ORIGINAL ARTICLE

Phylogenetic assessment of *Chaetomium indicum* and allied species, with the introduction of three new species and epitypification of *C. funicola* and *C. indicum*

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Abstract Historically, the *Chaetomium indicum* group contained six species with similar ascus and ascospore morphology. Large morphological variation in their branched ascomatal hairs often resulted in ambiguous species delimitation. In this study, morphological characters and the maximum growth temperature (MGT) are evaluated in light of phylogenetic relationships. Multigene phylogenetic analyses with ribosomal internal transcribed spacer (ITS), partial ribosomal large subunits (28S rDNA), β-tubulin, the translation elongation factor 1α (TEF1-α), and the largest subunit of RNA polymerase II (RPB1) recognize eight well-supported lineages within the monophyletic *C. indicum* group. The eight lineages correspond to five known species and three new species described in this paper (i.e., *C. pratense*, *C. subfunicola*, and *C. ramosissimum*). *Chaetomium dolichotrichum* is resurrected, as it appears to be a distinct species from *C. funicola*. *Chaetomium cancroideum*, though phylogenetically consistent with *C. funicola*, is retained as separate because of its distinct morphology. Both *C. funicola* and *C.indicum* are epitypified to stabilize the taxonomy.

Keywords Chaetomiaceae · Phylogeny · Taxonomy

Introduction

The genus *Chaetomium* Kunze is characterized by ostiolate ascomata covered by characteristic hairs, fasciculate and often evanescent asci, and single-celled, smooth and pigmented ascospores with germ pores (von Arx et al. 1986). *Chaetomium* colonizes a variety of substrates, and contains many species with cellulolytic capacity (Lee and Hanlin 1999). A few species have been reported to cause human infection (Koch and Haneke 1965; Abbott et al. 1995; Barron et al. 2003).

More than 400 species have been described in *Chaetomium* according to the statistics of Index Fungorum. Historically, ascomatal hairs have been considered important in distinguishing *Chaetomium* species (Chivers 1915; Skolko and Groves 1948, 1953; Ames 1961; Mazzucchetti 1965; Seth 1970), which can be straight (seta-like), flexuous, arcuate, undulate, circinate, spirally coiled or variously branched. After examining the ornamentation of the hairs in detail, Hawksworth and Wells (1973) concluded that the types of hair ornamentation lacked taxonomic value. Other authors suggested that the morphology of the hairs varied with culture conditions and the age of ascomata (Tschudy 1937; von Arx et al. 1984). Millner (1977) inferred that the growth responses of *Chaetomium* to temperature were useful in identifying closely related species. Subsequently, researchers paid increasing attention to the morphology of asci, ascospores and the germ pores (Dreyfuss 1976; Millner et al. 1977; von Arx et al. 1984; Cannon 1986). In a monographic study, von Arx et al. (1986) treated a number of species as synonyms that had been proposed primarily on the basis of the differences in ascomatal hairs. Their treatments have been extensively followed (Gené and Guarro 1996; Udagawa et al. 1997; de Cock and Hennebert 1997; Rodríguez et al. 2002; Doveri 2013). At present, less than 160 species are accepted within *Chaetomium* (Kirk et al. 2008; Asgari and Zare 2011; Doveri 2013).

Members of the *C. indicum* group have dichotomously branched ascomatal hairs. The existence of numerous variant forms of the hairs have complicated the classification of

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C. indicum Corda and allied species. Based on the presence or absence of unbranched terminal hairs and the characters of the branched hairs, Skolko & Grover (1948) circumscribed and accepted *C. cancroideum* Tschudy, *C. dolichotrichum* Ames, *C. erectum* Skolko & Groves, *C. funicola* Cooke, *C. indicum* and *C. reflexum* Skolko & Groves in the group. This treatment was followed by most subsequent mycologists (Ames 1961; Mazzucchetti 1965; Seth 1970). However, von Arx et al. (1986) definitely accepted only two species: *C. indicum* and *C. funicola*. *Chaetomium indicum* was described to show typical dichotomously branched ascomatal hairs, while *C. funicola* was described to produce two types of hairs: long, seta-like or occasionally apically branched hairs, and short, repeatedly dichotomously branched hairs. Based on this classification, von Arx et al. (1986) incorporated *C. dolichotrichum* into *C. funicola*. Meanwhile, the same authors tentatively kept three intermediates as the relatives of *C. indicum*, namely *C. cancroideum* with incurved branches of hairs, *C. erectum* with erect and only apically branched hairs, and *C. reflexum* with reflexed branches of hairs. All these taxa together with *C. indicum* and *C. funicola* have asci and ascospores with similar morphologies. Asexual states have not been found in this group.

Molecular phylogenetic studies of *Chaetomium* have been conducted by several authors (Lee and Hanlin 1999; Untereiner et al. 2001; Greif et al. 2009; Asgari and Zare 2011). However, because of the limited sampling in previous studies, the phylogenetic relationships among the majority of *Chaetomium* species remain unclear, and the current circumscriptions of these *Chaetomium* species lack phylogenetic evaluation.

The objectives of the present study were to recognize phylogenetic species within the *C. indicum* group, and to determine their evolutionary relationships. Based on a larger sampling, the phylogenetic position of this group in the whole genus is estimated by a three-gene phylogeny. The study also assessed the morphological characters in light of the phylogenetic relationships, and the potential use of maximum growth temperature (MGT) for species discrimination in the *C. indicum* group.

Material and methods

Cultures and morphology

Twenty-three strains representing six putative species were selected. The isolates studied are listed in Table 1. Eight strains were obtained from the CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands (CBS), including ex-type strains of *C. erectum* (CBS 140.56), *C. dolichotrichum* (CBS 162.48, preserved as *C. funicola*) and *C. reflexum*

(CBS 157.49), and other authentic strains of *C. indicum* (CBS 212.74 and CBS 860.68), *C. funicola* (CBS 159.52) and *C. cancroideum* (CBS 154.52 and CBS 136.38). Each of these strains has been used as a basis of description for each respective species by von Arx et al (1986). Other strains were isolated in China and preserved at the China General Microbiological Culture Collection Centre in the Institute of Microbiology (CGMCC), and five strains in CBS. Dried cultures of holotypes were preserved at the Herbarium Mycologicum Instituti Microbiologici Academiae Sinicae (HMAS).

All the strains were inoculated on cornmeal (3 %) agar (CMA), and incubated in the dark at 25 °C until the ascomata matured. The features of the ascomata, ascomatal hairs, ascomal wall structures, asci, and ascospores were microscopically examined.

Maximum growth temperature (MGT)

The MGT for all strains studied was determined in two phases. The first phase provided a rough estimate of MGT (with 5 °C intervals), and the second provided a finer estimate (with 1 °C intervals). In the first phase, agar discs of 3-mm-diam were cut from the border of a 1-week-old colony of each strain grown at 25 °C on CMA. Each inoculum disc was inverted onto a 90-mm-diam Petri dish containing potato dextrose agar (PDA). The dishes were kept at 25, 30, 35, 40, and 50 °C, with three replicate dishes for each combination of strain and temperature. After 4 days, the colony diameters were measured, and the MGT that supported growth was determined. In the second phase, the inoculated dishes were kept at the maximum temperature that supported growth in the first phase, and five higher temperatures with 1 °C intervals, respectively, i.e., if the maximum temperature supporting growth was 30 °C in the first phase, dishes were kept at 30, 31, 32, 33, and 34 °C in the second phase of the experiment. Based on the results of the second phase, the MGT of each strain was determined (Table 1).

DNA extraction, PCR amplification, sequence alignment and phylogenetic analyses

Total genomic DNA was extracted from cultures using the E.Z.N.A.™ HP Fungal DNA Kit (Omega) following the manufacturer's instruction with minor modifications. PCR-amplifications were conducted in a Thermo Hybaid Px2 Thermal Cycler (Thermo Hybaid Co., US) using the following primer sets: ITS4/ITS5 for the complete ITS1/5.8S/ITS2 regions (White et al. 1990), NL1/NL4 for D1/D2 domains of 28S rDNA (O'Donnell 1993), T1 (O'Donnell and Cigelnik 1997)/T222 (Bt2b) (Glass and Donaldson 1995) for partial β-tubulin (BTUB) gene, EF983/EF2218R (AFTOL, http://aftol.

Table 1 Isolates and their sequences employed in this study

Species	Isolate code[a]	Origin/Source	MGT[b] (°C)	GenBank accession number				
				28S rDNA	ITS rDNA	β-tubulin	TEF1-α	RPB1
Chaetomium cancroideum	CBS 136.38	Unknown	36	**HM449060**	**HM449046**	**JF772457**	**KC485009**	**KC485039**
C. cancroideum	CBS 154.52	Unknown	36	JX215348	JX215348	JX215349	**KC485010**	**KC485040**
C. dolichotrichum	CBS 162.48 (**T**)	U.S.A.	37	**HM449063**	**HM449049**	**JF772462**	**KC485023**	**KC485053**
C. dolichotrichum	CGMCC 3.14188	Corn straw, Xingtai, Hebei Province	37	**HM449061**	**HM449047**	**JF772454**	**KC485024**	**KC485054**
C. dolichotrichum	CGMCC 3.14189	Discarded cloth, Longjing, Jilin Province	37	**HM449062**	**HM449048**	**JF772455**	**KC485025**	**KC485055**
C. erectum	CBS 140.56 (**T**)	*Petroselinum sativum*, USA	41	**HM449058**	**HM449044**	**JF772458**	**KC485018**	**KC485048**
C. erectum	CGMCC 3.12900	Soil, Anqiu, Shandong Province	41	KC109760	KC109760	KC109778	**KC485019**	**KC485049**
C. funicola	CBS 159.52 (**Epitype**)	Germany	37	GU563354	GU563369	**JF772461**	**KC485013**	C485043
C. funicola	CGMCC 3.9459	Discarded shoe, Taibai, Shanxi Province	37	GU563355	GU563370	**JF772445**	**KC485011**	**KC485041**
C. funicola	CGMCC 3.12918	Sheep dung, Eling Lake, Qinghai Province	37	**JX867123**	**JX867123**	**JX867120**	**KC485012**	**KC485042**
C. funicola	IRAN 1201C [b]	Straw of *Triticum aestivum*, Bilesavar, Iran, GenBank		HM365248	HM365248	HM365301		
C. funicola	IRAN 1277C [b]	Seed of *Horedum vulgare*, Kaleybar, Iran, GenBank		HM365249	HM365249	HM365302		
C. indicum	CBS 212.74	Dung of herbivore, Kenya	34	GU563356	GU563364	**JF772463**	**KC485007**	**KC485037**
C. indicum	CBS 860.68	Air, Germany	34	GU563359	GU563365	**JF772464**	**KC485008**	**KC485038**
C. indicum	CGMCC 3.14182	Rhizosphere of *Panax Notoginseng*, Wenshan county, Yunnan Province	34	GU563358	GU563366	**JF772451**	**KC485006**	**KC485036**
C. indicum	CGMCC 3.14184 (**Epitype**)	Rhizosphere of *Panax Notoginseng*, Wenshan county, Yunnan Province	34	GU563360	GU563367	**JF772453**	**KC485005**	**KC485035**
C. pratense	CGMCC 3.14181 (**T**); CBS 133396	Soil, Huangnan, Qinghai Province	34	GU563357	GU563372	**JF772450**	**KC485017**	**KC485047**
C. ramosissimum	CGMCC 3.14183 (**T**); CBS 133398	Rhizosphere of *Panax Notoginseng*, Wenshan county, Yunnan Province	41	GU563361	GU563371	**JF772452**	**KC485021**	**KC485051**
C. ramosissimum	CGMCC 3.12930	Soil, Huanggang, Hubei Province	41	**HM449059**	**HM449045**	**JF772449**	**KC485020**	**KC485050**
C. ramosissimum	CGMCC 3.12898; CBS 133934	Soil, Anqiu, Shandong Province	41	KC109759	KC109759	KC109777	**KC485022**	**KC485052**
C. reflexum	CBS 157.49 (**T**)	Germinating seed, USA	36	**HM449055**	**HM449051**	**JF772460**	**KC485027**	**KC485057**
C. reflexum	CGMCC 3.14190	Soil, Huaian, Jiangsu Province	36	**HM449054**	**HM449050**	**JF772456**	**KC485026**	**KC485056**
C. subfunicola	CGMCC 3.12892 (**T**); CBS 133397	Soil, Shihezi, Xinjiang Autonomous Region	37	**JX867125**	**JX867125**	**JX867122**	**KC485014**	**KC485044**
C. subfunicola	CGMCC 3.9466; CBS 133935	Rhizosphere of *Panax Notoginseng*, Wenshan county, Yunnan Province	37	GU563353	GU563368	**JF772446**	**KC485016**	**KC485046**
C. subfunicola	CGMCC 3.12926	Soil, Huanggang, Hubei Province	37	**JX867124**	**JX867124**	**JX867121**	**KC485015**	**KC485045**
C. subfunicola	IMI 300511 [c]	*Citrus* sp., India, GenBank		HM365262	HM365262	HM365300		
C. globosum	CBS 148.51	Stored cotton, Washington DC, USA		GU563363	GU563374	**JF772459**	**KC485028**	**KC485058**
C. globosum	CGMCC 3.12922	Soil, Qingdao, Shandong Province		**HM449057**	**HM449053**	**JF772448**	**KC485029**	**KC485059**
Achaetomium strumarium	CBS 333.67 (**T**)	Soil, Lucknow, India		AY681170	AY681204	AY681238	KC503252	KC503253

The newly generated sequences in this study are shown in **bold**

org/) for partial translation elongation factor 1α (TEF1-α) gene, and RPB1-Af/RPB1-Cr (Matheny et al. 2002) for partial fragments of the largest subunit of RNA polymerase II (RPB1) genes, respectively. The PCR reaction (50 μL) contained 0.25 μM of each primer, 0.5 mM dNTP, 0.8 U of *Taq* DNA polymerase in 1× reaction buffer containing 2 mM MgCl2 with 2 μL of template. The PCR programs consisted of an initial denaturation of 5 min at 95 °C followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 49 °C (for ITS and RPB1) or 55 °C (for 28S rDNA D1/D2 domain, BTUB and TEF1-α) for 1 min and elongation at 72 °C for 2 min, and a final extension of 72 °C for 10 min. The purified PCR products were sequenced on an Applied Biosystems 3730Xl DNA Analyzer by SinoGenoMax Co. Ltd. (http://www.sinogenomax.com). The obtained sequences were deposited in GenBank (http://www.ncbi. nlm.nih.gov, Table 1).

Sequences of each gene partition were initially aligned using Clustal X 1.83 (Thompson et al. 1997), and were manually edited to optimize homology using BioEdit 5.0.9 (Hall 1999). All five gene regions were then concatenated into a combined dataset consisting of 23 target taxa, two *C. globosum* strains and one *Achaetomium* strain (Tree-Base ID: 13834). For ITS, 28S rDNA and BTUB gene regions, and 33 published sequences were available (Asgari and Zare 2011) to allow an expanded dataset (56 taxa, Tree-Base ID: 13833) for determining the phylogenetic positions of the members of this group in the whole *Chaetomium* genus. The datasets in NEXUS and PHYLIP formats from both five-gene and three-gene datasets were used for the phylogenetic analysis.

Bayesian phylogenetic analysis, maximum likelihood analysis and maximum parsimony were conducted. The best-fit model of evolution was determined by jModelTest 0.1.1 (Posada 2008). Bayesian analysis was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Four MCMC chains were run simultaneously for 6×10^5 or 2×10^6 generations for the two datasets. Trees were sampled from every 300th or 1000th generation. After the first 25 % of the trees were discarded, posterior probabilities were determined from a consensus tree generated from the remaining trees. Maximum likelihood analysis was performed using RAxML-VI-HPC 7.0.3 (Stamatakis 2006) with nonparametric bootstrapping using 1,000 replicates. In the maximum parsimony, all characters were equally weighted and unordered, and gaps were treated as missing data. Phylogenetic trees were inferred in PAUP 4.0b8 (Swofford 2001) by Maximum Parsimony (MP) using a heuristic search with 1,000 random sequence additions, tree bisection-reconnection (TBR) branch swapping, and MulTrees ON. The robustness of the trees was evaluated with 1,000 bootstrap replications. Trees were viewed in FigTree v1.1.2 (Rambaut 2009).

Results

Morphology

All strains examined in this study shared the following morphological characters: (1) ascospores typically shorter than 7.5 μm, ovate to broadly ovate or limoniform, bilaterally flattened and to appear ellipsoid, with an apical germ pore at the more attenuated end; (2) asci clavate, broadly clavate or obovate; (3) more or less dichotomously branched ascomatal hairs arising around the ostioles of spherical or ovate ascomata. Ascomatal wall is either textura intricata or angularis in surface view (Fig. 1).

MGT

Our results showed that all the tested strains within each species exhibit consistent MGTs (Table 1). The data of MGTs clearly split the *C. indicum* group into three value types (Table 2), with MGTs of 34 °C represented by *C. indicum* and *C. pratense*, 36–37 °C by *C. funicola*, *C. cancroideum*, *C. subfunicola*, *C. dolichotrichum* and *C. reflexum*, and 41 °C by *C. erectum* and *C. ramosissimum*.

Phylogeny

The three-gene alignment (ITS, 28S rDNA, BTUB) comprised 56 taxa and 1,972 characters, of which 406 were parsimony-informative. The alignment of five genes (ITS, 28S rDNA, BTUB, TEF1-α, RPB1) comprised 26 taxa and 3,630 characters, of which 800 were parsimony-informative. The best-fit models of evolution were estimated as the GTR-GAMMA model for the three-gene dataset and the TIM1+ GAMMA model for the five-gene dataset. Bayesian analysis was conducted with the number of rate categories set to six, rates set to gamma, and all the remaining parameters followed the default. Maximum likelihood (ML) analysis was conducted under the GTR-GAMMA model of evolution (Stamatakis 2006). For the three-gene dataset, MP analysis generated nine equally most parsimonious trees with one of the trees described with TL=869, CI=0.7883, RI=0.9045, RC=0.7130 and HI=0.2117. For the five-gene dataset, MP analysis generated four equally most parsimonious trees, with one of the trees described with TL=1696, CI=0.7471, RI=0.8853, RC= 0.6613 and HI=0.2837. The MP and ML analyses resulted in essentially concordant trees to that of Bayesian analysis. The Bayesian trees are presented (Figs. 5 and 6) with the respective MP and ML bootstrap proportions indicated.

Analyses based on both datasets recognized the monophyly of the *C. indicum* group that comprised eight well-supported clades (Figs. 4 and 5). *Chaetomium pratense*, *C. ramosissimum* and *C. subfunicola* were introduced as new species based on distinct phylogenetic relationships and

Fig. 1 Drawings of the wall structure of ascomata in surface view associated with this study. **a** textura intricata. **b** textura angularis
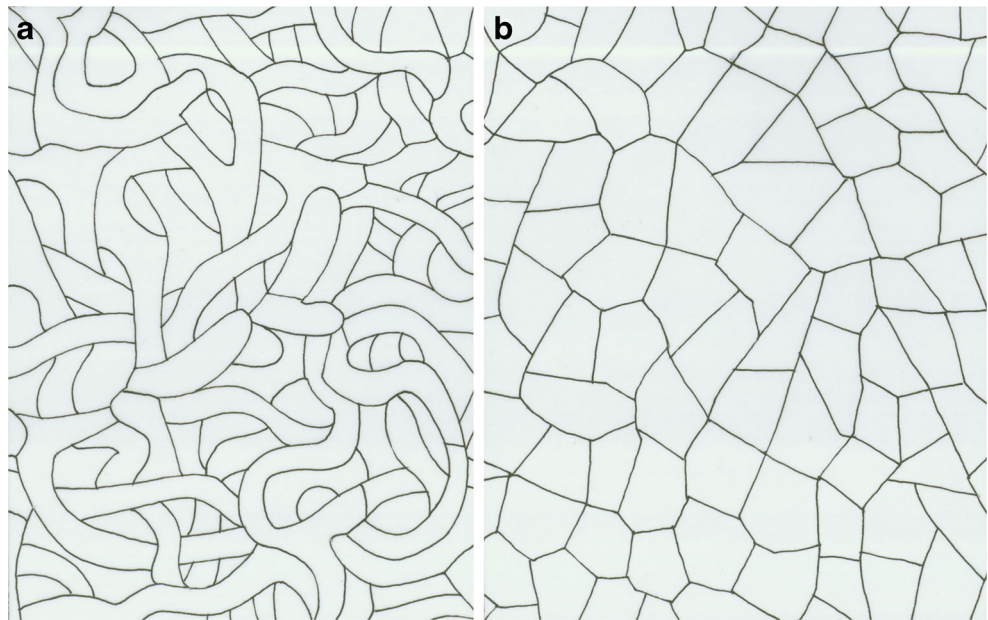
morphology. However, *Chaetomium cancroideum*, though it exhibits distinct morphology, appears non-separable from *C. funicola*.

Taxonomy

Chaetomium pratense X.W. Wang & L. Cai, sp. nov., Fig. 2a–d.

MycoBank MB 563348

Etymology: Referring to its habitat in the pasture.

Colonies yellow, aerial hyphae sparse or absent, with yellow or orange exudate. *Anamorph* absent. *Ascomata* superficial, spherical or ovate, 160–250 μm high, 110–200 μm diam., ostiolate. *Ascomatal wall* composed of brown, irregular or elongate cells (*textura intricata* or *epidermoidea*). *Terminal hairs* erect, dichotomously branched 3–5(7) times at wide (nearly straight) angles and starting primarily from the upper half part, punctulate or verrucose. *Lateral hairs* unbranched, seta-like, tapering towards tips. *Asci* fasciculate, clavate, stalked, without apical structures, 26–48 × 8–13 μm, 8–spored, evanescent. *Ascospores* brown, broadly ovate, bilaterally flattened, ellipsoid, 5–7.5 × 4.5–6 × 3–4 μm, with an apical germ pore at the more attenuated end.

Cardinal temperatures for growth: Minimum about 10 °C, maximum 34 °C, optimum 25 °C.

Holotype: China, Qinghai, Huangnan, from grassland soil, 8 June 2002, *X.–W. Wang*, HMAS 242921 (Holotype); culture ex–type CGMCC 3.14181.

Note: Phylogenetically, *Chaetomium pratense* is relatively close to *C. indicum*, *C. funicola*, and *C. subfunicola*. In morphology, *C. pratense* differs from *C. indicum* in producing larger and broadly ovate ascospores and the terminal hairs that start to branch from the upper half part; from *C. subfunicola* in having clavate asci and hairs not significantly different in length; from *C. funicola* in possessing broader ascospores and hairs not significantly different in length.

Chaetomium subfunicola X.W. Wang & L. Cai, sp. nov., Fig. 3a–h.

MycoBank MB 801733

*Etymology*: Referring to its phenotypic similarity to the species *C. funicola*.

Colonies white or yellowish, aerial hyphae white, with yellow to green exudate. *Anamorph* absent. *Ascomata* superficial, nearly spherical or ellipsoid, 150–250 μm high, 170–235 μm diam., ostiolate, with a brown wall of *textura intricata* or *epidermoidea*. *Terminal hairs* of two types verrucose: (1) longer, erectly extending above the cirrhus, dichotomously branched 2–8 times at the apices or seta-like, usually at acute angles and tapering towards a point; (2) shorter and dichotomously branched repeatedly, often obscured by cirrhi. *Asci* fasciculate, obovate or broadly clavate, 22–35 μm long, 10–14 μm diam. in spore-producing part, stalked, without apical structures, 8–spored, evanescent. *Ascospores* brown, ovate to broadly ovate, bilaterally flattened as ellipsoid, 5.5–7.5(10) × 4.5–5 (6) × 3–4 μm, with an apical germ pore at the more attenuated end.

Cardinal temperatures for growth: Minimum about 7 °C, maximum 37 °C, optimum 28 °C.

Holotype: China, Xinjiang, Shihezi University, from garden soil, 3 Aug. 2007, *F.–J. Liu*, HMAS 244194 (Holotype), culture ex–type CGMCC 3.12892.

Table 2 Comparison of phenotypic characters of species in the *Chaetomium indicum* group

Species	Ascomata		Terminal ascomatal hairs						Ascus Shape	Ascospores		MGT (°C)
	Diameter (µm)	Wall in surface	Length difference	Roughening	Stiffening	Branching start from	Branching angle at	Apical branches		shape	Length (µm)	
C. cancroideum	<150	angularis	Two types	verrucose	Not rigid	Lower or upper	Acute	incurved	Clavate	Ovate	≤6.5	36
C. dolichotrichum	<150	angularis	Two types	Longer hairs smooth	Not rigid	Lower, apical or none	Obtuse to straight	Erect	Clavate	Ovate	Up to 7.5	37
C. erectum	>150 on average	intricate	Not significant	verrucose	Conspicuous rigid	Upper half part	Acute to straight	Erect	Clavate	Ovate to limoniform	Up to 7.5	41
C. funicola	>150 on average	intricate	Two types	verrucose	Not rigid	Lower, apical or none	acute	Erect or incurved	Clavate	Ovate to ellipsoid	≤6.5	37
C. indicum	>150 on average	intricate	Not significant	verrucose	Not very rigid	Lower half part	acute to straight	Erect to recurved	Clavate	Ovate	≤6.5	34
C. pratense	>150 on average	intricate	Not significant	verrucose	Not rigid	Upper half part	acute	Erect	Clavate	Broadly ovate	Up to 7.5	34
C. ramosissimum	>150 on average	intricate	Not significant	verrucose	Conspicuous rigid	Lower half part	Acute to straight	Erect	Clavate	Ovate to limoniform	Up to 7.5	41
C. reflexum	<150	angularis	Not significant	verrucose	Not rigid	Upper half part	acute to straight	reflexed	Clavate	Ovate or inaequilaterally	≤6.5	36
C. subfunicola	>150 on average	intricate	Two types	verrucose	Not rigid	Lower, apical or none	acute	Erect	Obovate	Ovate or broadly ovate	Up to 7.5	37
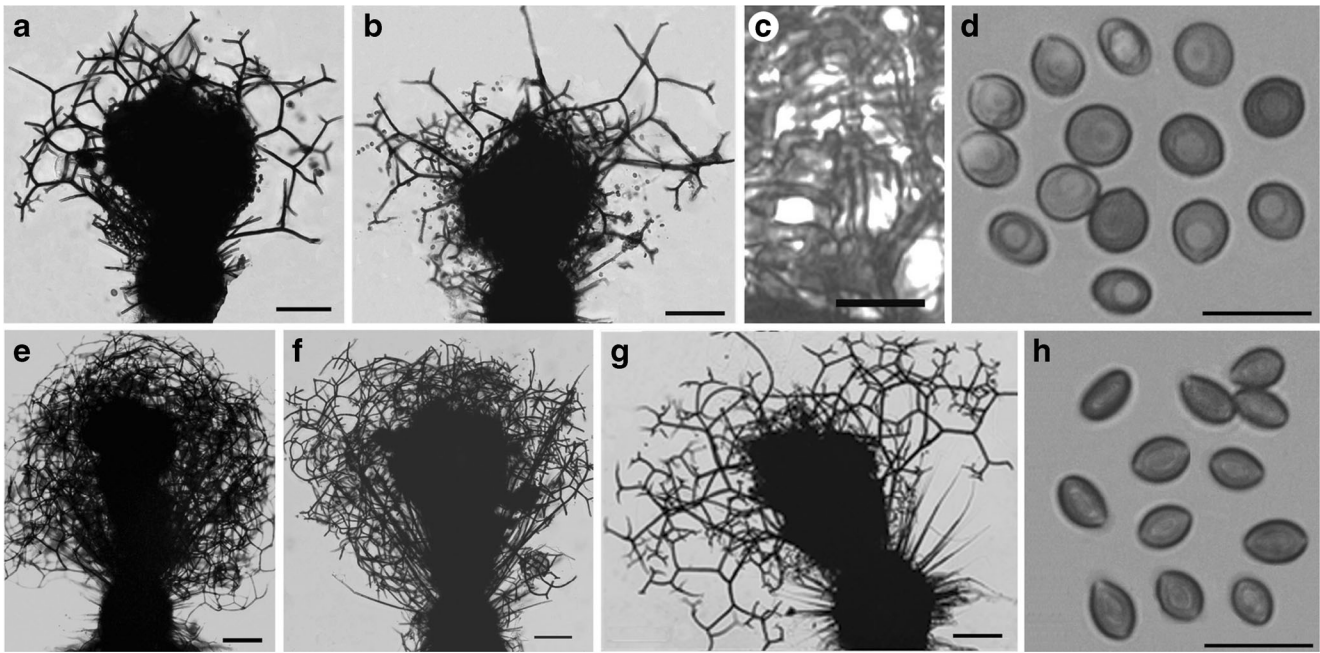
Fig. 2 **a–d** *Chaetomium pratense* (ex-type strain CGMCC 3.14181). **a-b** Ascoma. **c** Surface cellular texture of the ascomatal wall. **d** Ascospores. **e–h** *Chaetomium indicum*. **e-g** Ascomata (**e–f** ex-epitype strain CGMCC 3.14184, **g** CBS 860.68). **h** Ascospores (ex-epitype strain CGMCC 3.14184). Bars: **a, b, e, f, g** =100 μm; **c, d, h** =10 μm

Other strains examined: China, Yunnan, Wenshan county, from the rhizosphere of *Panax Notoginseng*, 11 Apr. 2003, *X.–Z. Liu*, culture CGMCC 3.9466; China, Hubei, Huanggang city, from the soil, 8 Nov. 2008, *F.–J. Liu*, culture CGMCC 3.12926.

Note: *Chaetomium subfunicola* is closely related to *C. funicola*, and distinguished by possessing obovate rather than clavate asci and broader or larger ascospores. The strain IMI 300511 was originally recognized as *C. funicola* by Asgari and Zare (2011). Though no morphological data of IMI 300511 are available, the strain is assigned here to *C. subfunicola* based on molecular evidence.

Chaetomium ramosissimum X. W. Wang & L. Cai, sp. nov., Fig. 4a–d.

MycoBank MB 801734

Eᴛʏᴍᴏʟᴏɢʏ: Referring to its terminal hairs more copiously branched than those of *C. erectum*.

Colonies yellow, aerial hyphae yellow, without exudate. *Anamorph* absent. *Ascomata* superficial, nearly spherical or ovate, 125–180 μm high, 110–170 μm diam., ostiolate, with a brown wall of *textura intricata* or *epidermoidea*. *Terminal hairs* erect, rigid, dark, copiously dichotomously branched more than four times at wide (nearly straight) angles and starting from the lower half part, punctulate or verrucose. *Lateral hairs* unbranched, seta-like, tapering. *Asci* fasciculate, clavate, stalked, without apical structures, 28–35 × 8–12 μm, 8–spored, evanescent. *Ascospores* brown, ovate to

limoniform, bilaterally flattened as ellipsoid, 5–7.5 × 4.5–5.5 × 3–4 μm, with an apical germ pore at the more attenuated end.

Cardinal temperatures for growth: Minimum about 10 °C, maximum 41 °C, optimum 30 °C.

Holotype: China, Yunnan, Wenshan county, from the rhizosphere of *Panax Notoginseng*, 11 April, 2003, *X.–Z. Liu*, HMAS 244195 (Holotype), culture ex–type CGMCC 3.14183.

Other strains examined: China, Hubei, Huanggan city, from the soil, 8 Nov. 2008, *F.–J. Liu*, culture CGMCC 3.12930; China, Shandong, Anqiu town, from the soil, 13 Aug. 2007, *F.–J. Liu*, culture CGMCC 3.12898.

Note: *Chaetomium ramosissimum* is closely related to *C. erectum*, but distinguished by the terminal hairs that branched copiously from the lower half part. *Chaetomium ramosissimum* also differs from the other species in the group by possessing limoniform ascospores and higher MGT.

Chaetomium dolichotrichum Ames, Mycologia 37: 145. 1945, Fig. 4i–l.

Specimen examined: USA, Tennessee, Great Smoky Mts., Cades Cove, 1945, *L. M. Ames*, culture ex–type CBS 162.48; CHINA, Hebei, Xingtai city, from corn straw, 16 Apr. 2009, *J. Li*, culture CGMCC 3.14188; CHINA, Jilin, Longjing county, from discarded cloth, 2 Nov. 2009, *J. Li*, culture CGMCC 3.14189.

Note: *Chaetomium dolichotrichum* produces ascomata with hairs differing markedly in length. Its longer hairs are usually two or more times as long as the shorter ones with no
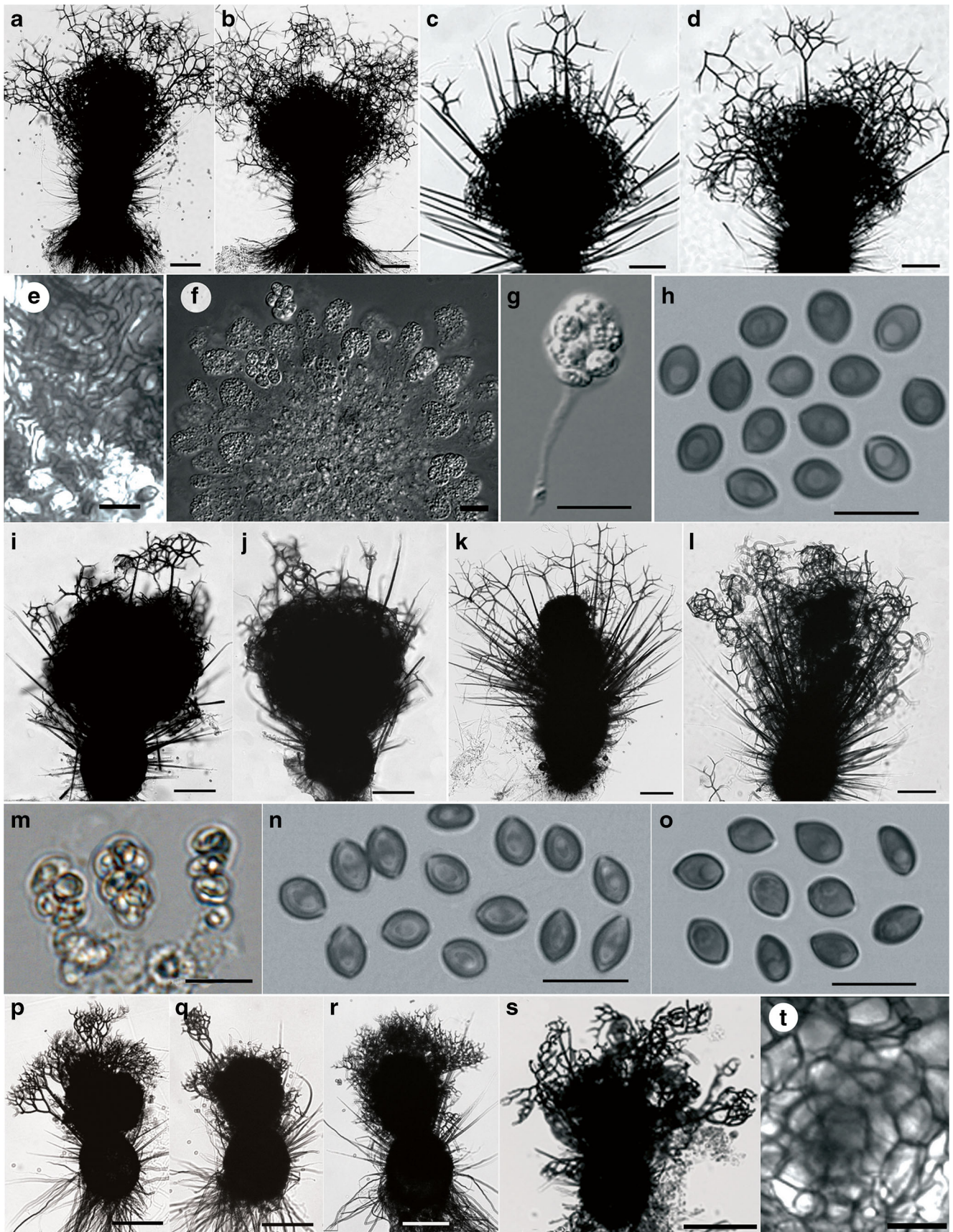
◄ **Fig. 3** **a-h** *Chaetomium subfunicola.* **a-d** Ascomata. (**a–b** ex-type strain CGMCC 3.12892, **c–d** CGMCC 3.9466). **e** Surface cellular texture of the ascomatal wall. **f–g** Asci. **h** Ascospores. **i–o** *Chaetomium funicola.* **i–l** Ascomata (**i–j** ex-epitype strain CBS 159.52, **k** CGMCC 3.12918. **l** CGMCC 3.9459). **m** Asci. **n–o** Ascospores (**n** ex-epitype strain CBS 159.52, **o** CGMCC 3.12918). **p–t** *Chaetomium cancroideum* (CBS 136.38). **p–s** Ascoma. **t** Surface cellular textures of the ascomatal wall. Bars: **a–d**, **i–l**, **p–s**=100 μm; **e–h**, **m–o**, **t**=10 μm

branches, and are tapering towards a point, or occasionally branched simply with long, terminal branches tapering towards a point (Figs. 4i-l, 5 and 6). Moreover, the longer hairs of *C. dolichotrichum* are smooth, which is quite unique in the *C. indicum* group, as all the other species produce verrucose hairs. Besides, *C. dolichotrichum* also produces smaller ascomata with the wall of *textura angularis* in contrast with *C. funicola*. The multigene phylogenies presented in this study
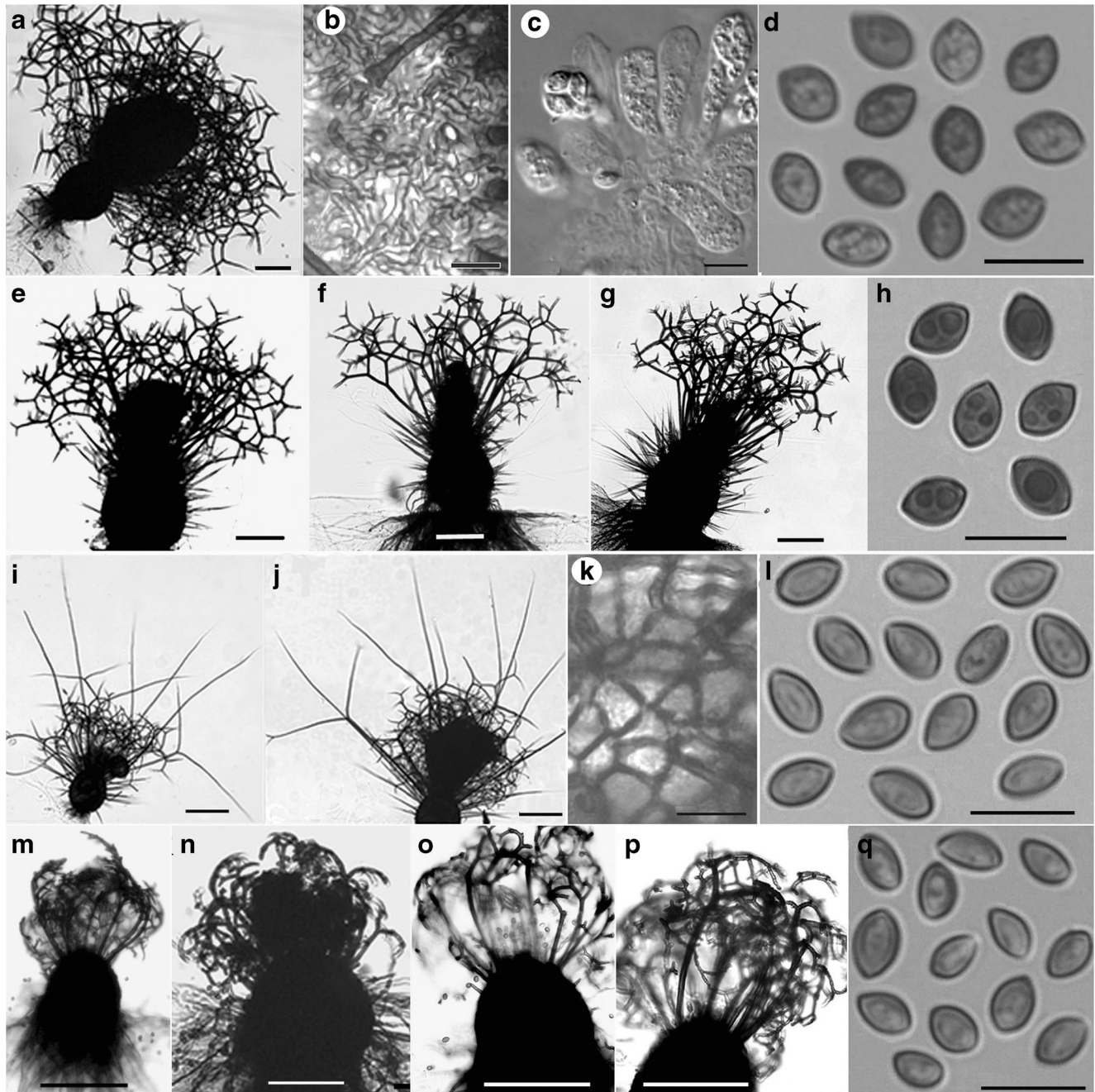

Fig. 4 **a–d** *Chaetomium ramosissimum* (ex-type strain CGMCC 3.14183). **a** Ascoma. **b** Surface cellular texture of the ascomatal wall. **c** Asci. **d** Ascospores. **e–h** *Chaetomium erectum* (ex-type strain CBS 140.56). **e–g** Ascoma. **h** Ascospores. **i–l** *Chaetomium dolichotrichum* (ex-type strain CBS 162.48). **i–j** Ascomata. **k** Surface cellular texture of the ascomatal wall. **l** Ascospores. **m–q** *Chaetomium reflexum* (ex-type strain CBS 157.49). **m–p** Ascoma. **q** Ascospores. Bars: **a**, **e–g**, **i–j**, **m–p**= 100 μm; **b–d**, **h**, **k–l**, **q** =10 μm
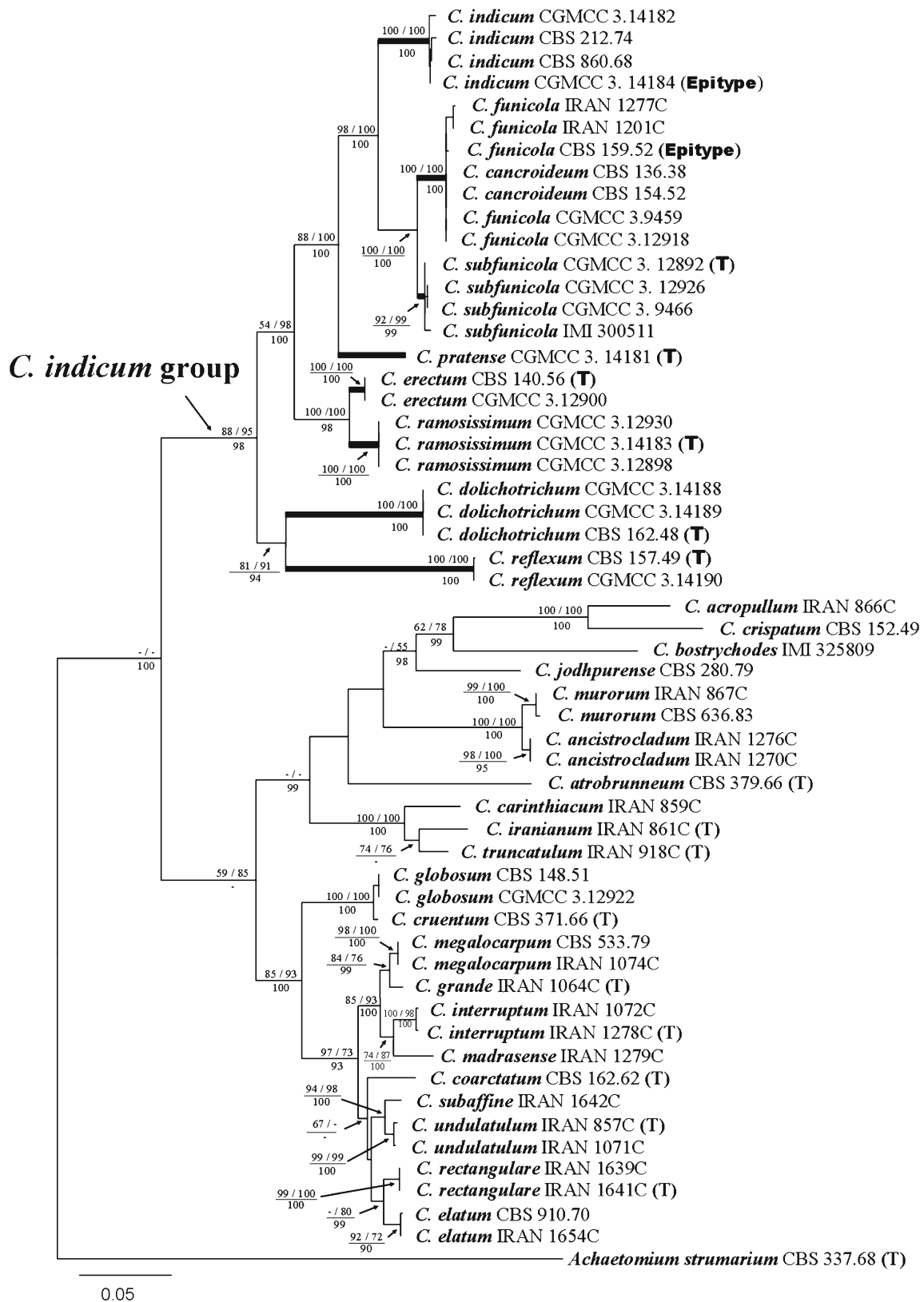
Fig. 5 Phylogram of Bayesian analysis based on the concatenation of ITS rDNA, 28S rDNA D1/D2 domain and the BTUB partition, with the confidence values of bootstrap proportions from MP analyses (before the *backslash*) and ML analyses (after the *backslash*) above branches, and the posterior probabilities from Bayesian analyses below branches. "-"means lacking statistic support (<50 for bootstrap proportions from ML or MP analyses; <90 for posterior probabilities from Bayesian analyses). The *C. indicum* group comprising eight well-supported species (indicated using *thickened branches*) is revealed as a monophyletic clade in the genus
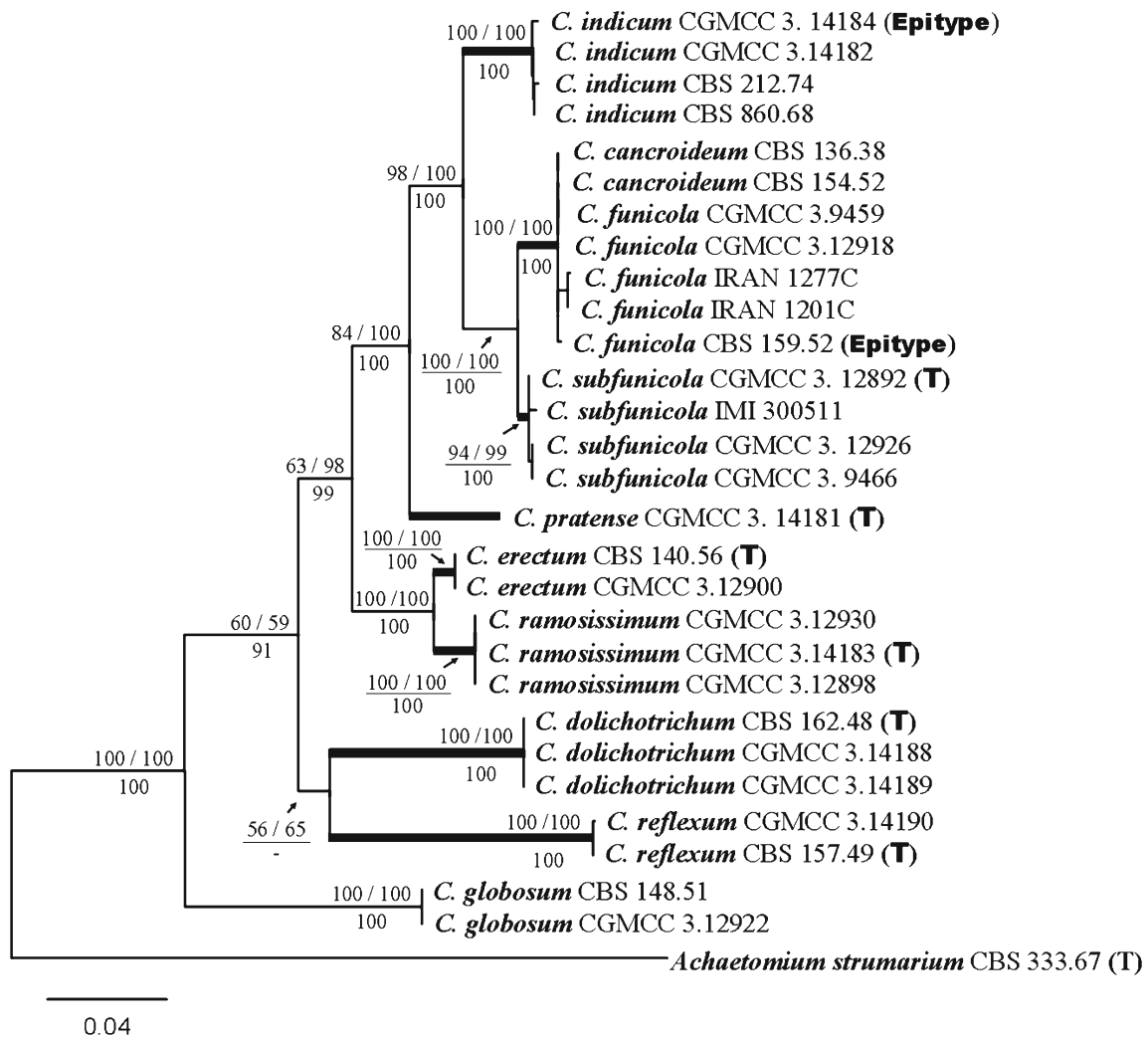
Fig. 6 Phylogram of Bayesian analysis based on the concatenation of five loci (ITS rDNA, 28S rDNA D1/D2 domain, BTUB, EF1-α and RPB1 gene partitions) with the confidence values of bootstrap proportions from MP analyses (before the *backslash*) and ML analyses (after the *backslash*) above branches, and the posterior probabilities from Bayesian analyses below branches. "-"means lacking statistic support (<50 for bootstrap proportions from ML or MP analyses; <90 for posterior probabilities from Bayesian analyses)

revealed *C. dolichotrichum* to be a distinct species from *C. funicola* and other species in the group. Consequently, the synonymy of *C. dolichotrichum* with *C. funicola* (von Arx et al. 1986) is rejected.

Chaetomium funicola Cooke, Grevillea 1: 176. 1873, Fig. 3i–o.

Description of the epitype: *Colonies* white, aerial hyphae white to yellowish, with yellow exudate. *Anamorph* absent. *Ascomata* superficial, nearly spherical to ellipsoid, 150–260 μm high, 150–220 μm diam., ostiolate, with a brown wall of *textura intricata* or *epidermoidea*. *Terminal hairs* typically showing two types: (1) longer, erectly extending above the cirrhus, dichotomously branched 2–5 times at the apices, with branches straight or incurved, or unbranched (seta-like); (2) shorter and dichotomously branched repeatedly, often obscured by cirrhi. *Asci* fasciculate, clavate to broadly clavate, 24–36 μm long, 8–12 μm diam. in spore-producing part, stalked, without apical structures, 8–spored, evanescent. *Ascospores* brown, ovate to broadly fusiform, bilaterally flattened as ellipsoid, 5–6.5 × 3.5–5 × 3–4 μm, with an apical germ pore at the more attenuated end.

Cardinal temperatures for growth: Minimum about 9 °C, maximum 37 °C, optimum 28 °C.

Specimens examined: **Britain**, British Museum, from twine, collection date unknown, *M. C. Cook*e, KEW(M) 189267, holotype, and an associated slide No. 144890 (Herbarium IMI, ex type collection). Germany, Bamberg, substrate unknown, *R. Pfleger*, deposited in CBS collection Sep. 1952, HMAS 244231 **epitype designated here** of *C. funicola*, culture ex–epitype CBS 159.52 (MBT 176686). China, Shanxi Province, Taibai, from discarded shoe, 12 June 2002, *X.–W. Wang*, culture CGMCC 3.9459. China, Qinghai Province,

Eling Lake, from sheep dung, 2 Aug. 2007, *F.–J. Liu*, culture CGMCC 3.12918.

Note: From the holotype of *C. funicola*, only ascospores and some ascomatal hairs on fragments of ascomata were observed that are morphologically similar to that of the epitype. The epitype is geographically close to that of the type locality.

Chaetomium cancroideum, represented by two authentic strains, CBS 136.38 (deposited in 1938 by Tschudy, who described this species) and CBS 154.52 (deposited by Ames in 1952), was found phylogenetically non-separable from *C. funicola*. Some morphological characters of *C. cancroideum* have also been uncovered that suggest its affinity to *C. funicola*. For example, parts of its ascomatal hairs are significantly longer than others (two types of ascomatal hairs). The incurved branches of ascomatal hairs, which had traditionally been used to distinguish *C. cancroideum* from *C. funicola*, occasionally can also be observed in *C. funicola*. However, *C. cancroideum* is easily distinguishable from *C. funicola* by its smaller ascomata with the wall of *textura angularis* (Fig. 3p-t). Because of this, *C. cancroideum* is retained as a separate species.

Chaetomium indicum Corda Icon. Fung. 4: 38. 1840, Fig. 2e–h.

Description of the epitype: *Colonies* yellow, aerial hyphae yellow, usually with yellow exudate. *Anamorph* absent. *Ascomata* superficial, spherical or ovate, 150–220 μm high, 150–200 μm diam., ostiolate. *Ascomatal wall* composed of brown, irregular, or elongated cells (*textura intricata* or *epidermoidea*). *Terminal hairs* erect, profusely dichotomously branched 4–6 (8) times at wide to acute angles and starting from the lower half part, punctulate or verrucose; *Lateral hairs* unbranched, seta-like, tapering towards tips. *Asci* fasciculate, clavate to broadly clavate, stalked, without apical structures, 20–34 × 9–13 μm, 8–spored, evanescent. *Ascospores* are brown, ovate, bilaterally flattened as ellipsoid, 4.5–6 × 3.5–5 × 3–3.5 μm, with an apical germ pore at the more attenuated end.

Cardinal temperatures for growth: Minimum about 7 °C, maximum 34 °C, optimum 25 °C.

Specimens examined: **Burma**, Hinter-indien region, from rotten paper, collection date unknown, *A. C. J. Corda*, PRM 155406, part of **holotype**. China, Yunnan Province, Wenshan, from the rhizosphere of *Panax Notoginseng*, 11 April, 2003, *X.–Z. Liu*, HMAS 244232 **epitype designated here** of *C. indicum*, culture ex–epitype CGMCC 3.14184 (MBT176607). **Kenya**, Mt. Kenya, from dung of herbivore, *R. S. Khan*, deposited in CBS collection Apr. 1974, CBS 212.74. **Germany**, Kiel-Kitzeberg, from air, *K. H. Domsch*, deposited in CBS collection Dec. 1968, CBS 860.68. China, Yunnan, Wenshan, from the rhizosphere of *Panax Notoginseng*, 11 April, 2003, *X.–Z. Liu*, CGMCC 3.14182.

Note: The holotype of *C. indicum* is morphologically similar to that of the epitype, particularly in ascospore

morphology. The epitype is geographically close to that of the type locality and phylogenetically consistent with the authentic strains CBS 860.68 and CBS 212.74; both have been examined by von Arx et al (1986). All the examined strains of *C. indicum* produce abundant and dichotomously branched, erect or recurved terminal hairs starting from the lower part (Fig. 1e-g). *Chaetomium indicum* is significantly lower in MGT (34 °C).

KEY TO THE SPECIES EXAMINED

1. Length of terminal hairs different enough to show two types of hairs. 2
1. Length of terminal hairs not significantly different. 5
2. Ascomatal wall *textura angularis* 3
2. Ascomatal wall *textura intricata* 4
3. Longer terminal hairs smooth with apices erect . . . . *C. dolichotrichum*
3. Terminal hairs all verrucose with apical branches incurved . . . . *C. cancroideum*
4. Asci clavate. . . . . *C. funicola*
4. Asci obovate. . . . . *C. subfunicola*
5. Terminal hairs arcuate with apical branches strongly reflexed . . . . *C. reflexum*
5. Terminal hairs erect with apical branches not reflexed 6
6. MGT 34 °C; terminal hairs typically not rigid. 7
6. MGT 41 °C; terminal hairs conspicuously rigid. 8
7. Ascospores ovate; terminal hairs starting to branch from lower half part . . . . *C. indicum*
7. Ascospores broadly ovate; terminal hairs starting to branch from upper half part . . . . *C. pratense*
8. Terminal hairs starting to branch from lower half part. . . . .*C. ramosissimum*
8. Terminal hairs starting to branch from upper half part. . . . . *C. erectum*

Discussion

The phylogenetic relationships of the eight species in the *C. indicum* group are well-presented by multigene phylogenies based on the sequences of five unrelated genes. *Chaetomium dolichotrichum*, represented by its ex-type strain, is morphologically distinct and phylogenetically separated from *C. funicola*, and therefore von Arx et al.'s synonymization is rejected. The five genes used in this study fail to separate *C. cancroideum* from *C. funicola*. The morphological variation of *C. cancroideum* from *C. funicola* suggests that during the evolution, the divergence of these genes may lag behind that of the morphology. Such incongruence between gene phylogeny and morphology was also recently reported in *C. cruentum* and *C. globosum* (Asgari and Zare

2011). These results indicate that the relationships among species of *Chaetomium* can be well resolved using the multigene phylogeny, but for a few species it may be necessary to find additional genes with higher resolving ability to recognize their phylogenetic differences.

Our microscopic examination demonstrated the morphological differences among species, including those in their ascomatal hair. Subtle infra-specific morphological variation in ascomatal hairs was also observed, particularly within the species *C. indicum* and *C. funicola*. These hairs may vary among strains, among generations derived from repeated subculturing, and even among different ascomata in the same culture. This may be interpreted as an adaptive response to the effect of external environments, since ascomatal hairs are the fungal structures directly exposed to the outer environments and serve to protect the ascomata against the environmental stresses (von Arx et al. 1986). By comparison, morphologies of ascospores and asci, as presented here in further detail, are more stable against environmental changes, and thus are more valuable in species delimitation.

Millner (1977) considered that growth responses to temperature had taxonomic value for identifying closely allied species, and designated *C. indicum*, *C. funicola* and *C. erectum* as 'transitional mesophiles' with mycelial growth at 15–37 °C. While von Arx et al. (1986) noted the growth rate of colonies at a specific temperature (28 °C) for each species, a recent study compared the optimum and maximum growth temperatures, as well as the growth rate of 50 strains belonging to 25 *Chaetomium* species. Their results revealed that all the tested strains within each specific species possessed a consistent MGT, whereas the optimum temperature and the growth rate were shown to vary within species and overlapped between species (Li et al. 2012). The present study gave further evidence for the potential usefulness of MGT in delimiting species (Table 1). Growth response of a *Chaetomium* species to the temperature 37 °C has also been used as an indicator of its potential for infecting humans (Abbott et al. 1995; Barron et al. 2003). *Chaetomium globosum* has been known as a causal agent of onychomycosis (Naidu et al. 1991; Stiller et al. 1992). An isolate from a clinic case of fatal brain abscess, which was originally attributed to *C. globosum* (Anandi et al. 1989), has been re-identified as *C. atrobrunneum* according to its morphology and ability to grow at 42 °C. The infection of *C. globosum* was suggested to be confined to cooler areas of the human body due to its restricted growth at 37 °C (Abbott et al. 1995). *Chaetomium funicola* has also been reported to cause subcutaneous infection (Koch and Haneke 1965), while the MGT at 37 °C rules out its potential for deeper infection like *C. globosum*.

In many cases, *Chaetomium* species from clinical specimens were left unrecognized because they are usually in sterile form (Abbott et al. 1995). In addition, some *Chaetomium* strains easily lose sporulating ability in culture

(Tschudy 1937; von Arx et al. 1986). In these cases, DNA sequence data are essential in determining their taxonomic placements and phylogenetic relationships.

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