki & Eisvand.

Sexual morph on Hapalocystis bicaudata Fuckel *on Ulmus glabra* Huds.: *Ascomata* pyriform, black, immersed singly or in valsoid groups beneath periderm above ascomata of its host, upright or oblique with convergent ostiolar necks, surrounded by subiculum, forming bumps, becoming visible through bark fissures. *Ostiolar necks* forming stout papillae. *Peridium* leathery, black, pseudoparenchymatous, 3-layered. *Hamathecium* consisting of branched paraphyses. *Asci* cylindrical, with a distinct ocular chamber, a slightly elongated stipe and a simple base, containing 8 uni- to partly biseriately arranged ascospores. *Ascospores* fusoid to subclavate, with the upper part slightly widened, first yellow, with 3 main septa, later brown, finally with numerous transverse and longitudinal septa, surrounded by a swollen sheath around each hemisphere (Jaklitsch et al. 2018).

Asexual morph on OA: *Conidiomata* pycnidial, brown, solitary or confluent, superficial, pycnidial wall of *textura angularis*, glabrous, subglobose to ovoid, ostiolate. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical to allantoid, guttulate (Valenzuela-Lopez et al. 2018).

Type species: *Allocucurbitaria botulispora* Valenzuela-Lopez *et al*.

Notes: The genus *Allocucurbitaria* (21 Nov. 2017, Stud. Mycol. 90: 51) is here emended to include the genus *Seltsamia* (21 Nov. 2017, Stud. Mycol. 90: 111) that clustered with the type species of *Allocucurbitaria*, *A. botulispora*, in the phylogenetic analyses of the present study (MLBS 100%, MPBS 96%, BPP 1.0). Features of the sexual morph of *Seltsamia* (Jaklitsch et

al. 2018) are therefore incorporated into the emended generic circumscription of *Allocucurbitaria*.

Allocucurbitaria ulmi (Jaklitsch & Voglmayr.) M. Mehrabi-Koushki & Eisvand, **comb. nov.**

MycoBank: MB 849859.

Basionym: *Seltsamia ulmi* Jaklitsch & Voglmayr, Stud. Mycol. 90: 113. 2017.

Description: Jaklitsch et al. (2018).

Typus: Norway, Aust-Agder, Froland kommune, Ytre Lauvrak, associated with *Hapalocystis bicaudata* on corticated twigs of *Ulmus glabra* (*Oleaceae*), 3 Oct. 2014, H. Voglmayr & W. Jaklitsch (holotype, WU 36957, ex-holotype culture CBS 143002 = L150).

Notes: *Seltsamia* was published on 21 Nov. 2017, with the type species *Seltsamia ulmi* (CBS 143002) isolated from corticated *Ulmus glabra* in Norway (Jaklitsch et al. 2018). The sexual morph is exceptional among the members of the *Cucurbitariaceae* because of swollen ascospore sheath.

Allocucurbitaria galinsogisoli M. Mehrabi-Koushki & Eisvand, **sp. nov.**

MycoBank: MB 851767.

Synonym: *Seltsamia galinsogisoli* T.Y. Zhang & Y.X. Zhang, *Scientific Reports* **9** (no. 8319): 2 (2019), *nom. inval*. Art. 40.7 (Shenzhen).

Etymology: Isolated from soil with *Galinsoga parviflora* Cav.

Typus: China, Liaoning province, Huludao city, from the rhizosphere of *Galinsoga parviflora* (*Asteraceae*), Sept. 2014, W. Weixun (holotype, CBS 140956, preserved as metabolically inactive culture; ex-type culture CBS 140956 = CGMCC 3.17981 = SYP-F-7336).

Diagnosis: *Conidiomata* pycnidial, subglobose to globose, brown to dark brown, 70–125 × 45–96 µm, covered with hyphal outgrows. *Conidiogenous cells* phialidic, hyaline, smooth-walled, 9–19.2 × 1.4–4.2 µm. Conidia aseptate, hyaline, smooth, cylindrical, slightly curved, 2.5–4.3 × 0.8–1.2 µm (Zhang et al. 2019).

Description and illustrations: Zhang et al. (2019).

Notes: *Allocucurbitaria galinsogisoli* was initially described as *Seltsamia galinsogisoli* Tianyuan Zhang & Yixuan Zhang (Zhang et al. 2019). However, this name is illegitimate (nom. inval., Art. 40.7), as no holotype specimen was designated. The species is newly described here as *A. galinsogisoli*, being phylogenetically distinct from other genera and species in *Cucurbitariaceae* (Fig. 1).

Neocucurbitaria pittospori (Crous) M. Mehrabi-Koushki & Eisvand, **comb. nov.**

MycoBank: MB 849861.

Basionym: *Parafenestella pittospori* Crous, Persoonia 43: 245. 2019.

Description: Crous et al. (2019b).

Typus: New Zealand, Auckland, Rotorua, leaf spots on *Pittosporum tenuifolium* Gaertn. (*Pittosporaceae*), 25 Aug. 2017, *R. Thangavel* (holotype, CBS H-24152, ex-type culture CPC 34462 = CBS 146026).

Notes: *Parafenestella pittospori* was introduced from *Pittosporum tenuifolium* in New Zealand (Crous et al. 2019b). The only isolate of this species (CPC 34462) is however phylogenetically distinct from other species of *Parafenestella*, but closely related to species of *Neocucurbitaria* (Fig. 1), where it is introduced as a new combination.

Nothocucurbitaria Eisvand & M. Mehrabi-Koushki, **gen. nov.**

MycoBank: MB 849862.

Etymology: *Notho* = fake, close to *Cucurbitaria* but different.

Conidiomata pycnidial, brown to olivaceous black, solitary or aggregated, globose to ellipsoidal, occasionally forming a short ostiolated neck, with some hyphal outgrowths or setose; pycnidial wall of *textura angularis*. *Conidiogenous cells* phialidic, subcylindrical to doliiform. *Conidia* aseptate, hyaline, smooth- and thin-walled, (sub-) cylindrical to allantoid, guttulate, straight or curved.

Type species: *Nothocucurbitaria izehica* Eisvand & M. Mehrabi-Koushki.

Nothocucurbitaria izehica Eisvand & M. Mehrabi-Koushki, **sp. nov.** (Fig. 2).

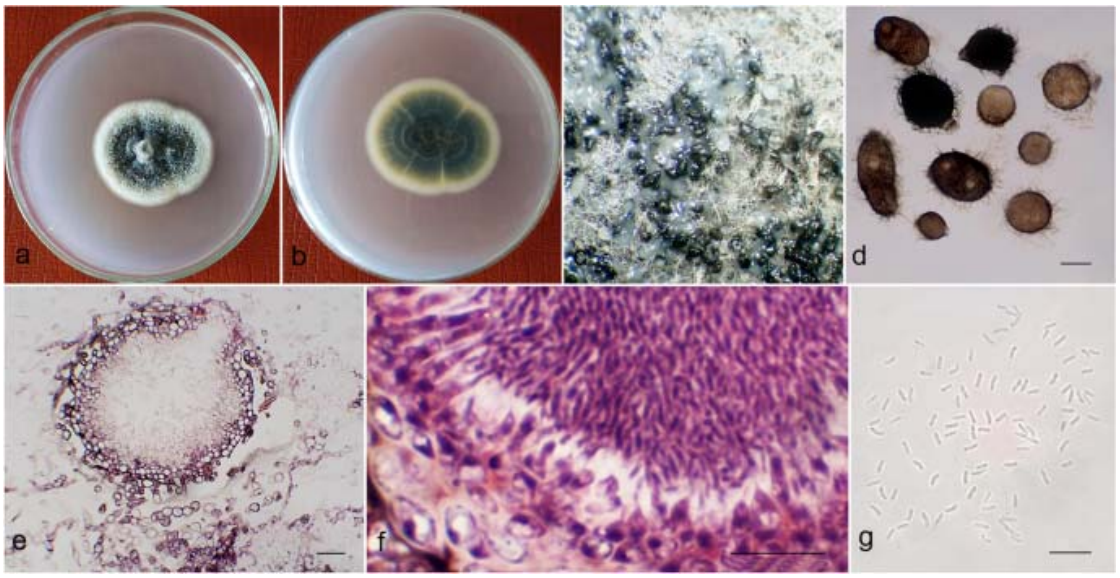

Fig. 2. *Nothocucurbitaria izehica* (IRAN 4856C). **a**, **b** Colony on PDA after 14 days at 25 °C (**a** top, **b** reverse). **c**, **d** Pycnidia. **e** Section of pycnidium. **f** Conidiogenous cells. **g** Conidia. Scale bars: **d** = 100 µm, **e** = 20 µm, **f**, **g** = 10 µm

MycoBank: MB 849863

Typus: Iran, Khuzestan Province, Izeh, isolated from stem canker of *Crataegus* sp. (*Rosaceae*), May. 2022, P. Eisvand (holotype, IRAN 18298 F; ex-type cultures, IRAN 4856C = SCUA-Is-A10).

Etymology: Pertaining to Izeh, the township in Iran where the fungus was collected.

Morphology on PDA: *Conidiomata* pycnidial, formed after a week of incubation in the light and dark, immersed or semi-immersed, solitary or aggregated, brown olivaceous to olivaceous black, globose, sub-globose, ellipsoidal or irregular in shape, with long and short hyphal outgrowths, ostiolate, 60–253 × (34–)44–113(–127) µm, 95% confidence limits = 115–126 × 70–76 µm (x ± SD = 120 ± 35 × 73 ± 17. µm, n = 140), occasionally forming a short ostiolated *neck*, 9–40 (–77) × 18–49 µm, 95% confidence limits = 17–24 × 28–32 µm (x ± SD = 20 ± 12 × 30 ± 7.5 µm, n = 50). *Ostioles* 1–2. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 4–7(–9) layers, outer layer brown pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to dolliform, 2.9–6.3 × 1.5–3 µm. *Conidia* cylindrical to allantoid, straight or slightly curved, rounded at the ends, smooth- and thin-walled, aseptate, with several distinct guttules, 2.5–5 × 0.5–1.75 µm, 95% confidence limits = 3.5–3.6 × 1–1.1 µm (x ± SD = 3.5 ± 0.5 × 1 ± 0.2 µm, n = 170). Conidial matrix cream to buff. *Sexual morph* and *chlamydospores* not observed.

Culture characteristics: Colonies on PDA reaching 37 mm diam. after 14 days of incubation at 25 ± 0.5 °C and 30 ± 0.5 °C, circular to ovoid with filiform margin, olivaceous black to dull black with greenish grey in the margin, floccose, often zonate with sectors; reverse olivaceous greenish grey, paler towards margin, with colourless concentric and sectoring lines.

Additional material examined: Iran, Khuzestan Province, Izeh, isolated from stem canker of *Crataegus* sp. (*Rosaceae*), Jun. 2022, P. Eisvand (SCUA-Is-A10-2).

Notes: *Nothocucurbitaria izehica* is phylogenetically closely related to *N. prunicola* (MLBS 100%, MPBS 100%, BPP 1.0). Nucleotide comparison of these species revealed a difference of 0.1.6% (7/434 bp) in the ITS region, 6% (20/329 bp) in *TUB2* and 4.4% (31/700 bp) in *RPB2*. Morphologically, *N. izehica* can be easily distinguished from *N. prunicola* by having a short ostiolated neck in some pycnidia and more elongated conidia (2.5–5 × 0.5–1.75 µm vs. (2–)3–3.5(–4) × 1.5(–2) µm). No ascomatal structures were observed on the collected specimens of this species and other new species *Parafenestella quercicola*. All attempts mentioned above to induce ascomatal production by these species on culture medium (supplemented with starch, peptone, malt, filter paper, and leaf and stem parts of several trees) failed, even after 2 months of incubation.

Nothocucurbitaria prunicola (Crous & Akulov) M. Mehrabi-Koushki & Eisvand, **comb. nov.**

MycoBank: MB 849864.

Basionym: *Neocucurbitaria prunicola* Crous & Akulov, Fungal Systematics and Evolution 3: 91. 2019.

Description: Crous et al. (2019a).

Typus: Ukraine, Ternopil region, Dniester Canyon N.P., forest, fallen twigs of *Prunus padus* L. (= *Padus avium*) (*Rosaceae*), 6 Oct. 2016, *A. Akulov* (holotype, CBS H-23824, ex-type culture CPC 33709 = CBS 145033).

Notes: This species, originally described by Crous et al. (2019a), was first placed in the genus *Neocucurbitaria* based on its phoma-like asexual morph and its phylogenetic relationship with other members of *Cucurbitariaceae*. Subsequently, it was transferred to *Allocucurbitaria* based on phylogenetic analyses of four loci including ITS, LSU, *TUB2* and *RPB2* (Magaña-Dueñas et al. 2021). However, more representative strains of all known genera in *Cucurbitariaceae* were used in the present phylogenetic analyses, in which this species clusters with new genus *Nothocucurbitaria*. Accordingly, this species was treated as *N. prunicola.*

Parafenestella quercicola Eisvand & M. Mehrabi-Koushki, **sp. nov.** (Fig. 3).

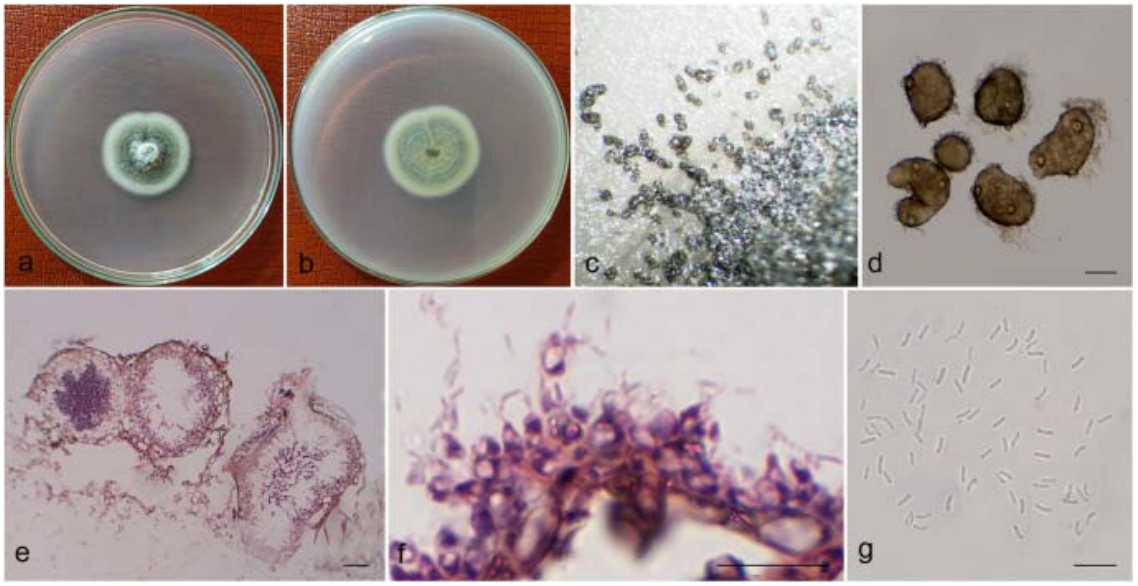

Fig. 3. *Parafenestella quercicola* (IRAN 4857C). **a**, **b** Colony on PDA after 14 days at 25 °C (**a** top, **b** reverse). **c**, **d** Pycnidia. **e** Section of pycnidium. **f** Conidiogenous cells. **g** Conidia. Scale bars: **d** = 100 µm, **e** = 20 µm, **f**, **g** = 10 µm

MycoBank: MB 849865

Typus: Iran, Khuzestan Province, Izeh, isolated from leaf spot of *Quercus brantii* Lindl. (*Fagaceae*), May. 2022, P. Eisvand (holotype, IRAN 18299 F; ex-type cultures, IRAN 4857C = SCUA-Is-B1).

Etymology: Name refers to the host genus *Quercus* from which it was isolated.

Morphology on PDA: *Conidiomata* pycnidial, formed after a week of incubation, immersed, semi-immersed or superficial, rarely on aerial mycelium, solitary or aggregated in clusters of 2 to several pycnidia, pale brown to brown, globose, sub-globose or ellipsoidal, sometimes irregular in shape, with some hyphal outgrowths, rarely glabrous, ostiolate, 43–184(–285) × 39–142(–161) µm, 95% confidence limits = 106–123 × 77–87 µm (x ± SD = 114 ± 40 × 82 ± 24 µm, n = 60), mostly forming a short ostiolated neck, 14–42 × 23–61 µm, 95% confidence limits = 25–29 × 34–38 µm (x ± SD = 27 ± 7 × 36 ± 8 µm, n = 60). *Ostioles* 1–2(–4), non-

papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 2–3(–5) layers, thin-walled, outer layer more pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform or lageniform, 3.5–5.5 × 1.8–3.3 µm. *Conidia* cylindrical to allantoid, straight or slightly curved, rounded at both ends, hyaline to grey, smooth- and thin-walled, aseptate (1.6–)2.3–4.6 × 0.5–1.4(–1.7) µm, 95% confidence limits = 3.4–3.5 × 0.88–0.95 µm (x ± SD = 3.4 ± 0.47 × 0.9 ± 0.2 µm, n = 130). Conidial matrix whitish to grey. *Sexual morph* and *chlamydospores* not observed.

Culture characteristics: Colonies on PDA reaching 31 mm diam. after 14 days of incubation at 25 ± 0.5 °C, and 30 mm diam at 30 ± 0.5 °C, circular with filiform margin, dull green to greenish glaucous, with age becoming darker at the centre due to the forming of concentric rings of sporulation, floccose; reverse greenish glaucous with paler margin, with light and dark concentric zones.

Additional material examined: Iran, Khuzestan Province, Izeh, isolated from leaf spot of *Quercus brantii* Lindl. (*Fagaceae)*, May 2022, P. Eisvand (SCUA-Is-B1-2).

Notes: *Parafenestella quercicola* is closely related to *P. tetratrupha* (Berk. & Broome) Jaklitsch & Voglmayr (MLBS 70%, MPBS 76%, BPP 0.92). A nucleotide comparison of these two species revealed a difference of 0.5% (2/431 bp) in the ITS region, 5.5% (18/330 bp) in *TUB2* and 2.4% (10/409 bp) in *TEF1α*. Morphologically, *Parafenestella quercicola* can be distinguished from *P. tetratrupha* by its less globose and smaller pycnidia (43–184 × 39–142 µm vs. 100–200 µm) having a short ostiolated neck, smaller conidiogenous cells (3.5–5.5 × 1.8–3.3 µm vs. (4.5–6.7 × 2.2–4 µm), and slightly curved conidia with rounded ends.

Discussion

In this study, we identified 12 isolates belonging to the family *Cucurbitariaceae* which were isolated from disease symptoms in two genera of forest trees in Khuzestan province, Iran. The family *Cucurbitariaceae* was reassessed using phylogenetic analyses based on DNA sequences from five nuclear regions (ITS, LSU, *TUB2*, *TEF1α*, and *RPB2*) to clarify generic and species concepts in this family. These genomic regions have been shown to be suitable to reflect phylogenetic relationships and delimit genera and species within *Cucurbitariaceae* (Wanasinghe et al. 2017; Jaklitsch et al. 2018; Valenzuela-Lopez et al. 2018; Jaklitsch and Voglmayr 2020; Su et al. 2022). The major clades of phylogenetic trees (Fig. 1) constructed in this study showed the same overall topology with those obtained in previous studies. Isolates in the present study were shown to represents one new genus, *Nothocucurbitaria*, and three new species, *Allocucurbitaria galinsogisoli*, *Nothocucurbitaria izehica*, and *Parafenestella quercicola*. Furthermore, the genus *Allocucurbitaria* was emended to accommodate the genus *Seltsamia* which clustered with the type species of *Allocucurbitaria*. Similarly, in phylogenetic analyses performed by Jaklitsch et al. (2018), the type species of both genera *Allocucurbitaria* and *Seltsamia* grouped in a strongly supported clade, but *Seltsamia* was introduced as a new genus based on morphological differences. The use of more strains of these two genera in the phylogenetic analyses showed that they should be synonymised. Accordingly, *S. ulmi* was recombined into *Allocucurbitaria*. Furthermore, *Allocucurbitaria prunicola* grouped in the new genus *Nothocucurbitaria*, and *Parafenestella pittospori* in the genus *Neocucurbitaria*, for which new combinations were introduced.

Comparative analysis of single-locus trees (not shown) revealed that the ITS and LSU phylogeny showed a low resolution at both generic and species level. The ITS phylogeny was

able to separate seven of the 11 generic lineages with sufficient support, including *Allocucurbitaria*, *Astragalicola*, *Cucurbitaria*, *Fenestella*, *Nothocucurbitaria*, *Paracucurbitaria*, and *Protofenestella*. The LSU phylogeny was only able to distinguish three generic lineages, including *Astragalicola*, *Cucurbitaria*, and *Paracucurbitaria*. The *TUB2* phylogeny was only able to distinguish nine of the 11 generic lineages and lacked resolution for *Allocucurbitaria* and *Neocucurbitaria*. The *TEF1α* tree was not able to distinguish the genera *Neocucurbitaria* and *Synfenestella* from other generic lineages in *Cucurbitariaceae*. The *RPB2* tree displayed the best resolution at the generic and species level among these genomic regions. This phylogenetic tree was able to distinguish all generic and most species lineages in *Cucurbitariaceae* (Fig. 1). Previous studies have been shown that the *RPB2* phylogeny results in much higher bootstrap and posterior probabilities support for most taxa of *Ascomycota* from class to species level (Liu et al. 1999; Chen et al. 2015; Crous et al. 2021). Because *RPB2* is a single-copy gene with a large size and modest rate of evolutionary changes, it generates a high phylogenetic resolution to distinguish different taxa of *Ascomycota* (Liu et al. 1999). However, there were some gaps in sequence data for some published species represented in our phylogenetic analyses, and thus, we could not include all of them in our analyses.

In present study, *Nothocucurbitaria izehica* was isolated from necrotic tissues of stem cankers in *Crataegus* sp. Species of *Crataegus* were reported to be host of several fenestelloid fungi from the family *Cucurbitariaceae*, including *Fenestella crataegi* (Niessl) Jaklitsch & Voglmayr, *Parafenestella austriaca* Jaklitsch & Voglmayr, and *P. rosacearum* Jaklitsch & Voglmayr on *C. monogyna* Jacq. (Jaklitsch and Voglmayr 2020). However, the fenestelloid fungi are phylogenetically and morphologically distinct from other genera in *Cucurbitariaceae*, including *Nothocucurbitaria* (Jaklitsch and Voglmayr 2020). Interestingly, another species of this genus, *N. prunicola*, has also been isolated from the fallen twigs of *Prunus padus* (Crous et al. 2019a). Because of insufficient data about the pathogenicity of these species, we cannot conclude that this new genus is pathogenic. Furthermore, *Cucurbitaria* and other cucurbitaria-like fungi, including *Allocucurbitaria*, *Paracucurbitaria*, and *Neocucurbitaria*, are mainly saprobic on bark, branches, leaves, stems, twigs, and/or wood of different trees and shrubs (Bubak and Kabat 1912; Doilom et al. 2013; Jaklitsch et al. 2018; Valenzuela-Lopez et al. 2018; Crous et al. 2019a; Su et al. 2022). A small number of them are opportunistic pathogens of animals and humans, including *A. botulispora* infecting human leg (Valenzuela-Lopez et al. 2016), *N. chlamydospora* H.Y. Jung & S.Y. Lee infecting *Hygia lativentris* Motschulsky (Hong et al. 2023), *N. irregularis* Valenz-Lopez et al. infecting human arm (Valenzuela-Lopez et al. 2018), and *N. keratinophila* (Verkley et al.) Valenz.-Lopez et al. and *N. unguis-hominis* (Punith. & M.P. English) Wanas. et al. infecting the skin and nails of humans (Punithalingam and English 1975; Verkley et al. 2010). The remaining species have been isolated from plant debris in freshwater, rhizosphere, soil, and sea water (Jaklitsch et al. 2018; Zhang et al. 2019; Magaña-Dueñas et al. 2021). These reports shows that saprophytism is the most common lifestyle reported among members of cucurbitaria-like taxa to date.

Other new species introduced in this study include *P. quercicola* from leaf spots of *Quercus brantii* in Zagrosian forests of Izeh, Iran. The genus *Quercus* also hosts several other species of *Cucurbitariaceae*, namely, *F. parafenestrata* Jaklitsch & Voglmayr on *Q. robur* L. and *Q. petraea* (Matt.) Liebl., *Neocucurbitaria juglandicola* Jaklitsch & Voglmayr on *Q. rubra*, *N. quercina* (Kabát & Bubák) Wanas. et al. on *Q. robur*, *N. cava* (Schulzer) Valenz.-Lopez et al. on *Q. cerris* L. and *Q. robur* (Valenzuela-Lopez et al. 2018; Jaklitsch and Voglmayr 2020; Jankowiak et al. 2022). *Parafenestella* is one of the more species-rich genera among the members of *Cucurbitariaceae*. Most species of *Parafenestella* are chiefly fungicolous,

parasitising (or colonising) the conidiomata or ascomata of other fungi. For example, some species are reported from *Cytospora* sp. on *Salix* spp., including *P. parasalicum* Jaklitsch & Voglmayr, *P. salicis* (Rehm) Jaklitsch & Voglmayr, *P. salicum* Jaklitsch & Voglmayr, and *P. vindobonensis* Jaklitsch & Voglmayr (Jaklitsch and Voglmayr 2020). Some of them are associated with perithecial ascomata on woody plants, including *P. alpina* Jaklitsch & Voglmayr, *P. austriaca* Jaklitsch & Voglmayr, *P. germanica* Jaklitsch & Voglmayr, *P. pseudoplatani* Jaklitsch & Voglmayr, *P. pseudosalicis* Jaklitsch & Voglmayr, and *P. rosacearum* (Jaklitsch et al. 2018; Jaklitsch and Voglmayr 2020). In addition, some species of *Parafenestella* were reported to be saprobic on dead stems and twigs, including *P. faberi* (J. Kunze ex Sacc.) Jaklitsch & Voglmayr (syn. *Fenestella mackenziei* Wanas. et al.) on *Rosa canina* L., *P. ostryae* (Wanas. et al.) Jaklitsch & Voglmayr on *Ostrya carpinifolia* Scop., *P. ulmi* W.X. Su et al. and *P. ulmicola* W.X. Su et al. on *Ulmus pumila* L., and *P. ontariensis* Ilyukhin & Markovsk. on *Acer negundo* L. (Wanasinghe et al. 2017; Jaklitsch and Voglmayr 2020; Ilyukhin et al. 2022; Su et al. 2022). Other fenestella-like fungi, including *Cucitella*, *Fenestella* and *Protofenestella*, have a similar lifestyle (Jaklitsch et al. 2018; Jaklitsch and Voglmayr 2020). All species colonise *Cytospora* spp. on different trees except the species *Cucitella opali* Jaklitsch & Voglmayr, *Fenestella fenestrata* (Berk. & Broome) J. Schröt., and *Protofenestella ulmi* Jaklitsch & Voglmayr which have been directly isolated from branches and twigs of *Acer opalus* Mill., *Alnus glutinosa* (L.) Gaertn., and *Ulmus* spp., respectively (Jaklitsch et al. 2018). These reports indicate that mycoparasitism and saprophytism are possibly a common lifestyle in fenestella-like fungi.

Some genera and species related to the family *Cucurbitariaceae* do not have DNA sequence data in GenBank, and therefore, their phylogeny and taxonomy remains unconfirmed. More molecular data and representative cultures of *Cucurbitariaceae* and allied taxa are needed to truly elucidate the taxonomy of the genera and species in this family and among all related taxa within *Pleosporales*. Given that the new species were isolated from plants exhibiting disease symptoms, their pathogenicity and ecology should be further investigated to clarify whether they are plant pathogens.

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Contributions

Payam Eisvand carried out sample preparation, fungal isolation and purification, morphometric and morphological determination, DNA isolation, PCR amplification, and contribution to describe the novel species. Mehdi Mehrabi-Koushki carried out the design and implementation of the research, DNA and phylogenetic analyses, and the writing of the manuscript. Pedro W. Crous contributed to the writing of the manuscript.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Competing interests

The authors declare no competing interests.

Data availability

New sequences generated in the current study are deposited in NCBI GenBank and new species in MycoBank. Combined DNA dataset used for phylogenetic analyses is included in a supplementary file.

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