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Endophytic *Colletotrichum* species from *Bletilla ochracea* (Orchidaceae), with descriptions of seven new speices

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Abstract Thirty-six strains of endophytic *Colletotrichum* species were isolated from leaves of *Bletilla ochracea* Schltr. (Orchidaceae) collected from 5 sites in Guizhou, China. Seventeen different species, including 7 new species (namely *C. bletillum*, *C. caudasporum*, *C. duyunensis*, *C. endophytum*, *C. excelsum-altitudum* and *C. guizhouensis* and *C. ochracea*), 8 previously described species (*C. boninense*, *C. cereale*, *C. destructivum*, *C. karstii*, *C. liriopes*, *C. miscanthi*, *C. parsonsiae* and *C. tofieldiae*) and 2 sterile mycelia were identified. All of the taxa were identified based on morphology and phylogeny inferred from multi-locus sequences, including the nuclear ribosomal internal transcribed spacer (ITS) region, partial genes of β-tubulin (TUB2), actin (ACT) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Comprehensive morphological descriptions and illustrations are provided for new species. Our investigation indicates a high diversity of *Colletotrichum* species in *B. ochracea*.

Keywords Fungal diversity · Phylogeny · Systematics · Taxonomy

Introduction

The Orchidaceae is one of the largest plant families with nearly 25,000 species (Jones 2006). Orchids are fascinating ornamental plants and important research materials for coevolution between plants and fungi because of their special symbiosis with mycorrhizal fungi (Zettler et al. 2004; Stark et al. 2009; Nontachaiyapoom et al. 2010). Recently, the fungal communities in leaves and roots of orchid *Bletilla ochracea* have been investigated and the results indicated that there is a high diversity of endophytic fungi, including species from the genus *Colletotrichum* Corda (Tao et al. 2008, 2012).

Endophytic fungi live asymptomatically and internally within different tissues (e.g. leaves, roots) of host plants (Ganley and Newcombe 2006; Promputtha et al. 2007; Hoffman and Arnold 2008). Some endophytes have been demonstrated to be able to enhance the competitive abilities and resistance to herbivores, pathogens, and various abiotic stresses for their hosts (Saikkonen et al. 1998; Newton et al. 2010; Saikkonen et al. 2010). *Colletotrichum* species are among the most commonly occurring pathogens and foliar endophytes of terrestrial plants and have been recorded from approximately 2,200 plant species (Farr and Rossman 2013). *Colletotrichum* pathogens are the principal cause of anthracnose, as well as causal agents of pre- and post-harvest fruit rots, damping-off of blossom and seedling blight diseases (Bailey and Jeger 1992). This genus was recently voted the eighth most important group of plant pathogenic fungi in the world, based on perceived scientific and economic importance (Dean et al. 2012). However, it has also been shown that particular *Colletotrichum* endophytes confer protective benefits to *Cacao* hosts by reducing disease incidence and damage caused by other plant pathogens (Arnold et al. 2003; Herre et al. 2007). Our understanding on endophytic *Colletotrichum* species remains very limited.

The nuclear ribosomal internal transcribed spacer (ITS) has been chosen as the universal barcode for the Kingdom of *Fungi* (Schoch et al. 2012), but it is also widely acknowledged that ITS does not provide sufficient resolution to differentiate species in *Colletotrichum* (Cai et al. 2009; Crouch et al. 2009a; Prihastuti et al. 2009; Yang et al. 2009; Phoulivong et al. 2010a). ITS sequences have been applied to resolve species of the 'gloeosporioides' complex (Sreenivasaprasad et al. 1993;

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Sreenivasprasad et al. 1996), but the resolution is not satisfactory (Crouch et al. 2009a). Phylogenetic species recognition criterion (Taylor et al. 2000) have been increasingly used in *Colletotrichum* to recognize and differentiate species. Up to now, significant progress has been achieved in the *Colletotrichum acutatum* species complex (Damm et al. 2012a), *Colletotrichum boninense* species complex (Damm et al. 2012b), and the *Colletotrichum gloeosporioides* species complex (Prihastuti et al. 2009; Phoulivong et al. 2010a; Weir et al. 2012), and some cryptic species in *Colletotrichum* have been disclosed using multilocus phylogenetics (Phoulivong et al. 2010b; Rojas et al. 2010; Damm et al. 2012a; Damm et al. 2012b; Weir et al. 2012).

The objective of this study was to investigate the endophytic *Colletotrichum* species associated with *Bletilla ochracea* (Orchidaceae) in differently geographic sites in Guizhou province, China.

Materials and methods

Sampling sites and treatments

Fifty intact plants of *Bletilla ochracea* were collected from 5 sites in Guizhou province, China during June–August of 2006 (Table 1). Ten healthy and intact plants with native soil in each site were packed and carefully transported to laboratory within 48 h. Three symptomless leaves of each plant were treated with gently running tap water to remove the surface debris and soil. They were surface-sterilized using 75 % ethanol for 1 min, 0.1 % $HgCl_2$ for 3 min, and washed for five times using sterile distilled water, finally dried on sterile filter paper (Newell 1976). The 5-mm-diam leaf discs treated as above were placed on potato dextrose agar (PDA) plates without antibiotics, which were used as control of microorganisms test.

Isolation and spore induction of endophytic *Colletotrichum*

Six leaf discs treated as above were placed on PDA, malt extract agar (MEA) (Stone et al. 2004) and modified Czapek Dox agar medium (Yamato et al. 2005). Ten repeats were made for each medium type. Streptomycin sulphate and chloramphenicol were added into the media to a final concentration of 100 mg/L and 50 mg/L to inhibit bacterial contamination. Plates were kept in the dark at room temperature (25 °C). When colonies appeared, they were transferred onto new PDA plates and incubated at 25 °C in 12 h light and 12 h dark for morphological examination. All endophytic isolates are deposited in China General Microbiological Culture Collection Center (CGMCC). The specimens of type strains were deposited in the Herbarium of Microbiology, Academia Sinica (HMAS).

Sporulation was induced on pine needle medium ("pine needle" and 1/10-strength PDA, hereafter abbreviated as "PNP") with exposure to 12 h near-UV light/12 h dark at 25 °C for 7 days, or up to 2 months (Su et al. 2012). Mycelial appressoria were produced and measured using a slide culture technique (Riddell 1950). Five-mm-diam plugs from the margin of actively growing cultures were placed onto PDA plates (Petri dishes diameter: 90 mm) for assessment of growth rates. Colonial diameters were measured at the seventh day (at sixth day for the fast growing cultures). Daily growth rate was calculated (mm/day). Colonial characters were described after 7 days growth on PDA (10 days for slow growing cultures).

Table 1 Sampling sites in Guizhou Province, China

Site[a]	Altitude (m)	Geographical locality (lat. N, long. E)	Sampling time
DYXB	990	26°15′ N,107°33′ E	13/Jul/2006
GYYL	1120	26°35′ N,106°48′ E	11/Jun/2006
QZPS	1310	26°30′ N,106°27′ E	16/Aug/2006
QXHS	1315	27°06′ N,106°00′ E	17/Jul/2006
SBJP	1635	26°26′ N,104°44′ E	28/Jun/2006

[a] Abbreviations of sampling sites: *DYXB* Xiaba mountain, Duyun; *GYYL* Yongle mountain, Guiyang; *QZPS* Pianshan mountain, Qingzhen; *QXHS* Hongshui mountain, Qianxi; *SBJP* Baijipo mountain, Shuicheng

DNA extraction, PCR amplification and sequencing

Fungal isolates were incubated on PDA at 25 °C for 7–10 days for DNA extraction. Total genomic DNA of the isolates was extracted using a modified protocol as outlined by Yang and Liu (2005). The ITS_1 and ITS_4 primers were used to amplify the ITS region following the procedure described by White et al. (1990). The primers of T1 and Bt-2b were used to amplify partial gene of β-tubulin (TUB2) (O'Donnell and Cigelnik 1997; Glass and Donaldson 1995). PCR protocol for TUB2 was performed as follows, an initial step of 3 min at 95 °C, 34 cycles of 1 min at 94 °C , 30 s at 56 °C , and 1 min at 72 °C, followed by 10 min at 72 °C. Primer pairs and PCR amplification conditions of partial genes of actin (ACT) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) followed the protocols as previously described in Prihastuti et al. (2009) and Crouch et al. (2009b). The PCR amplifications were performed in a 25 μl mixture containing 9.5 μl ddH_2O, 12.5 μl 2×PCR Master Mix (TIANGEN Co. China), 1 μl of DNA template, 1 μl of each primer (10 μM). DNA sequencing was performed at the SinoGenoMax Company Limited, Beijing.

Phylogenetic analysis

Sequences of our isolates, together with reference sequences obtained from GenBank (Table 2), were aligned using Clustal X 1.81 (Thompson et al. 1997) under the default settings. Phylogenetic tree was constructed using combined ITS, TUB2, ACT and GAPDH dataset.

Table 2 Sources of isolates and GenBank accession numbers used in this study

Species	Strain no.[1]	GenBank Accession number (ITS, TUB2, ACT, GAPDH)[2]			
		ITS	TUB2	ACT	GAPDH
C. acutatum	CBS 112996, ATCC 56816*	JQ005776	JQ005860	JQ005839	JQ005776
C. anthrisci	CBS 125334*	GU227845	GU228139	GU227943	GU228237
	CBS 125335	GU227846	GU228140	GU227944	GU228238
C. asianum	MFU 090233, ICMP 18580, CBS 130418*	FJ972612	JX010406	JX009584	JX010053
C. australe	CBS 116478*	JQ948455	JQ950106	JQ949776	JQ948786
C. bletillum	**CGMCC 3.15117***	**JX625178**	**JX625207**	**KC843542**	**KC843506**
C. boninense	MAFF 305972, CBS 123755*	JQ005153	JQ005588	JQ005501	JQ005240
	CSSX8	GQ485596	GQ849433	GQ856771	GQ856742
	CGMCC 3.15124	**JX625165**	**JX625193**	**KC843551**	**KC843490**
	CGMCC 3.15125	**JX625172**	**JX625201**	**KC843552**	**KC843492**
	CGMCC 3.15165	**KC244163**	**KC244156**	**KC843553**	**KC843493**
	CGMCC 3.15168	**KC244165**	**KC244158**	**KC843554**	**KC843491**
C. brasiliense	CBS 128501,ICMP 18607*	JQ005235	JQ005669	JQ005583	JQ005322
	CBS 128528	JQ005234	JQ005668	JQ005582	JQ005321
C. brevisporum	BCC 38876*	JN050238	JN050244	JN050216	JN050227
	MFLUCC 100182	JN050239	JN050245	JN050217	JN050228
C. brisbanense	CBS 292.67*	JQ948291	JQ949942	JQ949612	JQ948621
C. caudasporum	**CGMCC 3.15106***	**JX625162**	**JX625190**	**KC843526**	**KC843512**
C. caudatum	BRIP 15842	DQ195690	DQ195729	–	–
C. caudatum	BRIP 15849	DQ195691	DQ195730	–	–
C. cereale	CBS 129663	JQ005774	JQ005858	JQ005837	–
	CGMCC 3.15110	**JX625159**	**JX625186**	**KC843534**	**KC843517**
	CGMCC 3.15111	**JX625161**	**JX625188**	**KC843535**	**KC843518**
C. chlorophyti	CBS 142.79	GU227895	GU228189	GU227993	GU227895
	IMI 03806*	GU227894	GU228188	GU227992	GU227894
C. cliviae	CBS 125375*	GQ485607	GQ849440	GQ856777	GQ856756
C. coccodes	CBS 369.75*	HM171679	JQ005859	HM171667	HM171673
	CPOS1	GQ485588	GQ849444	GQ856787	GQ856744
C. cordylinicola	MFU 090551, ICMP 18579*	HM470246	JX010440	HM470234	HM470240
C. curcumae	IMI 288937*	GU227893	GU228187	GU227991	GU228285
C. cuscutae	IMI 304802*	JQ948195	JQ949846	JQ949516	JQ948485
C. dematium	CBS 125.25*	GU227819	GU228113	GU227917	GU228211
	CBS 125340	GU227820	GU228114	GU227918	GU228212
C. destructivum	CBS 149.34	AJ301942	JQ005848	JQ005827	–
	CGMCC 3.15127	**JX625169**	**JX625198**	**KC843545**	**KC843525**
	CGMCC 3.15128	**JX625171**	**JX625200**	**KC843544**	**KC843523**
	CGMCC 3.15129	**JX625174**	**JX625203**	**KC843546**	**KC843524**
C. dracaenophilum	CBS 118199*	JX519222	JX519247	JX519238	–
C. duyunensis	**CGMCC 3.15105***	**JX625160**	**JX625187**	**KC843530**	**KC843515**
C. echinochloae	MAFF 511473*	AB439811	–	–	–
C. eleusines	MAFF 511155*	JX519218	JX519243	JX519234	–
C. endophytum	**CGMCC 3.15107**	**HM751814**	**JX625196**	**KC843532**	**KC843520**
	CGMCC 3.15108*	**JX625177**	**JX625206**	**KC843533**	**KC843521**
C. eremochloae	CBS 129661*	JX519220	JX519245	JX519236	–
C. excelsum-altitudum	**CGMCC 3.15130***	**HM751815**	**JX625211**	**KC843548**	**KC843502**
	CGMCC 3.15131	**JX625182**	**JX625212**	**KC843549**	**KC843503**
C. falcatum	CGMCC 3.14187*	HM171677	HM171680	HM171665	–

Table 2 (continued)

Species	Strain no.[1]	GenBank Accession number (ITS, TUB2, ACT, GAPDH)[2]			
		ITS	TUB2	ACT	GAPDH
C. fioriniae	CBS 128517*	JQ948292	JQ949943	JQ949613	JQ948622
C. fructi	CBS 346.37*	GU227844	GU228138	GU227942	GU228236
C. fructicola	BPDI16, MFU 090228, ICMP 18581,CBS 130416*	JX010165	JX010405	FJ907426	JX010033
C. gloeosporioides	CBS 953.97	FJ972609	GQ849434	GQ856782	GQ856762
C. graminicola	CBS 130836, M1.001*	JQ005767	JQ005851	JQ005830	–
C. godetiae	CBS 133.44*	JQ948402	JQ950053	JQ949723	JQ948733
C. guajavae	IMI 350839*	JQ948270	JQ949921	JQ949591	JQ948600
C. guizhouensis	**CGMCC 3.15112***	**JX625158**	**JX625185**	**KC843536**	**KC843507**
	CGMCC 3.15113	**JX625164**	**JX625192**	**KC843537**	**KC843508**
	CGMCC 3.15114	**JX625168**	**JX625197**	**KC843538**	**KC843511**
	CGMCC 3.15115	**JX625170**	**JX625199**	**KC843539**	**KC843510**
	CGMCC 3.15167	**KC244164**	**KC244157**	**KC843540**	**KC843509**
C. hanaui	MAFF 305404*	JX519217	JX519242	–	–
C. hippeastri	CBS 125376*(CSSG1)	GQ485599	GQ849446	GQ856788	GQ856764
	CBS 125377(CSSG2)	GQ485598	GQ849445	GQ856789	GQ856765
C. horii	NBRC 7478, ICMP 10492	AY791890	GU133380	JX009438	GQ329681
C. jacksonii	MAFF 305460	JX519216	JX519241	JX519233	–
C. kahawae	IMI 319418*	JX010231	JX010444	JX009452	JX010012
C. karstii	CGMCC 3.14194*	HM585409	HM585428	HM581995	HM585391
	CORCK1	HM585406	HM585424	HM581991	HM585387
	CGMCC 3.15119	**JX625180**	**JX625209**	**KC843559**	**KC843496**
	CGMCC 3.15120	**JX625173**	**JX625202**	**KC843557**	**KC843495**
	CGMCC 3.15121	**JX625175**	**JX625204**	**KC843558**	**KC843498**
	CGMCC 3.15122	**JX625157**	**JX625184**	**KC843555**	**KC843494**
	CGMCC 3.15123	**JX625163**	**JX625191**	**KC843556**	**KC843497**
	CGMCC 3.15169	**KC244166**	**KC244159**	**KC843560**	**KC843499**
C. kinghornii	CBS 198.35*	JQ948454	JQ950105	JQ949775	JQ948785
C. lilii	CBS 109214*	GU227810	GU228104	GU227908	GU228202
	CBS 186.30	GU227811	GU228105	GU227909	GU228203
C. limetticola	CBS 114.14*	JQ948193	JQ949844	JQ949514	JQ948523
C. lineola	CBS 125337*	GU227829	GU228123	GU227927	GU228221
	CBS 125339	GU227830	GU228124	GU227928	GU228222
C. liriopes	CBS 119444*	GU227804	GU228098	GU227902	GU228196
	CGMCC 3.15170	**KC244167**	**KC244160**	**KC843543**	**KC843505**
C. lupini	CBS 109225*	DQ286119	JQ949806	JQ949476	JQ948485
C. melonis	CBS 159.84*	JQ948194	JQ949845	JQ949515	JQ948524
C. miscanthi	MAFF 510857*	JX519221	JX519246	JX519237	–
	CGMCC 3.15116	**HM751812**	**JX625189**	**KC843531**	**KC843519**
C. musae	MFLU10-0978	HQ596283	HQ596295	HQ596287	HQ596302
C. navitas	CBS 125086*	JQ005769	JQ005853	JQ005832	–
C. nicholsonii	MAFF 511115*	JQ005770	JQ005854	JQ005833	–
C. nymphaeae	CBS 515.78*	JQ948197	JQ949848	JQ949518	JQ948527
C. ochracea	**CGMCC 3.15102**	**JX625166**	**JX625194**	**KC843528**	**KC843514**
	CGMCC 3.15103	**JX625167**	**JX625195**	**KC843529**	**KC843516**
	CGMCC 3.15104*	**JX625168**	**JX625183**	**KC843527**	**KC843513**
C. parsonsiae	CBS 128525, ICMP 18590*	JQ005233	JQ005667	JQ005581	JQ005320
	CGMCC 3.15126	**JX625181**	**JX625210**	**KC843561**	**KC843500**

Table 2 (continued)

Species	Strain no.[1]	GenBank Accession number (ITS, TUB2, ACT, GAPDH)[2]			
		ITS	TUB2	ACT	GAPDH
C. paspali	MAFF 305403*	JX519219	JX519244	JX519235	–
C. paxtonii	IMI 165753*	JQ948285	JQ948285	JQ949606	JQ948615
C. phaseolorum	CBS 157.36	GU227896	GU228190	GU227994	GU228288
	CBS 158.36	GU227897	GU228191	GU227995	GU228289
C. rhombiforme	CBS 129953*	JQ948457	JQ950108	JQ949778	JQ948788
C. rusci	CBS 119206*	GU227818	GU228112	GU227916	GU228210
C. salicis	CBS 607.94*	JQ948460	JQ950111	JQ949781	JQ948791
C. siamense	MFU 090230, ICMP 18578, CBS 130417*	FJ972613	FJ907438	FJ907423	JX009924
C. simmondsii	BRIP 28519, CBS 122122*	JQ948276	JQ949927	FJ907428	FJ972580
C. spaethianum	CBS 167.49*	GU227807	GU228101	GU227905	GU228199
	CBS 100063	GU227808	GU228102	GU227906	GU228200
C. sublineoa	CBS 131301, S3.001*	JQ005771	JQ005855	JQ005834	–
C. thailandicum	BCC 38879*	JN050242	JN050248	JN050220	JN050231
	MFLUCC100192	JN050243	JN050249	JN050221	JN050232
C. tofieldiae	CBS 168.49	GU227802	GU228096	GU227900	GU228194
	CBS 495.85*	GU227801	GU228095	GU227899	GU228193
	CGMCC 3.15118	**JX625176**	**JX625205**	**KC843541**	**KC843504**
C. trichellum	CBS 118198	GU227813	GU228107	GU227911	GU228205
	CBS 448.90	GU227814	GU228108	GU227912	GU228206
C. tropicale	ICMP 18653, CBS 124949*	JX010264	JX010407	JX009489	JX010007
C. tropicicola	BCC 38877*	JN050240	JN050246	JN050218	JN050229
C. tropicicola	MFLUCC 100167	JN050241	JN050247	JN050219	JN050230
C. truncatum	CBS 151.35*	GU227862	GU228156	GU227960	GU228254
	CBS 120709	GU227877	GU228171	GU227975	GU228269
C. verruculosum	IMI 45525*	GU227806	GU228100	GU227904	GU228198
C. walleri	CBS 125472*	JQ948275	JQ949926	JQ949596	JQ948605
C. yunnanense	CGMCC AS3.9167, CBS 132135*	EF369490	JX519248	JX519239	–
***Colletotrichum* sp.**	**CGMCC 3.15171**	**HM751813**	**KC244161**	**KC843550**	**KC843501**
***Colletotrichum* sp.**	**CGMCC 3.15172**	**HM751816**	**KC244162**	**KC843547**	**KC843522**
Monilochaetes infuscans	CBS 869.96	JQ005780	JQ005864	JQ005843	–

[1] *BCC* BIOTEC Culture Collection (Thailand); *BRIP* Plant Pathology Herbarium, Department of Primary Industries, Queensland, Australia; *CBS* Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; *CGMCC* China General Microbial Culture Collection Center; *ICMP* International Collection of Microorganisms from Plants, Landcare Research, Auckland, New Zealand; *IMI* International Mycological Institute, CABI-Bioscience, Europe-UK, Egham, Bakeham Lane, UK; *MAFF* NIAS Genebank, Microorganism Section, Tsukuba, Japan; *MFLU* Mae Fah Luang University, Thailand

[2] *ITS* the nuclear ribosomal Internal transcribed spacer; *TUB2* β-tubulin; *ACT* actin; *GAPDH* glyceraldehyde-3-phosphate dehydrogenase

The isolated strains and newly generated sequences in this study are shown in bold

*indicates the ex-type cultures

Phylogenetic analyses were performed using PAUP v. 4.0 b10 (Swofford 2003). Ambiguously aligned regions were excluded from all analyses. Unweighted parsimony (UP) analysis was performed. Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Branches of zero length were collapsed and all equally most parsimonious trees were saved. Descriptive tree statistics such as tree length [TL], consistency index [CI], retention index [RI], rescaled consistency index [RC], and homoplasy index [HI], were calculated for trees generated. Robustness of clades was estimated by bootstrap analysis (Felsenstein 1985) with 1,000 replications. Trees were visualized in TreeView v. 1.6.6 (Page 1996).

A second phylogenetic analysis using Markov Chain Monte Carlo (MCMC) algorithm was conducted to generate trees with Bayesian posterior probabilities in MrBayes

v.3.1.2 (Ronquist and Huelsenbeck 2003). Nucleotide substitution models were determined using MrModeltest v.2.3 (Nylander 2004) for each gene region and included in the analyses. Two analyses of four MCMC chains were run from random trees for ten millions generations and sampled every 1,000 generations. The first 25 % of trees were discarded as the burn-in phase of each analysis and posterior probabilities determined from the remaining trees. Sequences derived in this study were deposited in GenBank.

Results

Phylogeny

The concatenated alignment of four loci from 124 strains comprised 1,710 characters including alignment gaps, of which 902 characters were parsimony informative, 89 variable and parsimony uninformative, and 719 constant. The parsimony analysis resulted in a most parsimonious tree (TL = 4608, CI = 0.4065, RI = 0.8511, RC = 0.3460, HI = 0.6068). The phylogram shows that 36 isolates from *Bletilla ochracea* belong to 17 distinct clades with high bootstrap support (Fig. 1), thus presumably representing different *Colletotrichum* species. The Bayesian tree agreed with the topology of the parsimonious tree, Bayesian posterior probability values ≥ 0.95 are shown at the nodes.

Taxonomy

Based on the multi-locus phylogeny and morphological characteristics, 36 endophytic isolates from orchid were identified as 17 species of *Colletotrichum* (Fig. 1, Table 3), including 7 new species (named as *C. bletillum*, *C. caudasporum*, *C. duyunensis*, *C. endophytum*, *C. excelsum-altitudum*, *C. guizhouensis* and *C. ochracea*), 8 previously described species (*C. boninense*, *C. cereale*, *C. destructivum*, *C. karstii*, *C. liriopes*, *C. miscanthi*, *C. parsonsiae* and *C. tofieldiae*), and 2 sterile mycelia.

Colletotrichum bletillum G. Tao, Z. Y. Liu & L. Cai, **sp. nov.**

MycoBank: MB803643

Figure 2a–o.

Etymology: Named after its host plant, *Bletilla ochracea.*

On PNP: Conidiomata not observed. Setae not observed. Conidiophores rare, formed directly on hyphae, hyaline, septate, sometimes branched. Conidiogenous cells hyaline, cylindrical, apex constricted, 11–26×2–2.5 μm, opening 1.5–2 μm diam, collarette distinct, funnel-shaped, 1–1.5 μm long. Conidia hyaline, smooth-walled, lunate, slightly curved, gradually obtuse on both sides, aseptate, 12.5–20.5×3–4.5 μm, mean ± SD=17.5±1.6×3.8±0.4 μm, *n*=31, L/W ratio = 4.6. Appressoria single, brown, ellipsoidal, ovoid to clavate or irregular with a crenate edge, 7.5–14 (−25)×5.5–10 μm, mean ± SD=11.9±3.2×7.9±1.2 μm, *n*=32, L/W ratio = 1.5.

Culture characteristics: Colonies on PDA reaching 71–84 mm diam in 7 d at 25 °C, white to pale grey aerial mycelia with circular margin, reverse grey to dark grey, sometimes slightly yellowish with age, few visible conidial masses. Mycelial growth rate 8.9–10.4 mm per day, mean ± SD=9.7±0.7 mm, *n*=5.

Material examined: CHINA, Guizhou Province, Shuicheng, Baijipo mountain, isolated from healthy leaves of *Bletilla ochracea*, 28 June 2006, Gang Tao (**Holotype** HMAS 244278 (dried culture); culture ex-holotype CGMCC 3.15117 = LC2340).

Notes: *Colletotrichum bletillum*, belonging to the "Spaethianum clade", is phylogenetically distinct and most closely related to *C. liriopes*, *C. tofieldiae* and *C. verruculosum* (Fig. 1) (Damm et al. 2009; Cannon et al. 2012). *Colletotrichum bletillum* is different from the three species as it failed to sporulate on SNA media (Damm et al. 2009) despite several attempts. Morphologically, *Colletotrichum bletillum* (on PNP medium) is different from *C. liriopes* and *C. tofieldiae* by producing conidia that are shorter and with lower L/W ratio (12.5–20.5×3.0–4.5 μm, L/W ratio = 4.6 in *C. bletillum* vs. (10.5–)16–23.5(−25.5)×(2.5–)3.5–4.5(−5) μm, L/W ratio = 5.0 in *C. liriopes*, and (12–)17–21(−23)×3–3.5(−4) μm, L/W ratio = 5.7 in *C. tofieldiae*) (Damm et al. 2009). Up to now, the "Spaethianum clade" contains only 7 species including the two new species *C. bletillum* and *C. guizhouensis* described in the present study.

Colletotrichum boninense Moriwaki, Toy. Sato & Tsukib., Mycoscience 44(1): 48 (2003)

Material examined: CHINA, Guizhou Province, Shuicheng, Baijipo mountain, isolated from healthy leaves of *Bletilla ochracea*, 28 June 2006, Gang Tao, culture CGMCC 3.15168 = LC2349; Qingzhen, Pianshan mountain, from healthy leaves of *B. ochracea*, 16 August 2006, Gang Tao, culture CGMCC 3.15125 = LC2327; Guiyang, Yongle mountain, from healthy leaves of *B. ochracea*, 11 June 2006, Gang Tao, culture CGMCC 3.15124 = LC2314; and Duyun, Xiaba mountain, from healthy leaves of *B. ochracea*, 13 July 2006, Gang Tao, culture CGMCC 3.15165 = LC2346.

Notes: *Colletotrichum boninense* was isolated as endophyte from leaves of *Bletilla ochracea* in this study. The conidial shape and dimension are exactly similar to the holotype of *Colletotrichum boninense* (Moriwaki et al. 2003). The appressoria are, however, slightly shorter than that from ex-holotype and ex-paratype cultures (Moriwaki et al. 2003). In the phylogram, our four endophytic strains confidently clustered together with the type strain of *C. boninense* (CBS123755) and strain CSSX8, which were reported as pathogens of orchid and *Crinum asiaticum*

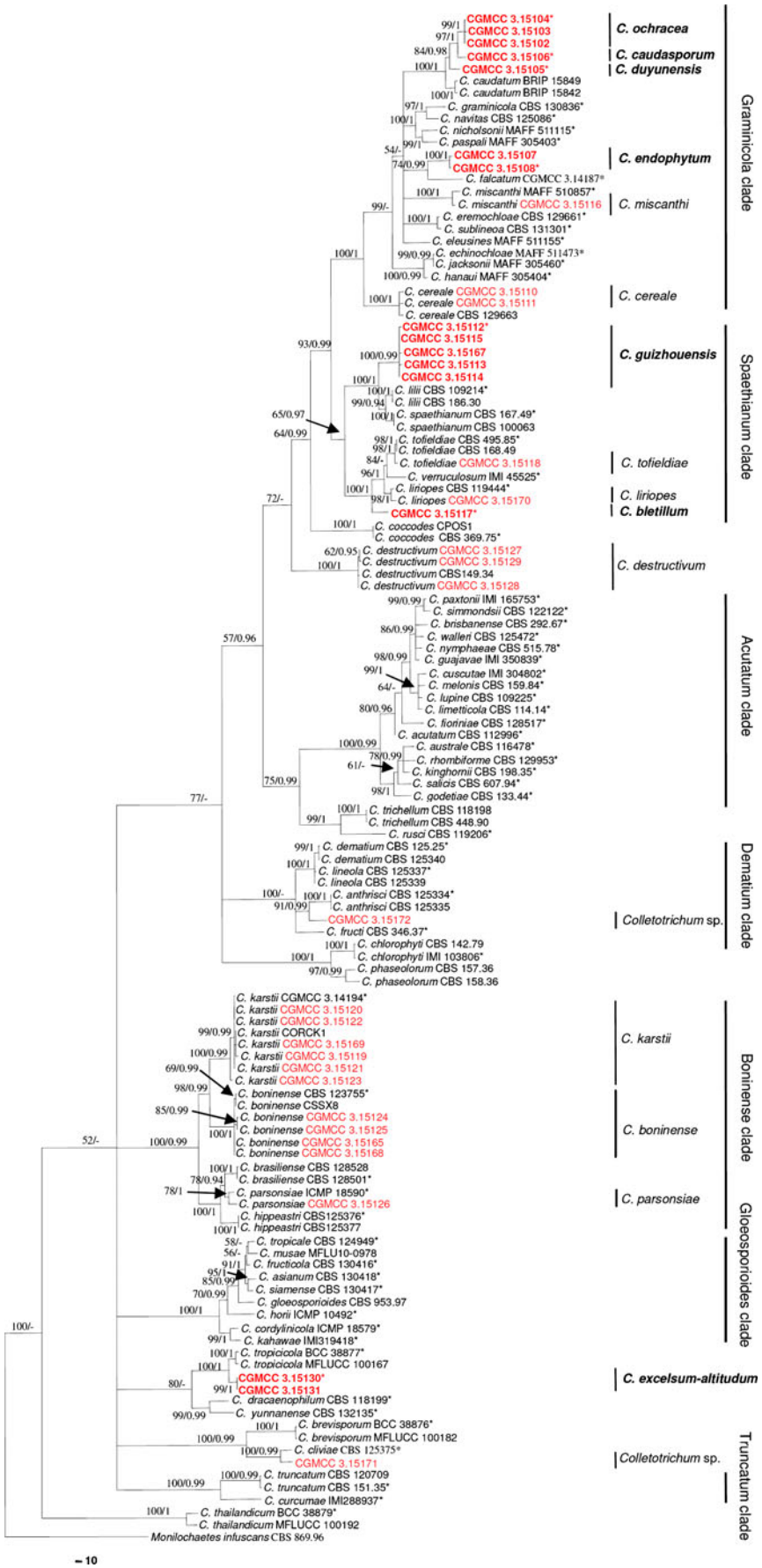

Fig. 1 Phylogenetic tree generated from a maximum parsimony analysis based on the combined ITS, TUB2, ACT and GAPDH sequence alignment, showing the phylogenetic relationships of endophytes from *Bletilla ochracea* (strain numbers are in red colour) with other related species. Values at the nodes represent parsimony bootstrap support values (> 50 %) and posterior probability values (≥ 0.95). Novel sequences are printed in bold and the scale bar indicates 10 changes. The tree is rooted with *Monilochaetes infuscans*. Asterisk (*) indicates the ex-type strains

Table 3 Morphological synopsis of endophytic *Colletotrichum* species from *Bletilla ochracea*

Species	Conidia shape and size on PNP (μm)	Appressoria shape and size on PNP (μm)	Colony characteristics on PDA	Mycelial growth rate PDA (mm/day)	References
C. bletillum	Falcate, slightly curved, gradually obtuse on both sides, aseptate, 12.5–20.5×3–4.5 μm, mean ± SD=17.5±1.6×3.8±0.4 μm, *n*=31, L/W ratio=4.6	Ellipsoidal to clavate or irregular with a crenate edge, sepia brown, 7.5–25×5.5–10 μm, mean ± SD=11.9±3.2×7.9±1.2 μm, *n*=32, L/W ratio=1.5	White to light grey aerial mycelia with circular around at margin, reverse grey to dark grey with age.	8.9–10.4 mm, mean ± SD=9.7±0.7 mm, *n*=5	This study
C. boninense	Cylindrical, with a hilum-like base, 12–15.5×4.5–6 μm, mean ± SD=13.8±0.9×5.1±0.4 μm, *n*=25, L/W ratio=2.7	Irregular, sepia to dark brown, 8.5–11×5–8.5 μm, mean ± SD=9.3±0.9×6.4±0.7 μm, *n*=20, L/W ratio=1.5	White aerial mycelia,reverse cream to reddish orange	7.8–9.1 mm, mean ± SD=8.5±0.5 mm, *n*=5	Moriwaki et al. 2003
C. caudasporum	Falcate, or fusiform, apices prolonged into a filiform, unbranched appendage, 18–33.5×3–5.5 μm, mean ± SD=25±3.5×4.3±0.6 μm, *n*=30, L/W ratio=5.8 (excluding appendage), conidial appendage 2.5–13.5 μm, mean ± SD=6.3±3.1 μm, *n*=30.	Ovate to ellipsoidal, sometimes clavate or lobed, 6–16×5–11 μm, mean ± SD=9.8±1.7×7±1.4 μm, *n*=50, L/W ratio=1.4.	White to grey, and cotton-like aerial mycelia, sometimes with feathery colonies at margin, reverse grey to dark grey or irregular sectors with slightly purplish pigment within the grey with age.	11.1–16.6 mm, mean ± SD=14.7±2.3 mm, *n*=5	This study
C. cereale	Falcate or fusiform, apices acute, 18–28.5×3–6 μm, mean ± SD=24.7±2.4×4.6±0.5 μm, *n*=33, L/W ratio=5.4	Ovate to ellipsoidal, rounded and smooth, or sometimes irregular or lobate, 7.5–21×5–10.5 μm, mean ± SD=12.6±3.1×7.4±1.1 μm, *n*=57, L/W ratio=1.7.	White to grey, and orange, cotton-like or fluffy aerial mycelia along agar surface, highly variable.	9.5–12.9 mm, mean ± SD=11±1.4 mm, *n*=5	Crouch et al. 2006
C. destructivum	Smooth, hyaline, fusiform to constricted, mostly cylindrical, 9–15.5×3–5 μm, mean ± SD=12±1.4×4±0.5 μm, *n*=41, L/W ratio=3	Thick-walled, medium brown, mostly globose,some clavste, smooth, sigle celled, 6–11×5–9.5 μm, mean ± SD=8.6±1.1×7.4±1.4 μm, *n*=40, L/W ratio=1.2.	Strawberry pink to carrot red, perithecia were sparse after 10–15 days, acervuli rarely formed.	8.9–9.7 mm, mean ± SD=9.2±0.3 mm, *n*=5	Manandhar et al. 1986
C. duyunensis	Cymbiform, falcate, apex prolonged into a filiform appendage, 15.2–28.5×3–5.5 μm, mean ± SD=22±3.1×4.3±0.5 μm, *n*=42, L/W ratio=5.1 (excluding appendage), conidial appendage 5.6–20.1 long, mean ± SD=10.9±5.2 μm (*n*=42).	Single, brown, subglobose to ellipsoidal, sometimes clavate or lobed, 7.2–16.3×5.2–10.4 μm, mean ±SD=11.3±2.3×7.8±1.3 μm, *n*=55, L/W ratio=1.5.	First white and becoming greyish white, dense, cottony, reverse pale yellowish to brown.	7–9 mm, mean ± SD=8±0.2 mm, *n*=5.	This study
C. endophytum	Falcate, strongly curved, apices obtuse, tapering much more towards the apex, 16–27.5×3.5–5.5 μm, mean ± SD=21.4±2.8×4.5±0.5 μm, *n*=48, L/W ratio=4.8.	Globose to subglobose, clavate, the edge entire, sometimes slightly lobed, 8.5–16×7–13 μm, mean ± SD=11.5±1.9×9.5±1.3 μm, *n*=22, L/W ratio=1.2.	White to yellowish green, grey, and felty aerial mycelia, reverse yellowish to grey, and dark grey with age.	8.9–9.6 mm, mean ± SD=9.2±0.2 mm, *n*=5	This study
C. excelsum-altitudum	Cylindrical, straight, sometimes slightly constricted near centre, ends broadly rounded, 12.5–16.5×5–7 μm, mean ± SD=14.8±0.8×5.8± 0.4 μm, *n*=61, L/W ratio=2.6.	Clavate, or irregular, sometimes deeply lobed, medium to dark brown, 7–14.5×5–10.5 μm, mean ± SD=10.9±2.1×6.5±1.4 μm, *n*=20, L/W ratio=1.7.	White to grey, and felty aerial mycelia, sometimes with sectors and circular around in colony, reverse yellowish to brown, and dark brown with age.	5.7–6.6 mm, mean ± SD=6±0.3 mm, *n*=5.	This study

Table 3 (continued)

Species	Conidia shape and size on PNP (μm)	Appressoria shape and size on PNP (μm)	Colony characteristics on PDA	Mycelial growth rate PDA (mm/day)	References
C. guizhouensis	Falcate, fusiform, straight to slightly curved, 16–23.5×3–4.5 μm, mean ± SD=19.5±1.9×3.6±0.3 μm, *n*=52, L/W ratio=5.4.	Ellipsoidal to clavate, sometimes slightly lobed, or irregular with a crenate edge, dark brown, 6–14.5×4.5–10.5 μm, mean ± SD=10.8±1.9×8.2±1.6 μm, *n*=28, L/W ratio=1.3.	White to light grey aerial mycelia, reverse off- white to slightly gray.	8–11 mm, mean ± SD= 9.4±1.1 mm, *n*=5.	This study
C. karstii	Cylindrical, straight, btuse at both apexes, base truncate, 12–16×6–7.5 μm, mean ± SD=13.8±0.9×6.8±0.4 μm, *n*=30, L/W ratio=2.1.	Circular to clavate, margin entire, sepia brown, 6.5–11.5×4.5–7.5 μm, mean ± SD=8.8±1.2×6.1±1.1 μm, *n*=20, L/W ratio=1.4.	White aerial mycelia, reverse reddish yellow.	7.2–9 mm, mean ± SD= 8.3±0.7 mm, *n*=5.	Yang et al. 2011
C. liriopes	Hyaline, smooth walled, falcate, fusiform, 10.5–24×2.5–5 μm, mean ± SD=18±4.8×3.5±2.4, *n*=40, L/W ratio=5.1.	Clavate to irregular, margin crenate to lobbed, medium to dark brown, 8.5–17×8–15 μm, mean ± SD= 13±2.8×8.2±2.3, *n*=40, L/W ratio=1.6.	Pale to dark grey aerial mycelia, reverse yellowish brown to dark brown with age.	8–9.5 mm, mean ± SD= 8.8±0.6 mm, *n*=5.	Damm et al. 2009
C. miscanthi	Hyaline, falcate or fusiform, apex acute, conidial base obtuse, 21–32×3.5–5 μm, mean ± SD=28±3.3×4.5±0.4, *n*=22, L/W ratio=6.2.	Ellipsoidal to subglobose, sometimes clavate, brown, 7–13×5–10.5 μm, mean ± SD=10±2×7±1.2, *n*=20, L/W ratio=1.4.	Pale to dark grey mycelium with medium grey rings and irregular felty edge, reverse grey to black with age.	9–10 mm, mean ± SD= 9.5±0.4 mm, *n*=5.	Crouch et al. 2009a
C. ochracea	Straight or slightly curved, one-celled, hyaline, guttulate, cylindrical with obtuse ends, 5.5−15×1.5−3.5 μm, mean±SD=9.4±2.4×2.6±0.4 μm, *n*=60, L/W ratio=3.6.	Single, dark brown, subglobose, clavate, the edge entire, 9.3−18.2×6.6−9.3 μm, mean ± SD=12.4±2.3×8±0.4 μm, *n*=20, L/W ratio=1.6.	White to yellowish, sparse, and with floccose aerial mycelia in centre, reverse slightly yellowish.	6−8 mm, mean ± SD= 6.8±0.3 mm, *n*=5.	This study
C. parsonsiae	Cylindrical, straight, sometimes slightly constricted near centre, broadly rounded at ends, 11.5–18×4.5–7.5 μm, mean ± SD=15.5±1.4×5.8±0.7 μm, *n*=38, L/W ratio=2.7.	Ellipsoidal to globose with a crenate edge, brown, 5.5–8.5×4.5–7 μm, mean ± SD=6.5±0.8×5.5±0.7 μm, *n*=20, L/W ratio=1.2.	White aerial mycelia, reverse off- white to slightly gray.	4.8–5.6 mm, mean ± SD= 5±0.3 mm, *n*=5.	Damm et al. 2012b
C. tofieldiae	Falcate, distinctly curved, both sides gradually tapering towards the round apex and round or truncate base, 15.5–23×3.5–5 μm, mean ± SD=19.8±1.8×4.2±0.3 μm, *n*=65, L/W ratio=4.7.	Ellipsoidal to clavate, entire edge, crenate or more or less lobed, aseptate, medium brown or dark brown to almost black, 9.5–16.5× 5.5–11.5 μm, mean ± SD=11.5±2.3×7.4±1.7 μm, *n*=20, L/W ratio=1.6.	White to grey , and cotton-like aerial mycelia,reverse grey to dark grey or irregular sectors with orange.	9.2–13.2 mm, mean ± SD= 10.8±1.5 mm, *n*=5.	Damm et al. 2009

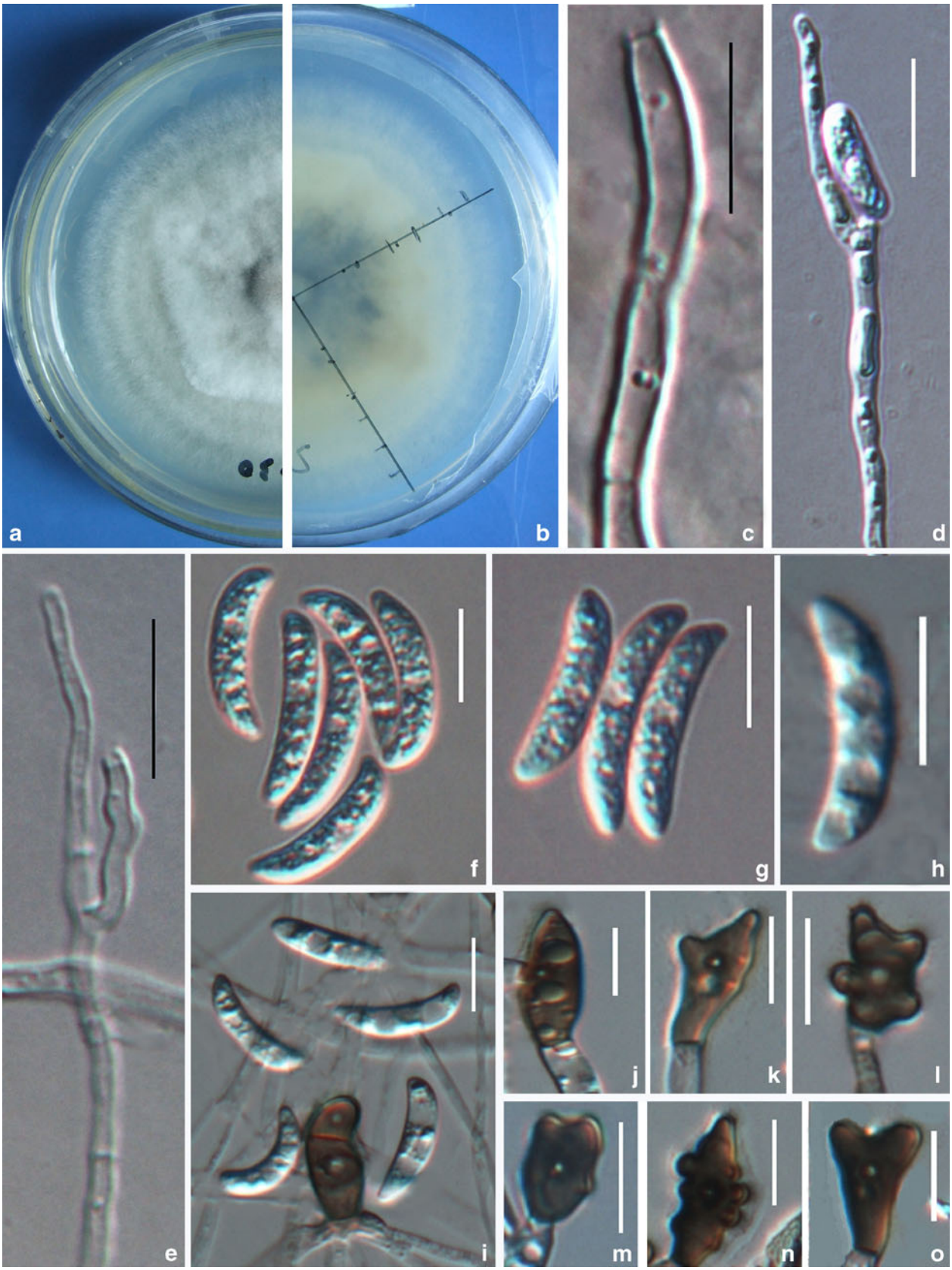

Fig. 2 *Colletotrichum bletillum* (from holotype). **a**–**b** Colonies on PDA in 7 days, upper (**a**) and reverse (**b**); **c**, **e** Conidiophores; **d**, **f**–**h** Conidia; **i** Conidia and appressoria; **j**–**o** Appressoria. *Scale Bars*: **c**–**o** = 10 μm

(Amaryllidaceae) (Moriwaki et al. 2003; Yang et al. 2009; Damm et al. 2012b) (Fig. 1).

Colletotrichum caudasporum G. Tao, Z. Y. Liu & L. Cai, **sp. nov.**

MycoBank: MB 803646

Figure 3a–q.

Etymology: Referring to the falcate conidia with a tail.

On PNP: Conidiomata not observed. Setae not observed. Conidiophores rare, directly formed on hyphae, hyaline, septate, occasionally branched. Conidiogenous cell clavate or cylindrical, apex more or less inflated, collarette not visible. Conidia cymbiform, fusiform, or falcate, apex prolonged into a filiform appendage, 18–30×3–5.5 μm, mean ± SD=25±3.5×4.3±0.6 μm, *n*=30, L/W ratio = 5.8 (excluding appendage), conidial appendage 2.5–13 long, mean ± SD=6.3±3.1 μm (*n*=30). Appressoria single, brown, ovate to ellipsoidal, sometimes clavate or lobed, 6.5–16×5–11.0 μm, mean ± SD=9.8±1.7×7±1.4 μm, *n*=50, L/W ratio = 1.4.

Culture characteristics: Colonies on PDA reaching 70–85 mm diam in 5 days at 25 °C, white to grey, and cotton-like aerial mycelia, sometimes with fimbriate margin, reverse grey to dark grey or irregular sectors with slightly purplish pigment with age. Mycelial growth rate 11–16.5 mm per day, mean ± SD=14.7±2.3 mm, *n*=5.

Material examined: CHINA, Guizhou Province, Duyun, Xiaba mountain, isolated from healthy leaves of *Bletilla ochracea*, 13 July 2006, Gang Tao (**Holotype** HMAS 244282 (dried culture); culture ex-holotype CGMCC 3.15106 = LC2311).

Notes: *Colletotrichum caudasporum* is morphologically similar to *Colletotrichum caudatum* (Sacc.) Peck, but could be distinguished from the later by the dimension of conidial appendage (2.5–13 μm vs. 10–16 μm) (Sutton 1980). In the phylogram, *C. caudasporum* appears in a distinct lineage from *C. caudatum* (Fig. 1). Although there is no type specimen and type-derived sequences of *Colletotrichum caudatum*, we can confirm that *C. caudasporum* is a distinct species from *C. caudatum* based on the comparisons to original descriptions. *Colletotrichum caudasporum* belongs to the "Graminicola clade" recognised as a distinct assemblage by Crouch et al. (2009a, b) and Cannon et al. (2012). The conidial appendage of *Colletotrichum caudasporum* is different from all other species in this clade. Several species in the "Graminicola clade" are economically important pathogens, including *C. falcatum* on sugarcane (*Saccharum*), *C. graminicola* on maize (*Zea*), *C. sublineola* on *Sorghum* species, *C. cereale* and *C. eremochloae* on cultivated turfgrasses (Wilson 1914; Crouch and Beirn 2009). However, *C. caudasporum* described here is endophytic.

Colletotrichum cereale Manns, Ohio Agric. Exp. Stn. Bull. 203: 207 (1909)

Material examined: CHINA, Guizhou Province, Duyun, Xiaba mountain, isolated from healthy leaves of *Bletilla ochracea*, 13 July 2006, Gang Tao, culture CGMCC 3.15110 = LC2306; Duyun, Xiaba mountain, isolated from healthy leaves of *B. ochracea*, 13 July 2006, Gang Tao, culture CGMCC 3.15111 = LC2308.

Notes: *Colletotrichum cereale* is the pathogen of grass of the subfamily Pooideae, and was re-described through a designation of epitype by Crouch et al. (2006). Both strains (CGMCC 3.15110 and CGMCC 3.15111) have similar conidia and appressoria to that of *C. cereale* epitype (Crouch et al. 2006). In the phylogram, they clustered together with the epitype of *C. cereale* (CBS129663) with highly supported bootstrap and posterior probabilities (100 %/1.00) (Fig. 1).

Colletotrichum destructivum Manandhar, J. B., Hartman, G. L. & Sinclair, J. B., Phytopathology 76 (3): 284 (1986)

Material examined: CHINA, Guizhou Province, Qianxi, Hongshui mountain, isolated from healthy leaves of *Bletilla ochracea*, 17 July 2006, Gang Tao, culture CGMCC 3.15127 = LC2320; Qianxi, Hongshui mountain, isolated from healthy leaves of *B. ochracea*, 17 July 2006, Gang Tao, culture CGMCC 3.15128 = LC2323; Qingzhen, Pianshan mountain, isolated from healthy leaves of *B. ochracea*, 16 August 2006, Gang Tao, culture CGMCC 3.15129 = LC2329.

Notes: *Colletotrichum destructivum* was reported as pathogen on lucerne (*Medicago sativa*) and soybean (*Glycine max*) (Manandhar et al. 1986; Latunde-Dada et al. 1999), and parasitise on a range of plants of Brassicaceae, Cuscutaceae, Lamiaceae and Solanaceae (Hyde et al. 2009). In the present study, three endophytic strains were identified as *C. destructivum* based on morphology and multi-locus phylogeny. In the phylogram, three strains clustered together with the type strain of *C. destructivum* (CBS149.34) with bootstrap support/posterior probability values of 100 %/1.00 (Fig. 1).

Colletotrichum duyunensis G. Tao, Z. Y. Liu & L. Cai, **sp. nov.**

MycoBank: MB 804546

Figure 4a–o.

Etymology: Referring to the Duyun county in China where this fungus was first collected.

On PNP: Conidiomata not observed. Setae not observed. Conidiophores rare, directly formed on hyphae, hyaline, septate, occasionally branched. Conidiogenous cell clavate or cylindrical, apex more or less inflated, collarette not visible. Conidia cymbiform, falcate, apex prolonged into a filiform appendage, 15.2–28.5×3–5.5 μm, mean ± SD=22±3.1×4.3±0.5 μm, *n*=42, L/W ratio = 5.1 (excluding appendage), conidial appendage 5.6–20.1 long, mean ± SD=10.9±5.2 μm (*n*=42). Appressoria single, brown, subglobose to ellipsoidal, sometimes clavate or lobed, 7.2–16.3×5.2–10.4 μm, mean ± SD=11.3±2.3×7.8±1.3 μm, *n*=55, L/W ratio = 1.5.

Culture characteristics: Colonies on PDA reaching 56 mm diam in 7 days at 25 °C, at first white and becoming greyish white, dense, cottony, reverse pale yellowish to

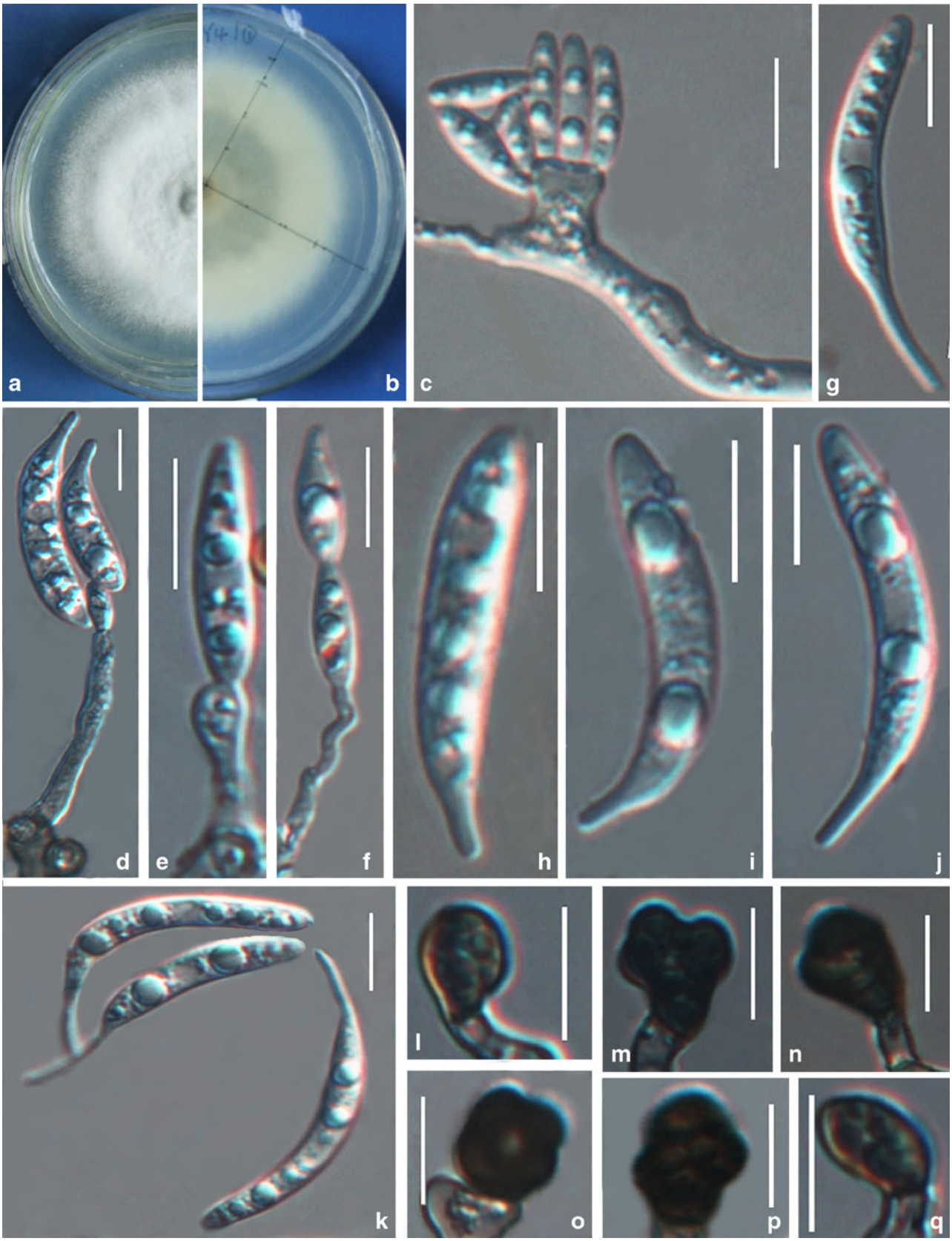

Fig. 3 *Colletotrichum caudasporum* (from holotype). **a**, **b** Colonies on PDA in 7 days, upper **a** and reverse **b**; **c**–**f** Conidiophores cells; **g**–**k** Conidia; **l**–**q** Appressoria. Scale Bars: **c**–**q** = 10 μm

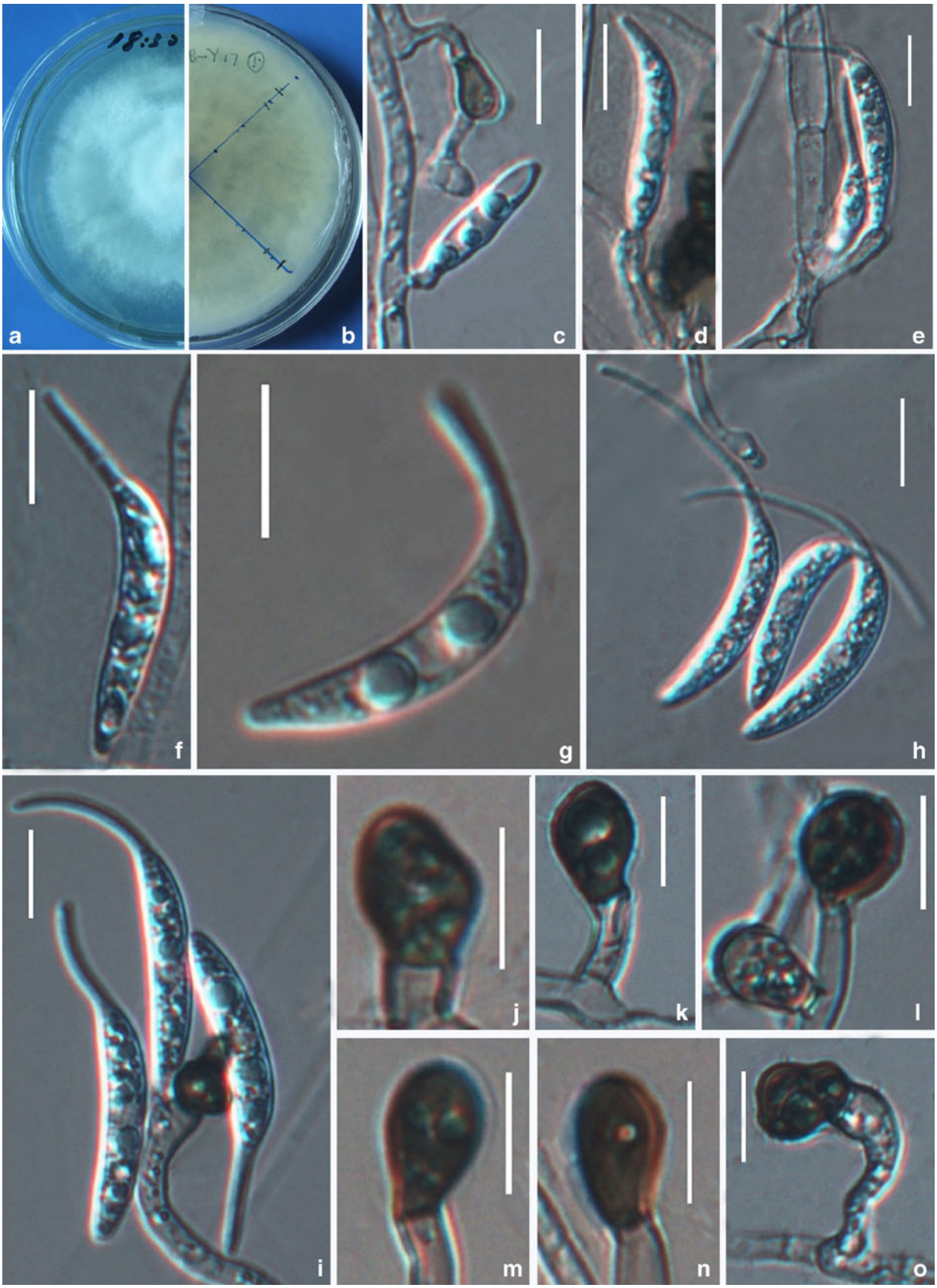

Fig. 4 *Colletotrichum duyunensis* (from holotype). **a**, **b** Colonies on PDA in 7 days, upper **a** and reverse **b**; **c**–**e** Conidiophores cells; **f**–**i** Conidia; **j**–**o** Appressoria. Scale Bars: **c**–**o** = 10 μm

brown. Mycelial growth rate 7−9 mm per day, mean ± SD=8±0.2 mm, *n*=5.

Material examined: CHINA, Guizhou Province, Duyun, Xiaba mountain, isolated from healthy leaves of *Bletilla ochracea*, 13 July 2006, Gang Tao (**Holotype** HMAS 244832 (dried culture); culture ex-holotype CGMCC 3.15105 = LC2307).

Notes: *Colletotrichum duyunensis* is a sister clade of *Colletotrichum caudatum* (Sacc.) Peck and the new species of *C. caudasporum* (Fig. 1). *C. duyunensis* shows highly morphological similarity to *C. caudatum* and *C. caudasporum* with a special conidial appendage, but could be distinguished from them by the dimension of conidial appendage (5.6–20.1 μm in *C. duyunensis* vs. 2.5–13 μm in *C. caudasporum*) and setae (absence of setae in *C. duyunensis* vs. abundant setae in *C. caudatum*) (Sutton 1980). In the phylogram, *C. duyunensis* appears in a distinct lineage from *C. caudasporum* and *C. caudatum* with 100 %/1.00 of bootstrap support and posterior probability values (Fig. 1).

Colletotrichum endophytum G. Tao, Z.Y. Liu & L. Cai, **sp. nov.**

MycoBank: MB 803647

Figure 5a–n.

Etymology: Referring to the endophytic life mode of this fungus.

On PNP: Conidiomata not observed. Setae scattered or in small groups under or among hyphae, straight or bent at the base, 2–3-septate, brown, basal cell pale brown, 47.5–113.5 μm long, base more or less inflated, tip usually acute. Conidiophores abundant, formed directly on hyphae, hyaline to pale brown, simple, sometimes branched, septate. Conidiogenous cell enteroblastic, clavate or cylindrical, sometimes elongate ampulliform, apices more or less constricted, 8.5–21.5×3–5 μm, opening 1.5–2.5 μm diam, collarette visible, 1–1.5 μm long. Conidia falcate, strongly curved, base obtuse, tapering much more towards apex, 16–27.5×3.5–5.5 μm, mean ± SD=21.4±2.8×4.5±0.5 μm, *n*=48, L/W ratio = 4.8. Appressoria single, medium to dark brown, globose to subglobose, clavate, the edge entire, sometimes slightly lobed, 8.5–16×7–13 μm, mean ± SD=11.5±1.9×9.5±1.3 μm, *n*=22, L/W ratio=1.2.

Culture characteristics: Colonies on PDA reaching 68–70 mm diam in 7 days at 25 °C, white to yellowish green, grey, and felty aerial mycelia, reverse yellowish to grey, and dark grey with age, visible conidial masses. Mycelial growth rate 8.9–9.6 mm per day, mean ± SD=9.2±0.2 mm, *n*=5.

Material examined: CHINA, Guizhou Province, Shuicheng, Baijipo mountain, isolated from healthy leaves of *Bletilla ochracea*, 28 June 2006, Gang Tao (**Holotype** HMAS 244280 (dried culture); culture ex-holotype CGMCC 3.15108 = LC2338); Guiyang, Yongle mountain, isolated from healthy leaves of *B. ochracea*, 11 June 2006, Gang Tao, culture CGMCC 3.15107 = LC2318.

Notes: Strains representing *Colletotrichum endophytum* clustered in a sister clade to *C. falcatum* (Fig. 1). *C. endophytum* produces shorter conidia than *C. falcatum* (8.5–21.5×3–5 μm vs. 15.5–26.5×4–5 μm). *C. endophytum* could be further differentiated from *C. falcatum* by producing relatively abundant seta, and strongly curved or falcate conidia (Sutton 1980; Prihastuti et al. 2010).

Colletotrichum excelsum-altitudum G. Tao, Z.Y. Liu & L. Cai, **sp. nov.**

MycoBank: MB 803648

Figure 6a–p.

Etymology: Referring to the high altitude site where this species was first collected.

On PNP: Conidiomata acervular, conidiophores and setae formed on a basal cushion, hyaline to pale brown, clavate or cylindrical. Setae straight or sometimes slightly bent, 3–5-septate, brown, 70–114 μm long, basal cell pale brown, cylindrical, tip usually acute. Conidiophores abundant, hyaline to pale brown, septate, unbranched. Conidiogenous cell clavate or cylindrical, apex sometimes constricted, 8.5–25×4–5 μm, collarette hardly visiable. Conidia cylindrical, straight, sometimes slightly constricted near centre, both ends broadly rounded, 13–16.5×5–7 μm, mean ± SD=14.8±0.8×5.8±0.4 μm, *n*=61, L/W ratio=2.6. Appressoria clavate, or irregular, sometimes deeply lobed, medium to dark brown, 7–14.5×5–10.5 μm, mean ± SD=10.9±2.1×6.5±1.4 μm, *n*=20, L/W ratio=1.7. Teleomorph not produced in culture after 3 months.

Culture characteristics: Colonies on PDA reaching 41–46 mm diam in 7 days at 25 °C, white to grey, and felty aerial mycelia, sometimes with sectors and circular margin in colonies, reverse yellowish to brown, and dark brown with age. Mycelial growth rate 5.7–6.6 mm per day, mean ± SD=6±0.3 mm, *n*=5.

Material examined: CHINA, Guizhou Province, Shuicheng, Baijipo mountain, isolated from healthy leaves of *Bletilla ochracea*, 28 June 2006, Gang Tao (**Holotype** HMAS244279 (dried culture); culture ex-holotype CGMCC 3.15130 = LC2344); Shuicheng, Baijipo mountain, isolated from healthy leaves of *B. ochracea*, 28 June 2006, Gang Tao, culture CGMCC 3.15131 = LC2345.

Notes: The cylindrical conidia of *C. excelsum-altitudum* resemble that of *C. boninense* and species in *C. gloeosporioides* complex. However, the phylogram reveals its close affinity to *C. tropicicola* (Fig. 1). *C. excelsum-altitudum* can be distinguished from *C. tropicicola* by the dimensions of conidia and appressoria (conidia 13–16.5×5–7, appressoria 7–14.5×5–10.5 vs. conidia 15–19×6–7 μm, appressoria 13–24×7–8 μm) (Noireung et al. 2012).

Colletotrichum guizhouensis G. Tao, Z.Y. Liu & L. Cai, **sp. nov.**

MycoBank: MB 803649

Figure 7a–s.

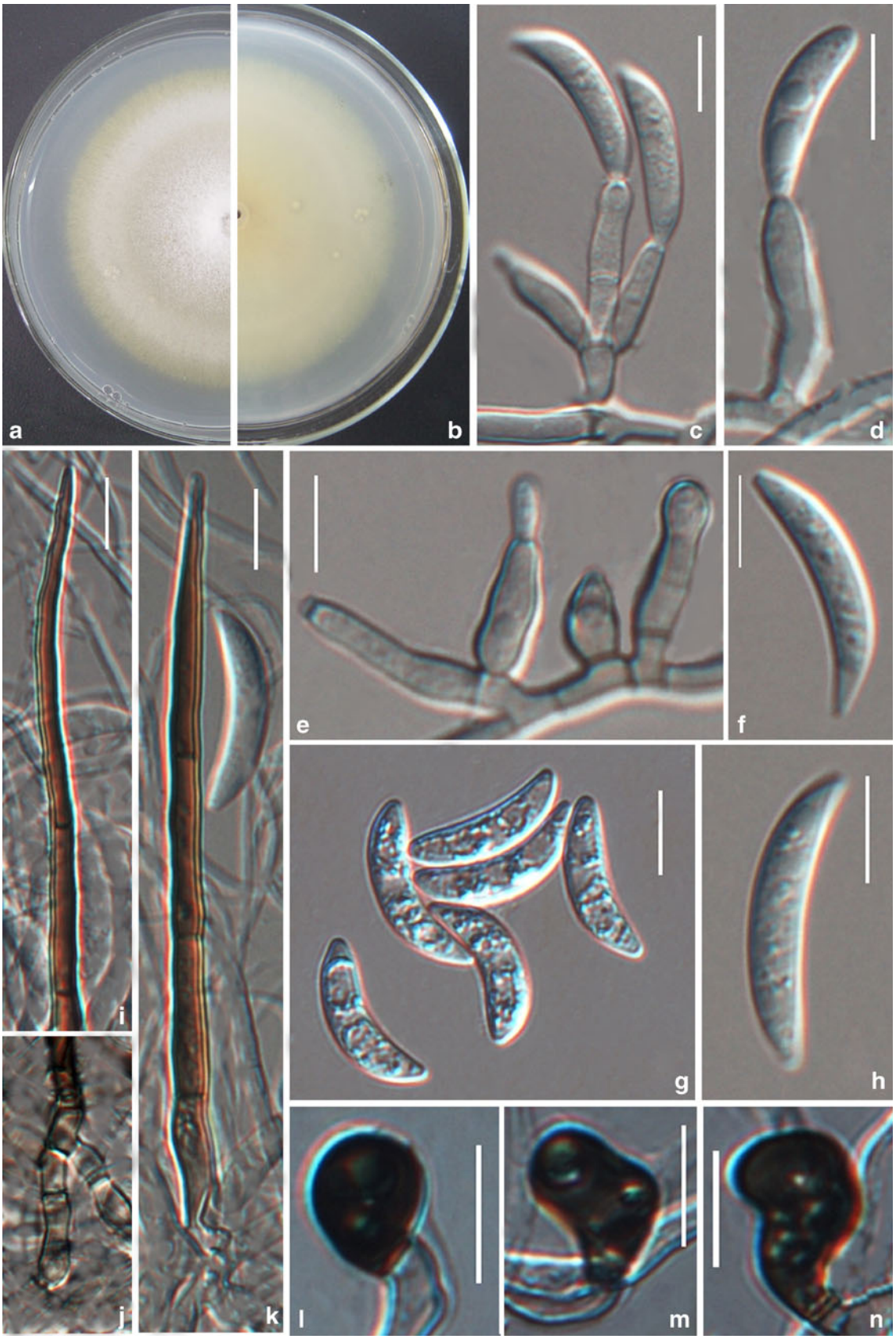

Fig. 5 *Colletotrichum endophytum* (holotype). **a**, **b** Colonies on PDA in 7 days, upper **a** and reverse **b**; **c**–**e** Conidiophores; **f**–**h** Conidia; **i**–**k** Setae, **i**, **j** tip and base of a seta; **l**–**n** Appressoria. Scale Bars: **c**–**n** = 10 μm

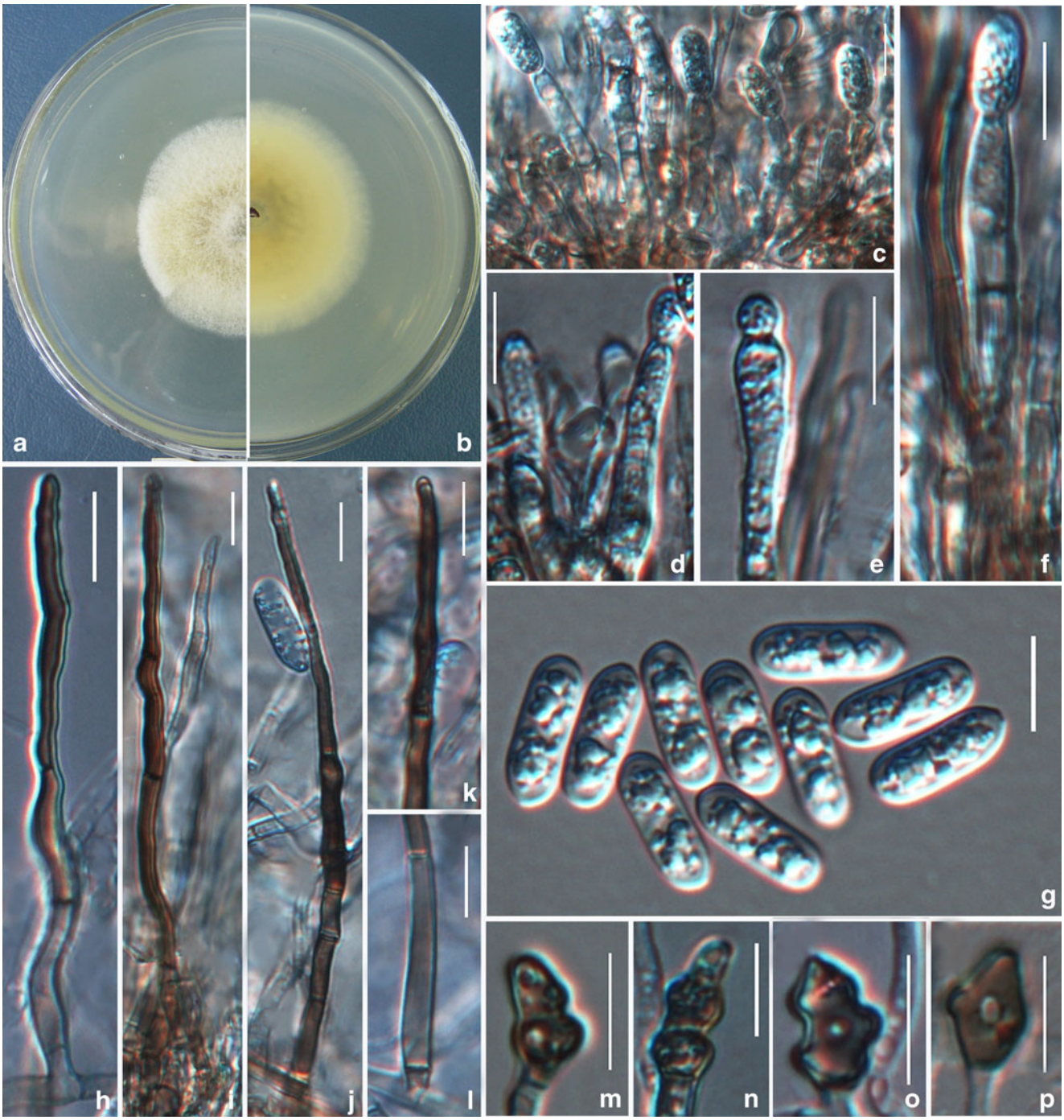

Fig. 6 *Colletotrichum excelsum-altitudum* (from holotype). **a**, **b** Colonies on PDA in 7 days, upper **a** and reverse **b**; **c**–**f** Conidiophores; **g** Conidia; **h**–**l** Setae; **m**–**p** Appressoria. Scale Bars: **c**–**p** = 10 μm

Etymology: Named after Guizhou province where it was first collected.

On PNP: Conidiomata acervular, few developed, setae and few conidiophores formed from a basal cushion of hyaline to pale brown and clavate cells. Setae straight or sometimes slightly bent, 2–4-septate, dark brown, septation hardly visible, 65.5–170 μm long, basal cell brown to dark brown, basel cell cylindrical, tip acute. Conidiophores formed from a cushion of pale brown cells or directly on hyphae, abundant, hyaline to pale brown, usually branched. Conidiogenous cell slightly bent, apex sometimes constricted to acute, clavate, 12–25×2–2.5 μm, collarette not observed. Conidia falcate, fusiform, straight to slightly curved, 16–23.5×3–4.5 μm, mean ± SD=19.5±1.9×3.6±0.3 μm, *n*=52, L/W ratio=5.4. Appressoria ellipsoidal to clavate, sometimes slightly lobed, or irregular with a crenate edge, dark brown, 6–

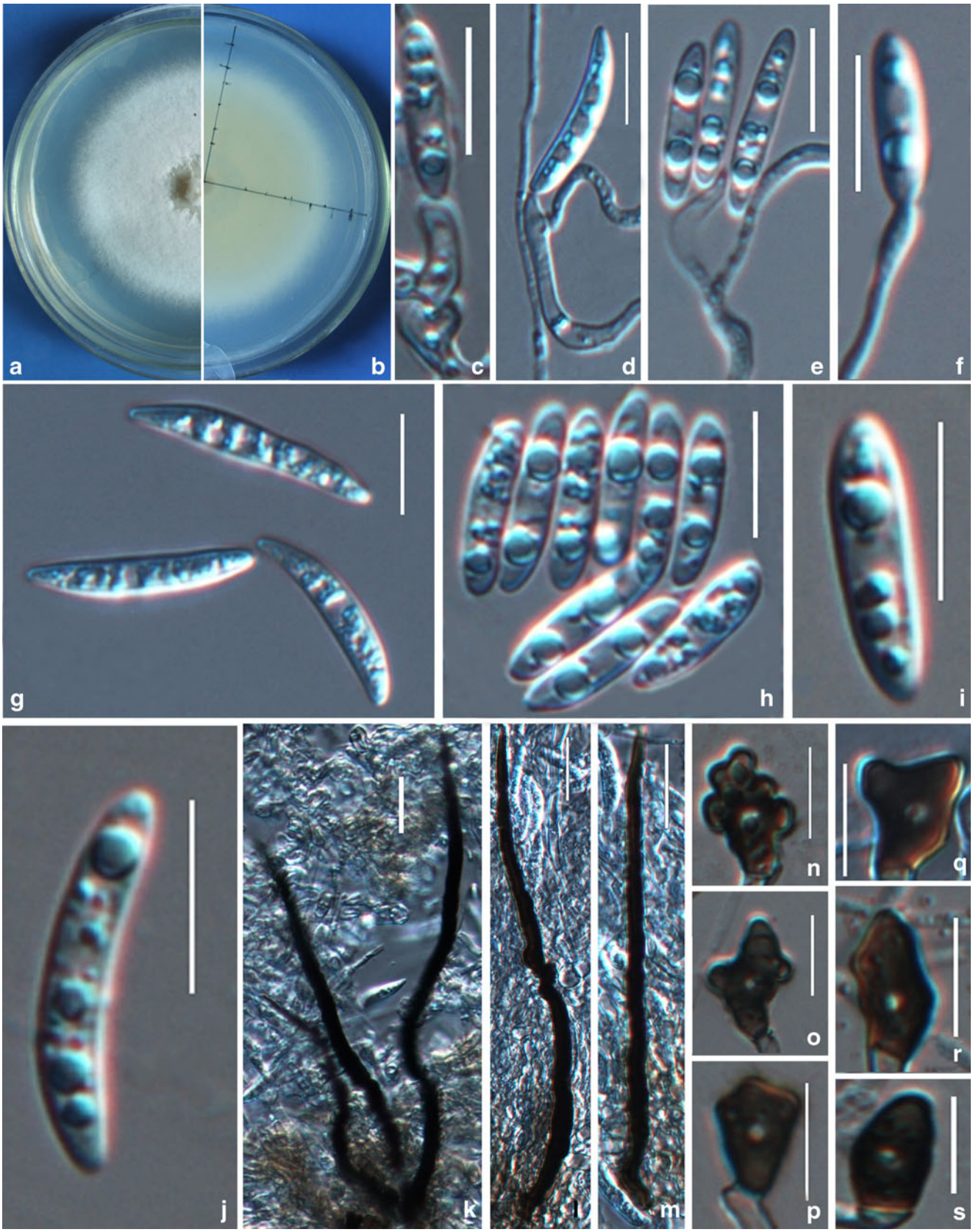

Fig. 7 *Colletotrichum guizhouensis* (from holotype). **a**, **b**. Colonies on PDA in 7 days, upper **a** and reverse **b**; **c**–**f** Conidiophores; **g**–**j** Conidia; **k**–**m** Setae; **n**–**s** Appressoria. Scale Bars: **c**–**j**, **n**–**s** = 10 μm; **k**–**m** = 20 μm

14.5×5–11 μm, mean ± SD=10.8±1.9×8.2±1.6 μm, *n*=28, L/W ratio=1.3.

Culture characteristics: Colonies on PDA reaching 56–74 mm diam in 7 days at 25 °C, white to light grey aerial mycelia, reverse off-white to slightly gray. Mycelial growth rate 8–11 mm per day, mean ± SD=9.4±1.1 mm, *n*=5.

Material examined: CHINA, Guizhou Province, Duyun, Xiaba mountain, isolated from healthy leaves of *Bletilla ochracea*, 13 July 2006, Gang Tao (**Holotype** HMAS244281 (dried culture); culture ex-holotype CGMCC 3.15112 = LC2305); Yongle mountain Guiyan, isolated from healthy leaves of *B. ochracea*, 11 June 2006, Gang Tao, culture CGMCC 3.15113 = LC2313; Qianxi, Hongshui mountain, isolated from healthy leaves of *B. ochracea*, 17 July, 2006, Gang Tao, culture CGMCC 3.15167 = LC2348; Qianxi, Hongshui mountain, isolated from healthy leaves of *B. ochracea*, 17 July, 2006, Gang Tao, culture CGMCC 3.15114 = LC2319; Qianxi, Hongshui mountain, isolated from living leaves of healthy *B. ochracea*, 17 July, 2006, Gang Tao, culture CGMCC 3.15115 = LC2322.

Notes: *Colletotrichum guizhouensis* is morphologically similar to *C. lilii* and *C. spaethianum* but differs from them in having darker and longer setae (65.5–170 μm in *C. guizhouensis* vs. 20–70 μm in *C. lilii* and 30–90 μm in *C. spaethianum*) (Damm et al. 2009).

Colletotrichum karstii Y.L. Yang, Z.Y. Liu, K.D. Hyde & L. Cai, Cryptogamie Mycologie 32: 241 (2011)

Material examined: CHINA, Guizhou Province, Shuicheng, Baijipo mountain, isolated from healthy leaves of *Bletilla ochracea*, 28 June 2006, Gang Tao, culture CGMCC 3.15119 = LC2342; Shuicheng, Baijipo mountain, isolated from healthy leaves of *B. ochracea*, 28 June 2006, Gang Tao, culture CGMCC 3.15169 = LC2350; Qingzhen, Pianshan mountain, isolated from healthy leaves of *B. ochracea*, 16 August 2006, Gang Tao, culture CGMCC 3.15120 = LC2328; Qingzhen, Pianshan mountain, isolated from healthy leaves of *B. ochracea*, 16 August 2006, Gang Tao, culture CGMCC 3.15121 = LC2332; Duyun, Xiaba mountain, isolated from healthy leaves of *B. ochracea*, 13 July 2006, Gang Tao, culture CGMCC 3.15122 = LC2304; Duyun, Xiaba mountain, isolated from leaves of healthy *B. ochracea*, 13 July 2006, Gang Tao, culture CGMCC 3.15123 = LC2312.

Notes: *Colletotrichum karstii* was described by Yang et al. (2011), and has been known as pathogen and endophyte from the leaf and root of *Calanthe argenteo-striata*, *Eria coronaria* and *Pleione bulbocodioides* (Orchidaceae) in China (Yang et al. 2011). In this study, six strains isolated from leaves of *Bletilla ochracea* were identified as *C. karstii* on the basis of morphological and phylogenetic comparison to the holotype (Yang et al. 2011). Recently, many previous records cited as *C. boninense* have been confirmed to be *C. karstii* (Damm et al. 2012b), including strains used in Moriwaki et al. (2003), Farr et al. (2006) and Lubbe et al. (2004). Some isolates from *Passiflora edulis* in Brazil that caused anthracnose fruits (Tozze et al. 2010) were also re-identified to be *C. karstii*.

Colletotrichum liriopes Damm, P.F. Cannon & Crous, Fungal Diversity 39: 71 (2009)

Material examined: CHINA, Guizhou Province, Shuicheng, Baijipo mountain, isolated from healthy leaves of *Bletilla ochracea*, 28 June 2006, Gang Tao, culture CGMCC 3.15170 = LC2351.

Notes: *Colletotrichum liriopes* has been known as a pathogen from herbaceous host of *Liriope muscari* (Damm et al. 2009), and a common endophyte and pathogen from leaves and roots of orchid plants (Yang et al. 2011). In the present study, the strain CGMCC 3.15170 was identified as *C. liriopes* on the basis of morphology and molecular phylogeny (Fig. 1).

Colletotrichum miscanthi J.A. Crouch, B.B. Clarke, J.F. White & B.I. Hillman, Mycologia 101: 729 (2009)

Material examined: CHINA, Guizhou Province, Duyun, Xiaba mountain, isolated from healthy leaves of *Bletilla ochracea*, 13 July 2006, Gang Tao, culture CGMCC 3.15116 = LC2310.

Notes: *Colletotrichum miscanthi* belongs to the "Graminicola clade" which consists of grass-associated *Colletotrichum* species (Crouch et al. 2009a). In the original description of *C. miscanthi*, the morphology of appressoria was not provided (Crouch et al. 2009a). Our strain was identified to be *C. miscanthi* based on morphology and multi-locus phylogeny (Crouch et al. 2009a, Fig. 1). The hyphal appressoria were successfully induced on the PNP under the 12 h near-UV light and 12 h dark and described and illustrated based on strain CGMCC 3.15116 (Table 3, Fig. 8).

Colletotrichum ochracea G. Tao, Z.Y. Liu & L. Cai, **sp. nov.**

MycoBank: MB804547

Figure 9a–o.

Etymology: Named after its host plant, *Bletilla ochracea.*

On PNP: Conidiomata not observed. Setae absent. Conidiophores hyaline, smooth, formed directly on hyphae, septate, branched. Conidiogenous cell straight or slightly curved, enteroblastic, cylindrical, tapering towards the apex, 9−39×1.5−3 μm, opening 1−1.5 μm diam, collarette absent. Conidia straight or slightly curved, one-celled, hyaline, guttulate, cylindrical with obtuse ends, 5.5−15×1.5−3.5 μm, mean ± SD=9.4±2.4×2.6±0.4 μm, *n*=60, L/W ratio=3.6. Appressoria single, dark brown, subglobose, clavate, the edge entire, 9.3−18.2×6.6−9.3 μm, mean ± SD=12.4±2.3×8±0.4 μm, *n*=20, L/W ratio=1.6.

Culture characteristics: Colonies on PDA reaching 48 mm diam in 7 days at 25 °C, white to yellowish, sparse, and with floccose aerial mycelia in centre, reverse slightly yellowish. Mycelial growth rate 6−8 mm per day, mean ± SD=6.8±0.3 mm, *n*=5.

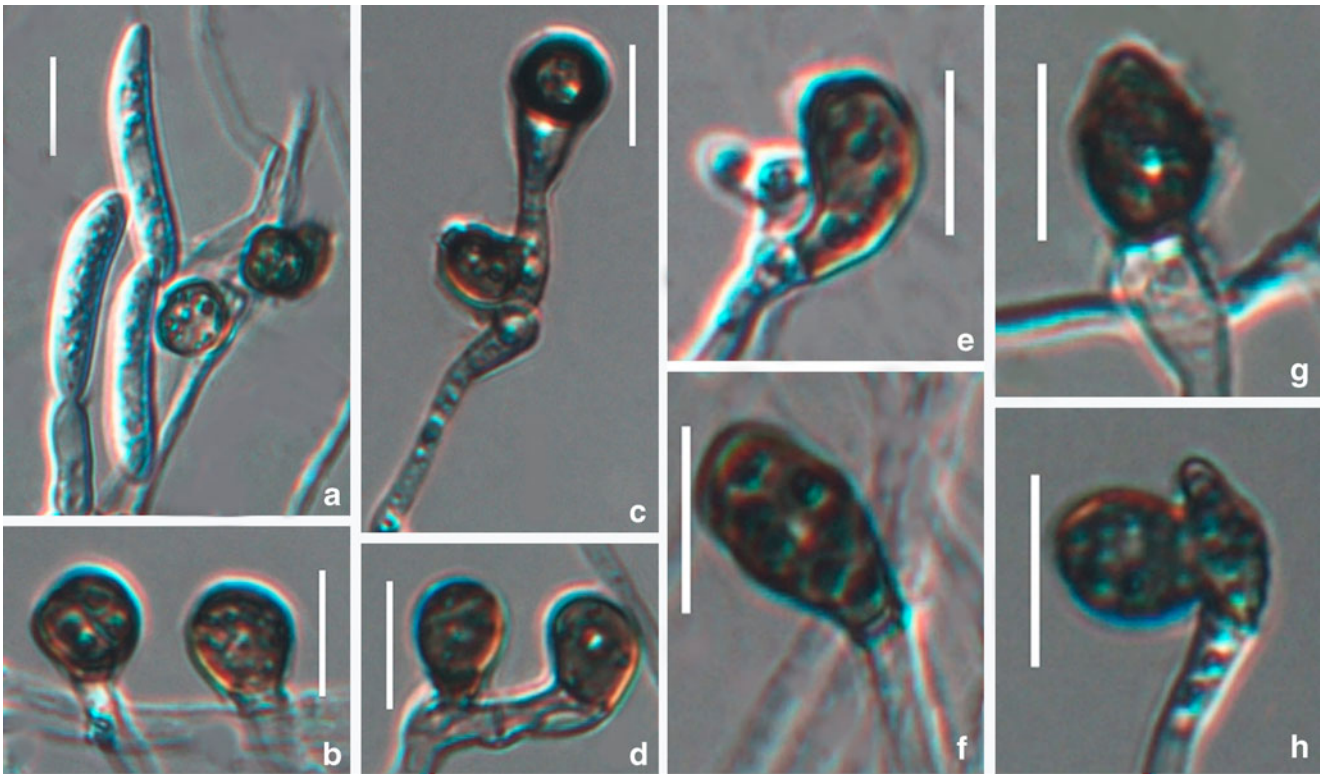

Fig. 8 Hyphal appressoria of *Colletotrichum miscanthi* (from CGMCC 3.15116). **a** Conidia and Appressoria; **b**–**h** Appressoria. Scale Bars: **a**–**h** = 10 μm

Material examined: CHINA, Guizhou Province, Duyun, Xiaba mountain, isolated from healthy leaves of *Bletilla ochracea*, 13 July 2006, Gang Tao (**Holotype** HMAS244831 (dried culture); culture ex-holotype CGMCC 3.15104 = LC2303); Guiyang, Yongle mountain, isolated from healthy leaves of *B. ochracea*, 11 June 2006, Gang Tao, culture CGMCC 3.15102 = LC2315; Guiyang, Yongle mountain, isolated from healthy leaves of *B. ochracea*, 11 June 2006, Gang Tao, culture CGMCC 3.15103 = LC2317.

Notes: *Colletotrichum ochracea* is phylogenetically closely related to *C. duyunensis*, another new species described in this study (Fig. 1). Although they are similar in producing curved conidia, *C. ochracea* can be easily distinguished from *C. duyunensis* by absence of conidial appendage and conidial sizes (5.5−15×1.5−3.5 μm in *C. ochracea* vs. 15.2–28.5×3–5.5 μm in *C. duyunensis*).

Colletotrichum parsonsiae Damm, P.F. Cannon, Crous, P.R. Johnst & B. Weir, Studies in Mycology 73: 27 (2012)

Material examined: CHINA, Guizhou Province, Shuicheng, Baijipo mountain, isolated from healthy leaves of *Bletilla ochracea*, 28 June 2006, Gang Tao, culture CGMCC 3.15126 = LC2343.

Notes: *Colletotrichum parsonsiae* is hitherto only known as leaf endophyte from *Parsonsia capsularis* from New Zealand (Damm et al. 2012b) and *B. ochracea* from China (this study).

Colletotrichum tofieldiae (Pat.) Damm, P.F. Cannon & Crous, Fungal Diversity 39: 77 (2009)

Material examined: CHINA, Guizhou Province, Qingzhen, Pianshan mountain, isolated from healthy leaves of *Bletilla ochracea*, 16 August 2006, Gang Tao, culture CGMCC 3.15118 = LC2336.

Notes: *Colletotrichum tofieldiae* was originally found in China and also known as pathogen of many plants in Europe (Damm et al. 2009). In the present study, the endophytic strain CGMCC 3.15118 was identified as *C. tofieldiae* based on morphology and multi-locus phylogeny (Fig. 1), and is the first report of *C. tofieldiae* as endophyte.

Discussion

Diversity and significance of endophytic *Colletotrichum* species

In the assessment of Hyde et al. (2009), 66 species of the genus *Colletotrichum* were recognized as "names in current use". Recently, a further 52 species have been introduced (Damm et al. 2012a; Damm et al. 2012b; Noireung et al. 2012; Weir et al. 2012; Doyle et al. 2013). In our study, 7 new species were described, adding the number of accepted *Colletotrichum* species to over 120. Among these *Colletotrichum* species, 30 were known as endophytes (Table 4).

Unlike mycorrhizal fungi that colonize plant roots and grow into the rhizosphere, endophytes inhabit the entire

Fig. 9 *Colletotrichum ochracea* (from holotype). **a**, **b** Colonies on PDA in 7 days, upper **a** and reverse **b**; **c**–**f** Conidiogenous cells; **g**–**k** Conidia; **l**–**o** Appressoria. Scale Bars: **c**–**o** = 10 μm

Table 4 *Colletotrichum* species that have been isolated from healthy plant tissues

Colletotrichum species	Hosts	Geographical limits	Notes	References
C. aotearoa	*Boehmeria* sp. or native plants (leaf endophyte)	China, New Zealand	Pathogen of native plants in New Zealand	Wang et al. 2010; Weir et al. 2012.
C. beeveri	*Pleione bulbocodioides* (Orchidaceae), *Podocar paceae* (root and leaf endophyte)	China, New Zealand	Pathogen of *Brachyglottis repanda* in New Zealand.	Joshee et al. 2009; Yang et al. 2011; Damm et al. 2012b
C. bletillum	*Bletilla ochracea* (Orchidaceae) (leaf endophyte)	China		This study
C. boninense	*Bletilla ochracea* (Orchidaceae) (leaf endophyte)	China	Pathogen of *Crinum asiaticum, Crinum asiaticum* var. *sinicum, Leucospermum* sp., *Solanum betaceum* in Australia, Japan and New Zealand.	Moriwaki et al. 2003; Damm et al. 2012b; Present study
C. caudasporum	*Bletilla ochracea* (Orchidaceae) (leaf endophyte)	China		This study
C. cereale	*Bletilla ochracea* (Orchidaceae) (leaf endophyte)	China	Pathogen of Turfgrass and Corn in North America.	Crouch et al. 2006; Present study
C. crassipes	*Pleione bulbocodioides* (Orchidaceae) (root endophyte)	China	Pathogen of the orchids in China.	Sutton 1980; Yang et al. 2011
C. destructivum	*Bletilla ochracea* (Orchidaceae) (leaf endophyte)	China	Pathogen of *Glycine max* (soybean) in USA.	Manandhar et al. 1986; Present study
C. dacrycarpi	*Dacrycarpus dacrydioides* (leaf endophyte)	New Zealand		Damm et al. 2012b
C. duyunensis	*Bletilla ochracea* (Orchidaceae) (leaf endophyte)	China		This study
C. endophytum	*Bletilla ochracea* (Orchidaceae) (leaf endophyte)	China		This study
C. excelsum-altitudum	*Bletilla ochracea* (Orchidaceae) (leaf endophyte)	China		This study
C. fioriniae	*Mangifera indica* (stem endophyte)	Australia	Pathogen of *Fiorinia externa, Persea americana*, *Rubus* sp., *Vaccinium* sp. (blueberry), *Vitis vinifera* in Australia Portugal and USA.	Damm et al. 2012a; Marcelino et al. 2009
C. fructicola	*Tetragastris panamensis* and *Theobroma cacao* (leaf endophyte)	Panama	Pathogen of *Camellia sinensis Coffea arabica, Dioscorea alata, Ficus edulis, Fragaria ananassa, Limonium* spp., *Malus domestica, Persea americana, Pyrus pyrifolia* in Thailand, Israel, Australia, Germany, Japan, Brazil, USA, Nigeria and Indonesia	Prihastuti et al. 2009; Rojas et al. 2010.
C. fructivorum	*Vaccinium macrocarpon* (stem endophyte)	USA	Pathogen of *Vaccinium macrocarpon* in USA and Canada.	Doyle et al. 2013
C. guizhouensis	*Bletilla ochracea* (Orchidaceae) (leaf endophyte)	China		This study
C. karstii	*Bletilla ochracea* (Orchidaceae), *Musa acuminate, Pleione bulbocodioides* (Orchidaceae) (root endophyte)	Thailand, China	Pathogen of *Diospyros australis, Annona cherimola, Leucospermum* sp., *Musa* sp., orchids in Australia, China, Mexico anf Thailand.	Damm et al. 2012b; Photita et al. 2005; Yang et al. 2011; Present study
C. liriopes		China		

Table 4 (continued)

Colletotrichum species	Hosts	Geographical limits	Notes	References
	Bletilla ochracea (Orchidaceae), *Pleione bulbocodioides* (Orchidaceae) (leaf and root endophyte)		Pathogen of *Eria Coronaria* and *Liriope muscari* in China and Mexico.	Damm et al. 2009; Yang et al. 2011; This study
C. miscanthi	*Bletilla ochracea* (Orchidaceae) (leaf endophyte)	China	Pathogen of *Miscanthus sinensis* in Japan.	Crouch et al. 2009a; This study
C. musae	*Musa acuminate* (leaf endophyte)	Thailand	Pathogen of *Musa balbisiana, M. cavendishii*, *M. paradisiaca* and *M. sapientum* throughout the world.	Pereira et al. 1999; Photita et al. 2001, 2005; Hyde et al. 2009; Su et al. 2011; Weir et al. 2012
C. ochracea	*Bletilla ochracea* (Orchidaceae) (leaf endophyte)	China		This study
C. orchidophilum	*Dendrobium nobile* (Orchidaceae)	China	Pathogen of plants of Orchidaceae in Germany, Panama, USA and UK.	Yuan et al. 2009; Damm et al. 2012a
C. parsonsiae	*Bletilla ochracea* (Orchidaceae), *Parsonsia capsularis* (leaf endophyte)	China, New Zealand		Damm et al. 2012b; This study
C. rhexiae	*Vaccinium macrocarpon* (fruit endophyte)	USA	Pathogen of *Vaccinium macrocarpon* in USA.	Doyle et al. 2013
C. siamense	*Coffea Arabica*	Thailand	Pathogen of *Carica papaya, Coffea arabica, Dioscorea rotundata, Hymenocallis americana, Jasminium sambac, Malus domestica, Persea Americana* and *Vitis vinifera* in Africa, Australia, China, Nigeria, South Thailand, USA and Vietnam.	Prihastuti et al. 2009; Yang et al. 2009; Wikee et al. 2011; Weir et al. 2012
C. simmondsii	*Actinidia chinensis* (stem endophyte)	Australia	Pathogen of *Capsicum frutescens*, *Carica papaya*, *Cyphomandra betaceae*, *Fragaria* × *ananassa*, *Litchi chinensis*, *Lycopersicon esculentum*, *Mangifera indica*, *Nephelium lappaceum*, *Persea americana*, *Vaccinium Corymbosum* in Australia.	Shivas and Tan 2009
C. temperatum	*Vaccinium macrocarpon* (stem endophyte)	USA	Pathogen of *Vaccinium macrocarpon* in USA.	Doyle et al. 2013
C. tofieldiae	*Bletilla ochracea* (Orchidaceae) (leaf endophyte)	China	Pathogen of *Tofieldia* sp., *Dianthus* p., *Lupinus polyphyllus* and *Tofieldia calyculata* in China, Germany, Switzerland and United Kingdom.	Damm et al. 2009; This study
C. tropicale	*Cordia aliodora, Theobroma cacao, Trichilia tuberculata* and *Viola surinamensis* (leaf endophyte)	Panama	Pathogen of *Annona muricata* and *Litchi chinensis* in Japan and Panama.	Rojas et al. 2010; Weir et al. 2012
C. yunnanense	*Buxus* sp. (Buxaceae) (leaf endophyte)	China		Liu et al. 2007.

plant tissues and may grow within leaves, roots and stems (Carroll 1988; Stone et al. 2004), and have been disclosed from every major lineage of land plants distributed from the tropics to the tundra (Arnold and Engelbrecht 2007). Over the last few years, the endophytic fungi have been revealed for their important ecological roles and potential applications in the biocontrol of plant diseases (Vasiliauskas et al. 2007; Maciá-Vicente et al. 2008). A good example of mutualism between *Colletotrichum* species and its host plants is the endophytic *C. fioriniae* that can be used as natural protectants against insect herbivory (Marcelino et al. 2008). While some strains of *C. gloeosporioides* sensu lato are also known to be able to protect *Theobroma cacao* against *Phytophthora* pathogens (Arnold et al. 2003; Mejía et al. 2008; Rojas et al. 2010).

Colletotrichum species known as endophyts and pathogens from Orchidaceae

Previous studies of fungal communities associated with orchid mainly focused on the mycorrhizal fungi (Taylor and Bruns 1999; Kristiansen et al. 2001; Taylor et al. 2003; Selosse et al. 2009). Recent investigations on non-mycorrhizal fungi of Orchidaceae showed that orchids host a high diversity of endophytes (Tao et al. 2008, 2012; Yang et al. 2011). All the currently known endophytes in the genus *Colletotrichum*, with their host and distribution information are summarized in Table 4.

The common *Colletotrichum* pathogens of Orchidaceae include *C. boninense*, *C. crassipes*, *C. crossandrae*, *C. gloeosporioides*, *C. lujae*, *C. orchidearum*, *C. stanhopeae* and *C. vanillae* (Allescher et al. 1902; Patel et al. 1953; von Arx 1957; Sutton 1980; Moriwaki et al. 2003; Hyde et al. 2009; Farr and Rossman 2013), which cause brown to black spot on leaves, flowers or stalks (Teoh 2005). Some species appear to be host-specific, e.g. *C. graminicola* on *Zea mays* (Sutton 1966; Hyde et al. 2009) but most species infect more than one host (Table 4).

In this paper, 17 endophytic *Colletotrichum* species from *Bletilla ochracea* (Orchidaceae) were identified, including 7 new species, 8 previously described species, and 2 mycelia sterilia (Table 3, Fig. 1). These species were isolated as endophytes, but some of them have been known as pathogens on same or different hosts. Species such as *C. boninense*, *C. destructivum*, *C. karstii, C. miscanthi*, *C. musae* and *C. tofieldiae* have been known as causal agents of anthracnose of other host plants (Photita et al. 2001; Moriwaki et al. 2003; Damm et al. 2009; Yang et al. 2011). *Colletotrichum musae* isolated from healthy leaves and roots of *Musa acuminata* (Pereira et al. 1999; Photita et al. 2001, 2005) can also lead to post-harvest disease of many varieties of banana (Anthony et al. 2004). These examples provide additional support for the hypothesis that endophytes can be latent pathogens (Photita et al. 2001; Romero et al. 2001; Photita et al. 2004).

Induction of sporulation in endophytic *Colletotrichum* species

Most of the descriptions in this study were based on PNP medium because these fungi failed to sporulate in commonly used media such as PDA and SNA. In endophyte studies, the mycelia sterilia account for 4.5–54 % to the total isolates (Fisher et al. 1994; Guo et al. 1998; Sánchez Márquez et al. 2008; Sun and Guo 2012). Although molecular phylogenetics has been popularized in fungal taxonomy, morphological characters are still essential (Hyde et al. 2010) in species recognition and identification. Spores and fruiting structures are the most important morphological characters to be used for classifying fungi into genera or differentiating closely related species. Su et al. (2012) demonstrated that sporulation was significantly improved for *Colletotrichum and Diaporthe* species under the special media such as $CaCO_3$ water agar, pine needle agar and 1/10-strength PDA in combination with exposure to near-UV light. In the present study, most of our endophytic fungi failed to sporulate on PDA at 25 °C. Sporulations were induced by using pine needle medium, in combination with the exposure to 12 h' near-UV light and 12 h' dark. Most strains have been successfully induced except two that remain sterile (CGMCC 3.15171 and CGMCC 3.15172).

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