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

Mehdi Mehrabi1 · Bita Asgari1 · Rasoul Zare1

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Abstract

We describe *Allocanariomyces tritici* gen et sp. nov. and *Achaetomium aegilopis* sp. nov. as seed endophytes of *Triticum boeoticum* and *Aegilops triuncialis*, respectively, in the west and northwestern Iran using morphological traits and sequences of the internal transcribed spacer regions 1 and 2 including the intervening 5.8S nuclear ribosomal DNA (ITS), partial nuclear 28S ribosomal DNA (LSU), β-tubulin (*TUB2*), and the second-largest subunit of DNA-directed RNA polymerase II (*RPB2*) gene. *Chaetomium carinthiacum*, *C. iranianum*, and *C. truncatulum* are also combined here under the new genus, *Parachaetomium*. *Allocanariomyces* is differentiated from *Canariomyces*, its closest relative, by having solitary and glabrous ascomata, cells of the perithecial wall forming a *textura epidermoidea*, stalked asci, densely granular ascospores with a distinct subapical germ pore, and producing only solitary conidia. *Parachaetomium* is characterized by distinctly ostiolate ascomata and equi- or inequilaterally fusiform, typically less than 13-μm-long ascospores with an oblique or subapical germ pore. *Achaetomium aegilopis* is mainly distinguished from *A. strumarium*, its closest relative, by possessing brown, ascomata, hyaline ascomatal hairs covered with hyaline crystals, hyaline chlamydospores, and lacking an anamorph.

Keywords Ascomycetes · Endophytic fungi · 2 new genera · 2 new species · New combinations · Phylogeny · Sordariales · Taxonomy

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

The Chaetomiaceae was placed in the Chaetomiiales 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). Hawksworth and Wells (1973) placed the family in the Sordariales, and this classification was supported by DNA sequence-based phylogenetic analyses (Lee and Hanlin 1999; Zhang et al. 2006; Lumbsch and Huhndorf 2010).
Morphological characteristics of perithecia, hair/setae, asci, ascospores, anamorphs, and cultures and some physiological traits have been used to delimit genera and species in the Chaetomiaceae (von Arx et al. 1984; Asgari and Zare 2011; Wang et al. 2016a, 2019a, b). Because of the plasticity of these characteristics, solid taxonomic concepts were established only with the help of phylogenetic analyses. Wang et al. (2016b) reevaluated 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). 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). More recently, one additional genus was added to the family, namely, Batnamyces Nourmeur (Noumeur et al. 2020). Greif et al. (2009) investigated the taxonomy of the genus Chaetomidium using partial nuclear 28S ribosomal DNA (LSU), β-tubulin (TUB2) gene, and the second-largest subunit of the DNA-directed RNA polymerase II (RPB2) gene sequence data. The results of their analyses showed that Chaetomidium is polyphyletic. Furthermore, the genus Chaetomidium was rejected (Wang et al. 2016b).

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), C. iranianum from leaves of Hordeum vulgare L. and C. truncatulum from cysts of Heterodera schachtii Schmidt, and C. carinhiacum (Sörgel 1961; von Arx et al. 1986) are combined here under the new genus, Parachaetomium.

**Materials and methods**

**Isolation and identification**

Fungal isolates were obtained from seeds of wheat (*Triticum aestivum* L.) and its wild relatives (*Triticum boeoticum* Boiss. and *Aegilops triuncialis* L.) collected from the 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 surface-disinfected with 50% H₂SO₄ for 30 min and 2% sodium hypochlorite for 20 min as described by Florea et al. (2015). Seeds were then rinsed three times with sterile water, drained on sterile filter paper, and placed on potato dextrose agar (PDA, Merck, Germany) plates containing 150 mg/L of penicillin G (Jiangxi Dongfeng Pharmaceutical Co., Ltd., China) and streptomycin sulfate (Sigma-Aldrich, Inc., USA). The plates were sealed, incubated for 2 months at 25 °C, and examined periodically for the growth of fungal endophytes. Potato carrot agar (PCA; Domsch et al. 2007) was used to induce sporulation. Single-ascospore cultures were obtained by serial dilutions and transferring a single germinating ascospore to a new PDA plate.

Colonies growth and characters were determined on PDA, OA (Sigma-Aldrich, Inc., USA), and PCA at 25 °C. Colony colors were determined with the color charts of Rayner (1970). Microscopic characters were recorded from colonies grown on PCA. Microscopic features, such as the shape of ascomata, ascomatal hairs, and ascospores, were determined in lactic acid mounts. Ornamentations of ascomatal hairs, structures of the ascomatal wall, the shape of asci, and guttulation of ascospores were determined in water mounts (Asgari and Zare 2011). Photographs were taken using a DinoCapture 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 Ianici Agriculturae “IRAN,” Iranian Research Institute of Plant Protection (Tehran). Ex-type cultures are deposited at the Iranian Fungal Culture Collection (IRAN…C) of “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, followed by grounding in liquid nitrogen by a mini pestle. DNA extraction was performed according to Liu et al. (2000). The following primers were used for PCR amplification and sequencing: RPB2AM-1bf/RPB2AM-7R (Miller & Huhndorf 2005) for the RPB2 gene; ITS1/ITS4 (White et al. 1990) for the internal transcribed spacer regions 1 and 2 including the intervening 5.8S nuclear ribosomal DNA (ITS) and LROR/LR3 (Rehner and Samuels, 1995) for the D1/D2 domains of the LSU; and Bt2a/Bt2b (Glass and Donaldson 1995) for the partial TUB2 gene.

The PCR reaction (25 μL) contained 1 μL of each primer (10 pmol/μL, Takapouzist Inc.), 1.0 μL of genome DNA (30 ng/μL), 2.5 μL of 10× high yield PCR buffer (Jena Bioscience, Germany), 0.3 μL of *Taq* polymerase (5 units/μL, Jena Bioscience, Germany), 1 μL of MgCl₂ (25 mM), 0.5 μL of dNTPs (10 mM), and 17.7 μL of sterile distilled water.

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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 by Microsynth Company, Switzerland. The purified DNA samples were submitted for sequencing to a capillary sequencing machine (ABI 3730XL, Applied Biosystem, Foster City, CA) of the same company.

**Sequence alignment and phylogenetic analyses**

New sequences generated in this study were checked with FinchTV v. 1.4.0 (Geospiza Inc.). Separate ITS, LSU, TUB2, and RPB2 sequences were subjected to the BLAST search engine tool of NCBI for verification and selection of taxa for subsequent phylogenetic analyses. 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) (see supplementary materials, Table 1). The alignments were obtained using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh et al. 2019) and manually optimized with MEGA6 (Tamura et al. 2013). Sequences of the ITS region, partial LSU, TUB2, and RPB2 were analyzed individually and in combination. Maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI) analyses were used to estimate phylogenetic relationships.

Maximum likelihood analysis was performed with RAxML-HPC2 on XSEDE v.8.2.10 (Stamatakis 2014) on the CIPRES Science Gateway portal (Miller et al. 2010) using the GTRGAMMA substitution model. Non-parametric bootstrap iterations were run with 1000 replications. Maximum parsimony analyses were performed by PAUP* v. 4.0b10 (Swofford 2002) and Bayesian inference analyses with MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001) according to 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). For the Bayesian inference, the best evolutionary model for each locus was determined by comparing the Akaike information criterion in jModelTest 2 (Darriba et al. 2001) as new genera and Achaeonium aegilopsis as a new species within the family Chaetomiaceae, concordant with morphological traits.

**Results**

**Phylogeny**

A partition homogeneity test in PAUP v. 4.0b10 (Swofford 2002) did not show any significant divergence (P = 0.1), indicating that the individual datasets were congruent and produced trees with a similar topology. Therefore, the four datasets were combined in a single analysis, with Podospora fimicola Ces. (CBS 482.64) and Triangularia bambusae (J.F.H. Beyma) Boedijn (CBS 352.33) used as outgroups (Wang et al. 2019b). The ML analysis of the combined dataset yielded a best-scoring tree (Fig. 1) with a final ML optimization likelihood value of −47,790.561115. The matrix had 1636 distinct alignment patterns, with 23.76% of undetermined characters or gaps. Parameters for the GTRGAMMA model of the combined ITS, LSU rDNA, TUB2, and RPB2 were as follows: estimated base frequencies A = 0.228173, C = 0.284233, G = 0.281294, and T = 0.206300; substitution rates AC = 1.254869, AG = 3.400447, AT = 1.496079, CG = 1.498467, CT = 5.257741, and GT = 1.000000; gamma distribution shape parameter α = 0.297987. The phylogenetic tree obtained in this study showed similar results to previous studies (Wang et al. 2019a,b, Noumeur et al. 2020). The MP dataset contained 3057 positions, of which 1601 were constant, 240 parsimony uninformative, and 1216 parsimony informative. Parsimony analysis resulted in a single most parsimonious tree of 10,547 steps with a CI of 0.270, HI of 0.730, RI of 0.558, and RC of 0.150. For BI, the GTR+I+G model was selected as optimal for ITS, LSU, TUB2, and RPB2 based on the result of the jModelTest. The BI tree resulted in 20,002 trees after 1,000,000 generations. The first 5000 trees, representing the burn-in phase of the analysis, were discarded, while the remaining 15,002 trees were used for calculating posterior probabilities in the majority rule consensus tree. The BI and MP trees had the same topology as the ML tree. Therefore, the information obtained from the three analyses were combined, and the tree from ML analysis is illustrated (Fig. 1).

Members of the Chaetomiaceae, included in our phylogenetic analyses, were divided into 39 genera similar to Wang et al. (2019b) (Fig. 1). This analysis supports the position of Allocanariomyces and Parachaeotium as new genera and Stolonocarpus in having non-ostiolate perithecia and often fusiform

**Taxonomy**

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

*MycoBank*: MB 835853

**Etymology**: In reference to the morphological resemblance to Canariomyces.

**Diagnosis**: Reminisce Canariomyces and Stolonocarpus in having non-ostiolate perithecia and often fusiform
| Taxon                        | Strain   | Origin                                      | GenBank accession numbers | References                        |
|-----------------------------|----------|---------------------------------------------|---------------------------|-----------------------------------|
| Achaetomium aegilopis (T)   | IRAN 3453C | Seed of Aegilops triuncialis, Sanandaj, Iran | MT568844 MT568841 MT568852 – | This study                        |
| Achaetomium globosum (T)    | CBS 332.67 | Rhizosphere, Lucknow, India                | KX976695 KX976570 KX976911 – | Wang et al. 2016a                   |
| Achaetomium lippiae (T)     | URM 7547  | Lipia gracilis, Brazil                      | KY855414 KY855413 KY855412 – | Crous et al. 2017                   |
| Achaetomium luteum (T)      | CBS 544.83 | Rosa stem, Lahore, Pakistan                | KX976697 KX976572 KX976913 KX976795 | Wang et al. 2016a                   |
| Achaetomium luteum (T)      | CBS 618.68 | Rhizosphere of Cucurbita sp., Delhi, India  | KX976696 KX976571 KX976912 KX976794 | Wang et al. 2016a                   |
| Achaetomium macrosporum (T) | CBS 532.94 | Mangrove mud, Japan                        | KX976699 KX976574 KX976915 KX976797 | Wang et al. 2016a                   |
| Achaetomium macrosporum (T) | CBS 152.97 | Leaf litter, Uttar Pradesh, India          | KX976698 KX976573 KX976914 KX976796 | Wang et al. 2016a                   |
| Achaetomium strumarium (T)  | CBS 333.67 | Soil, Lucknow, India                       | AY681170 AY681204 AY681238 KC503254 | Wang et al. 2016a                   |
| Acrophialophora ellipsoidea | CBS 102.61 | Soil, Belgium                               | MK926786 MK926786 MK926886 MK876748 | Wang et al. 2019b                   |
| Acrophialophora naiviana    | CBS 100.60 | Farm soil, India                           | MK926793 MK926793 MK926893 MK876755 | Wang et al. 2019b                   |
| Allocanariomyces tritici    | IRAN 4014C | Seed of Triticum boeoticum, Hashtrud, Iran | MT568843 MT568840 MT568851 MT568846 | This study                        |
| Allocanariomyces tritici    | IRAN 3450C | Seed of Triticum boeoticum, Hashtrud, Iran  | MT568842 MT568839 MT568850 MT568845 | This study                        |
| Amesia atrobrunnea          | CBS 175.84 | Tent rope, Solomon Islands                 | KX976701 KX976576 KX976918 KX976800 | Wang et al. 2016a                   |
| Arcopilus aureus            | CBS 153.52 | VA, USA                                     | KX976707 KX976582 KX976924 KX976806 | Wang et al. 2016a                   |
| Arcopilus cupreus           | CBS 560.80 | Dung of moose, Mietta Hot Springs, Canada  | KX976709 KX976584 KX976926 KX976808 | Wang et al. 2016a                   |
| Botryotrichum globosum (T)  | CBS 144474 | Root of Globularia alypum, Algeria         | MT075917 MT075917 MT075919 MT075918 | Noumeur et al. 2020                 |
| Botryotrichum piluliferum   | CBS 654.79 | Pastry, Enschede, The Netherlands          | KX976722 KX976597 KX976939 KX976821 | Wang et al. 2016a                   |
| Brachychaeta versatilea     | CBS 414.73 | Soil, Thailand                              | MK926797 MK926797 MK926897 MK876759 | Wang et al. 2016b                   |
| Canariomyces arenarius (T)  | CBS 507.74 | Desert soil, Egypt                         | MK926798 MK926798 MK926898 KM655438 | Wang et al. 2019b                   |
| Canariomyces microsporus (T)| CBS 276.74 | Desert soil, Egypt                         | MK926799 MK926799 MK926899 MK876760 | Wang et al. 2016b                   |
| Canariomyces notabilis (T)  | CBS 548.83 | Litter of Phoenix canariensis, Spain       | MK926800 MK926802 MK926902 MK876763 | Wang et al. 2016b                   |
| Canariomyces subthermophilus (T) | CBS 509.74 | Desert soil, Egypt                         | MK926804 MK926804 MK926904 MK876975 | Wang et al. 2016b                   |
| Canariomyces vonarxii       | CBS 160.80 | Dried flower of Hibiscus, Sudan            | MK926805 MK926805 MK926905 MK876917 | Wang et al. 2016b                   |
| Chrysanthotrichum lentum    | CBS 397.66 | Dust, USA                                   | KX976731 KX976609 KX976951 KX976830 | Wang et al. 2016a                   |
| Chrysanthotrichum lentum    | DTO 318-H9 | Dust, USA                                   | KX976731 KX976609 KX976951 KX976830 | Wang et al. 2016a                   |
| Chaetomium globosum (nT)    | CBS 160.62 | Compost, Germany                           | KT214596 KT214565 KT214742 KT214666 | Wang et al. 2016b                   |
| Chaetomium grande (T)       | IRAN 1064C, CBS126780 | Leaf of Triticum aestivum, Naghadeh, Iran | HM365253 HM365253 HM365273 – | Asgari and Zare 2011                |
| Chaetomium subfiniti (T)    | CBS 370.66 | Paper and vegetable material, Wales        | FJ666354 KT214562 KT214739 JX666385 | Wang et al. 2016b                   |
| Chaetomium undulatum (T)    | IRAN 887C, CBS 126775 | Leaf of Hordeum vulgare, Bonab, Iran   | HM365251 HM365251 HM365279 – | Asgari and Zare 2011                |
| Chrysanthotrichum leptolentum (T) | CBS 339.67 | Soil, South Africa                         | MK926809 MK926809 MK926809 MK926809 | Wang et al. 2016b                   |
| Chrysanthotrichum leptolentum (T) | CBS 126.85 | Dung of elephant, Kenya                     | MK926810 MK926810 MK926910 MK926910 | Wang et al. 2016b                   |
| Chrysanthotrichum leptolentum (T) | CBS 732.71 | Dung of deer, India                         | MK926813 MK926813 MK926913 MK926913 | Wang et al. 2016b                   |
| Collariella bostrychodes    | CBS 163.73 | Dung of antelope, East Africa              | KX976738 KX976641 KX976983 KX976837 | Wang et al. 2016a                   |
| Collariella carteri (T)     | CBS 128.85 | Air, British Columbia, Canada              | KX976742 KX976647 KX976989 KX976841 | Wang et al. 2016a                   |
| Condenascus tortuosus       | CBS 610.97 | Soil, India                                 | MK926817 MK926817 MK926917 MK926917 | Wang et al. 2016b                   |
| Corynascella hisicola       | CBS 379.74 | Soil, Piedmont, NC, USA                    | KX976752 KX976657 KX976999 KX976851 | Wang et al. 2016a                   |
| Corynascella hisicola (T)   | CBS 337.72 | Soil, Piedmont, NC, USA                    | KX976751 KX976656 KX976998 KX976850 | Wang et al. 2016a                   |
| Taxon                                           | Strain      | Origin                          | GenBank accession numbers | References                                    |
|------------------------------------------------|-------------|---------------------------------|---------------------------|-----------------------------------------------|
| *Corynascus novoguineensis* (T)                | CBS 359.72  | Soil, Papua New Guinea          | MH872213 HQ871762 –       | van den Brink et al. 2012, Vu et al. 2019    |
| *Corynascus sepedonium*                       | CBS 111.69  | Soil, Allahabad, India          | KX976777 KX977027 KX977024| van den Brink et al. 2012, Wang et al. 2016a|
| *Crassicarpon thermophilum* (T)               | CBS 406.69  | Mushroom compost, PA, USA       | KX976776 HQ871794 KX977024| van den Brink et al. 2012, Wang et al. 2016a|
| *Dichotomulopsis funicola* (eT)               | CBS 159.52  | Germany                         | GU563354 GU563369 JF772461| Wang et al. 2014, Wang et al. 2016a          |
| *Dichotomulopsis indicus* (eT)                | CGMCC 3.14184| Rhizosphere of *Panax notoginseng*, Yunnan, China | GU563360 GU563367 JF772453| van den Brink et al. 2012, Wang et al. 2016a|
| *Florophus chivorsi*                          | CBS 558.80  | Dung of moose, Canada           | MK926818 MK926818 MK926918| Wang et al. 2019b                             |
| *Humicola fuscoatra*                          | CBS 118.14  | Soil, Norway                    | KX976769 KX976675 KX977022| Wang et al. 2016a                             |
| *Humicola homopilata*                         | CBS 157.55  | Filter paper in soil, Norway    | LT993582 LT993582 LT993663| Wang et al. 2019a                             |
| *Hyposphaerella fragilis* (T)                 | CBS 456.73  | Rhizosphere of *Pennisetum typhoideum* in garden soil, India | KX976791 KX976693 KX977042| van den Brink et al. 2012, Wang et al. 2016a|
| *Madurella fahali*                            | CBS 129176  | Mycetoma of a man’s foot, Sudan | MK926819 MK926819 MK926919| Wang et al. 2019b                             |
| *Madurella tropicana*                         | CBS 201.38  | Man foot, Indonesia             | MK926824 MK926824 MK926924| Wang et al. 2019b                             |
| *Melanocarpus albomyces*                      | CBS 747.70  | Coal pit refuse, UK             | KX976774 KX976680 KX977022| Wang et al. 2016a                             |
| *Melanocarpus albomyces*                      | CBS 638.94  | Chicken nest straw, NV, USA     | KX976773 KX976679 KX977021| Wang et al. 2016a                             |
| *Microthelaeua asii*                          | CBS 165.75  | Root of *Avena sativa*, Ukraine | MK926826 MK926826 MK926926| Wang et al. 2019b                             |
| *Mycothermus thermophilum* (T)                | CBS 145.77  | Hay, Newmarket, UK              | KM655351 HQ871775 HQ977026| van den Brink et al. 2012, Wang et al. 2016a|
| *Ovatospora brasiliensis*                     | CBS 625.91  | Chicken nest straw, NV, USA     | LT993604 LT993604 LT993685| Wang et al. 2019a                             |
| *Ovatospora mollicella* (T)                   | CBS 130174  | Soil, Colombia                  | KX976780 KX976682 KX977030| Wang et al. 2016a                             |
| *Parachaeomium carinthiacum* (IRAN 889C, CBS 126669) | Leaf of *Hordeum vulgare*, Sabah, Iran | HM365265 HM365265 HM365299| Wang et al. 2016a                             |
| *Parachaeomium iranicum* (IRAN 861C, CBS 126670) | Leaf of *Hordeum vulgare*, Sabah, Iran | HM365257 HM365257 HM365297| Wang et al. 2016a                             |
| *Podospora fimicola* (eT)                     | CBS 482.64  | Dung of cow, Switzerland        | MK926862 MK926862 MK926962| Wang et al. 2019b                             |
| *Pseudothielavia arxii* (T)                   | CBS 603.97  | Soil, Chile                     | MK926830 MK926830 MK926930| Wang et al. 2019b                             |
| *Pseudothielapia terricola*                   | CBS 165.88  | Barren soil, NC, USA            | KX976792 KX976694 KX977045| Wang et al. 2019a                             |
| *Remersonia thermophil*                       | CBS 643.91  | Compost, The Netherlands        | LT993610 LT993610 LT993691| Wang et al. 2019a                             |
| *Staphylostichum coccosporum* (T)             | CBS 364.58  | Soil, Zaire                    | LT993620 LT993620 LT993701| Wang et al. 2019a                             |
| *Staphylostichum longicolium* (T)             | CBS 119.57  | Soil, Madagascar                | LT993621 LT993621 LT993702| Wang et al. 2019a                             |
| *Stellatospora terricola*                     | CBS 811.95  | Paddy soil, Japan              | MK926835 MK926835 MK926935| Wang et al. 2019b                             |
| *Stolonocarpus gigasporus* (T)                | CBS 112062  | Dung of *Cameles dromedarius*, Egypt | MK926836 MK926836 MK926936| Wang et al. 2019b                             |
| *Subramaniula asteroides* (T)                 | CBS 123294  | Keratitis of *Homo sapiens*, USA | JX280731 HQ906667 KP900703| de Hoog et al. 2013, Ahmed et al. 2016, Wang et al. 2016a|
| *Subramaniula thielavioides* (T)              | CBS 122.78  | Dung of nilgai, Delhi Zoo, India | KP970654 KP862597 KP900708 KP900670| Ahmed et al. 2016, Wang et al. 2016a|
| *Thermothelomyces thermophila*                | CBS 669.85  | Cellulase, USA                  | KX976778 HQ871767 KX977028| van den Brink et al. 2012, Wang et al. 2016a|
ascospores. Distinguished from other genera by cells of the perithecial wall arranged in a textura epidermoidea, densely granulated ascospores showing a distinct subapical germ pore, and humicola-like anamorph.

Ascomata superficial, globose to subglobose, non-translucent, solitary, non-ostiolate, glabrous, connected to the agar by rhizoidal hyphae; tissue type in ascomatal wall 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 granulate, with a distinct, subapical germ pore. Anamorph humicola-like.

Type species: Allocanariomyces tritici Mehrabi, Asgari & Zare

Notes: The four-locus phylogenetic analyses (Fig. 1) showed that Allocanariomyces is a monotypic genus forming a single lineage within the Chaetomiaceae (ML = 100%/MP = 100%/BI = 1.00). It was grouped within the clade accommodating Batnamyces, Canariomyces, Madurella, and Stolonocarpus (Fig. 1, 99%/85%/1.00). Allocanariomyces is also morphologically similar to Canariomyces (von Arx 1984; Wang et al. 2019b). Both genera have non-ostiolate perithecia, fusiform ascospores, and a humicola-like anamorph. 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 non-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 species only produce solitary conidia.

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

(Fig. 2)

MycoBank: MB 835854

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

Ascomata maturing on PCA within 20 days, at first hyaline, then becoming black, non-translucent, superficial, globose to subglobose, solitary, non-ostiolate, glabrous, 100–130 μm diam. Rhizoids poorly developed, brown, septate, up to 60 μm long and 1–2.5 μm diam. Ascomatal wall pale brown, with cells arranged in a textura epidermoidea when observed 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, gray-brown, fusiform, with attenuated ends, densely granulated, 13–22.8 × 9–16 μm (av. = 18 × 11.8 μm, n = 30), with a distinct, subapical germ pore. Conidia arising terminally or laterally from hyaline to brown aerial hyphae or short branches of hyphae up to 1 μm long.
Fig. 1 RAxML tree based on analyses of a combined dataset of ITS, LSU, TUB2, and RPB2 sequences. Bootstrap support values for ML (ML-BS) and MP (MP-BS) ≥ 50% and Bayesian posterior probabilities (PP) ≥ 0.90 are shown as ML/MP/BI above or below the nodes. The branches with full statistical support (ML-BS = 100%; MP-BS = 100%; PP = 1.0) are thickened. Newly described taxa are shown in red typeface. The scale bar shows the expected number of changes per site. The tree is rooted in *Podospora fimicola* and *Triangularia bambusae* (Podosporaceae). (T), ex-type strain; (eT), ex-epitype strain; (nT), ex-neotype strain.
blastic, globose to pyriform, hyaline to brown, smooth, solitary, 3–9 × 3–4.5 μm (av. = 4.9 × 3.6 μm, n = 20).

Culture characteristics: 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 days at 25 °C, circular, flat, at first hyaline, becoming buff (45); reverse of the same color. Colonies on PDA attaining 15 mm diam. in 7 days at 25 °C, circular to slightly irregular, slightly raised, wrinkled at the center, glabrous, dense, often deeply immersed into the agar, buff (45); reverse of the same color. Colonies on OA attaining 9 mm diam. in 7 days at 25 °C, circular, flat, usually fasciculate at the center and glabrous towards the periphery, grayish-white; reverse buff (45).

Holotype: Iran, East Azerbaijan province, Hashtrud, 37° 30′ 17.6″ N, 46° 59′ 42.11″ E, seed endophyte of Triticum boeoticum, Sept. 6, 2018, M. Mehrabi (IRAN 17711F); ex-type culture, IRAN 3450C.

Other strain examined: Iran, East Azerbaijan province, Hashtrud, 37° 30′ 17.6″ N, 46° 59′ 42.11″ E, seed endophyte of Triticum boeoticum, Sept. 6, 2018, M. Mehrabi (IRAN 4014C).

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

MycoBank: MB 835855

**Etymology:** Name refers to a genus morphologically similar to, but phylogenetically distinguishable from *Chaetomium*.

**Diagnosis:** For morphological similarities and differences between *Parachaetomium* and other genera in the Chaetomiaceae, see below.

*Ascomata* gray or olivaceous in reflected light, globose to subglobose or ovate, solitary, distinctly ostiolate, with a wide apical pore, non-translucent, typically up to 200 μm diam.; cells of ascomatal wall arranged in textura intricata, t. angularis or t. irregularis in surface view. *Ascomatal hairs* long, flexuous, wavy or regularly coiled, some short, arcuate, verrucose, spiny, or wart-like. *Asci* fasciculate, fusiform or clavate, short-stalked, eight-spored, evanescent. *Ascospores* biseriate or irregularly distributed in ascus, pale grayish- or bluish-brown to dark olivaceous-brown, one-celled, smooth, equi- or inequilaterally fusiform, typically 13 μm long, with an oblique or subapical, occasionally apical germ pore. *Anamorph* not observed.

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

Notes: In our phylogenetic analysis (Fig. 1), ex-type strains of the newly combined taxa, *Parachaetomium carinthiacum*, *P. iranianum*, and *P. truncatulum* were grouped together and formed a well-supported monophyletic lineage (98%/95%/1.00). The new genus *Parachaetomium* grouped within a clade including members of *Chaetomium*, *Corynascus*, *Crassicarpon*, *Dichotomopilus*, *Myceliophthora*, and *Thermotheleomyces* (100%/61%/1.00, Fig. 1).
Colonies on PDA growing rapidly, attaining 60 mm diam. in 4 days at 25 °C, circular, cottony, consisting of submerged and aerial mycelium, at first subhyaline, becoming buff (45); reverse of the same color. Colonies on OA at 25 °C similar as those on PDA.

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

Notes: *Achaetomium aegilopis* and *A. strumarium* have similarly sized perithecia, asci, and ascospores. *Achaetomium aegilopis* is distinguished from *A. strumarium* by its 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 × 6–7.5 μm, fusiform to rhomboid in *A. strumarium*), hyaline chlamydospores (absent in *A. strumarium*), and lack of an anamorph (sporothrix-like in *A. strumarium*) (Cannon 1986).

**Discussion**

Phylogenetic analyses based on ITS, LSU rDNA, *tub2*, and *rpb2* sequence data showed that the new genera...
Allocanariomyces and Parachaetomium were placed in distinct lineages in the family Chaetomiaceae (Fig. 1). Allocanariomyces formed a strongly supported monophyly with a lineage of Batnamyces, Canariomyces, Madurella, and Stolonocarpus. Batnamyces, typified by B. globulariicola, is distinguished by the lack of reproductive structures (Noumeur et al. 2020). Species of Madurella, a group of 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). Stolonocarpus, typified by S. gigasporus (Wang et al. 2019b), also produces non-ostiolate perithecia and fusiform ascospores, but it is distinct from Allocanariomyces in having perithecia covered by hyphae-
like, flexuous and brown hairs, perithecial walls composed of irregular and angular or elongated cells, cylindrical, stalked, and larger asci (usually over 20 μm long), not granular-ornamented ascospores with an apical germ pore and by the absence of anamorph.

**Chaetomium**, somehow resembling **Parachaetomium** in ascomatal wall anatomy and hairs, has limoniform to globose, 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**, consistently produces an anamorph that is characterized by broadly ellipsoidal, smooth-walled conidia with a wide, truncate base (Marin-Felix et al. 2015). **Crassicarpus** produces spherical to cuneiform, smooth-walled conidia, and non-ostiolate perithecia. Its ascomatal wall is composed of cells that form a textura angularis, and ascospores have a germ pore at each end (Marin-Felix et al. 2015). **Corynascus** is characterized by spherical, mostly ornamented conidia, non-ostiolate perithecia, cells of the perithecial wall with ornamented walls and arranged in a textura epidermoidea, and ascospores with one germ pore at each end. **Thermoctomyces** produces perithecia and conidia similar to **Corynascus**, but its ascospores have a single germ pore (Marin-Felix et al. 2015). **Dictotomopillus** 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 subapical 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, cells of the perithecial wall forming a textura intricata, cylindrical asci, and opaque, dark brown, spherical, ellipsoidal to limoniform ascospores with an apical germ pore (Rodriguez et al. 2004). Wang et al. (2019a, b), using sequence data of ITS, LSU rDNA, **TUB2**, and **RPB2**, demonstrated that **Achaetomium** forms a monophyletic lineage in the Chaetomiaceae. There are 26 species epithets in Index Fungorum (2020); however, some were transferred to other genera such as **Chaetomium**, **Subramanula**, and **Pseudothielavia**, and the names of some others have been synonymized with earlier described **Achaetomium** species (Wang et al. 2019a, b).

**Achaetomium aegilopis** 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 included species of **Achaetomium** grouped to form the highly supported clade (100%/100%/1.00). The inferred sister group relationship of **Achaetomium** and **Microthielavia ovispora** was, however, not statistically supported. This is in agreement with previous studies by Wang et al. (2019b) and Noumeur et al. (2020). Although ex-type strains of **A. aegilopis** and **A. strumarium** were grouped with high bootstrap and posterior probability support (100%/100%/1.00), **A. aegilopis** is clearly separated from **A. strumarium** by its morphology. Based on a MegaBlast search in GenBank, the ITS and **TUB2** sequences of **A. aegilopis** have 98% (513/522) and 96% (401/419) homology to **A. strumarium** (CBS 333.67), respectively. Attempts to amplify the **RPB2** gene from the ex-type culture of **A. aegilopis** were not successful.

Morphologically, **A. aegilopis** is different from **A. luteum** in perithecia size (116.2–182.6 × 99.6–157.7 μm), asci (37–40.7 μm), and ascospores (8.8–10.3 × 3.7–6.6 μm) (Rai et al. 1964); from **A. macrosporum** in asci size (55–80 × 12–19 μm) and ascospores (16.5–21.5 × 10–13.5 μm) (Cannon 1986); from **A. globosum** in asci size (60–75 × 9–14.5 μm) and ascospores (9–15 × 8–11 μm) (Rai et al. 1964; Cannon 1986); and from **A. umbonatum** in asci size (45–50 × 7.5–16.5 μm) and ascospores (13.5–17 × 9.5–11.5 × 7–9.5 μm) (Rodriguez et al. 2004). **Achaetomium aegilopis** is also distinguished from another closely related 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).

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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 read and approved the final manuscript.

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Data availability 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 electronic supplementary material and TreeBASE (http://www.treebase.org).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.
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