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A new species of *Collodiscula* (Xylariaceae) from China

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Abstract

A *Collodiscula* isolate, found on a bamboo stalk in China, differs from *C. japonica* by having smaller ascospores. On the basis of morphology and molecular phylogeny it is described as a new species, *Collodiscula bambusae sp. nov.*

Key words: ascomycetes, taxonomy, Xylariales

Introduction

Collodiscula I. Hino & Katum. was introduced as a monotypic genus by Hino & Katumoto (1955) and later referred to the Sphaeriaceae (Hino 1961). However, based on features such as the stromatal ontogeny, heavily carbonized stromata, amyloid ascus apical apparatus, and short stipitate asci it is now included in the Xylariaceae (Samuels & Rossman 1992, Læssøe & Spooner 1994, Kang *et al.* 1999). Jaklitsch & Voglmayr (2012) provided a phylogenetic study based on LSU and ITS sequences and confirmed that the genus *Collodiscula* belongs to Xylariaceae. Samuels *et al.* (1987) gave a detailed description of the sexual morph, and *Acanthodochium collodisculae* was identified as the asexual state of *C. japonica*. *Collodiscula japonica* has been reported from Chinese mainland (Jaklitsch & Voglmayr 2012), Japan (Hino & Katumoto 1955), Russia (Vasiljeva 1998) and Taiwan (Ju & Rogers 1999).

A species of *Collodiscula* was found in Guizhou Province, China that differed from *C. japonica* by having smaller ascospores. Phylogenetic analysis also indicated that this species was distinct and it described as *C. bambusae sp. nov.*

Materials and methods

Morphological studies and isolation

Specimens of bamboo with ascocarps of an unknown fungus were collected from Guizhou Province, China and taken to the laboratory in plastic bags. The methodology used for morphological examination of fungi growing on the bamboo followed that used by Stadler *et al.* (2004). Materials were mounted in water and Melzer's iodine reagent for examination. Asci and ascospores were examined by light microscopy (BX41, Olympus). At least 20 propagules were measured, length and width ranges were recorded. Material was deposited in the herbarium of Guizhou University (GZUH).

DNA extraction, PCR amplification and sequencing

A culture was initiated from perithecial contents of freshly collected stromata, propagated and studied as described by Stadler *et al.* (2004) on potato dextrose agar (PDA) medium at 25˚C. Total genomic DNA was extracted from fresh cultures using a modified protocol of Doyle & Doyle (1987) and Lee & Taylor (1990). DNA preparations were stored at -20 °C until used for PCR.

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DNA sequencing and alignment

The ITS and 5.8S region of rDNA (ITS) molecule was amplified using primer pairs ITS4 and ITS5 (White *et al.* 1990). Large subunit nuclear ribosomal DNA (LSU) was amplified with primer pairs LROR and LR5 (Vilgalys & Hester 1990), RNA polymerase II second largest subunit (RPB2) gene was amplified with primer pairs fRPB2-5F and fRPB2-7cr, and β-tubulin gene was amplified with primer pairs T–1 and T–22 (Tanaka *et al.* 2009, Hsieh *et al.* 2010). PCR was performed with the 25 μL reaction system consisting of 19.75 μL of double distilled water, 2.5 μL of 10× Taq buffer with $MgCl_2$, 0.5 μL of dNTP (10 mM each), 0.5 μL of each primer (10 μM), 0.25 μL Taq DNA polymerase (5 U/μl), and 1.0 μL of DNA template. The thermal cycling program followed Maharachchikumbura *et al.* (2012).

Phylogenetic analyses

Two separate phylogenetic analyses were performed on two separate datasets. Combination sequence data were manually adjusted using BioEdit (Hall 1999), to allow maximum alignment and maximum sequence similarity. Maximum parsimony analysis (MP) were performed using PAUP (Phylogenetic Analysis Using Parsimony) v.4.0b10 (Swofford 2002). Ambiguously aligned regions were excluded and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Maxtrees were set up to 5,000, branches of zero length were collapsed and all multiple parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated by 1,000 bootstrap replications resulting from maximum parsimony analysis (Felsenstein 1985).

TABLE 1. Strains used in phylogenetic analyses and their corresponding GenBank accession numbers.

Species	Strain	Type status/References	GenBank accession numbers			
			ITS	LSU	RPB2	β-tubulin
Amphirosellinia fushanensis	HAST 91111209	Ex-type (Hsieh *et al.* 2010)	GU339496		GQ848339	GQ495950
Amphirosellinia nigrospora	HAST 91092308	Ex-type (Hsieh *et al.* 2010)	GU322457		GQ848340	GQ495951
Amphisphaeria umbrina	HKUCC 994, CBS 172.96, Mt2	Jaklitsch & Voglmayr 2012 Schoch *et al.* 2009	AF009805	AF452029	FJ238348	
Anthostomella brabeji	CBS 110128	Jaklitsch & Voglmayr 2012 Stadler *et al.* 2013	EU552098	EU552098		
Apiospora montagnei	AFTOL 951, H3-83	Jaklitsch & Voglmayr 2012	JN688916	DQ471018	DQ470921	
Apiospora sinensis	HKUCC 3143	Jaklitsch & Voglmayr 2012	AY083831	AY083831		
Arthrinium marii	CBS 114803	Crous & Groenewald 2013	KF144899	KF144945		
Arthrinium sacchari	ATCC76303	Jaklitsch & Voglmayr 2012	AF393679	ATCC76303		
Arthrinium phaeospermum	CBS 114317, HKUCC 3395	Jaklitsch & Voglmayr 2012		KF144953		
Astrocystis bambusae	HAST 89021904	Ex-type (Hsieh *et al.* 2010)	GU322449		GQ844836	GQ495942
Astrocystis mirabilis	HAST 94070803	Ex-type (Hsieh *et al.* 2010)	GU322448		GQ844835	GQ495941
Bartalinia robillardoides	BRIP 14180	Jaklitsch & Voglmayr 2012	AF405301	AF382366	DQ368653	
Biscogniauxia arima	WSP 122	Ex-type (Hsieh *et al.* 2010)	EF026150		GQ304736	AY951672
Biscogniauxia nummularia	BCC 1101, H86	Jaklitsch & Voglmayr 2012		AB376691	FR715504	
Clypeosphaeria uniseptata	HKUCC6349, Mt28	Jaklitsch & Voglmayr 2012	AF009808	DQ810219		
Collodiscula japonica	CBS 124266	Jaklitsch & Voglmayr 2012	JF440974	JF440974		
Collodiscula bambusae	GZUH0102	This study	KP054279	KP054280	KP276675	KP276674
Creosphaeria sassafras	CM AT-018	Authentic (Tang *et al.* 2009)	AJ390425	DQ840056		

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TABLE 1 (Continued)

Species	Strain	Type status/References	GenBank accession numbers			
			ITS	LSU	RPB2	β-tubulin
Daldinia concentrica	CBS 113277, ATCC 36659	Spatafora & Blackwell 1993	AY616683	U47828	FR715506	KC977274
Diatrype disciformis	AFTOL 927	Trouillas *et al.* 2001	AJ302437	DQ470964	DQ470915	
Discoxylaria myrmecophila	169 (JDR)	Hsieh *et al.* 2010	GU322433			GQ487710
Entoleuca mammata	100 (JDR)	Hsieh *et al.* 2010	AJ246235		GQ844782	GQ470230
Euepixylon sphaeriostomum	261 (JDR)	Hsieh *et al.* 2010	GU292821			GQ470224
Eutypa consobrina	CBS122677	Jaklitsch & Voglmayr 2012	EU552126	EU552126		
Graphostroma platystoma	CBS 270.87, AFTOL-ID 1249	Jaklitsch & Voglmayr 2012	JX658535	DQ836906		
Hyponectria buxi	UME 31430	Jaklitsch *et al.* 2012		AY083834		
Hypoxylon fragiforme	MUCL 51264, STMA07069, HKUCC 1022	Authentic (Seifert *et al.* 2003)	KM186294	KM186295		
Kretzschmaria guyanensi	HAST 89062903	Hsieh *et al.* 2010	GU300079		GQ844792	GQ478214
Melogramma campylosporum	MBU	Jaklitsch & Voglmayr 2012	JF440978	JF440978		
Muscodor albus	MSU 2081	Ex-type (Seifert *et al.* 2003)	AF324336	HM034864	FJ480345	
Nemania maritima	HAST 89120401	Ex-type (Hsieh *et al.* 2010)	GU292822	DQ840074	DQ631946	GQ470225
Nemania serpens	HAST 235 , FR AT 114	Authentic (Hsieh *et al.* 2010)	GU292820	DQ840075	GQ844773	GQ470223
Pestalosphaeria hansenii	ATCC48245	Jaklitsch & Voglmayr 2012	AF377290			
Podosordaria mexicana	176 (WSP)	Hsieh *et al.* 2010	GU324762		GQ853039	GQ844840
Poronia pileiformis	88113001 (WSP)	Ex-epitype (Hsieh *et al.* 2010)	GU324760		GQ853037	GQ502720
Rhopalostroma angolense	MUCL52664, CBS 126414	Authentic (Stadler *et al.*, 2010b)	FN821965	KM186298	KM186297	KM186299
Rosellinia merrillii	HAST 89112601	Hsieh *et al.* 2010	GU300071		GQ844781	GQ470229
Rosellinia necatrix	HAST 89062904, HKUCC 9037	Authentic (Hsieh *et al.* 2010)	EF026117	AY083824	GQ844779	EF025603
Rostrohypoxylon terebratum	CBS 119137	Ex-type (Fournier *et al.* 2010)	DQ631943	DQ840069	DQ631954	DQ840097
Ruwenzoria pseudoannulata	MUCL 51394	Ex-type (Stadler *et al.* 2010b)	GU053568			
Sordaria fimicola	CBS 723.96, CBS 508.50	Miller & Huhndorf 2005, Tang *et al.* 2009	AY681188	AY681160	DQ368647	DQ368618
Stilbohypoxylon elaeicola	JDR 173	Hsieh *et al.* 2010	EF026148		GQ844826	EF025616
Subramaniomyces fusisaprophyticus	CBS 418.95	Jaklitsch & Voglmayr 2012	EU040241	EU040241		
Thamnomyces camerunensis	MUCL 51396	Ex-type (Stadler *et al.* 2010a)	FN428828			

...continue on the next page

TABLE 1 (Continued)

Species	Strain	Type status/References	GenBank accession numbers			
			ITS	LSU	RPB2	β-tubulin
Truncatella angustata	ICMP 7062	Jaklitsch & Voglmayr 2012	AF405306	AF382383		
Xylaria bambusicola	WSP 205, BCC 23659	Ex-type (Hsieh *et al.* 2010; Okane *et al.* 2008)	EF026123	AB376825	GQ844802	AY951762
Xylaria grammica	HAST 479	Hsieh *et al.* 2010, Chen *et al.* 2013	JQ862677	JQ862638	GQ844813	GQ487704
Xylaria hypoxylon	CBS 122620	Authentic (Stadler *et al.* 2013)	AM993141	KM186301	KM186302	KM186300

NOTE: Abbreviations: **AFTOL:** Assembling the Fungal Tree of Life; **ATCC**: American Type Culture Collection, Virginia, USA; **AT**: Taxa collected and identified by Alvin M. C. Tang; **BCC:** BIOTEC Culture Collection, Bangkok, Thailand; **CBS**: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **HKUCC** Hong Kong University Culture Collection, Hong Kong, China; **HAST**: Herbarium, Research Center for Biodiversity, Academia Sinica, Taipei; **JDR**: Herbarium of Jack D. Rogers; **MSU**: Montana State University mycological collection, U.S.A.; **MUCL**: Mycothèque de l'Université catholique de Louvain, Germany; **WSP**: Washington State University, U.S.A.

Results

A species of *Collodiscula* (GZUH0102) was isolated in pure culture and subjected to morphological and molecular analyses.

Combined analysis of LSU and ITS rDNA

The alignment file resulted in a data set comprising 1,682 characters including gaps. Of these characters, 1,251 were constant and parsimony-uninformative. A best scoring MP tree is shown (Fig. 1) and bootstrap support (BS) values of MP (equal to or above 50% based on 1,000 replicates) are shown on the upper branches (TL=1986, CI=0.575, RI=0.532, RC=0.306, HI=0.425). Isolate GZUH0102 grouped with *Collodiscula japonica* (CBS 124266) with high bootstrap support (100%) in Xylariaceae.

Combined analysis of ITS, LSU, RPB2 and β–tubulin genes

The combined data set of ITS, LSU, RPB2 and β-tubulin genes comprised sequences from 32 taxa with *Sordaria fimicola* (CBS 723.96) as the outgroup taxon. The dataset consisted of 4,778 characters after alignment, of which 1,778 were conserved, 1,104 were variable and 1,896 were parsimony informative. A best scoring MP tree is shown (Fig. 2) and bootstrap support (BS) values of MP (equal to or above 50% based on 1,000 replicates) are shown on the upper branches (TL=11298, CI=0.468, RI=0.378, RC=0.177, HI=0.532). Our strain GZUH0102 grouped with *Collodiscula japonica* (CBS 124266) with high bootstrap support (89%) in a sister clade to *Astrocystis* spp. (100%) in Xylariaceae.

Taxonomy

Collodiscula bambusae Q.R. Li & J.C. Kang, *sp. nov*. (Fig. 3) MycoBank MB 810668

Differs from *Collodiscula japonica* mainly by its smaller, yellowish brown ascospores.

Type—CHINA. Guizhou Province: Guiyang, saprobic on the stalk of bamboo, March 2014, Q.R. Li (GZUH0102, holotype); *Ibid.,* (MFLU 15-0391, isotype), ex-type living cultures, MFLUCC 15-0398.

Saprobic on the stalk of bamboo, forming on the host surface. Sexual state: stromata scattered or gregarious, solitary, superficial, pulvinate to nearly semiglobose, 0.5–0.8 mm diam., 0.3–0.6 mm high, containing 1–3 perithecia. Surface convex or flattened, dark, smooth, with a central papillate of black ostiole. External stromatal layer black, carbonaceous,

easily chipped away to reveal the thin, black perithecia. Base surrounded by a black crustose ring on the host surface. Perithecia globose to subglobose. Paraphyses hyaline, septate. Asci cylindrical, 8-spored, overlapping uniseriate, 110–170 × 8–11 µm (mean 144 × 9.5 µm, n=30) with a J+, wedge-shaped apical apparatus, 2.5–3.5 µm (mean 3 µm, n=30) high, 1.5–2.5 µm (mean 2 µm, n=30) diam. Ascospores 15–17.5 × 4.5–5.5 µm (mean 17 × 5 µm, n=30), fusoid, inaequilateral, with one median slightly constricted septum, with narrow rounded ends, yellowish brown, smooth, lacking sheath and germ slit. Asexual state: unknown.

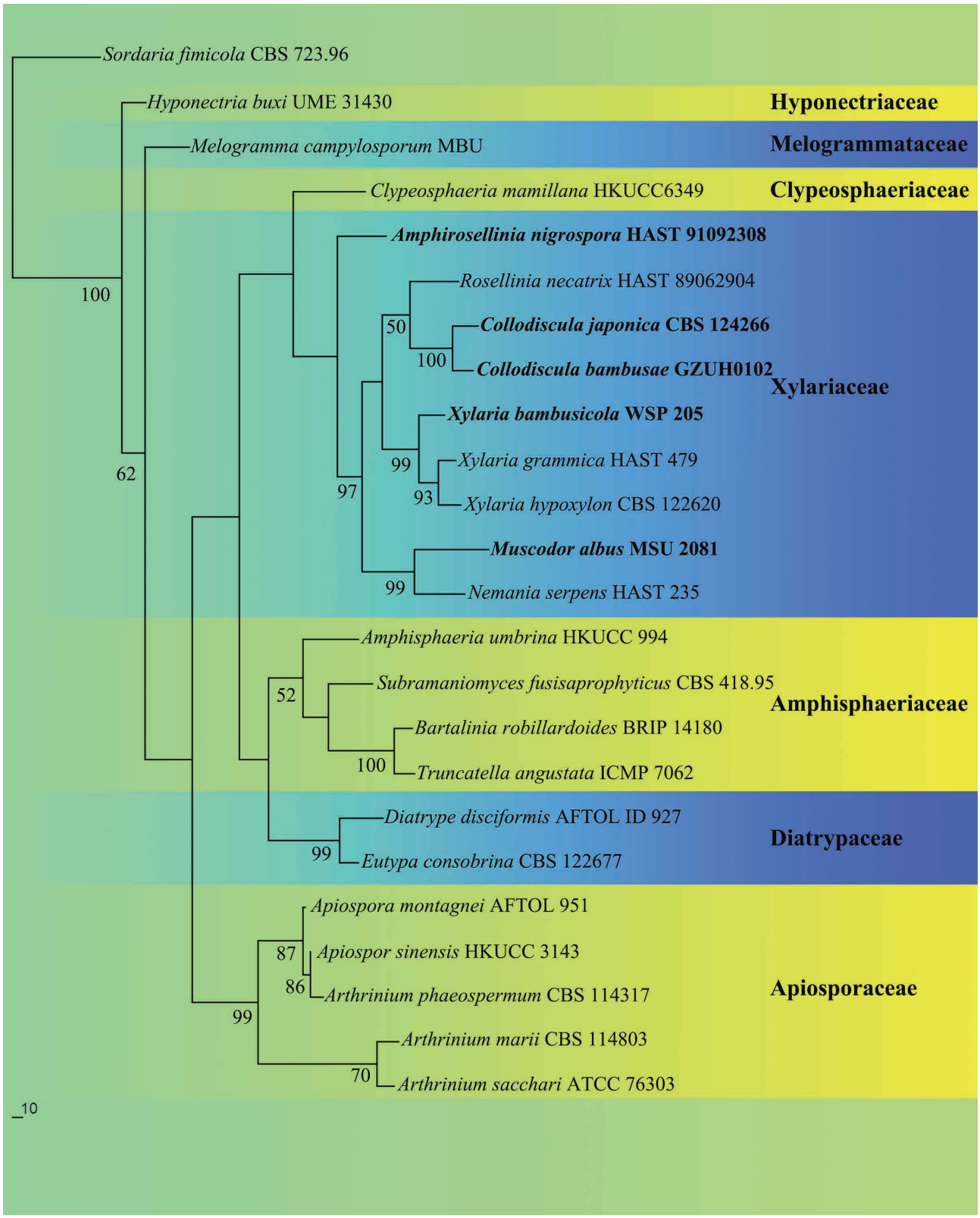

FIGURE 1. Topology showing the most parsimonious tree of ITS and LSU genes regions. Bootstrap values higher than 50% are shown. The tree is rooted with *Sordaria fimicola*. Sequence from type strains are in bold.

Habitat/Distribution:—Known to inhabit stalk of bamboo, Guizhou Province, China.

Etymology:—In reference to the host, bamboo.

Other material examined:—CHINA. Guiyang Province: Guiyang city, saprobic on the stalk of bamboo, 20 March 2014, Q.R. Li (GZUH0108!).

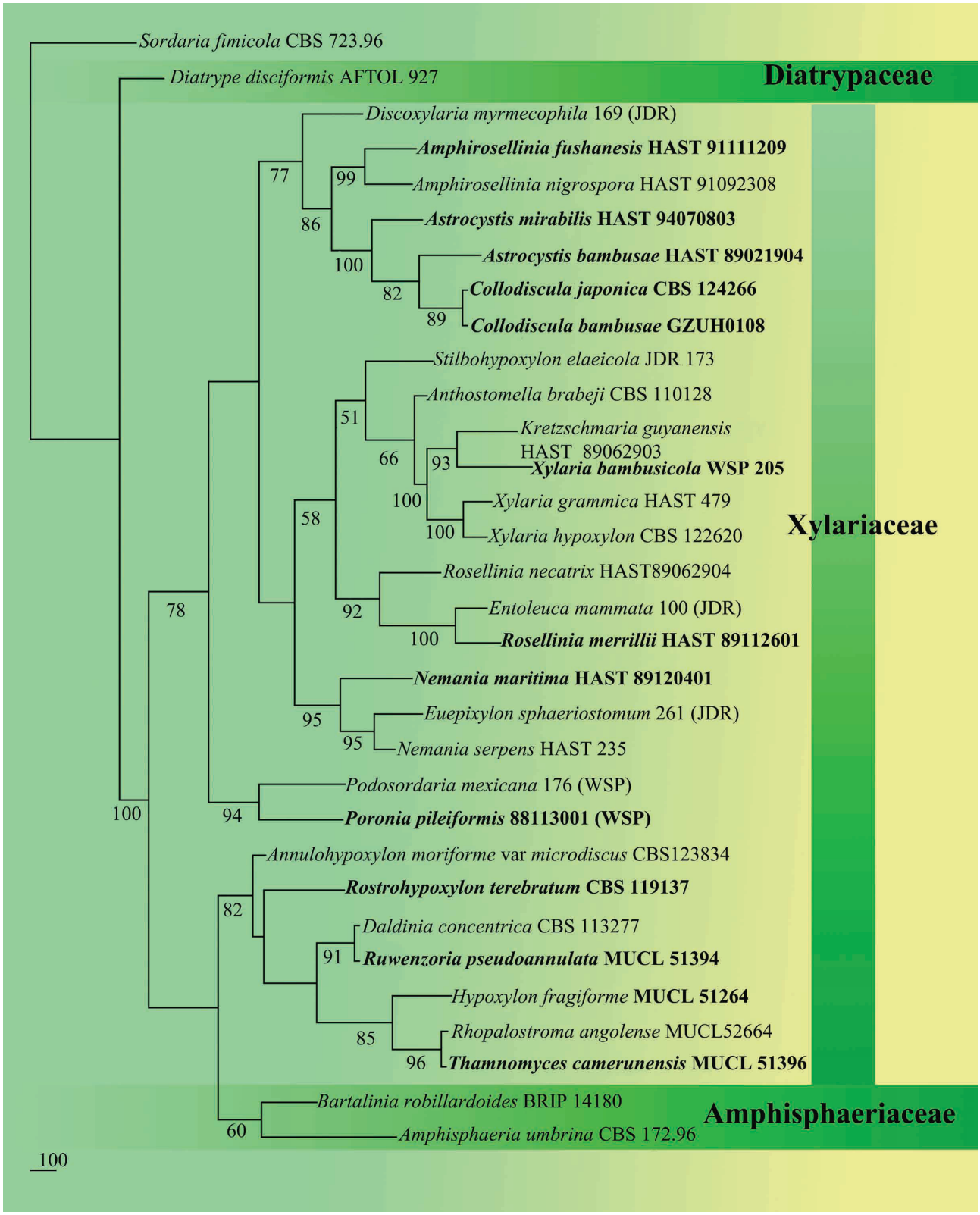

FIGURE 2. Topology showing the most parsimonious tree of ITS, LSU, RPB2 and β-tubulin genes regions. Bootstrap values higher than 50% are shown. The tree is rooted with *Sordaria fimicola*. Sequence from type strains are in bold.

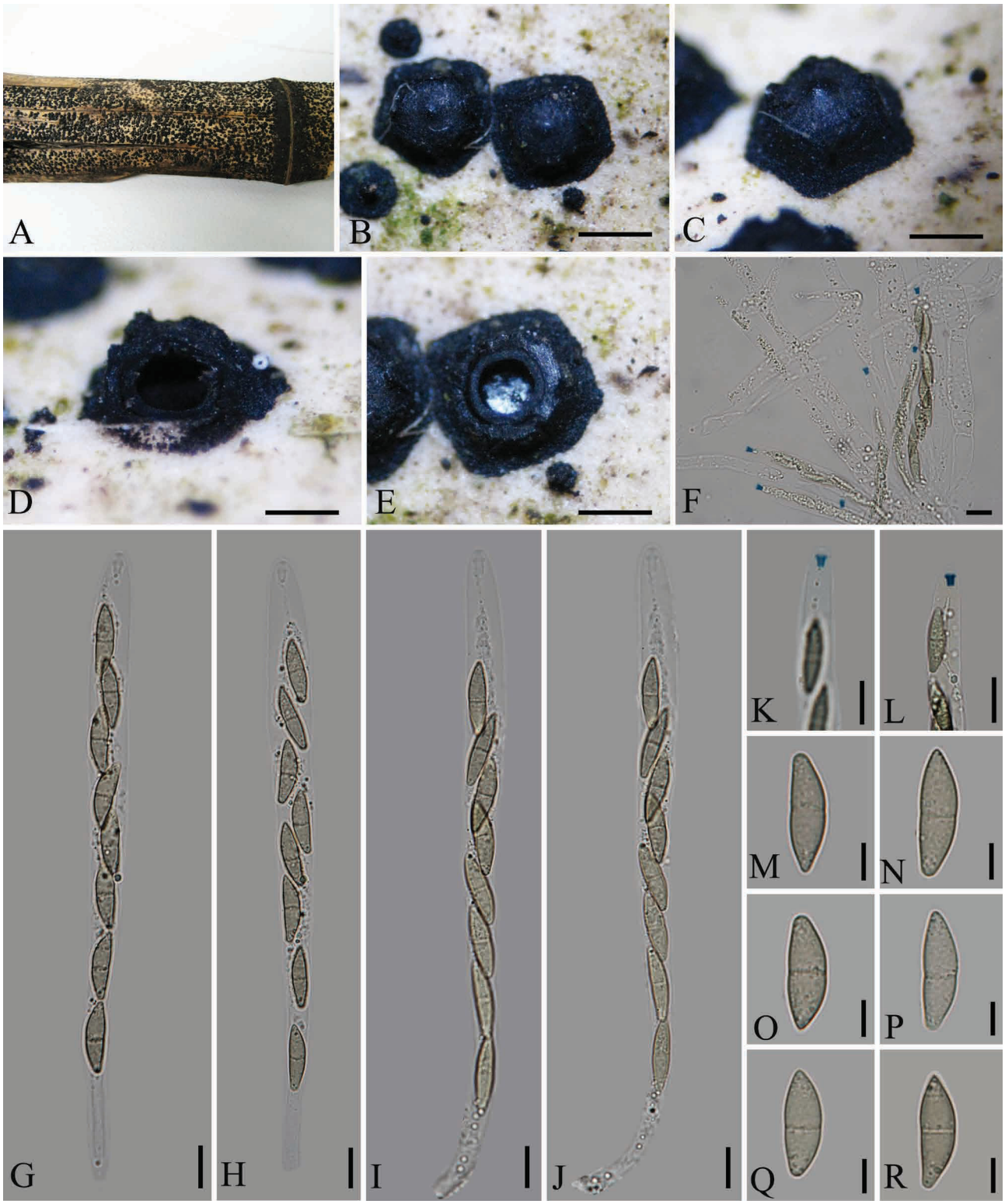

FIGURE 3. *Collodiscula bambusae*. A. Fresh material. B, C. Ascomata on the surface of host. D, E. Section of ascoma. F. Paraphyses. G–J. Mature asci with ascospores. K, L. Ascus apical apparatus (stained in Melzer's reagent). M–R. Ascospores. Scale bars: B–E=200 µm, F–L=10 µm, M–R=5 µm.

Discussion

Collodiscula was reported as a new genus from bamboo culms in Japan (Hino & Katumoto 1955). *Collodiscula* is characterised by possessing superficial, stromatic ascomata, brown septate ascospores, which lack a germ slit, and

large, J+, wedge-shaped ascal apical apparatus (Hino & Katum 1955). Currently, there is only one species in the genus. Samuels *et al.* (1987) studied the type material of *C. japonica*, gave a detailed description and reported its asexual state, *Acanthodochium collodisculae*. Kang *et al.* (1999) and Jaklitsch & Voglmayr (2012) placed *Collodiscula* in Xylariaceae.

In the molecular analyses of ITS, LSU, RPB2 and β–tubulin genes *Collodiscula* showed a very close relationship with *Astrocystis*. *Astrocystis* is a genus mostly confined to monocotyledons and has uni- or rarely multi-peritheciate stromata, which may develop beneath the host cuticle and appear superficial. The asci have a relatively short stipe and the ascal apical apparatus is relatively small, amyloid and stopper-shaped (Smith & Hyde 2001). *Astrocystis* also has a *Acanthodochium* asexual state (Samuels *et al.* 1987). However, *Collodiscula* species have septate ascospores, whereas those of *Astrocystis* are aseptate.

Collodiscula japonica has ascospores measuring 18–24 × 4.5–5.5 µm with one median not or slightly constricted septum, fusoid, inaequilateral, with rounded ends, rarely one end pinched, yellowish brown to dark brown, initially with a hyaline minute globose basal cell, smooth, with two guttules in each cell and thin hyaline sheath (Jaklitsch & Voglmayr 2012). *Collodiscula bambusae* has smaller ascospores (15–17.5 × 4.5–5.5 µm) without guttule and sheath. Phylogenetic analysis of ITS, LSU, RPB2 and β–tubulin genes and ITS–LSU also indicated that *C. bambusae* was distinct from *C. japonica*.

Acknowledgments

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