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Setophoma spp. on *Camellia sinensis*

F. Liu[1], J. Wang[1,2], H. Li[3], W. Wang[4], L. Cai[1, 2*]

[1]State key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China
[2]College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China
[3]College of Life Sciences, Hebei University, Baoding, Hebei Province, 071002, China
[4]Shandong Hetian Wang Biological Technology Co., Ltd., WeiFang, 261300, China

*Corresponding author: cail@im.ac.cn

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Abstract: During our investigation of *Camellia sinensis* diseases (2013–2018), a new leaf spot disease was found in seven provinces of China (Anhui, Fujian, Guangxi, Guizhou, Jiangxi, Tibet and Yunnan), occurring on both arboreal and terraced tea plants. The leaf spots were round to irregular, brown to dark brown, with grey or tangerine margins. Multi-locus (LSU, ITS, *gapdh*, *tef-1α*, *tub2*) phylogenetic analyses combined with morphological observations revealed four new species belonging to the genus *Setophoma*, *i.e. S. antiqua*, *S. longinqua*, *S. yingyisheniae* and *S. yunnanensis*. Of these four species, *S. yingyisheniae* was found to be present on diseased terraced tea plants in six of the seven sampled provinces (excluding Yunnan). The other three species only occurred on arboreal tea plants in Yunnan Province. In addition to the four species isolated from diseased leaves, *S. endophytica sp. nov*. was isolated from healthy leaves of terraced tea plants.

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INTRODUCTION

During our investigation of diseases of tea plants (*Camellia sinensis*) cultivated in China in 2013, a new leaf spot disease was found to cause severe yield losses in Guangxi Province. The associated leaf spots were circular to irregular, brown to dark brown in colour, with grey or tangerine margins. A subsequent collection effort of similarly affected tea plant leaves has been ongoing in many tea plantations located in six provinces of China, *i.e*. Anhui, Fujian, Guizhou, Jiangxi, Tibet and Yunnan. Preliminary morphological observations and molecular analyses identified the associated fungi as *Setophoma* spp. To our knowledge, this is the first report of *Setophoma* on tea plants. Considering the potential commercial yield losses in tea plantations and the limited knowledge of this disease, accurate identification of the causal organisms is of great importance.

The genus *Setophoma* (*Phaeosphaeriaceae*) was introduced to accommodate *Phoma terrestris* and *Pyrenochaeta sacchari* (de Gruyter *et al.* 2010). Species of *Setophoma* are characterised as having setose pycnidia, phialidic conidiogenous cells and hyaline, ellipsoidal to subcylindrical, aseptate conidia (de Gruyter *et al.* 2010, Quaedvlieg *et al.* 2013). According to Index Fungorum and MycoBank, four additional *Setophoma* species have been described since the genus was introduced in 2010. The currently recognised species are: *S. chromolaenae*, *S. cyperi, S. poaceicola*, *S. sacchari*, *S. terrestris*, and *S. vernoniae*. All except *S. terrestris* are reported to occur on unique host plants (Table 1).

The aim of the present study was to investigate the taxonomic and phylogenetic relationships of *Setophoma* spp. associated with tea plants based on multi-locus phylogenetic analyses, morphological comparison, host association and geographical distribution.

MATERIALS AND METHODS

Isolates

Isolates were obtained from either diseased or healthy tea plant tissues collected from 18 locations in seven provinces of China, following the single spore isolation and tissue isolation methods described in Liu *et al.* (2015). Type specimens of new species were deposited in the Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS), with the ex-type living cultures being deposited in the China General Microbiological Culture Collection Center (CGMCC).

DNA extraction, PCR amplification and phylogenetic analyses

Total genomic DNA was extracted from fresh mycelia using the CTAB method. Five partial loci including the large subunit of the nrDNA (LSU), the 5.8S nuclear ribosomal RNA gene with the two flanking internally transcribed spacer regions (ITS), partial glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), translation elongation factor 1-alpha (*tef-1α*) and β-tubulin (*tub2*) were amplified and sequenced using the following primer pairs: ITS1/ITS4 for ITS (White *et al.* 1990), LR0R/LR5 for LSU (Vilgalys & Hester 1990, Rehner & Samuels 1994), gpd1/gpd2 for *gapdh* (Berbee *et al.* 1999), EF-1/EF-2 for *tef-1α* (O'Donnell *et al.*

Table 1. Host and distribution of *Setophoma* species.

Species	Host	Distribution	References
S. antiqua	*Camellia sinensis*	China	This study
S. chromolaenae	*Chromolaena odorata*	Brazil	Quaedvlieg *et al.* (2013)
S. cyperi	*Cyperus sphaerocephalus*	South Africa, Eastern Cape	Crous *et al.* (2016)
S. endophytica	*Camellia sinensis*	China	This study
S. longinqua	*Camellia sinensis*	China	This study
S. poaceicola	Grass	Thailand	Thambugala *et al*. (2017)
S. sacchari	*Saccharum officinarum*	Brazil	de Gruyter *et al.* (2010)
S. terrestris	*Allium cepa*	North America, Senegal	de Gruyter *et al*. (2010)
	Allium sativum	United States	de Gruyter *et al.* (2010)
	Brassica sp.	Canada, Alberta	Yang *et al*. (2017)
	Cucurbita maxima	USA, Oregon	Rivedal *et al.* (2018)
	Cucurbita moschata	Japan	Ikeda *et al*. (2012)
	Solanum lycopersicum	Canada, Ontario	Johnston-Monje *et al*. (2017)
S. vernoniae	*Vernonia polyanthes*	Brazil	Crous *et al*. (2014)
S. yingyisheniae	*Camellia sinensis*	China	This study
S. yunnanensis	*Camellia sinensis*	China	This study

Table 2. GenBank accession numbers from NCBI database.

Name	Strain/specimen No.[a]	LSU	ITS
Acericola italica	MFLUCC 13-0609*	MF167429	MF167428
Allophaeosphaeria muriformia	MFLUCC 13-0349*	KP765681	KP765680
Allophaeosphaeria subcylindrospora	MFLUCC 13-0380*	KT314183	KT314184
Amarenomyces ammophilae	CBS 114595	GU301859	KF766146
Ampelomyces quisqualis	CBS 129.79*	EU754128	HQ108038
Bhatiellae rosae	MFLUCC 17-0664*	MG828989	MG828873
Chaetosphaeronema achilleae	MFLUCC 16-0476	KX765266	KX765265
Chaetosphaeronema hispidulum	CBS 216.75	KF251652	KF251148
Coniothyrium carteri	CBS 105.91	KF251712	KF251209
Dactylidina dactylidis	MFLUCC 14-0966*	MG829002	MG828886
Dematiopleospora rosicola	MFLU 16-0232*	MG829006	MG828888
Dematiopleospora salsolae	MFLUCC 17-0828*	MG829007	MG828889
Didymocyrtis consimilis	Gardiennet 12041	KT383796	KT383813
Didymocyrtis ramalinae	Ertz 16399	KT383802	KT383838
Embarria clematidis	MFLUCC 14-0976*	MG828987	MG828871
Galiicola pseudophaeosphaeria	MFLUCC 14-0524*	KT326693	KT326692
Hawksworthiana alliariae	MFLUCC 13-0070	KX494877	KX494876
Hawksworthiana clematidicola	MFLUCC 14-0910*	MG829011	MG828901
Italica achilleae	MFLUCC 14-0959*	MG829013	MG828903
Juncaceicola achilleae	MFLUCC 13-0606*	KX449526	KX449525
Juncaceicola luzulae	MFLUCC 16-0780*	KX449530	KX449529
Juncaceicola typharum	CBS 296.54	KF251695	KF251192
Leptospora galii	KUMCC 15-0521	KX599548	KX599547
Leptospora rubella	CPC 11006	DQ195792	DQ195780
Muriphaeosphaeria galatellae	MFLUCC 14-0614*	KT438329	KT438333
Neosetophoma samarorum	CBS 138.96*	KF251664	KF251160
Neostagonospora caricis	CBS 135092*	KF251667	KF251163
Neostagonospora elegiae	CBS 135101*	KF251668	KF251164

Table 2. (Continued).

Name	Strain/specimen No.[a]	LSU	ITS
Neosulcatispora agaves	CPC 26407*	KT950867	KT950853
Nodulosphaeria hirta	MFLUCC 13-0867	KU708845	KU708849
Nodulosphaeria scabiosae	MFLUCC 14-1111*	KU708846	KU708850
Ophiobolopsis italica	MFLUCC 17-1791*	MG520959	MG520939
Ophiobolus artemisiae	MFLUCC 14-1156*	KT315509	KT315508
Ophiobolus disseminans	MFLUCC 17-1787*	MG520961	MG520941
Ophiosimulans tanaceti	MFLUCC 14-0525	KU738891	KU738890
Paraophiobolus arundinis	MFLUCC 17-1789*	MG520965	MG520945
Paraophiobolus plantaginis	MFLUCC 17-0245*	KY815010	KY797641
Paraphoma chrysanthemicola	CBS 172.70	KF251669	KF251165
Paraphoma radicina	CBS 102875	KF251677	KF251173
Paraphoma rhaphiolepidis	CBS 142524*	KY979813	KY979758
Paraphoma vinacea	UMPV001 = BRIP 63684	KU176888	KU176884
Parastagonospora caricis	CBS 135671	KF251680	KF251176
Parastagonospora nodorum	CBS 110109	KF251681	KF251177
Parastagonospora poagena	CBS 136776*	KJ869174	KJ869116
Parastagonosporella fallopiae	CBS 135981*	MH460545	MH460543
	CCTU 1151.1	MH460546	MH460544
Phaeosphaeria oryzae	CBS 110110*	KF251689	KF251186
Phaeosphaeria papayae	CBS 135416	KF251690	KF251187
Phaeosphaeriopsis glaucopunctata	CBS 653.86	KF251702	KF251199
Poaceicola arundinis	MFLU 15-0702*	KU058726	KU058716
Poaceicola italica	MFLUCC 13-0267*	KX910094	KX926421
Populocrescentia forlicesenensis	MFLU 15-0651*	KT306952	KT306948
Pseudoophiobolus achilleae	MFLU 17-0925*	MG520966	MG520946
Pseudoophiobolus mathieui	MFLUCC 17-1784	MG520969	MG520949
Pseudophaeosphaeria rubi	MFLUCC 14-0259*	KX765299	KX765298
Sclerostagonospora rosicola	MFLUCC 15-0129*	MG829068	MG828957
Septoriella allojunci	MFLU 15-0701*	KU058728	KU058718
Septoriella phragmitis	CPC 24118 = CBS 140065*	KR873279	KR873251
Setophoma chromolaenae	CBS 135105*	KF251747	KF251244
Setophoma cyperi	CPC 25702 = CBS 141450*	KX228337	KX228286
Setophoma poaceicola	MFLUCC 16-0880*	KY550386	KY568988
Setophoma sacchari	MFLUCC 12-0241	KJ476147	KJ476145
	CBS 333.39*	MH867535	MH856038
Setophoma terrestris	CBS 335.87	KF251750	KF251247
	CBS 377.52	KF251751	KF251248
Setophoma vernoniae	CPC 23123 = CBS 137988*	KJ869198	KJ869141
Sulcispora pleurospora	MFLUCC 14-0995*	KP271444	KP271443
Tintelnotia destructans	CBS 127737*	KY090664	KY090652
Tintelnotia opuntiae	CBS 376.91*	GU238123	KY090651
Vagicola vagans	CBS 604.86	KF251696	KF251193
Wojnowicia lonicerae	MFLUCC 13-0737*	KP684151	KP744471
Wojnowicia rosicola	MFLUCC 15-0128*	MG829091	MG828979
Wojnowiciella eucalypti	CPC 25024*	KR476774	KR476741
Xenoseptoria neosaccardoi	CBS 120.43	KF251783	KF251280
	CBS 128665*	KF251784	KF251281

[a]Ex-type strains are marked with asterisk *.

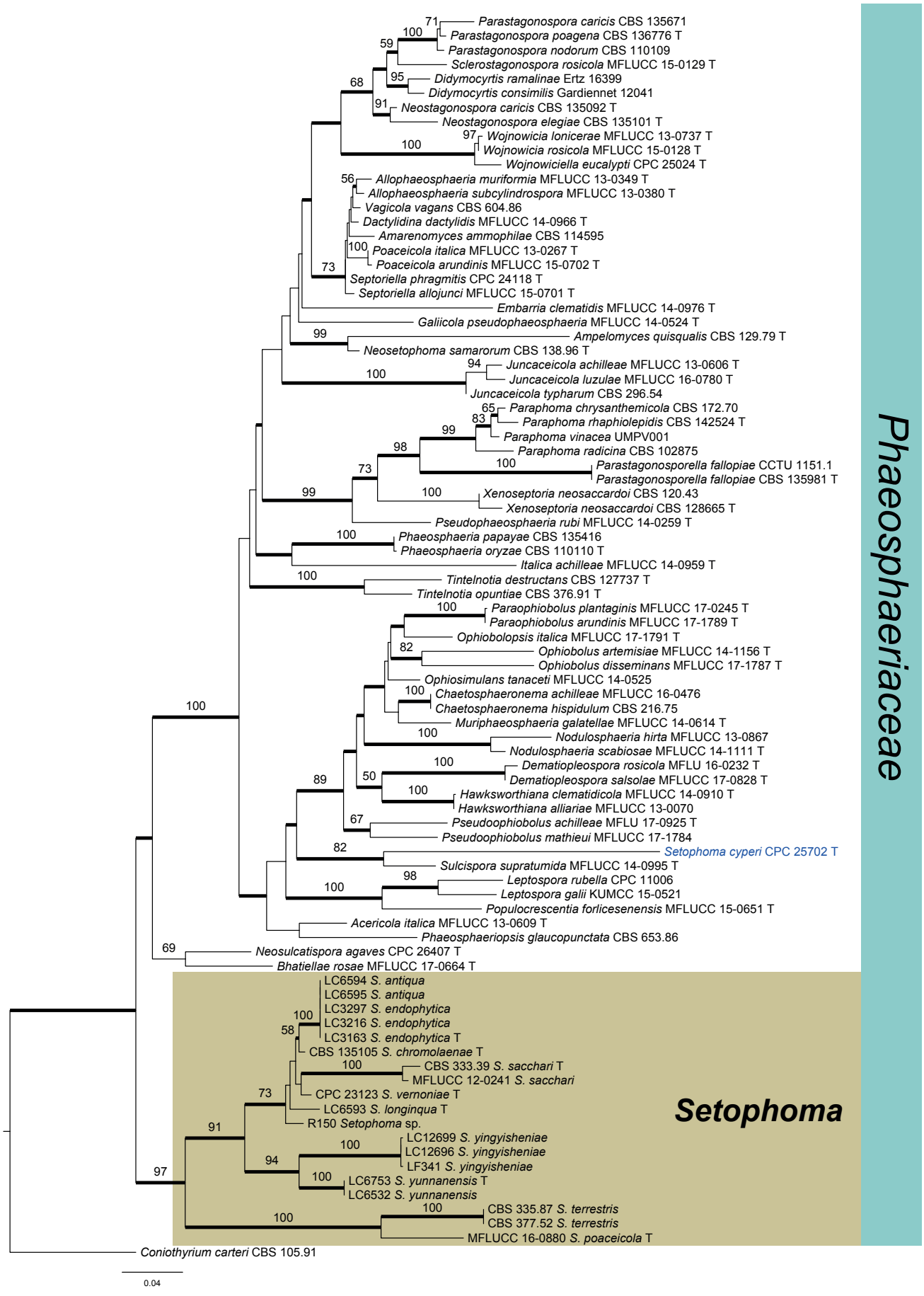

Fig. 1. Overview phylogenetic tree of *Phaeosphaeriaceae* (50 % majority rule consensus) resulting from a Bayesian analysis of the combined LSU and ITS sequence alignment. Bayesian posterior probabilities (PP > 0.95) are emphasized by thickened branches, maximum likelihood bootstrap support values (≥ 50 %) are shown at the nodes. The scale bar represents the expected number of changes per site. Ex-type cultures are indicated with "T" behind the taxa labels. The tree was rooted to *Coniothyrium carteri* (CBS 105.91).

1998) and T1/Bt2b for *tub2* (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997). Amplicons for LSU, ITS, *tef-1α* and *tub2* were generated according to Liu *et al.* (2019) while amplicons for *gapdh* were generated according to Liu *et al.* (2016). Amplicons were sequenced with both forward and reverse primers by the Omegagenetics Company, Beijing, China. MEGA v. 7.0.21 was used to generate consensus sequences.

DNA sequences (ITS and LSU) used to infer generic affiliation within the *Phaeosphaeriaceae* were downloaded from the NCBI database (Table 2). Sequence datasets of ITS, *gapdh*, *tub2* and *tef-1α* (Table 3) were used for species delimitation in *Setophoma*. Sequence alignments were made using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html) and were then manually edited in MEGA v. 7.0.21. Subsequently, individual alignments of above-mentioned loci were concatenated using Mesquite v. 3.4. The Maximum Likelihood (ML) and Bayesian analysis (BA) methods were used for phylogenetic inferences of both the single gene and concatenated alignments as described in Liu *et al.* (2019). The resulting trees were plotted using FIGTREE v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree). All the alignments derived from this study were deposited in TreeBASE (https://treebase.org/) (S24160).

Morphology

Cultures were cultivated on both 2 % malt extract agar (MEA) and potato dextrose agar (PDA) (Difco™, Becton, Dickinson and Company, Sparks, MD, USA) at 25 °C in a 12 h day/night regime. Growth rates were measured after 10 d in the dark. Colony colours were rated following the colour charts of Rayner (1970). In order to observe wall structures of mature conidiomata, sections were made using a Leica CM1950 freezing microtome. Morphological observations of reproductive structures were made in lactic acid and observed using a Nikon Eclipse 80i microscope using differential interference contrast (DIC) illumination. At least 30 measurements per structure were taken and the mean value, standard deviation and minimum–maximum values are given, with the extreme measurements given in parentheses.

RESULTS

DNA phylogeny

The concatenated alignment of LSU and ITS sequences comprised a total length of 1 397 characters (LSU: 818, ITS: 579) including alignment gaps, 486 of which were unique site patterns. The ML search resolved a best tree with a InL of -14098.620225. Fixed base frequency for LSU and ITS was set in the Bayesian analysis (BA). The BA lasted for 1 305 000 generations and the 50 % consensus tree and posterior probabilities were calculated from 3 908 trees, generated during two runs. The ML tree confirmed the same tree topology as that generated by the Bayesian analysis and it also revealed that strains isolated in this study clustered together with the generic type of *Setophoma* (*S. terrestris*), in the basal clade of Fig. 1. However, the ex-type strain of another known *Setophoma* species, *S. cyperi* (CPC 25702 = CBS 141450) clustered outside *Setophoma s. str.*, and was more closely related to *Sulcispora pleurospora* (MFLUCC 14-0995) (Fig. 1).

To better infer species delimitation in *Setophoma*, sequences of four loci (ITS, *tef-1α*, *gapdh* and *tub2*) were concatenated for further phylogenetic analyses. Strains of *S. sacchari*, *S. vernoniae* and *S. poaceicola* were excluded from this analysis due to their incomplete sequence datasets. The final alignment contained a total of 2 504 characters (ITS: 524, *tef-1α*: 875, *gapdh*: 550, *tub2*: 539) including alignment gaps, of which 872 were unique site patterns. The ML search revealed a best tree with an InL of -10791.633622. Fixed base frequency was set in the Bayesian analysis. The BA lasted for 1 315 000 generations and the 50 % consensus tree and posterior probabilities were calculated from 1 656 trees generated during two runs. The topology of the phylogenetic trees generated from both ML and BA methods were congruent (Fig. 2). Strains associated with tea plants clustered in five different clades.

Taxonomy

A total of 94 isolates from diseased leaves and 17 isolates from healthy leaves were isolated from arboreal and terraced tea plants. Based on ITS sequence similarity and colonial morphology, 35 representative isolates were selected for further microscopic observation and phylogenetic analyses. These 35 strains formed five distinct and novel phylogenetic clades representing five new species (Fig. 2). These are described and illustrated below.

Setophoma Gruyter, Aveskamp & Verkley, *Mycologia* **102**: 1077. 2010. ***emend.*** F. Liu & L. Cai.

Ascomata immersed or semi-immersed, uniloculate, globose to subglobose, brown to dark brown, solitary or gregarious, centrally ostiolate, papillate, wall of *textura angularis* or *prismatica*. *Pseudoparaphyses* hyaline, frequently septate. *Asci* bitunicate, fissitunicate, cylindrical to cylindrical-clavate, smooth-walled. *Ascospores* fusiform with rounded ends, hyaline, overlapping or irregularly biseriate, septate, usually widest at the second cell from apex, smooth-walled, guttulate. *Conidiomata* pycnidial, solitary to confluent, on upper surface or submerged in agar, globose to subglobose, setose or not, with papillate ostioles, honey to olivaceous or olivaceous black; *pycnidial wall* of pseudoparenchymatal cells. *Conidiophores* reduced to conidiogenous cells lining inner cavity, or simply branched. *Conidiogenous cells* hyaline, smooth, phialidic, discrete. *Conidia* aseptate, globose, subglobose, ellipsoidal to subcylindrical to subfusoid, guttulate.

Type species: *S. terrestris* (H.N. Hansen) Gruyter *et al.*

Notes: The genus *Setophoma* was introduced to accommodate phoma-like species with setose conidiomata by de Gruyter *et al.* (2010). However, the new species of *S. antiqua*, *S. endophytica* and *S. yunnanensis,* proposed in this study, lack setae. We therefore broadened the generic concept of the genus *Setophoma* to also accommodate species lacking setose conidiomata.

Table 3. Collection details and GenBank accession numbers of isolates included in this study.

Species	Strain No.[a]	Habitat	Location	GenBank accession no.[b]				
				LSU	ITS	*tub2*	*tef-1α*	*GAPDH*
Didymella pinodella	CBS 531.66	*Trifolium pretense*	USA	**GU238017**	**FJ427052**	**FJ427162**	**MK525067**	**MK532379**
Setophoma antiqua	LC6594	*Camellia sinensis*, pathogen	Yunnan, China	**MK511947**	**MK511909**	**MK524999**	**MK525070**	**MK525034**
	LC6595	*Camellia sinensis*, pathogen	Yunnan, China	**MK511948**	**MK511910**	**MK525000**	**MK525071**	**MK525035**
	CGMCC 3.19525 = LC6596*	*Camellia sinensis*, pathogen	Yunnan, China	-	**MK511911**	**MK525001**	**MK525072**	**MK525036**
Setophoma chromolaenae	CBS 135105*	*Chromolaena odorata*	Brazil	KF251747	KF251244	KF252728	KF253195	-
Setophoma endophytica	LC13538	*Camellia sinensis*, endophyte	Jiangxi, China	-	**MK511923**	**MK525012**	**MK525084**	**MK525047**
	LF2067	*Camellia sinensis*, endophyte	Jiangxi, China	-	**MK511924**	**MK525013**	**MK525085**	**MK525048**
	CGMCC 3.19528 = LC3163*	*Camellia sinensis*, endophyte	Jiangxi, China	**MK511956**	**MK511931**	**MK525020**	**MK525092**	**MK525053**
	LC3164	*Camellia sinensis*, endophyte	Jiangxi, China	**MK511957**	**MK511932**	**MK525021**	**MK525093**	**MK525054**
	LC3165	*Camellia sinensis*, endophyte	Jiangxi, China	-	**MK511933**	**MK525022**	**MK525094**	**MK525055**
	LC3216	*Camellia sinensis*, endophyte	Jiangxi, China	**MK511959**	**MK511938**	**MK525026**	**MK525099**	**MK525060**
	LC3297	*Camellia sinensis*, endophyte	Jiangxi, China	**MK511962**	**MK511941**	**MK525029**	**MK525102**	**MK525063**
Setophoma longinqua	CGMCC 3.19524 = LC6593*	*Camellia sinensis*, pathogen	Yunnan, China	**MK511946**	**MK511908**	**MK524998**	**MK525069**	-
	LC13481	*Camellia sinensis*, pathogen	Yunnan, China	-	**MK511925**	**MK525014**	**MK525086**	-
	LC13482	*Camellia sinensis*, pathogen	Yunnan, China	-	**MK511926**	**MK525015**	**MK525087**	-
Setophoma sp.	LC12841	Carbonatite in cave	Guizhou, China	-	**MK511927**	**MK525016**	**MK525088**	**MK525049**
	LC12842	Carbonatite in cave	Guizhou, China	-	**MK511928**	**MK525017**	**MK525089**	**MK525050**
	CGMCC 3.19526 = LC7511	Carbonatite in cave	Guizhou, China	**MK511965**	**MK511944**	**MK525032**	**MK525105**	**MK525066**
Setophoma terrestris	CBS 335.29 = MUCL 9892 = LC6449*	*Allium sativum*	USA	KF251749	KF251246	KF252729	KF253196	-
	CBS 335.87	*Allium cepa*	Senegal	KF251750	KF251247	KF252730	KF253197	-
	CBS 377.52	*Allium cepa*	-	KF251751	KF251248	KF252731	KF253198	-
Setophoma yingyisheniae	LC6739	*Camellia sinensis*, pathogen	Guizhou, China	-	**MK511912**	**MK525002**	**MK525073**	-
	LC12696	*Camellia sinensis*, pathogen	Anhui, China	**MK511950**	**MK511914**	-	**MK525075**	**MK525038**
	LC12699	*Camellia sinensis*, pathogen	Anhui, China	**MK511951**	**MK511915**	**MK525004**	**MK525076**	**MK525039**
	LC13477	*Camellia sinensis*, pathogen	Fujian, China	**MK511952**	**MK511916**	**MK525005**	**MK525077**	**MK525040**
	LC13478	*Camellia sinensis*, pathogen	Fujian, China	-	**MK511917**	**MK525006**	**MK525078**	**MK525041**
	CGMCC 3.19527 = LC13479*	*Camellia sinensis*, pathogen	Guangxi, China	-	**MK511918**	**MK525007**	**MK525079**	**MK525042**

Table 3. (Continued).

Species	Strain No.[a]	Habitat	Location	GenBank accession no.[b]				
				LSU	ITS	*tub2*	*tef-1α*	*GAPDH*
	LC13480	*Camellia sinensis*, pathogen	Guangxi, China	**MK511953**	**MK511919**	**MK525008**	**MK525080**	**MK525043**
	LF2026	*Camellia sinensis*, pathogen	Guangxi, China	-	**MK511920**	**MK525009**	**MK525081**	**MK525044**
	LF2027	*Camellia sinensis*, pathogen	Guangxi, China	**MK511954**	**MK511921**	**MK525010**	**MK525082**	**MK525045**
	LF2032	*Camellia sinensis*, pathogen	Guangxi, China	-	**MK511922**	**MK525011**	**MK525083**	**MK525046**
	LC3133	*Camellia sinensis*, pathogen	Jiangxi, China	**MK511955**	**MK511929**	**MK525018**	**MK525090**	**MK525051**
	LC3137	*Camellia sinensis*, pathogen	Jiangxi, China	-	**MK511930**	**MK525019**	**MK525091**	**MK525052**
	LC3176	*Camellia sinensis*, endophyte	Jiangxi, China	-	**MK511934**	-	**MK525095**	**MK525056**
	LC3181	*Camellia sinensis*, endophyte	Jiangxi, China	**MK511958**	**MK511935**	**MK525023**	**MK525096**	**MK525057**
	LC3185	*Camellia sinensis*, endophyte	Jiangxi, China	-	**MK511936**	**MK525024**	**MK525097**	**MK525058**
	LC3197	*Camellia sinensis*, endophyte	Jiangxi, China	-	**MK511937**	**MK525025**	**MK525098**	**MK525059**
	LC3236	*Camellia sinensis*, pathogen	Jiangxi, China	**MK511960**	**MK511939**	**MK525027**	**MK525100**	**MK525061**
	LC3276	*Camellia sinensis*, pathogen	Jiangxi, China	**MK511961**	**MK511940**	**MK525028**	**MK525101**	**MK525062**
	LC3334	*Camellia sinensis*, pathogen	Jiangxi, China	**MK511963**	**MK511942**	**MK525030**	**MK525103**	**MK525064**
	LC3499	*Camellia sinensis*, endophyte	Guangxi, China	**MK511964**	**MK511943**	**MK525031**	**MK525104**	**MK525065**
Setophoma yunnanensis	LC6532	*Camellia sinensis*, pathogen	Yunnan, China	**MK511945**	**MK511907**	**MK524997**	**MK525068**	**MK525033**
	CGMCC 3.19529 = LC6753*	*Camellia sinensis*, pathogen	Yunnan, China	**MK511949**	**MK511913**	**MK525003**	**MK525074**	**MK525037**

[a]Ex-type strains are marked with star *; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; LC: working collection of Lei Cai, housed at the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; MUCL: Mycothèque de l'Université catholique de Louvain, Louvain-la-Neuve, Belgium.

[b]Newly generated sequences are indicated in bold.

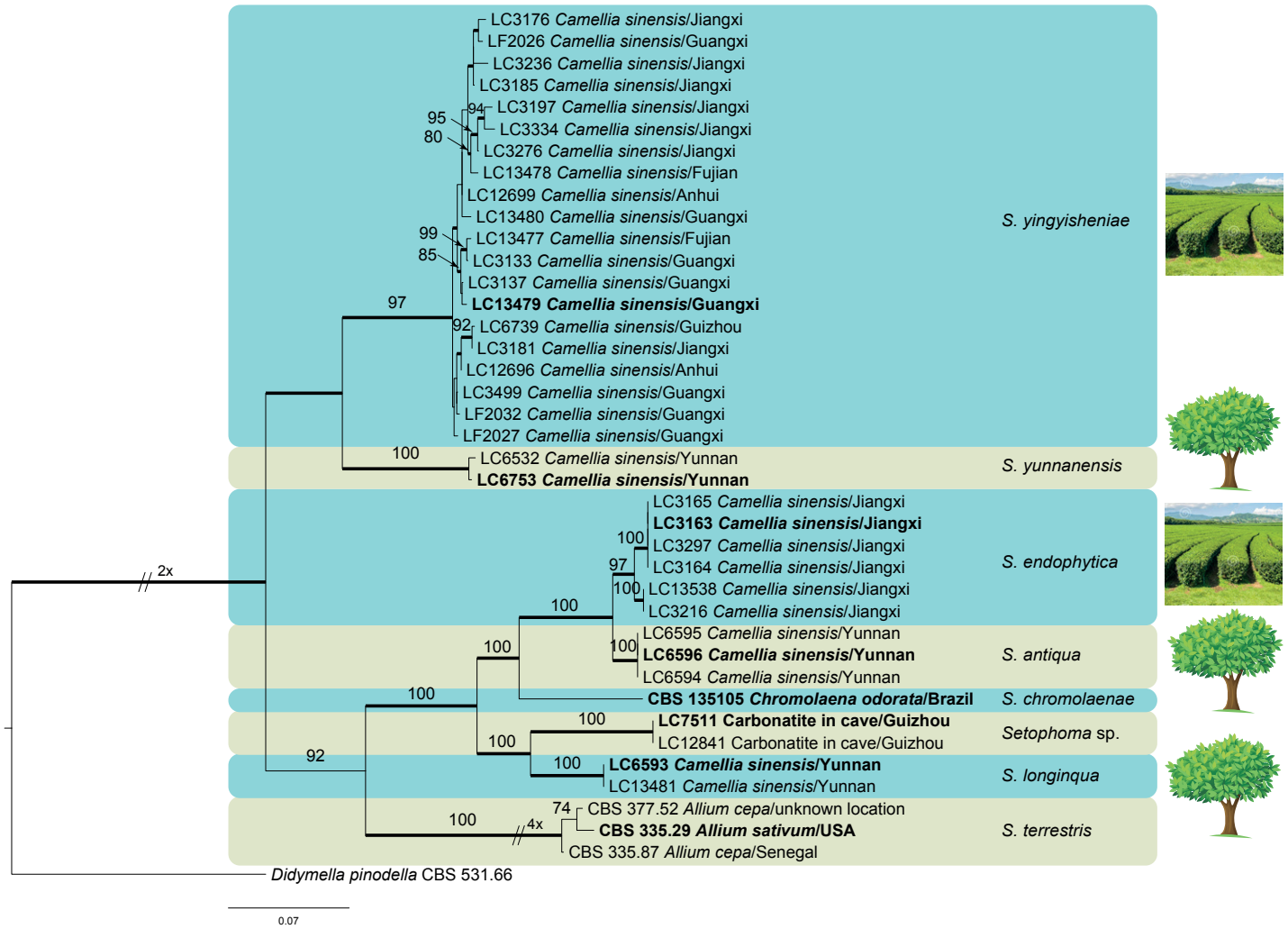

Fig. 2. Phylogenetic tree of *Setophoma* resulting from a Bayesian analysis of the combined ITS, *gapdh*, *tef-1α* and *tub2* sequence alignment. Bayesian posterior probabilities (PP > 0.95) are emphasized by thickened branches, maximum likelihood bootstrap support values (> 50 %) are shown at the nodes. The scale bar represents the expected number of changes per site. The taxa names consist of strain number, host or substrate, and location. Ex-type strains are represented in bold. *Setophoma yingyisheniae* and *S. endophytica* were isolated from terraced tea plants, *S. yunnanensis*, *S. antiqua* and *S. longinqua* were isolated from arboreal tea plants (illustrated by photos on the right side of the figure). The tree was rooted to *Didymella pinodella* (CBS 531.66).

Setophoma antiqua F. Liu & L. Cai, ***sp. nov.*** MycoBank MB829900. Fig. 3.

Etymology: From the Latin "*antiquus*" = old, referring to its host, old tea plant.

Sexual morph: Unknown. *Asexual morph*: *Aerial mycelia* hyaline or pale brown, branched, septate. *Conidiomata* pycnidial, scattered or gregarious, immersed or semi-immersed, globose to subglobose, olivaceous, 100–250 µm diam. *Pycnidial wall* pale to dark brown, 4–15 µm wide, with 3–4 layers, walls of *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining inner cavity. *Conidiogenous cells* single, phialidic, unbranched, aseptate, hyaline, smooth, ampulliform, 3–5.5 × 2.5–5 µm (av. = 3.8 ± 0.6 × 3.2 ± 0.7 µm). *Conidia* hyaline, aseptate, ellipsoid with rounded ends or allantoid, smooth-walled, 2.5–5 × 1.5–2.5 µm (av. = 3.7 ± 0.5 × 1.8 ± 0.2 µm).

Culture characteristics: On PDA, umbonate with entire edge, luteous in the centre, grey to deep grey at the edge, reverse deep grey, reaching 33–36 mm diam after 10 d at 25 °C. On MEA, flat with entire edge, front and reverse pale olivaceous in the centre and pale grey at the edge, reaching 24–29 mm diam after 10 d at 25 °C.

Typus: **China**, Yunnan Province, Xishuangbanna, Mengla County, on old/arboreal *Camellia sinensis*, 18 Apr. 2015, *F. Liu* (**holotype** HMAS 248083, culture ex-type CGMCC 3.19525 = LC6596 = LF1239).

Additional materials examined: **China**, Yunnan Province, Xishuangbanna, Mengla County, on old/arboreal *Camellia sinensis*, 18 Apr. 2015, *F. Liu*, living cultures LC6594 = LF1237, LC6595 = LF1238.

Notes: Based on the multi-locus phylogeny (Fig. 2), *S. antiqua* is phylogenetically related to *S. endophytica* and has high LSU (100 %), ITS (99 %) and *gapdh* (99 %) sequence similarities with the later. However, both species can be well differentiated from each other based on their *tub2* (502/518, 97 % sequence similarity) and *tef-1α* (561/613, 92 % sequence similarity) sequences. In addition, the habitat of both species are different. *Setophoma antiqua* was isolated from diseased leaf spots of arboreal tea plant from an unmanaged forest on the mountain in Yunnan Province, while *S. endophytica* was isolated from

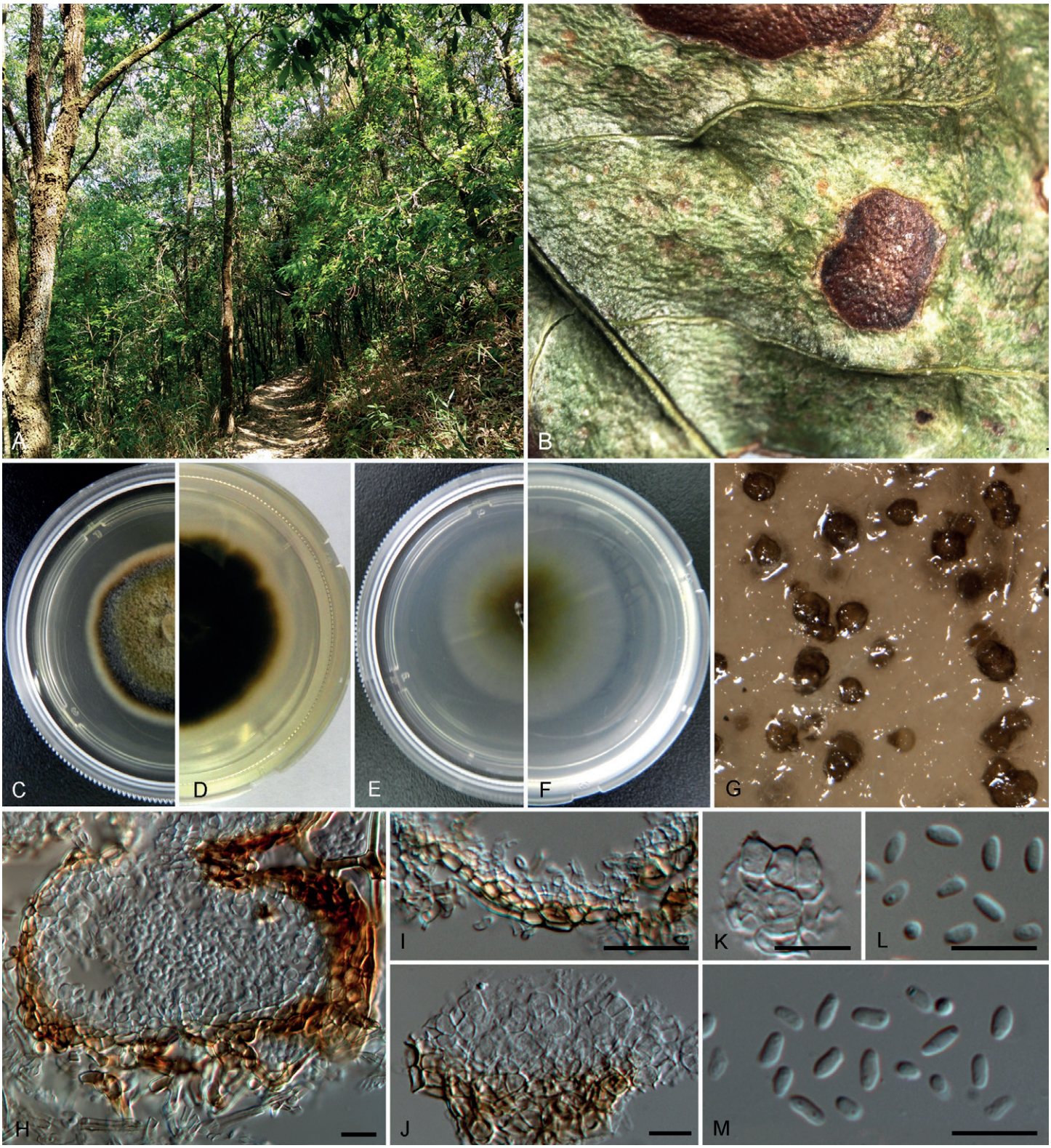

Fig. 3. *Setophoma antiqua* (ex-type CGMCC 3.19525 = LC6596). **A.** Sampling environment. **B.** Symptom of diseased leaf. **C, D.** Front and back of colony on PDA. **E, F.** Front and back of colony on MEA. **G.** Conidiomata. **H–J.** Vertical sections of conidiomata. **K.** Conidiogenous cells. **L, M.** Conidia. Scale bars = 10 µm.

healthy leaves of terraced tea plant from an open national forestry park in Jiangxi Province.

Setophoma endophytica F. Liu & L. Cai, ***sp. nov.*** MycoBank MB829902. Fig. 4.

Etymology: Named after its original habitat as an endophyte.

Sexual morph: Unknown. *Asexual morph*: *Aerial mycelia* hyaline, pale brown, smooth, branched, septate. *Conidiomata* immersed, olivaceous, globose to subglobose, scattered, 109–200 µm diam. *Pycnidial wall* pale brown, with 4–5 layers, 15–25 µm wide, walls of *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining inner cavity. *Conidiogenous cells* hyaline, aseptate, smooth, ampulliform, rarely irregular, 3.5–

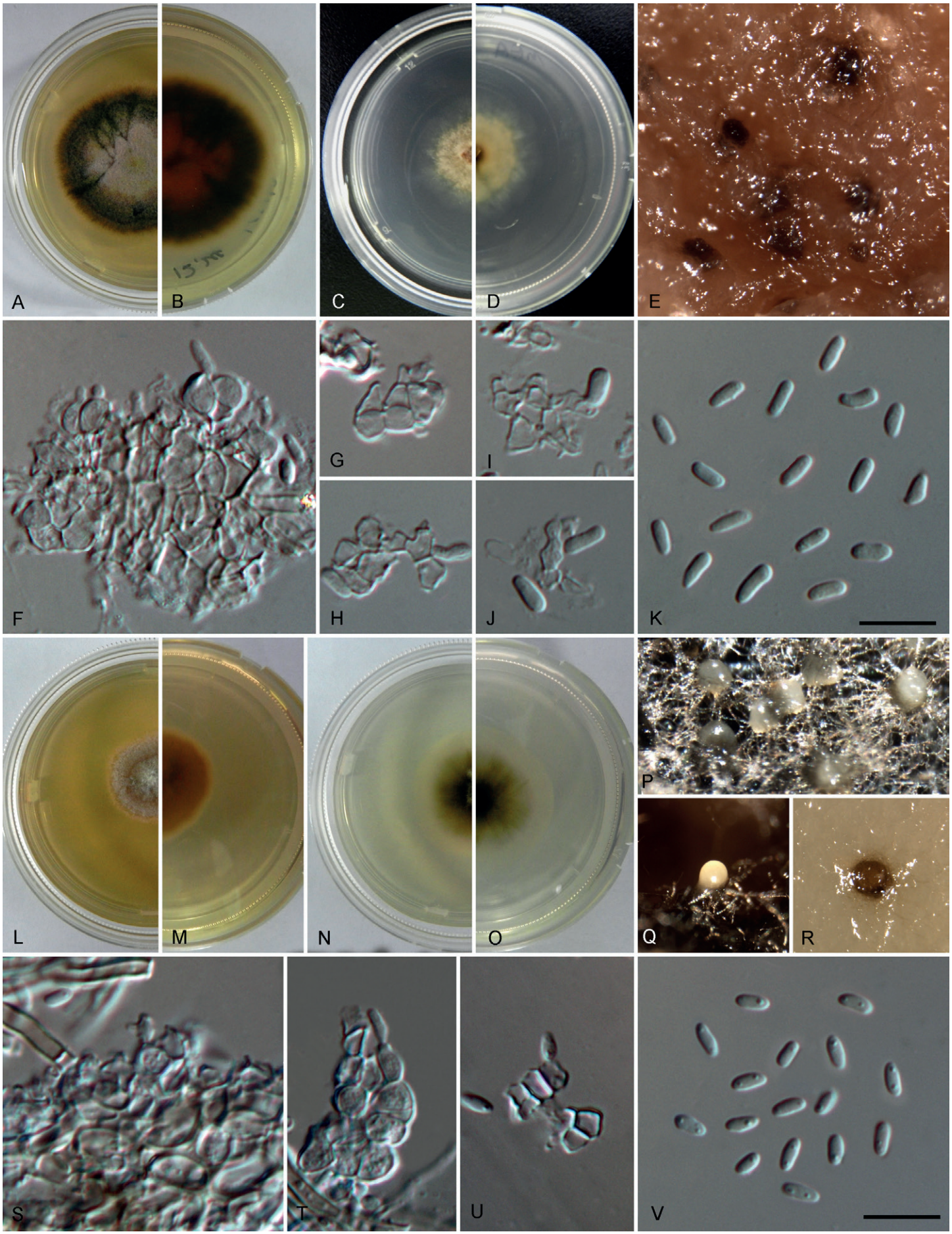

Fig. 4. *Setophoma endophytica* (**A–K.** ex-type CGMCC 3.19528 = LC3163, **L–V.** LC3216). **A, B, L, M.** Front and back of colonies on PDA. **C, D, N, O.** Front and back of colonies on MEA. **E, P–R.** Conidiomata. **F–J, S–U.** Conidiogenous cells and conidia. **K, V.** Conidia. Scale bars = 10 µm.

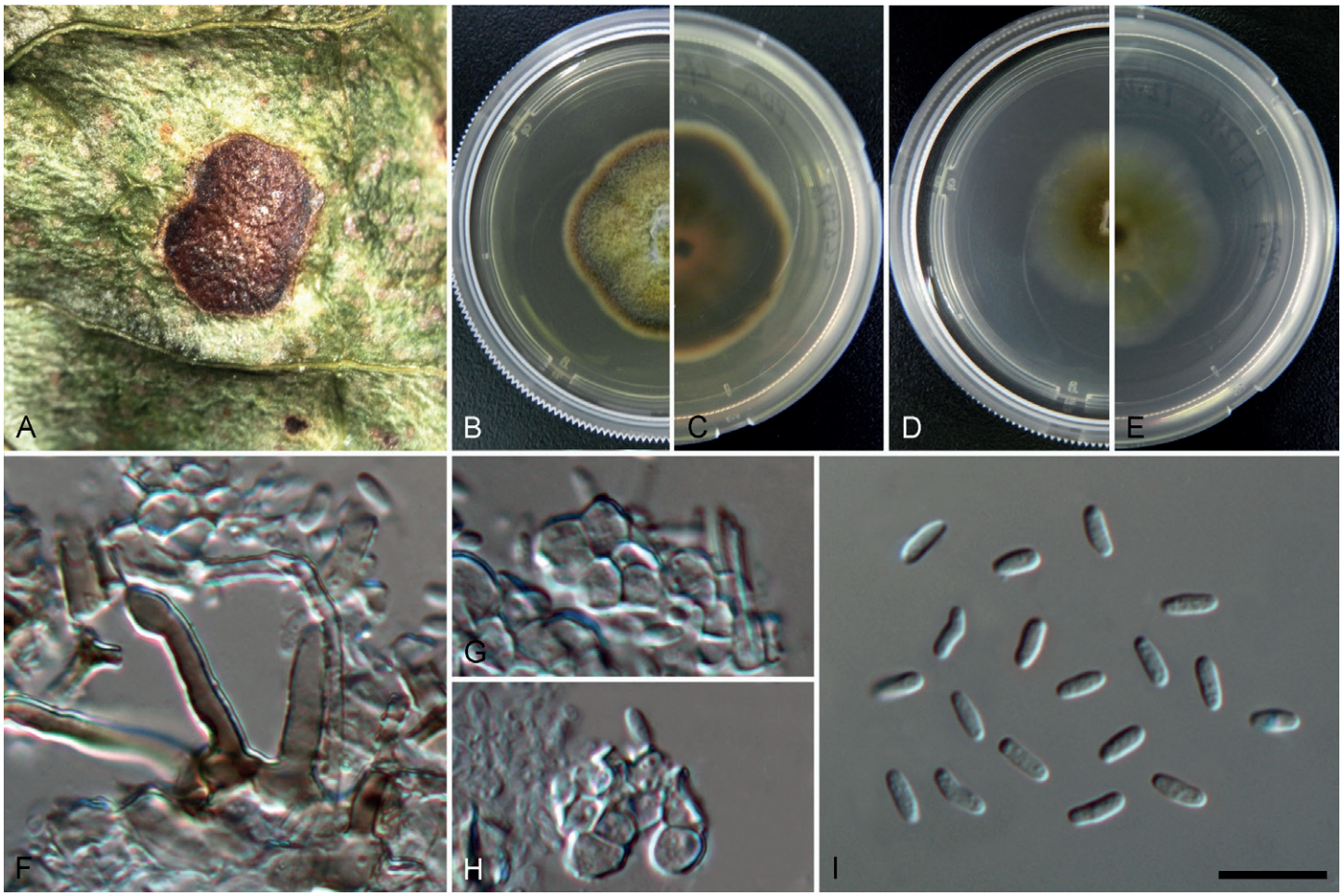

Fig. 5. *Setophoma longinqua* (ex-type CGMCC 3.19524 = LC6593). **A.** Symptom of diseased leaf. **B, C.** Front and back of colony on PDA. **D, E.** Front and back of colony on MEA. **F.** Setae. **G, H.** Conidiogenous cells and conidia. **I.** Conidia. Scale bars = 10 µm.

6.5 × 3–5.5 µm (av. = 4.9 ± 0.4 × 3.6 ± 0.3 µm). *Conidia* hyaline, aseptate, smooth, cylindrical to reniform, with rounded ends, straight or slightly curved, 4–5.5 × 1.5–2 µm (av. = 4.8 ± 0.2 × 1.7 ± 0.1 µm).

Culture characteristics: On PDA, low convex with entire edge, pale grey in the centre and olivaceous at the edge, reverse dark brown in the centre and black at the edge, reaching 28–30 mm diam after 10 d at 25 °C. On MEA, umbonate with entire edge, front and reverse brown in the centre and buff at the edge, reaching 22–25 mm diam after 10 d at 25 °C.

Typus: **China**, Jiangxi Province, Ganzhou, Yangling National Forest Park, on healthy leaves of *Camellia sinensis*, 24 Apr. 2013, *F. Liu*, YLBE3 (**holotype** HMAS 248081, culture ex-type CGMCC 3.19528 = LC3163 = LF372).

Additional materials examined: **China**, Jiangxi Province, Ganzhou, Yangling National Forest Park, on healthy leaves of *Camellia sinensis*, 24 Apr. 2013, *F. Liu*, YLBE3, living cultures LC3164 = LF373, LC3265 = LF374, LC3297 = LF519; on healthy leaves of *Camellia sinensis*, 24 Apr. 2013, *F. Liu*, YLBE1, living cultures LC3216 = LF428, LC13538 = LF2066.

Notes: *Setophoma endophytica* was isolated from healthy leaves of terraced tea plants from an open national forestry park in Jiangxi Province. Whether it is actually pathogenic to tea plant needs further research. Although strains of *S. endophytica* form two subclades in the multi-locus phylogeny (Fig. 2), they were recognised as one species because of their same geographical origin, similar morphological characters, and high sequence similarity (100 % in ITS, 99 % in *gapdh*, 98 % in *tef-1α* and *tub2*). For the differences between *S. endophytica* and its closely related species, see the notes under *S. antiqua*.

Setophoma longinqua F. Liu & L. Cai, ***sp. nov.*** MycoBank MB829904. Fig. 5.

Etymology: From the Latin *longinqua* = remote, refers to the remote mountain where the species was collected.

Sexual morph: Unknown. *Asexual morph*: *Aerial mycelia* hyaline or grey-brown, smooth, branched. *Conidiomata* pycnidial, subglobose to ovoid, with an opening at apex, 100–200 µm diam. *Pycnidial wall* pale brown, with 2–3 layers, walls of *textura angularis*. *Setae* erect, solitary, unbranched, aseptate or septate, smooth, medium-brown to brown, subcylindrical, apex rounded. *Conidiophores* reduced to conidiogenous cells lining the inner cavity. *Conidiogenous cells* phialidic, unbranched, aseptate, hyaline, ampulliform, smooth, 3.5–4.5 × 3–4 µm (av. = 3.9 ± 0.2 × 3.6 ± 0.2 µm). *Conidia* aseptate, smooth, hyaline, with small guttules, cylindrical or subcylindrical with round or obtuse ends, sometimes allantoid, 4–5.5 × 1.5–2 µm (av. = 4.9 ± 0.3 × 1.8 ± 0.2 µm).

Culture characteristics: On PDA, flat with undulate edge, luteous coloured in the centre and brown at the edge, reverse sepia, reaching 27–28 mm diam after 10 d at 25 °C. On MEA, flat with roughly entire edge, surface and reverse isabelline, reaching 21–22 mm diam after 10 d at 25 °C.

Typus: **China**, Yunnan Province, Xishuangbanna, Mengla County, on *Camellia sinensis*, 18 Apr. 2015, *F. Liu*, GFZCWS001P (**holotype** HMAS 248082, culture ex-type CGMCC 3.19524 = LC6593 = LF1236).

Additional materials examined: **China**, Yunnan Province, Xishuangbanna, Mengla County, on *Camellia sinensis*, 18 Apr. 2015, *F. Liu*, GFZCWS001P, living cultures LC13481 = LF2068, LC13482 = LF2069.

Notes: *Setophoma longinqua* was isolated from the same symptomatic leaf as *S. antiqua*, but they are phylogenetically distinct (Figs 1, 2). It is morphologically distinct from *S. antiqua* by producing seta and longer conidia (av. = 4.9 × 1.8 µm vs. 3.7 × 1.8 µm). Based on the multi-locus phylogeny (Fig. 2), the most closely related species to *S. longinqua* is an undescribed carbonatite associated species, but only with low sequence similarities of 95 % on ITS, 92 % on *tef-1α*, and 90 % on *tub2*.

Setophoma yingyisheniae F. Liu & L. Cai, ***sp. nov*.** MycoBank MB829903. Fig. 6.

Etymology: Named after the collector, Yingyi Shen, who was also the first to report Setophoma leaf spot disease on tea plants.

Sexual morph: Unknown. Sterile on cultural media. Lesions on plant leaves subglobose or irregular, initially off-white to brownish yellow, becoming dark grey in the centre, separated from the healthy tissue by a black margin. *Conidiomata* pycnidial, dark brown or black, globose or subglobose, with an opening at apex, 60–200 µm diam. *Pycnidial wall* brown, walls of *textura angularis*. *Setae* 8.5–10 µm wide, erect, solitary, unbranched, septate, dark brown, apex rounded. *Conidiophores* hyaline, often with two branches. *Conidiogenous cells* hyaline, oblong, 3–5.5 × 1.5–2.5 µm (av. = 4.2 ± 0.6 × 2.1 ± 0.2 µm). *Conidia* hyaline, aseptate, smooth, subglobose, reniform, or cylindrical, 3.5–6 × 1.5–2.5 µm (av. = 4.4 ± 0.5 × 2.2 ± 0.2 µm).

Typus: **China**, Guangxi Province, Guilin, Longsheng County, altitude 1 200 m, on *Camellia sinensis*, 21 Sep. 2016, *Y.Y. Shen*, GXGL01a (**holotype** HMAS 248086, culture ex-type CGMCC 3.19527 = LC13479 = LF1986).

Additional materials examined: **China**, Anhui Province, Bengbu County, Jianping Mountain, on *Camellia* sp., 2015, *F. Liu*, AHTEA07, living culture LC12696 = LF1529; on *Camellia* sp., 2015, *F. Liu*, AHTEA08, living culture LC12699 = LF1532; Fujian Province, Wuyishan City, Xingtianpu, 27.31'25"N, 118.1'59"E, 160 m, on *C. sinensis*, 22 Aug. 2016, *F. Liu*, WYSH3, living culture LC13477 = LF1935; on *C. sinensis*, 22 Aug. 2016, *F. Liu*, WYSH4, living culture LC13478 = LF1943; Guangxi Province, Guilin, Longsheng County, altitude 1 200 m, on *Camellia sinensis*, 21 Sep. 2016, *Y.Y. Shen*, GXGL02, living cultures LC13480 = LF2017, LF2026; on *C. sinensis*, 21 Sep. 2016, *Y.Y. Shen*, GXGL04, living cultures LF2027, LF2032; Guilin, Tea Science and Research Institute of GuiLin, on healthy twig of *Camellia* sp., Sep. 2013, *T.W. Hou*, living culture LC3499 = LF727; Guizhou Province, on *Camellia* sp., 2015, *F. Liu*, GZSXD001P, living culture LC6739 = LF1420; Jiangxi Province, Ganzhou City, Yangling National Forest Park, on *C. sinensis*, 24 Apr. 2013, *F. Liu*, YLA2, living culture LC3133 = LF341; on healthy leaves of *C. sinensis*, 24 Apr. 2013, *F. Liu*, YLBE5, living culture LC3176 = LF386; Nanchang City, Meiling, on healthy leaves of *C. sinensis*, Apr. 2013, *F. Liu*, MLE002, living culture LC3197 = LF407.

Notes: *Setophoma yingyisheniae* was isolated from both symptomatic and asymptomatic leaves of terraced (shrubby) tea plants from five provinces, *i.e.* Anhui, Fujian, Guangxi, Guizhou and Jiangxi. It is phylogenetically related to *S. yunnanensis* (Fig. 2, 96 % sequence similarity on ITS, 88 % on *gapdh*, 87 % on *tef-1α* and 93 % on *tub2*), but differs from the later in the host (terraced vs. arboreal tea plant) and habitat (plantation vs. unmanaged forest, Figs 6A, 7B). With respect to the conidiogenesis, the conidiophores of *S. yunnanensis* are simple and often reduced to single and ampulliform or globose conidiogenous cells, while these are branched in *S. yingyisheniae* and produces oblong conidiogenous cells. In addition, *S. yingyisheniae* differs from *S. yunnanensis* by producing seta in culture.

Setophoma yunnanensis F. Liu & L. Cai, ***sp. nov*.** MycoBank MB829905. Fig. 7.

Etymology: Named after the location where it was collected from, Yunnan Province.

Sexual morph: Unknown. *Asexual morph*: Sterile on cultural media. *Conidiomata* pycnidial, on diseased leaves black, globose or subglobose, with an opening at apex, 100–200 µm diam. *Pycnidial wall* brown, with 3–5 layers, 13–35 µm wide, walls of *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining inner cavity. *Conidiogenous cells* hyaline, smooth, phialidic, ampulliform or globose, aseptate, 2.5–5 × 2–4.5 µm (av. = 3.5 ± 0.5 × 3.1 ± 0.8 µm). *Conidia* hyaline, aseptate, granular to guttulate, ellipsoid or cylindrical, 3.5–5 × 2–3 µm (av. = 4.3 ± 0.4× 2.4 ± 0.2 µm).

Culture characteristics: On PDA, flat with entire edge, pale grey in the centre, buff at the edge, reverse blackish yellow in the centre and buff at the edge, reaching 31–34 mm diam after 10 d at 25 °C; On MEA, flat with entire edge, front and reverse blackish green, reaching 29 mm diam after 10 d at 25 °C.

Typus: **China**, Yunnan Province, Xishuangbanna, Mengla County, Laomansa, on *Camellia sinensis*, 19 Apr. 2015, *F. Liu*, LMS001P (**holotype** HMAS 248084, culture ex-type CGMCC 3.19529 = LC6753 = LF1434).

Additional material examined: **China**, Yunnan Province, Xishuangbanna, Mengla County, Daqishu, on *Camellia sinensis*, 19 Apr. 2015, *F. Liu*, DQS001P, living culture LC6532 = LF1167.

Notes: The leaf spots of arboreal tea plants where *S. yunnanensis* was isolated from were scattered, grey to brown in colour. *Setophoma yunnanensis* is phylogenetically related to *S. yingyisheniae* (Fig. 2, 96 % sequence similarity on ITS, 88 % on *gapdh*, 87 % on *tef-1α* and 93 % on *tub2*), but differs both morphologically and geographically from the latter (see notes under *S. yingyisheniae*).

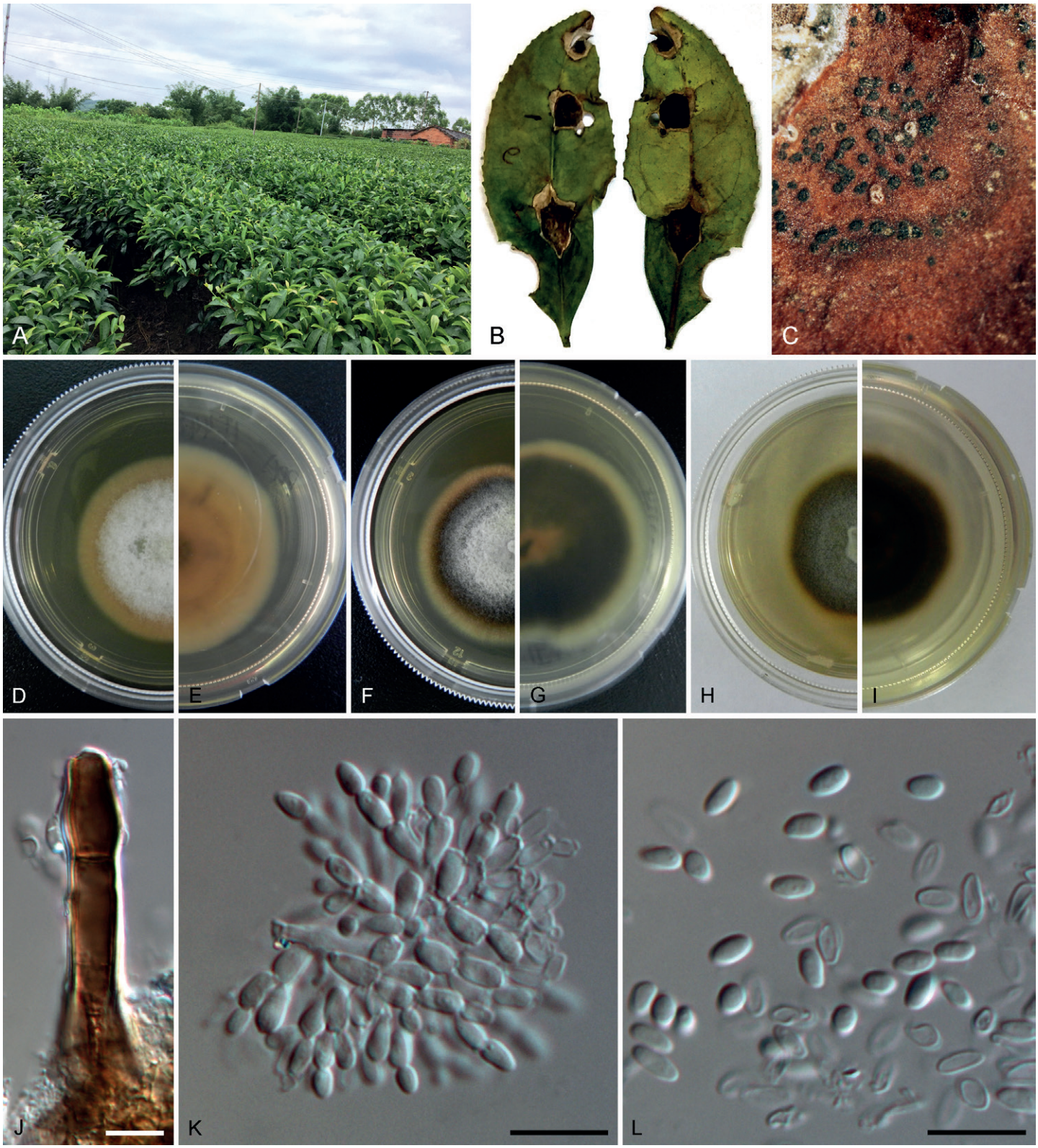

Fig. 6. *Setophoma yingyisheniae* (**B, C, J–L.** holotype GXGL01a, **D, E.** LC3236, **F, G.** LC3334, **H, I.** LC3133). **A.** Representative sampling environment-terraced tea plant. **B.** Front and back of diseased leaves. **C.** Conidiomata on leaf. **D–I.** Front and back of colonies on PDA. **J.** Seta. **K.** Conidiophores, conidiogenous cells and conidia. **L.** Conidia. Scale bars = 10 µm.

DISCUSSION

Leaf spots on tea trees appeared to be caused by several previously undescribed *Setophoma* species. These were found on both old and young tea leaves collected from several Chinese provinces. Phylogenetic analyses provided a clear resolution for these novel *Setophoma* species (Figs 1, 2). Although the genus *Setophoma* was only recently proposed and its relatives in *Pleosporales* have already been partly reassessed (e.g. Aveskamp *et al.* 2010, de Gruyter *et al.* 2010, Woudenberg *et al.* 2013), a large number of published phoma-like names still remained untreated. Therefore, in order to avoid proposing new *Setophoma* names for the old published taxa, we compared our species with all known *Pleosporales* species associated with *Camellia*

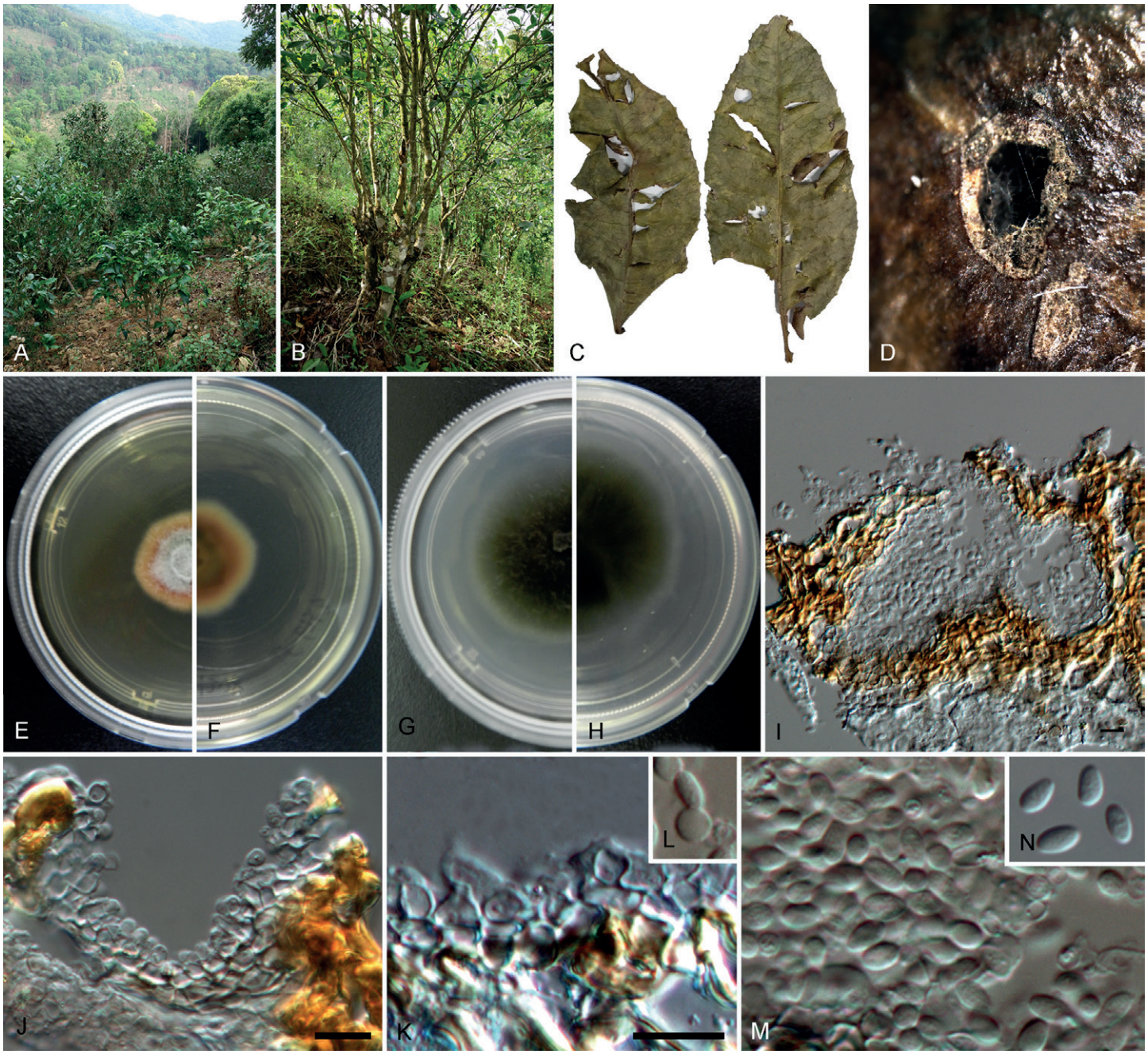

Fig. 7. *Setophoma yunnanensis* (ex-type CGMCC 3.19529 = LC6753). **A, B.** Sampling environment and arboreal tea plants. **C, D.** Symptom of diseased leaves. **E, F.** Front and back of colony on PDA. **G, H.** Front and back of colony on MEA. **I–K.** Vertical section of conidiomata. **L.** Conidiogenous cell and conidium. **M, N.** Conidia. Scale bars = 10 µm.

from China, *i.e. Alternaria alternata*, *A. longipes*, *A. brassicae*, *Coniothyrium palmarum*, *Deuterophoma* sp., *Fusicladium theae*, *Hendersonia theae*, *Phoma camelliae*, *P. chinensis*, *P. herbarium var. herbarum*, *Piggotia* sp., *Remotididymella destructiva* and *Stagonosporopsis cucurbitacearum* (Farr & Rossman 2019). Most of these species are clearly distinctive from *Setophoma* by either morphological or molecular data, but this could unfortunately not be determined for *Phoma camelliae* and *P. chinensis,* as no reliable information regarding type specimen or publication information could be found for these species. These two names were thus ignored in this study and the taxonomic treatments of *P. camelliae* and *P. chinensis* await further study.

Of the four novel species isolated from the tea plant leaf spots, three species (*S. antiqua*, *S. longinqua* and *S. yunnanensis*) were only isolated from old and arboreal tea plants (~100–300-yr-old, with less disease management and anthropogenic interference, Figs 3A, 7A, B) in Yunnan Province. In contrast, the fourth species, *S. yingyisheniae*, was widely distributed in all investigated provinces except Yunnan, and to our knowledge it only occurs on terraced tea plants (~50-yr-old), with intensive disease management and anthropogenic interference (Fig. 6A). *Setophoma yingyisheniae* has been isolated from both symptomatic and asymptomatic leaves tissues, indicating an alternative lifestyle ranging from endophytic to plant pathogenic. Morphologically, *S. yingyisheniae* differs from other *Setophoma* spp. in producing branched conidiophores, while other species in the genus produce solitary conidiophores. In contrast, *S. endophytica* was only isolated from healthy leaves, and whether it actually causes disease awaits to be determined.

A recently published species, *Setophoma cyperi* (Crous *et al.*

2016) should be excluded from the genus *Setophoma*, as it does not cluster in *Setophoma s. str.* (Fig. 1). *Setophoma cyperi* appears to be more closely related to *Sulcispora pleurospora* (Fig. 1). The definitive generic placement of *S. cyperi* should be clarified in future work when a broader sampling of *Phaeosphaeriaceae* species becomes available.

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