

# Description of *Allocanariomyces* and *Parachaetomium*, two new genera, and *Achaetomium aegilopis* sp. nov. in the *Chaetomiaceae*

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## Research

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# Abstract

We describe *Allocanariomyces tritici* gen et sp. nov. and *Achaetomium aegilopis* sp. nov. as seed endophytes of wheat and its poaceous relatives in the west and northwestern provinces of Iran using morphological traits and sequences of ITS region, partial LSU rDNA,  $\beta$ -tubulin and the second largest subunit of DNA-directed RNA polymerase II genes. *Chaetomium iranianum*, *C. truncatulum* and *C. carinthiacum* are also combined here under the new genus, *Parachaetomium*. *Allocanariomyces* is differentiated from the closest genus, *Canariomyces* by having solitary, glabrous perithecia with walls of *textura epidermoidea*, stalked asci, densely granular-ornamented ascospores with a distinct subapical germ pore, and producing only solitary conidia. *Parachaetomium* has fusiform or navicular ascospores not bilaterally flattened, compared to *Chaetomium* with limoniform to globose, bilaterally flattened ascospores. *Achaetomium aegilopis* is mainly distinguished from *A. strumarium*, the closest relative, by possessing brown, often scattered perithecia, hyaline perithecial hairs covered with many hyaline crystals, hyaline chlamydospores, and lacking of the asexual morph.

## Introduction

The family *Chaetomiaceae* was introduced by Winter (1885), as Chaetomiea, with *Chaetomium* Kunze as the type genus. Members of this family occur worldwide and live as saprobes on various substrates including dung, seeds, paper, plant debris, soil, air and wood (Wang et al. 2016a, b). Endophytic, parasitic (Violi et al. 2007), and mycoparasitic (Marin-Felix et al. 2015) representatives have also been reported. Furthermore, some species have been found as human opportunistic pathogens (Abbott et al. 1995; de Hoog et al. 2013; Ahmed et al. 2015).

The family *Chaetomiaceae* is mainly characterized by perithecia ostiolate or non-ostiolate, solitary to gregarious, superficial or immersed, mostly covered with hair/setae, rarely glabrous; asci clavate to cylindrical, pedicellate, 4–8-spored, unitunicate, evanescent; ascospores brown to black, and opaque when mature, ellipsoidal, globose, subglobose, oval, fusiform or triangular, aseptate, with thick, smooth walls, and single or sometimes two germ pores (Maharachchikumbura et al. 2016). Several asexual morphs are linked to the *Chaetomiaceae*, including acremonium-like, botryotrichum-like, chrysonilia-like, chrysosporium-like, humicola-like, myceliophthora-like, scytalidium-like and trichocladium-like (Asgari and Zare 2011; Cannon 1986; Wang et al. 2016a, b; 2019a, b).

The *Chaetomiaceae* historically was placed in the *Chaetomiales* by Ames (1963), Alexopoulos (1962) and Mukerji (1968). The family then was transferred to the order *Sphaeriales* by Barr (1976) and Müller and von Arx (1973), while Hawksworth and Wells (1973) placed it in *Sordariales*. Subsequent molecular phylogenetic studies indicate *Chaetomiaceae* belongs to the order *Sordariales* (Lee and Hanlin 1999; Zhang et al. 2006; Lumbsch and Huhndorf 2010).

Morphological characteristics of perithecia, hair/setae, asci, ascospores, anamorphs, cultures and some physiological traits have been used to delimit members of the *Chaetomiaceae* (von Arx et al. 1984; Asgari and Zare 2011; Wang et al. 2016a; 2019a, b). However, these features may show considerable variation within the established taxa, making the taxonomy of these fungi unsatisfactory. Several molecular approaches have been applied to address this problem. Wang et al. (2016b) re-evaluated generic and species concepts within *Chaetomium globosum* Kunze species complex based on phylogenetic inference from six loci and morphological characters. They resurrected six species that had been treated as synonyms of *C. globosum* by von Arx et al. (1986). Furthermore, the genus *Chaetomidium* was rejected. Based on the phylogenetic analyses together with morphological studies, Wijayawardene et al. (2017) recognized 24 genera within the *Chaetomiaceae*. Wang et al. (2016a; 2019a, b) additionally expanded the *Chaetomiaceae*, and proposed several new genera. They also restricted the genus

*Thielavia* to its type species, *T. basicola*, and transferred it to the *Ceratostomataceae* (*Melanosporales*). Greif et al. (2009) investigated taxonomy of the genus *Chaetomidium* using LSU, *tub2* and *rpb2* sequence data. The results of their analyses showed that *Chaetomidium* is polyphyletic.

In an investigation on fungal endophytes of wheat and its relatives (*Poaceae*) in the west and northwestern provinces of Iran, 2018–2019, three strains belonging to the *Chaetomiaceae* were isolated. Based on morphological characteristics and multilocus phylogeny, a new genus, *Allocanariomyces*, is established and a new species of *Achaetomium* is described. Two species of *Chaetomium* previously described by Asgari and Zare (2011) from northwestern provinces of Iran, *C. iranianum* (on *Hordeum vulgare* leaves) and *C. truncatulum* (on *Heterodera schachtii* cysts), and *C. carinthiacum* (Sörgel 1961; von Arx et al. 1986) are combined here under the new genus, *Parachaetomium*.

## Methods

### Isolation and identification

Fungal isolates were obtained from seeds of wheat (*Triticum aestivum*) and its wild relatives (*Triticum boeoticum* and *Aegilops triuncialis*) collected from west and northwestern provinces of Iran, 2018–2019. The spikelets were detached from symptomless plants and immediately placed in paper bags, labeled, and transferred to the laboratory. Seeds were manually separated from each spikelet and fungal endophytes were recovered from the seeds using a surface-sterilization technique described by Florea et al. (2015). Seeds were surface sterilized with 50% H<sub>2</sub>SO<sub>4</sub> for 30 min and 2% sodium hypochlorite for 20 min, respectively, each followed by three times rinsing in sterile water. After draining of seed samples on sterile filter paper, they were placed on potato dextrose agar (PDA, Merck, Germany) plates containing 150 mg/l of each penicillin G (Jiangxi Dongfeng Pharmaceutical Co., Ltd., China) and streptomycin sulfate, Sigma-Aldrich, Inc., USA). The plates were sealed, incubated for two months at 25 °C, and examined periodically for growth of fungal endophytes. Potato carrot agar (PCA; Domsch et al. 2007) was used to induce fungal sporulation. Single-ascospore cultures were obtained by serial dilutions and transferring a single germinating ascospore to a new Petri dish containing PDA.

Colony growth and characters were determined on PDA, Oatmeal agar (OA; Sigma-Aldrich, Inc., USA) and PCA at 25 °C. The microscopic characters were recorded for colonies on PCA. Microscopic features, such as shape of perithecia, perithecial hairs and ascospores, were determined in Lactic acid mounts. Ornamentations of perithecial hairs, structures of the perithecial wall, shape of asci and guttulation of ascospores were determined in water mounts. Photographs were taken using a Dino Capture 2.0 image software installed on an Olympus BH-2 microscope (Olympus, Tokyo, Japan). Macroscopic observations were carried out using an Olympus SZH stereo microscope.

Holotypes are preserved at the Fungus Reference Collection (IRAN...F) of Herbarium Ministerii Iranici Agriculturae "IRAN", Iranian Research Institute of Plant Protection (Tehran). Ex-type cultures are deposited at the Iranian Fungal Culture Collection (IRAN...C) of the "IRAN" Herbarium.

### DNA extraction, amplification and sequencing

Fresh fungal mycelium (500 mg) was scraped from the margin of a PDA plate and transferred into a 1.5 mL centrifuge tube and was ground using liquid nitrogen. DNA extraction was performed according to Liu et al. (2000). The following primers used for PCR amplification and sequencing: RPB2AM-1bf/RPB2AM-7R (Miller and Huhndorf

2005) for the second largest subunit of DNA-directed RNA polymerase II (*rpb2*) gene; ITS1/ITS4 (White et al. 1990) for the ITS region and LROR/LR3 (Rehner and Samuels 1995) for the D1/D2 domains of the LSU rDNA; Bt2a/Bt2b (Glass and Donaldson 1995) for the partial beta-tubulin (*tub2*) gene.

The PCR reaction (25  $\mu$ L) contained 1  $\mu$ L of each primer (10 pmol/ $\mu$ L, Takapouzist Inc.), 1.0  $\mu$ L genomic DNA (30 ng/ $\mu$ L), 2.5  $\mu$ L 10  $\times$  high yield PCR buffer (Jena Bioscience, Germany), 0.3  $\mu$ L *Taq* polymerase (5 units/ $\mu$ L, Jena Bioscience, Germany), 1  $\mu$ L MgCl<sub>2</sub> (25 mM), 0.5  $\mu$ L dNTPs (10 mM), and 17.7  $\mu$ L sterile distilled water. PCR amplification of all regions was carried out using a Mycycler Thermal Cycler (Bio-Rad, USA) according to Mehrabi et al. (2016) except the annealing temperature that was set at 56 °C for the *rpb2* gene. The PCR products were purified in Microsynth Company, Switzerland. The purified DNA samples were then submitted for sequencing to a capillary sequencing machine (ABI 3730XL, Applied Biosystem, Foster City, CA) of the same company.

## Sequences alignment and phylogenetic analyses

New sequences generated in this study were checked with FinchTV v. 1.4.0 (Geospiza Inc.). The alignments were obtained using MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server/index.html>) (Kato et al. 2019) and manually optimized with MEGA6 (Tamura et al. 2013). Sequences of the ITS region, partial LSU rDNA, *tub2* and *rpb2* were analyzed individually and in combination. Phylogenetic analyses were performed with Maximum Parsimony (MP) and Bayesian inference (BI) as described previously (Mehrabi et al. 2018). Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI), homoplasy index (HI), and rescaled consistency index (RC). Trees were drawn with FigTree v. 1.4.0 (Rambaut 2012). To determine whether the sequences for the four regions could be combined in one dataset, the partition homogeneity test (PHT) was applied from PAUP v4.0b10 (Swofford 2003). The sequences generated in this study were deposited in GenBank (Table 1). The finalized alignment and tree were deposited in TreeBASE (<http://treebase.org>), submission ID: 26422.

Table 1  
Isolates used in the phylogenetic analysis

Taxon	Strain	Origin	GenBank accession numbers			
			LSU	ITS	<i>tub2</i>	<i>rpb2</i>
<i>Achaetomium aegilopsis</i> (T)	IRAN 3453C	Seed of <i>Aegilops triuncialis</i> , Sanandaj, Iran	MT568844	MT568841	MT568852	-
<i>Achaetomium globosum</i> (T)	CBS 332.67	Rhizosphere, Lucknow, India	KX976695	KX976570	KX976911	KX976793
<i>Achaetomium lippiae</i> (T)	URM 7547	<i>Lippia gracilis</i> , Brazil	KY855414	KY855413	KY855412	-
<i>Achaetomium luteum</i>	CBS 544.83	Rosa stem, Lahore, Pakistan	KX976697	KX976572	KX976913	KX976795
<i>Achaetomium luteum</i>	CBS 618.68	Rhizosphere of <i>Cucurbita</i> , Delhi, India	KX976696	KX976571	KX976912	KX976794
<i>Achaetomium macrosporum</i>	CBS 532.94	Mangrove mud, Japan	KX976699	KX976574	KX976915	KX976797
<i>Achaetomium macrosporum</i> (T)	CBS 152.97	Leaf litter, Uttar Pradesh, India	KX976698	KX976573	KX976914	KX976796
<i>Achaetomium strumarium</i> (T)	CBS 333.67	Soil, Lucknow, India	AY681170	AY681204	AY681238	KC503254
<i>Allocanariomyces tritici</i>	IRAN 4014C	Seed of <i>Triticum boeoticum</i> , Hashtrud, Iran	MT568843	MT568840	MT568851	MT568846
<i>Allocanariomyces tritici</i> (T)	IRAN 3450C	Seed of <i>Triticum boeoticum</i> , Hashtrud, Iran	MT568842	MT568839	MT568850	MT568845
<i>Arcopilus aureus</i>	CBS 153.52	Virginia, USA	KX976707	KX976582	KX976924	KX976806
<i>Arcopilus cupreus</i>	CBS 560.80	Dung of moose, Mietta Hot Springs, Canada	KX976709	KX976584	KX976926	KX976808
<i>Arcopilus flavigenus</i> (T)	CBS 337.67	Soil, Johannesburg, South Africa	KX976712	KX976587	KX976929	KX976811

Sequences with underlined numbers are generated in this study, others are from GenBank. (T) = ex-type strain; (eT) = ex-epitype strain; (nT) = ex-neotype strain. CBS = Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; IRAN...C = Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran; Others are not registered abbreviations.

Taxon	Strain	Origin	GenBank accession numbers			
			LSU	ITS	<i>tub2</i>	<i>rpb2</i>
<i>Arcopilus fusiformis</i>	CBS 484.85	Dung of rodent, Newberry Mts., Nevada, USA	KX976710	KX976585	KX976927	KX976809
<i>Berkeleyomyces basicola</i>	CBS 341.33	Pathogenic on <i>Primula</i> sp., Netherlands	MK926784	MK926784	MK926884	MK876746
<i>Canariomyces arenarius</i> (T)	CBS 507.74	Desert soil, Egypt	MK926798	MK926798	MK926898	KM655438
<i>Canariomyces microsporus</i> (T)	CBS 276.74	Desert soil, Egypt	MK926799	MK926799	MK926899	MK876760
<i>Canariomyces notabilis</i> (T)	CBS 548.83	Litter of <i>Phoenix canariensis</i> , Spain	MK926802	MK926802	MK926902	MK876763
<i>Canariomyces subthermophilus</i> (T)	CBS 509.74	Desert soil, Egypt	MK926804	MK926804	MK926904	MK876764
<i>Canariomyces vonarxii</i> (T)	CBS 160.80	Dried flower of <i>Hibiscus</i> , Sudan	MK926805	MK926805	MK926905	MK876765
<i>Carteria arctostaphyli</i> (T)	CBS 229.82	<i>Arctostaphylos uva-ursi</i> , Switzerland	MK926807	MK926807	MK926907	MK876767
<i>Chaetomium angustispirale</i> (T)	CBS 137.58	<i>Fraxinus</i> sp., Tellerman forest, Russia	JN209862	JN209862	JN256141	KF001824
<i>Chaetomium cervicicola</i>	DTO 318-G6	Dust, Mexico	KX976728	KX976603	KX976945	KX976827
<i>Chaetomium citrinum</i> (T)	CBS 693.82	Rice field soil, Tochigi, Japan	KT214617	KT214587	KT214764	KT214691
<i>Chaetomium coarctatum</i> (T)	CBS 162.62	Seed of <i>Cappanula medium</i> , St. Petersburg, Russia	JN209863	JN209863	JN256142	KF001802
<i>Chaetomium cochliodes</i> (eT)	CBS 155.52	Animal dung, USA	KC109754	KC109754	KC109772	KF001811
<i>Chaetomium elatum</i>	DTO 318-H9	Dust, USA	KX976731	KX976609	KX976951	KX976830
<i>Chaetomium fimeti</i> (eT)	CBS 139034	Soil, Germany	KT214593	KT214559	KT214736	KT214663

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Taxon	Strain	Origin	GenBank accession numbers			
			LSU	ITS	<i>tub2</i>	<i>rpb2</i>
<i>Chaetomium globosum</i> (nT)	CBS 160.62	Compost, Germany	KT214596	KT214565	KT214742	KT214666
<i>Chaetomium grande</i> (T)	IRAN 1064C, CBS126780	Leaf of <i>Triticum aestivum</i> , Naghadeh, Iran	HM365253	HM365253	HM365273	-
<i>Chaetomium interruptum</i> (T)	IRAN 1278C, CBS 126660	Seed of <i>Triticum aestivum</i> , Hadishahr, Iran	HM365246	HM365246	HM365277	-
<i>Chaetomium madrasense</i> (T)	CBS 315.74	Rhizosphere of <i>Pennisetum typhoides</i> , Tamil Nadu, India	KC109751	KC109751	KC109769	KF001831
<i>Chaetomium megalocarpum</i> (eT)	CBS 149.59	Leaf of <i>Ficus carica</i> , Greece	KC109744	KC109744	KC109762	KF001828
<i>Chaetomium nozdrenkoae</i> (T)	CBS 163.62	Soil, Novosibirsk region, Russia	KT214590	KT214556	KT214733	KT214660
<i>Chaetomium olivaceum</i>	CBS 418.80A	Nilgai dung, Delhi, India	JN209914	JN209914	JN256184	KF001806
<i>Chaetomium pilosum</i> (T)	CBS 335.67	Seed of <i>Triticum aestivum</i> , Perth, Australia	FJ666356	KT214586	KT214763	FJ666387
<i>Chaetomium rectangulare</i> (T)	IRAN 1641C, CBS 126778	Leaf of <i>Hordeum vulgare</i> , Salmas, Iran	HM365239	HM365239	HM365285	-
<i>Chaetomium spirochaete</i> (eT)	CBS 730.84	Animal dung, Great Smokey Mountains, USA	JN209921	JN209921	JN256191	KF001819
<i>Chaetomium subaffine</i> (T)	CBS 637.91	Cereal, USSR	JN209929	JN209929	JN256199	KF001817
<i>Chaetomium subfimetii</i> (T)	CBS 370.66	Paper and vegetable material, Wales	FJ666354	KT214562	KT214739	FJ666385

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Taxon	Strain	Origin	GenBank accession numbers			
			LSU	ITS	<i>tub2</i>	<i>rpb2</i>
<i>Chaetomium tectifimeti</i> (T)	CBS 142032	Dust, USA	KX976737	KX976640	KX976982	KX976836
<i>Chaetomium undulatulum</i> (T)	IRAN 857C, CBS 126775	Leaf of <i>Hordeum vulgare</i> , Bonab, Iran	HM365251	HM365251	HM365279	-
<i>Chrysanthotrichum lentum</i> (T)	CBS 339.67	Soil, South Africa	MK926809	MK926809	MK926909	MK876769
<i>Chrysanthotrichum leptotentum</i> (T)	CBS 126.85	Dung of elephant, Kenya	MK926810	MK926810	MK926910	MK876770
<i>Chrysanthotrichum peruvianum</i> (T)	CBS 732.68	High mountain tundra soil, Peru	MK926812	MK926812	MK926912	MK876772
<i>Collariella bostrychodes</i>	CBS 163.73	Dung of antelope, East Africa	KX976738	KX976641	KX976983	KX976837
<i>Collariella carteri</i> (T)	CBS 128.85	Air, British Columbia, Canada	KX976742	KX976647	KX976989	KX976841
<i>Collariella causiiformis</i> (T)	CBS 792.83	Sweatband of helmet liner, Solomon Islands	KX976741	KX976646	KX976988	KX976840
<i>Collariella gracilis</i> (T)	CBS 146.60	Soil, Tsu, Mie, Japan	KX976743	KX976648	KX976990	KX976842
<i>Collariella robusta</i> (T)	CBS 551.83	Litter, Portland Parish, Jamaica	KX976747	KX976652	KX976994	KX976846
<i>Condenascus tortuosus</i>	CBS 610.97	Soil, India	MK926817	MK926817	MK926917	MK876777
<i>Corynascus novoguineensis</i> (T)	CBS 359.72	Soil, Papua New Guinea	MH872213	HQ871762	-	HQ871838
<i>Corynascus sepedonium</i> (T)	CBS 111.69	Soil, Allahabad, India	KX976777	HQ871751	KX977027	HQ871827
<i>Corynascus verrucosus</i>	CBS 137791	Soil, Great Smoky Mountains National Park, Tennessee	LK932704	LK932699	-	LK932732

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Taxon	Strain	Origin	GenBank accession numbers			
			LSU	ITS	<i>tub2</i>	<i>rpb2</i>
<i>Crassiacarpon thermophilum</i> (T)	CBS 406.69	Mushroom compost, Pennsylvania, USA; MT	KX976776	HQ871794	KX977024	HQ871815
<i>Dichotomopilus dolichotrichus</i> (T)	CBS 162.48	Great Smoky Mts., USA	HM449063	HM449049	JF772462	KX976852
<i>Dichotomopilus erectus</i> (T)	CBS 140.56	<i>Petroselinum sativum</i> , USA	HM449058	HM449044	JF772458	KX976854
<i>Dichotomopilus funicola</i> (eT)	CBS 159.52	Germany	GU563354	GU563369	JF772461	KX976856
<i>Dichotomopilus fusus</i> (T)	CBS 372.66	Leaf litter, Bataan, Costa Rica	KX976754	KX976660	KX977002	KX976859
<i>Dichotomopilus reflexus</i> (T)	CBS 157.49	Germinating seed, Toledo, Ohio, USA	HM449055	HM449051	JF772460	KX976873
<i>Madurella fahalii</i> (T)	CBS 129176	Mycetoma of a man's foot, Sudan	MK926819	MK926819	MK926919	MK876780
<i>Madurella pseudomycetomatis</i> (T)	CBS 129177	Mycetoma of a man's lower jaw, China	MK926821	MK926821	MK926921	MK876782
<i>Madurella tropicana</i> (T)	CBS 201.38	Man foot, Indonesia	MK926824	MK926824	MK926924	MK876785
<i>Melanocarpus albomyces</i>	CBS 747.70	Coal pit refuse, UK	KX976774	KX976680	KX977022	KX976887
<i>Melanocarpus albomyces</i> (T)	CBS 638.94	Chicken nest straw, Nevada, USA	KX976773	KX976679	KX977021	KX976886
<i>Melanocarpus tardus</i> (T)	CBS 541.76	Cotton jacket, Switzerland	KX976775	KX976681	KX977023	KX976888
<i>Microascus trigonosporus</i> (T)	CBS 218.31	USA	HG380436	LM652443	LM652655	DQ470908
<i>Microthielavia ovispora</i> (T)	CBS 165.75	Root of <i>Avena sativa</i> , Ukraine	MK926826	MK926826	MK926926	MK876787
<i>Myceliophthora lutea</i> (nT)	CBS 145.77	Hay, Newmarket, UK	KM655351	HQ871775	KX977026	HQ871816
<i>Myceliophthora thermophila</i>	CBS 669.85	Cellulase, USA	KX976778	HQ871767	KX977028	HQ871806

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Taxon	Strain	Origin	GenBank accession numbers			
			LSU	ITS	<i>tub2</i>	<i>rpb2</i>
<i>Ovatospora brasiliensis</i>	CBS 130174	Soil, Colombia	KX976780	KX976682	KX977030	KX976895
<i>Ovatospora medusarum</i> (T)	CBS 148.67	Soil, Zaire	KX976782	KX976684	KX977032	KX976897
<i>Ovatospora mollicella</i> (T)	CBS 583.83	Dung of spotted skunk, Washington, USA	KX976783	KX976685	KX977033	KX976898
<i>Ovatospora unipora</i> (T)	CBS 109.83	Soil, Egypt	KX976787	KX976689	KX977037	KX976902
<i>Parachaetomium carinthiacum</i>	IRAN 859C, CBS 126669	Leaf of <i>Hordeum vulgare</i> , Sarab, Iran	HM365265	HM365265	HM365299	MT568847
<i>Parachaetomium iranianum</i> (T)	IRAN 861C, CBS 126670	Leaf of <i>Hordeum vulgare</i> , Sarab, Iran	HM365257	HM365257	HM365297	MT568848
<i>Parachaetomium truncatulum</i> (T)	IRAN 918C, CBS 126782	Cyst of <i>Heterodera schachtii</i> , Urmia, Iran	HM365263	HM365263	HM365298	MT568849
<i>Stellatospora terricola</i> (T)	CBS 811.95	Paddy soil, Japan	MK926835	MK926835	MK926935	MK876797
<i>Stolonocarpus gigasporus</i> (T)	CBS 112062	Dung of <i>Camelus dromedarius</i> , Egypt	MK926836	MK926836	MK926936	MK876798
<i>Thermothielavioides terrestris</i>	CBS 492.74	Soil, Japan	MK926838	MK926838	MK926938	MK876800
<i>Thermothielavioides terrestris</i> (T)	CBS 117535	Soil, UK	MK926837	MK926837	MK926937	MK876799
<i>Trichocladium asperum</i> (eT)	CBS 903.85	Acidic soil, Germany	LT993632	LT993632	LT993713	LT993551

Sequences with underlined numbers are generated in this study, others are from GenBank. (T) = ex-type strain; (eT) = ex-epitype strain; (nT) = ex-neotype strain. CBS = Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; IRAN...C = Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran; Others are not registered abbreviations.

Taxon	Strain	Origin	GenBank accession numbers			
			LSU	ITS	<i>tub2</i>	<i>rpb2</i>
<i>Trichocladium gilmaniellae</i> (T)	CBS 388.75	Salt-marsh soil, Kuwait	LT993638	LT993638	LT993719	LT993557
<i>Trichocladium griseum</i> (nT)	CBS 119.14	Soil, Norway	LT993639	LT993639	LT993720	LT993558

Sequences with underlined numbers are generated in this study, others are from GenBank. (T) = ex-type strain; (eT) = ex-epitype strain; (nT) = ex-neotype strain. CBS = Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; IRAN...C = Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran; Others are not registered abbreviations.

## Results

To clarify the relationships of the newly described genera and species within the *Chaetomiaceae* we conducted phylogenetic analyses using sequences of LSU (1-583 bp) and ITS rDNA (584–1303 bp), *tub2* (1304–2436 bp) and *rpb2* (2437–3432 bp) individually (not shown) and combined (Figs. 1). The sequences generated in this study were aligned against sequences of members of the *Chaetomiaceae*, mostly from Wang et al. (2016a, 2019a, b) and Asgari and Zare (2011) (Table 1).

A partition homogeneity test in PAUP 4.0b10 (Swofford 2003) did not show any significant divergence ( $P = 0.1$ ), indicating that the individual datasets were congruent and produced trees with similar topology. Therefore, the four datasets were combined in a single analysis, with *Microascus trigonosporus* (CBS 218.31) and *Berkeleyomyces basicola* (CBS 341.33), as outgroups (Wang et al. 2019b). The combined dataset of ITS, LSU, *tub2* and *rpb2* contained 3432 positions, of which 1691 were constant, 551 parsimony uninformative and 1190 parsimony informative. Parsimony analysis resulted in 12 parsimonious trees of 9831 steps with a CI of 0.323, HI of 0.677, RI of 0.626 and RC of 0.202. The BI tree revealed by MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001) had the same topology of the MP tree. Therefore, the two datasets were combined in a single analysis.

Members of the *Chaetomiaceae*, included in our phylogenetic analysis of the combined ITS/LSU/*tub2*/*rpb2*, were divided into 23 genera similar to Wang et al. (2019b, Fig. 1). This analysis supports the position of *Allocanariomyces* and *Parahaetomium* as new genera and *Achaetomium aegilopsis* as a new species within the family *Chaetomiaceae*, concordant with morphological traits.

## Discussion

The four-locus phylogenetic analyses (Fig. 1) showed that *Allocanariomyces* is a monotypic genus forming a single lineage within the *Chaetomiaceae* (100%/1.00). It was grouped within the clade including members of *Canariomyces*, *Madurella* and *Stolonocarpus* (Fig. 1) (89%/0.99). *Allocanariomyces* is also morphologically similar to *Canariomyces* (von Arx 1984; Wang et al. 2019b). Both genera have non-ostiolate perithecia, fusiform ascospores, and humicola-like asexual morph. However, *Canariomyces* (Wang et al. 2019b; Hyde et al. 2013) is distinct from *Allocanariomyces* in having solitary to aggregated perithecia often covered by subhyaline to brown aerial hyphae, perithecial walls of *textura angularis*, asci without visible stalks and not granular-ornamented ascospores with a subapical or apical germ pore. Besides, in the type species of *Canariomyces*, *Can. notabilis*, conidia are often arranged in basipetal chains (von Arx 1984), while *Allocanariomyces* only produces solitary conidia.

*Stolonocarpus*, typified by *S. gigasporus* (Wang et al. 2019b), also produces non-ostiolate perithecia, but it is distinguished from *Allocanariomyces* by having perithecia covered by hypha-like, flexuous, brown hairs, perithecial wall composed of irregular, angular or elongated cells, cylindrical, stalked asci, larger (usually over 20 µm long), not granular-ornamented ascospores with an apical germ pore and by the absence of asexual morph. Species of *Madurella*, a group of etiologic fungi causing human mycetoma, often do not sporulate, grow restrictedly in culture and produce buff, cinnamon, sienna or orange exudates diffusing into the agar (see Wang et al. 2019b).

In our phylogenetic analysis (Fig. 1), ex-type strains of the new combinations, *Parachaetomium truncatulum*, *P. iranianum* and *P. carinthiacum* were grouped together and formed a well-supported monophyletic lineage (100%/1.00). This clade was clearly separated from the adjacent clade accommodating species of *Chaetomium*, *Corynascus*, *Crassicarpon*, *Myceliophthora* and *Thermothelomyces* (Fig. 1). All these genera have significantly different morphologies. Our analysis also resolved the relationship between members of *Dichotomopilus* and the above mentioned clade.

*Chaetomium*, the closest genus to *Parachaetomium*, has limoniform to globose or irregular, bilaterally flattened ascospores, with one or two (occasionally three or even four) apical, subapical or lateral germ pores (Wang et al. 2016a). *Myceliophthora*, the other genus resembling *Parachaetomium*, permanently produces asexual morph that is characterized by broadly ellipsoidal, smooth-walled conidia with a wide, truncate base (Marin-Felix et al. 2015). *Crassicarpon* produces spherical to cuneiform, smooth-walled conidia, and non-ostiolate perithecia with walls of *textura angularis* and ascospores with a germ pore at each end (Marin-Felix et al. 2015). *Corynascus* is characterized by spherical, mostly ornamented conidia, and non-ostiolate perithecia with walls of *textura epidermoidea* consisting of cells with ornamented walls and ascospores with one germ pore at each end. *Thermothelomyces* produces perithecia and conidia similar to *Corynascus*, but its ascospores have a single germ pore (Marin-Felix et al. 2015). *Dichotomopilus* is characterized by ostiolate perithecia with walls of *textura intricata* or *t. epidermoidea* in surface view, or of *textura angularis* in a few species, seta-like, dichotomously branched terminal hairs, and bilaterally flattened ascospores with an apical or sub-apical germ pore (Wang et al. 2016a).

*Achaetomium* was established by Rai et al. (1964) based on *A. globosum* as the type species. The genus is characterized by ostiolate, tomentose, globose to pyriform perithecia with walls of *textura intricata*, cylindrical asci, and opaque, dark brown, spherical, ellipsoidal to limoniform ascospores with an apical germ pore (Rodríguez et al. 2004). Wang et al. (2019a, b), using sequence data of ITS, LSU rDNA, *tub2* and *rpb2*, demonstrated that *Achaetomium* is a monophyletic lineage in the family *Chaetomiaceae*. This genus comprises 26 species (Index Fungorum 2020), from which some are transferred to other genera such as *Chaetomium*, *Subramaniula*, *Pseudothielavia* and some have been synonymized (Wang et al. 2019a, b).

*Achaetomium aegilopsis* conforms well with the genus *Achaetomium* by its morphology. This was further supported by our phylogenetic analysis of the combined ITS, LSU rDNA, *tub2* and *rpb2* sequence data (Fig. 1). All species of *Achaetomium*, included in our phylogenetic analysis (Fig. 1), were grouped to form the highly supported clade (100%/0.87), a sister to a clade including members of *Chrysanthotrichum*, *Thermothielavioides* and *Arcopilus*. This is in agreement with previous studies by Wang et al. (2019b). Although ex-type strains of *A. aegilopsis* and *A. strumarium* were grouped with high bootstrap support (99%/0.99), *A. aegilopsis* is clearly separated from *A. strumarium* by its morphology.

Based on a MegaBlast search in GenBank, the ITS and *tub2* sequences of *A. aegilopsis* has 98% (513/522) and 96% (401/419) homology to *A. strumarium* (CBS 333.67), respectively. Attempts to amplify *rpb2* gene from the ex-type culture were not successful. *Achaetomium aegilopsis* is close to *A. strumarium* in size of perithecia, asci and

ascospores, but it is distinguished from it by having brown, often scattered perithecia (pinkish brown, often aggregated in *A. strumarium*), hyaline perithecial hairs covered with many hyaline crystals (pale brown, not crystal-covered in *A. strumarium*), brown, smooth rhizoids (dark pinkish brown, usually covered with a conspicuous brown gelatinous coat in *A. strumarium*), slightly larger, often fusiform ascospores ( $11-13 \times 6-7.5 \mu\text{m}$ , fusiform to rhomboid in *A. strumarium*), hyaline chlamydospores (absent in *A. strumarium*), and lacking of the asexual morph (sporothrix-like in *A. strumarium*) (Cannon 1986).

Morphologically, *A. aegilopsis* is also different from *A. luteum* in perithecia size ( $116.2-182.6 \times 99.6-157.7 \mu\text{m}$ ), asci ( $37-40.7 \mu\text{m}$ ) and ascospores ( $8.8-10.3 \times 3.7-6.6 \mu\text{m}$ ) (Rai et al. 1964); from *A. macrosporum* in asci size ( $55-80 \times 12-19 \mu\text{m}$ ) and ascospores ( $16.5-21.5 \times 10-13.5 \mu\text{m}$ ) (Cannon 1986); from *A. globosum* in asci size ( $60-75 \times 9-14.5 \mu\text{m}$ ) and ascospores ( $9-15 \times 8-11 \mu\text{m}$ ) (Rai et al. 1964; Cannon 1986) and from *A. umbonatum* in asci size ( $45-50 \times 7.5-16.5 \mu\text{m}$ ) and ascospores ( $13.5-17 \times 9.5-11.5 \times 7-9.5 \mu\text{m}$ ) (Rodríguez et al. 2004). *Achaetomium aegilopsis* is also distinguished from another close species, *A. lippiae*, by having perithecial hairs (absent in *A. lippiae*), fusiform ascospores (limoniform in *A. lippiae*) and hyaline chlamydospores (brown in *A. lippiae*) (Crous et al. 2017).

## Taxonomy

*Allocanariomyces* Mehrabi, Asgari & Zare, **gen. nov.**

MycoBank: MB 835853

### Etymology

In reference to the morphological resemblance to *Canariomyces*

*Type species: Allocanariomyces tritici* Mehrabi, Asgari & Zare

*Diagnosis: Allocanariomyces* is closely related to *Condenascus*, *Hyalosphaerella*, *Microthielavia*, *Parathielavia* and *Pseudothielavia* in the *Chaetomiaceae*, all having glabrous, non-ostiolate perithecia and often fusiform ascospores with one apical, subapical or oblique germ pore. However, it is distinguished by its densely granular-ornamented ascospores and humicola-like asexual morph.

*Description: Sexual morph* perithecial. *Perithecia* superficial, globose to subglobose, non-translucent, solitary, non-ostiolate, glabrous, connected to the substrate by the rhizoidal hyphae. *Perithecial wall* of *textura epidermoidea* in surface view. *Asci* evanescent, spherical, ovate or pyriform, stalked, eight-spored. *Ascospores* arranged irregularly in the ascus, one-celled, brown, fusiform, densely granular-ornamented, with a distinct, subapical germ pore.

### Asexual morphs

humicola-like.

*Allocanariomyces tritici* Mehrabi, Asgari & Zare, **sp. nov.**

MycoBank: MB 835854

(Fig. 2)

### Etymology

Named after the host genus from which this fungus was isolated.

*Type:* **Iran**, *East Azerbaijan province*: Hashtrud, 37°30'17.6" N, 46°59'42.11" E, seed of *Triticum boeoticum*, 6 Sept. 2018, *M. Mehrabi* (IRAN 17711F – holotype; IRAN 3450C – ex-type culture).

*Description:* *Sexual morph* perithecial. *Perithecia* maturing within 20 d, at first hyaline, then becoming black, non-translucent, superficial, globose to subglobose, solitary, non-ostiolate, glabrous, 100–130 µm diam. *Rhizoides* poorly developed, brown, septate, up to 60 µm long and 1–2.5 µm diam. *Perithecial wall* pale brown, of *textura epidermoidea* in surface view. *Asci* spherical, ovate or pyriform, eight-spored, thin-walled, evanescent, spore-bearing part 20–36 × 18–25 µm (av. = 28 × 22 µm, n = 20), with stalks 5–10 µm long. *Ascospores* one-celled, grey-brown, fusiform, with attenuated ends, densely granular-ornamented, 13–22.8 × 9–16 µm (av. = 18 × 11.8 µm, n = 30), with a distinct, subapical germ pore.

*Humicola-like morph:* *Conidia* arising terminally or laterally from hyaline to brown aerial hyphae or short branches of hyphae up to 1 µm long, blastic, globose to pyriform, hyaline to brown, smooth, solitary, 3–9 × 3–4.5 µm (av. = 4.9 × 3.6 µm, n = 20).

*Cultures:* *Mycelium* composed of branched, septate, smooth, hyaline hyphae, partly becoming brown in advancing regions, 1.5–3.7 µm wide. *Colonies* on PCA attaining 38 mm diam. in 7 d, circular, flat, at first hyaline, becoming buff (45); reverse of the same colour. *Colonies* on PDA attaining 15 mm diam. in 7 d, circular to slightly irregular, slightly raised, wrinkled at center, glabrous, dense, often deeply immersed into the agar, buff (45); reverse of the same colour. *Colonies* on OA attaining 9 mm diam. in 7 d, circular, flat, usually fasciculate at the center and glabrous towards the periphery, greyish white; reverse buff (45).

## Ecology

Endophytic fungus isolated from *Triticum boeoticum* seeds.

## Distribution

At present, the new species is known only from Hashtrud, East Azerbaijan province, Iran.

*Additional specimen examined:* **Iran**, *East Azerbaijan province*: Hashtrud, 37°30'17.6" N, 46°59'42.11" E, seed of *Triticum boeoticum*, 6 Sept. 2018, *M. Mehrabi* (IRAN 4014C).

*Parachaetomium Mehrabi*, Asgari & Zare, **gen. nov.**

MycoBank: MB 835855

## Etymology

Name refers to a genus similar to, but different from *Chaetomium*.

*Type species:* *Parachaetomium iranianum* (Asgari & Zare) Mehrabi, Asgari & Zare

## Diagnosis

For similarities and differences of the new genus with others in the *Chaetomiaceae*, see above.

*Description:* *Perithecia* globose to subglobose or ovate, solitary, distinctly ostiolate, non-translucent, rarely exceeding 200 µm diam; *perithecial wall* of *textura intricata* or indistinct *t. angularis* in surface view. *Perithecial hairs* ranging from long, flexuous, wavy or spirally coiled to short, often arcuate, verrucose or warty, distinctly septate. Asci fasciculate, fusiform or clavate, short-stalked, eight-spored, evanescent. Ascospores arranged biserially or irregularly in the ascus, one-celled, smooth, equi- or inaequilaterally fusiform, rarely exceeding 13 µm long, with an oblique or subapical, occasionally apical germ pore.

### Asexual morphs

Absent

*Parachaetomium iranimum* (Asgari & Zare ) Mehrabi, Asgari & Zare, **comb. nov.**

MycoBank: MB 835856

*Basionym:* *Chaetomium iranimum* Asgari & Zare, *Mycologia* **103**: 877 (2011)

*Description:* Asgari and Zare (2011: 877–878)

*Parachaetomium truncatum* (Asgari & Zare ) Mehrabi, Asgari & Zare, **comb. nov.**

MycoBank: MB 835857

*Basionym:* *Chaetomium truncatum* Asgari & Zare, *Mycologia* **103**: 877 (2011)

*Description:* Asgari and Zare (2011: 877–879)

*Parachaetomium carinthiacum* (Sörgel) Mehrabi, Asgari & Zare, **comb. nov.**

MycoBank: MB 835858

*Basionym:* *Chaetomium carinthiacum* Sörgel, *Arch. Mikrobiol.* **40**: 393 (1961)

*Descriptions:* Sörgel (1961: 393) and von Arx et al. (1986: 18)

*Achaetomium aegilopsis* Mehrabi, Asgari & Zare, **sp. nov.**

MycoBank: MB 835859

(Fig. 3)

### Etymology

Named after the host genus from which this fungus was isolated.

### Diagnosis

The species is mainly characterized by opaque, brown perithecia, hyaline perithecial hairs covered with hyaline crystals, fusiform ascospores often with one small guttule, and hyaline to subhyaline chlamydospores.

*Type: Iran, Kurdistan province: Sanandaj, 35°17'24.83" N, 47°05'25.2" E, seed of Aegilops triuncialis, 6 Aug. 2018, M. Mehrabi (IRAN 17712F – holotype; IRAN 3453C – ex-type culture).*

*Description: Sexual morph perithecial. Perithecia maturing within 7 d, brown, superficial, globose to subglobose, with hyaline exudates, scattered or occasionally aggregated, ostiolate, ostiolar neck absent, tomentose, 152–200 µm diam. Rhizoids poorly developed, brown, septate, up to 50 µm long and 1–3 µm diam. Perithecial wall of textura intricata in surface view. Perithecial hairs hypha-like, hyaline, pale brown in mass, delicate, flexuous or undulate, branched, 2–3.7 µm diam, covered with many hyaline crystals. Asci cylindrical, eight-spored, thin-walled, evanescent, spore-bearing part 64–80 × 7–9 µm (av. = 70 × 8 µm, n = 20), with stalks 10–15 µm long. Ascospores arranged uniseriately in the ascus, one-celled, brown, fusiform, smooth-walled, 9.7–15 × 6–8 µm (av. = 11.7 × 7 µm, n = 30), with a distinct, apical germ pore, containing one or occasionally two small guttules. Chlamydospores hyaline to subhyaline, globose, ellipsoid, clavate, ovate or irregularly shaped, terminal and intercalary, usually two or more catenate, 7.6–14.7 × 3.7–8.4 µm (av. = 11 × 6 µm, n = 20).*

### Asexual morphs

Absent

*Cultures: Mycelium* composed of branched, septate, smooth, subhyaline hyphae, 1.6–5 µm wide. *Colonies* on PCA growing rapidly, attaining 60 mm diam. in 4 d, circular, flat, felty, with aerial mycelium, at first hyaline, becoming buff (45); reverse of the same colour. *Colonies* on PDA growing rapidly, attaining 60 mm diam. in 4 d, circular, cottony, consisting of submerged and aerial mycelium, at first subhyaline, becoming buff (45); reverse of the same colour. *Colonies* on OA at 25 °C had the same morphology of PDA.

### Ecology

Endophytic fungus isolated from *Aegilops triuncialis* seeds.

### Distribution

Currently only known from Sanandaj, Kurdistan province, Iran.

### Notes

For similarities and differences of *Achaetomium aegilopis* with other species of the genus, see above.

## Conclusions

In this study, novel taxa within the family *Chaetomiaceae*, mostly originated from the *Poaceae* in the west and northwestern provinces of Iran, are described based on morphological and molecular data (sequences of the ITS region, partial LSU rDNA,  $\beta$ -tubulin and the second largest subunit of DNA-directed RNA polymerase II genes). *Allocanariomyces tritici* gen et sp. nov. and *Achaetomium aegilopis* sp. nov. are introduced as seed endophytes of *Triticum boeoticum* and *Aegilops triuncialis*, respectively. *Chaetomium iranianum* (on *Hordeum vulgare* leaves) and *C. truncatum* (on *Heterodera schachtii* cysts), both described by Asgari and Zare (2011), and *C. carinthiacum* (Sörgel 1961; von Arx et al. 1986) are also combined here under the new genus, *Parachaetomium*. The association between morphological and molecular data is discussed.

## Abbreviations

BI: Bayesian inference; CBS: Westerdijk Fungal Biodiversity Institute; CI: Consistency index; HI: Homoplasy index; eT: Ex-epitype strain; IRAN: Herbarium Ministerii Iranici Agriculturae; MP: Maximum parsimony; nT: Ex-neotype strain; OA: Oatmeal agar; PCA: Potato carrot agar; PDA: Potato dextrose agar; RC: Rescaled consistency index; RI: Retention index; *rpb2*: The second largest subunit of DNA-directed RNA polymerase II gene; T: Ex-type strain; TL: Tree length; *tub2*: The partial beta-tubulin gene

## Declarations

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### Adherence to national and international regulations

Not applicable.

### Authors' contributions

Samples were collected by B. Asgari and M. Mehrabi. Experiments, data analysis and original draft preparation were conducted by M. Mehrabi. B. Asgari carried out data analysis and review and editing of the manuscript. R. Zare designed the research and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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### Availability of data and materials

All sequence data generated in this study (Table 1) are available at GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>). Alignments can be accessed via TreeBase (<http://www.treebase.org>).

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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## Figures

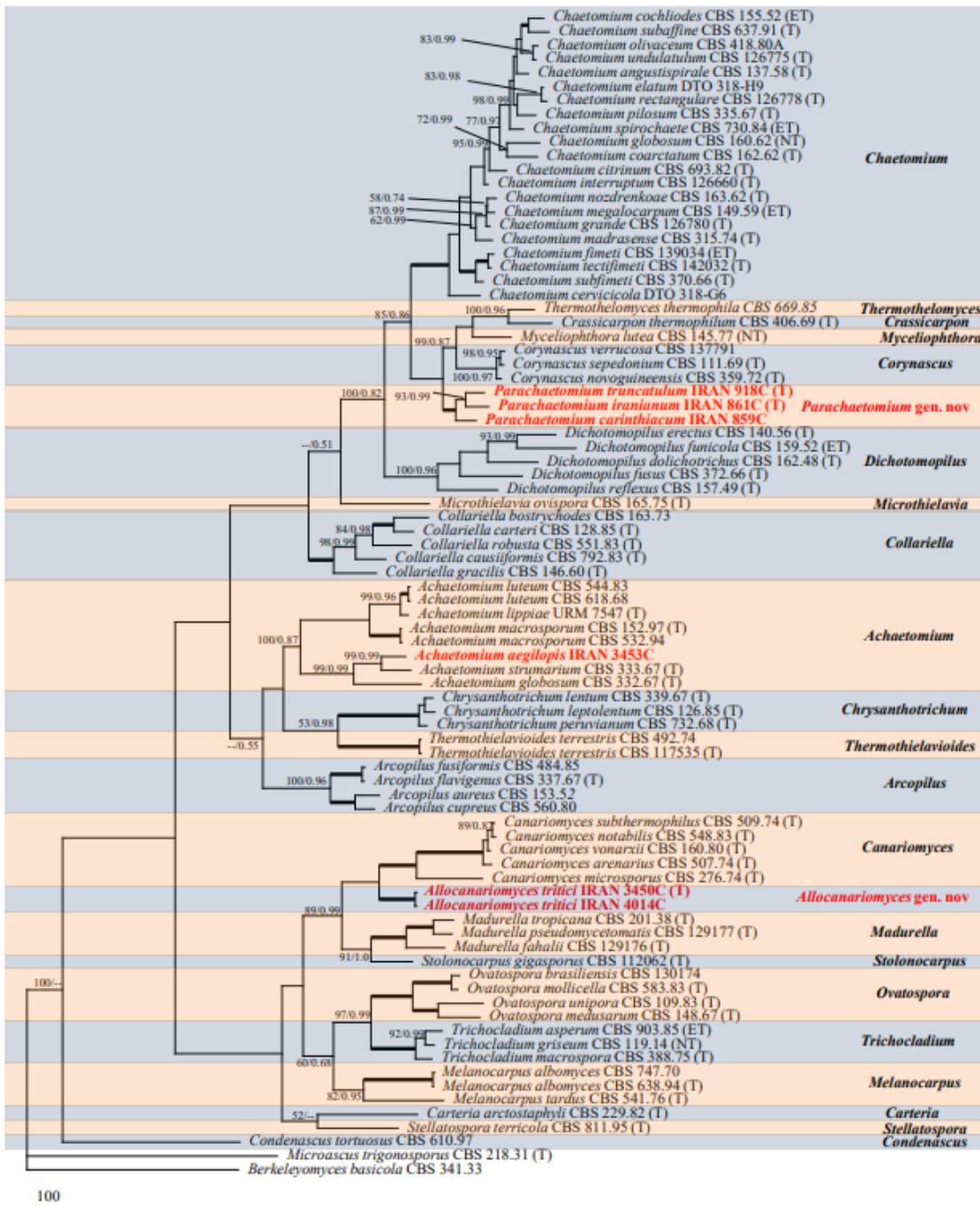
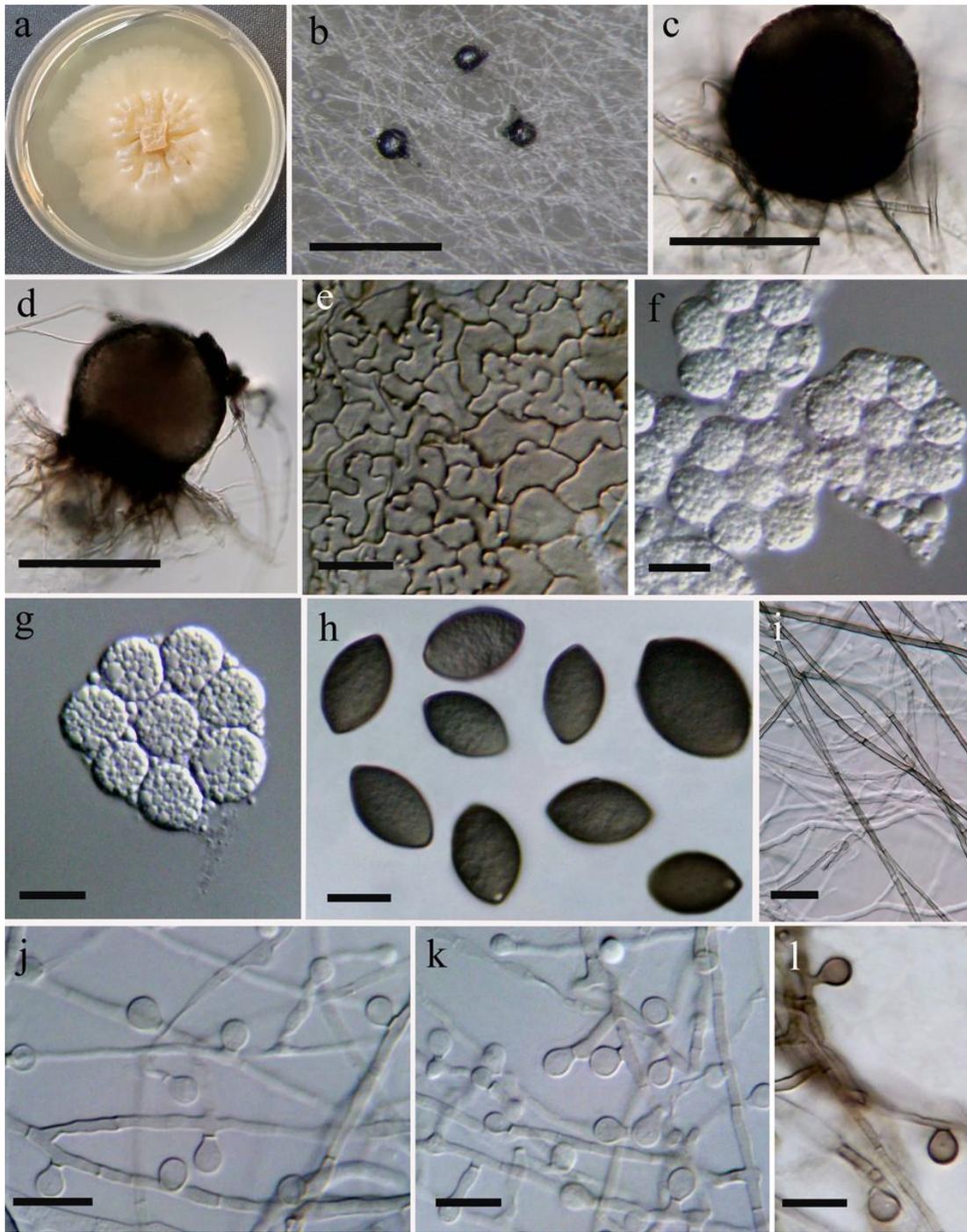


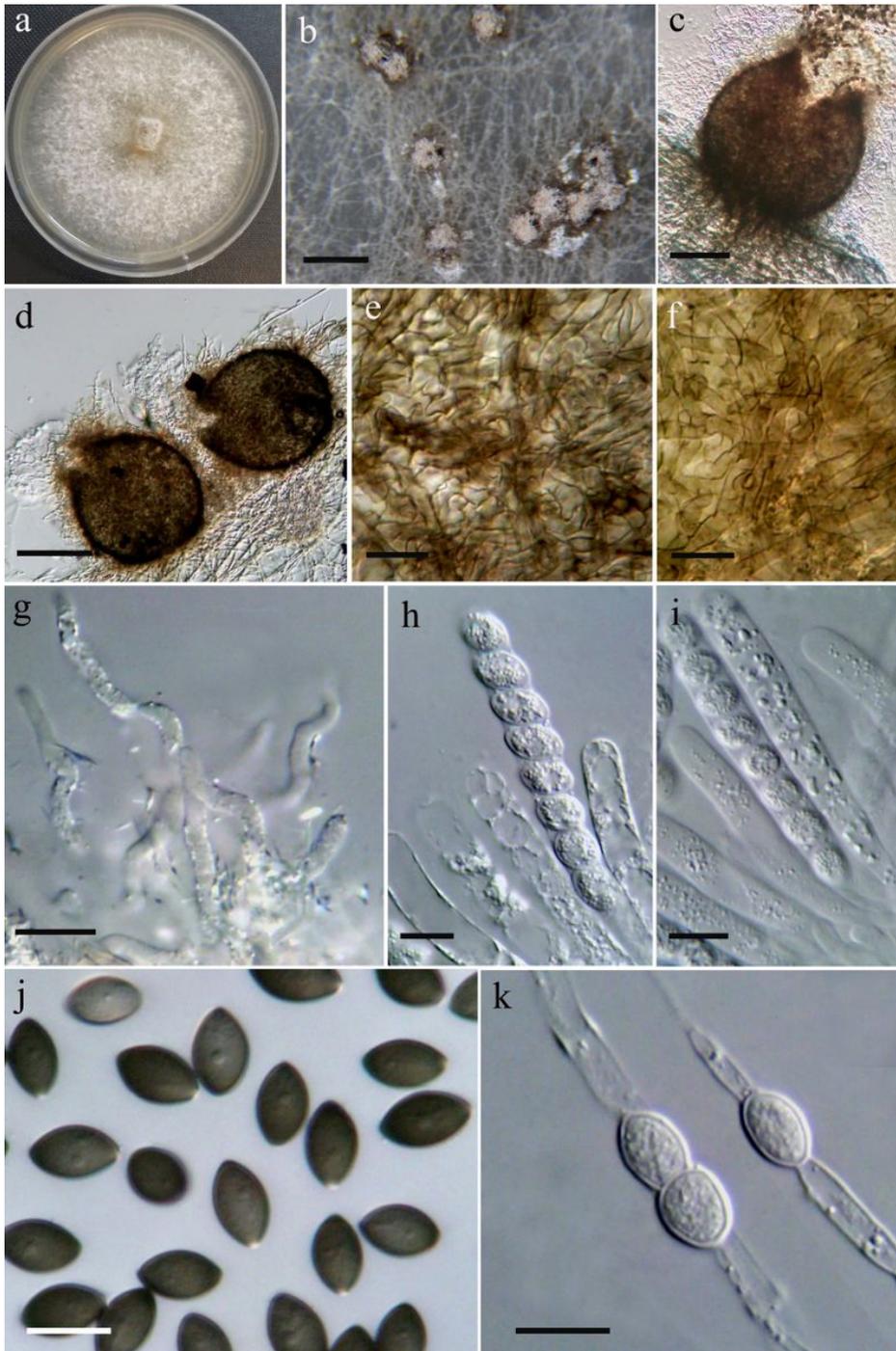
Figure 1

Consensus tree inferred from maximum parsimony analysis of the Chaetomiaceae, based on combined dataset of ITS, LSU, tub2 and rpb2. Bootstrap support values for maximum parsimony (left)  $\geq 50\%$  and Bayesian posterior probabilities (right)  $\geq 0.50$  are shown above or below the nodes. Dashes replace non-significant values ( $< 50\%$ ). Thickened branches represent 100% bootstrap support and 1.00 posterior probabilities. Newly described taxa are shown in red highlight. The tree is rooted to *Microascus trigonosporus* and *Berkeleyomyces basicola* (*Microascales*). (T) = ex-type strain, (eT) = ex-epitype strain, (nT) = ex-neotype strain



**Figure 2**

*Allocanariomyces tritici* IRAN 3450CT. a Colony on PDA after one month in 25 °C. b Part of the colony on PCA, showing mature perithecia. c–d Perithecia. e Outer surface of perithecial wall. f–g Asci. h Ascospores. i Hyaline and brown hyphae. j–l Conidia. l. Scale bars: b = 500 µm; c–d = 100 µm; e–h, j–l = 10 µm; i = 20 µm



**Figure 3**

*Achaetomium aegilopsis* IRAN 3453CT. a Colony on PDA after one week in 25 °C. b Part of the colony on PCA, showing mature perithecia. c–d Perithecia. e–f Outer surface of perithecial wall. g Perithecial hairs. h–i Asci. j Ascospores. k Chlamydospores. Scale bars: b = 500 µm; c–d = 100 µm; e–k = 10 µm