



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.

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 μ L reaction system consisting of 19.75 μ L of double distilled water, 2.5 μ L of 10 \times Taq buffer with MgCl₂, 0.5 μ L of dNTP (10 mM each), 0.5 μ L of each primer (10 μ M), 0.25 μ L Taq DNA polymerase (5 U/ μ l), and 1.0 μ L 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 |
| <i>Amphirosellinia fushanensis</i> | HAST 91111209 | Ex-type (Hsieh <i>et al.</i> 2010) | GU339496 | | GQ848339 | GQ495950 |
| <i>Amphirosellinia nigrospora</i> | HAST 91092308 | Ex-type (Hsieh <i>et al.</i> 2010) | GU322457 | | GQ848340 | GQ495951 |
| <i>Amphisphaeria umbrina</i> | HKUCC 994, CBS 172.96, Mt2 | Jaklitsch & Voglmayr 2012 Schoch <i>et al.</i> 2009 | AF009805 | AF452029 | FJ238348 | |
| <i>Anthostomella brabeji</i> | CBS 110128 | Jaklitsch & Voglmayr 2012 Stadler <i>et al.</i> 2013 | EU552098 | EU552098 | | |
| <i>Apiospora montagnei</i> | AFTOL 951, H3-83 | Jaklitsch & Voglmayr 2012 | JN688916 | DQ471018 | DQ470921 | |
| <i>Apiospora sinensis</i> | HKUCC 3143 | Jaklitsch & Voglmayr 2012 | AY083831 | AY083831 | | |
| <i>Arthrimum marii</i> | CBS 114803 | Crous & Groenewald 2013 | KF144899 | KF144945 | | |
| <i>Arthrimum sacchari</i> | ATCC76303 | Jaklitsch & Voglmayr 2012 | AF393679 | ATCC76303 | | |
| <i>Arthrimum phaeospermum</i> | CBS 114317, HKUCC 3395 | Jaklitsch & Voglmayr 2012 | | KF144953 | | |
| <i>Astrocystis bambusae</i> | HAST 89021904 | Ex-type (Hsieh <i>et al.</i> 2010) | GU322449 | | GQ844836 | GQ495942 |
| <i>Astrocystis mirabilis</i> | HAST 94070803 | Ex-type (Hsieh <i>et al.</i> 2010) | GU322448 | | GQ844835 | GQ495941 |
| <i>Bartalinia robillardoides</i> | BRIP 14180 | Jaklitsch & Voglmayr 2012 | AF405301 | AF382366 | DQ368653 | |
| <i>Biscogniauxia arima</i> | WSP 122 | Ex-type (Hsieh <i>et al.</i> 2010) | EF026150 | | GQ304736 | AY951672 |
| <i>Biscogniauxia nummularia</i> | BCC 1101, H86 | Jaklitsch & Voglmayr 2012 | | AB376691 | FR715504 | |
| <i>Clypeosphaeria uniseptata</i> | HKUCC6349, Mt28 | Jaklitsch & Voglmayr 2012 | AF009808 | DQ810219 | | |
| <i>Collodiscula japonica</i> | CBS 124266 | Jaklitsch & Voglmayr 2012 | JF440974 | JF440974 | | |
| <i>Collodiscula bambusae</i> | GZUH0102 | This study | KP054279 | KP054280 | KP276675 | KP276674 |
| <i>Creosphaeria sassafras</i> | CM AT-018 | Authentic (Tang <i>et al.</i> 2009) | AJ390425 | DQ840056 | | |

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TABLE 1 (Continued)

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

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TABLE 1 (Continued)

| Species | Strain | Type status/References | GenBank accession numbers | | | |
|------------------------------|-----------------------|---|---------------------------|----------|----------|------------------|
| | | | ITS | LSU | RPB2 | β -tubulin |
| <i>Truncatella angustata</i> | ICMP 7062 | Jaklitsch & Voglmayr 2012 | AF405306 | AF382383 | | |
| <i>Xylaria bambusicola</i> | WSP 205, BCC 23659 | Ex-type (Hsieh <i>et al.</i> 2010; Okane <i>et al.</i> 2008) | EF026123 | AB376825 | GQ844802 | AY951762 |
| <i>Xylaria grammica</i> | HAST 479 | Hsieh <i>et al.</i> 2010, Chen <i>et al.</i> 2013 | JQ862677 | JQ862638 | GQ844813 | GQ487704 |
| <i>Xylaria hypoxylon</i> | CBS 122620 | Authentic (Stadler <i>et al.</i> 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, $110\text{--}170 \times 8\text{--}11 \mu\text{m}$ (mean $144 \times 9.5 \mu\text{m}$, $n=30$) with a J+, wedge-shaped apical apparatus, $2.5\text{--}3.5 \mu\text{m}$ (mean $3 \mu\text{m}$, $n=30$) high, $1.5\text{--}2.5 \mu\text{m}$ (mean $2 \mu\text{m}$, $n=30$) diam. Ascospores $15\text{--}17.5 \times 4.5\text{--}5.5 \mu\text{m}$ (mean $17 \times 5 \mu\text{m}$, $n=30$), fusoid, inaequilateral, with one median slightly constricted septum, with narrow rounded ends, yellowish brown, smooth, lacking sheath and germ slit. Asexual state: unknown.

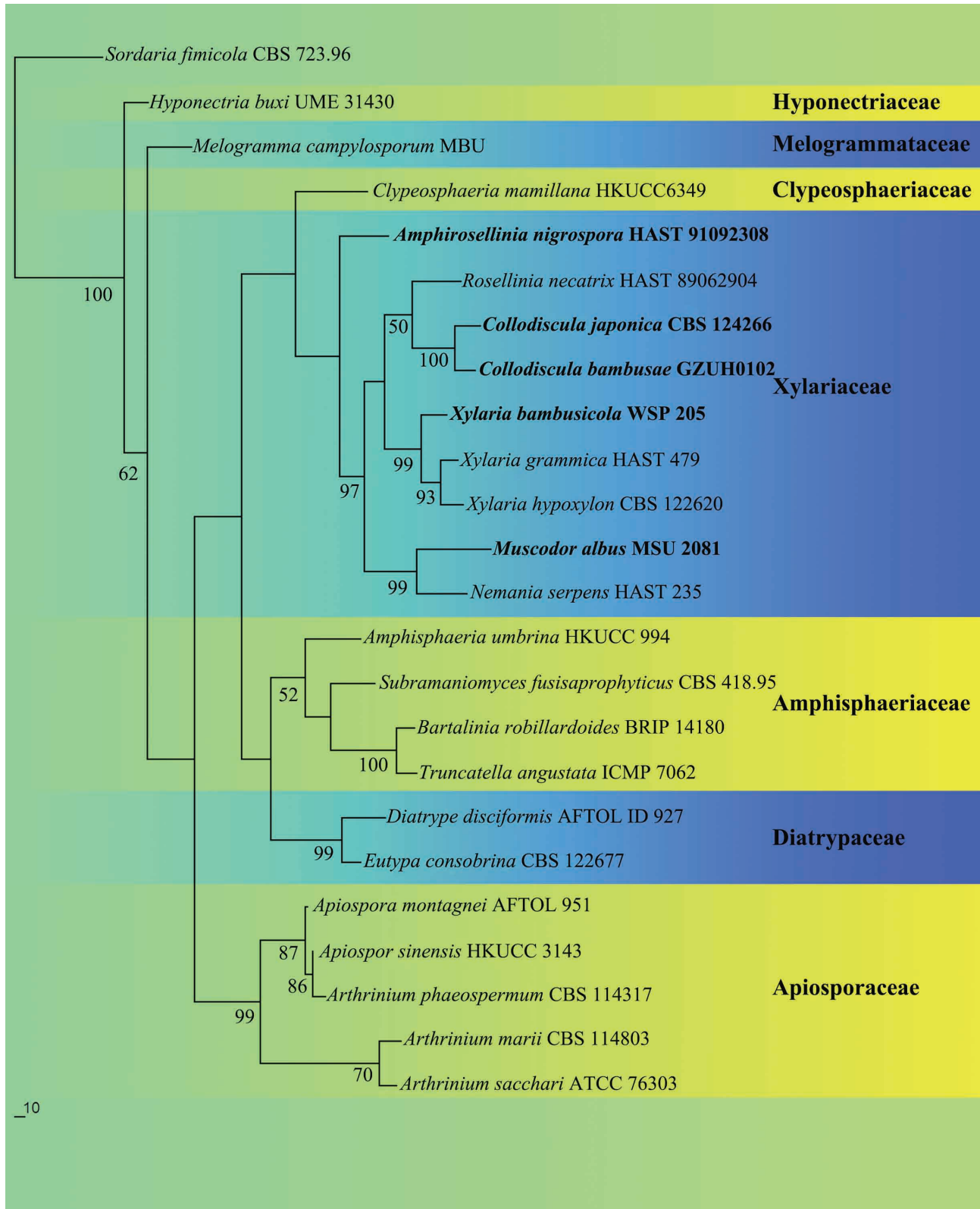


FIGURE 1. Topology showing the most parsimonious tree of ITS and LSU genes regions. Bootstrap values higher than 50% are shown. The tree is rooted with *Sordaria fimicola*. Sequence from type strains are in bold.

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!).

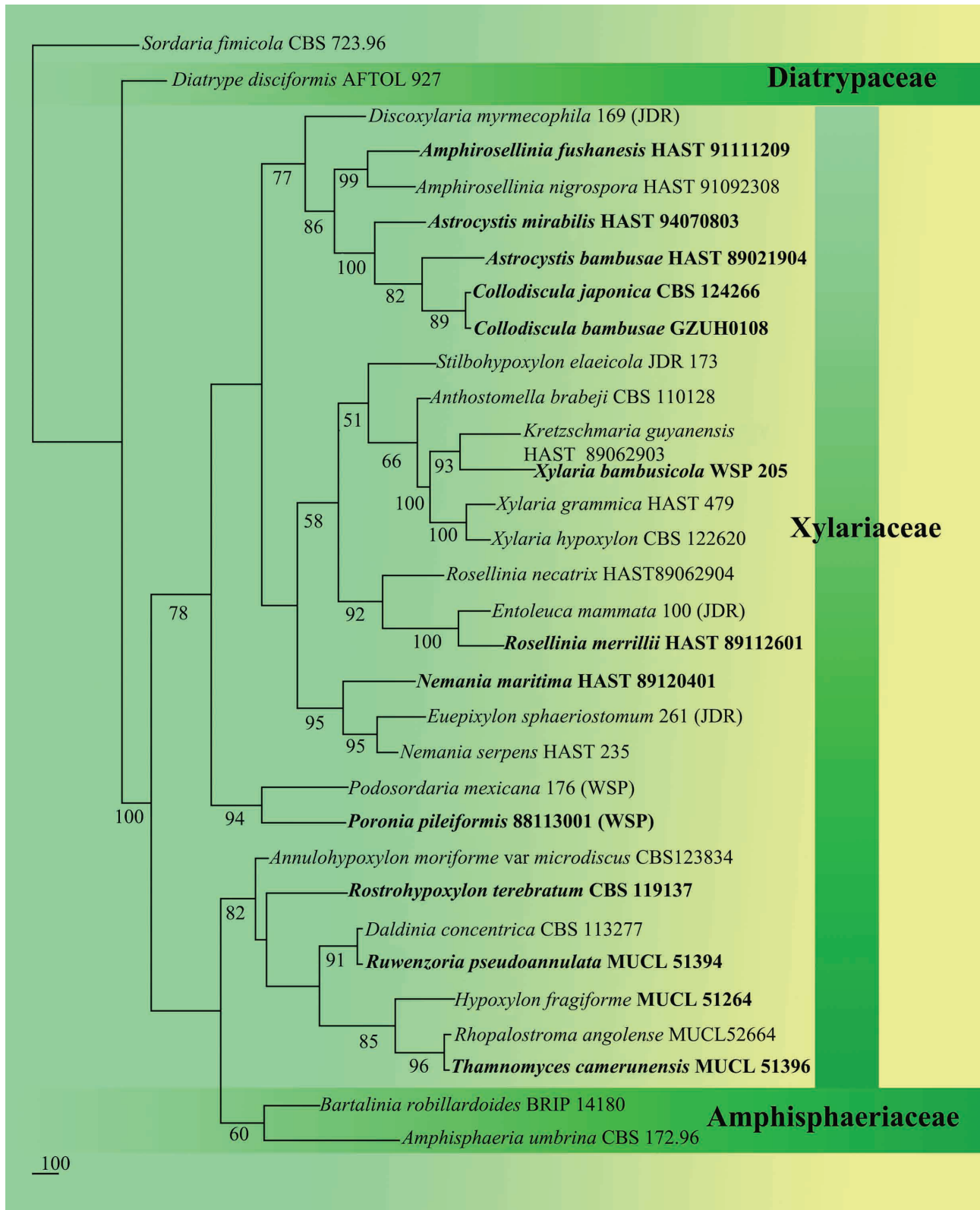


FIGURE 2. Topology showing the most parsimonious tree of ITS, LSU, RPB2 and β -tubulin genes regions. Bootstrap values higher than 50% are shown. The tree is rooted with *Sordaria fimicola*. Sequence from type strains are in bold.

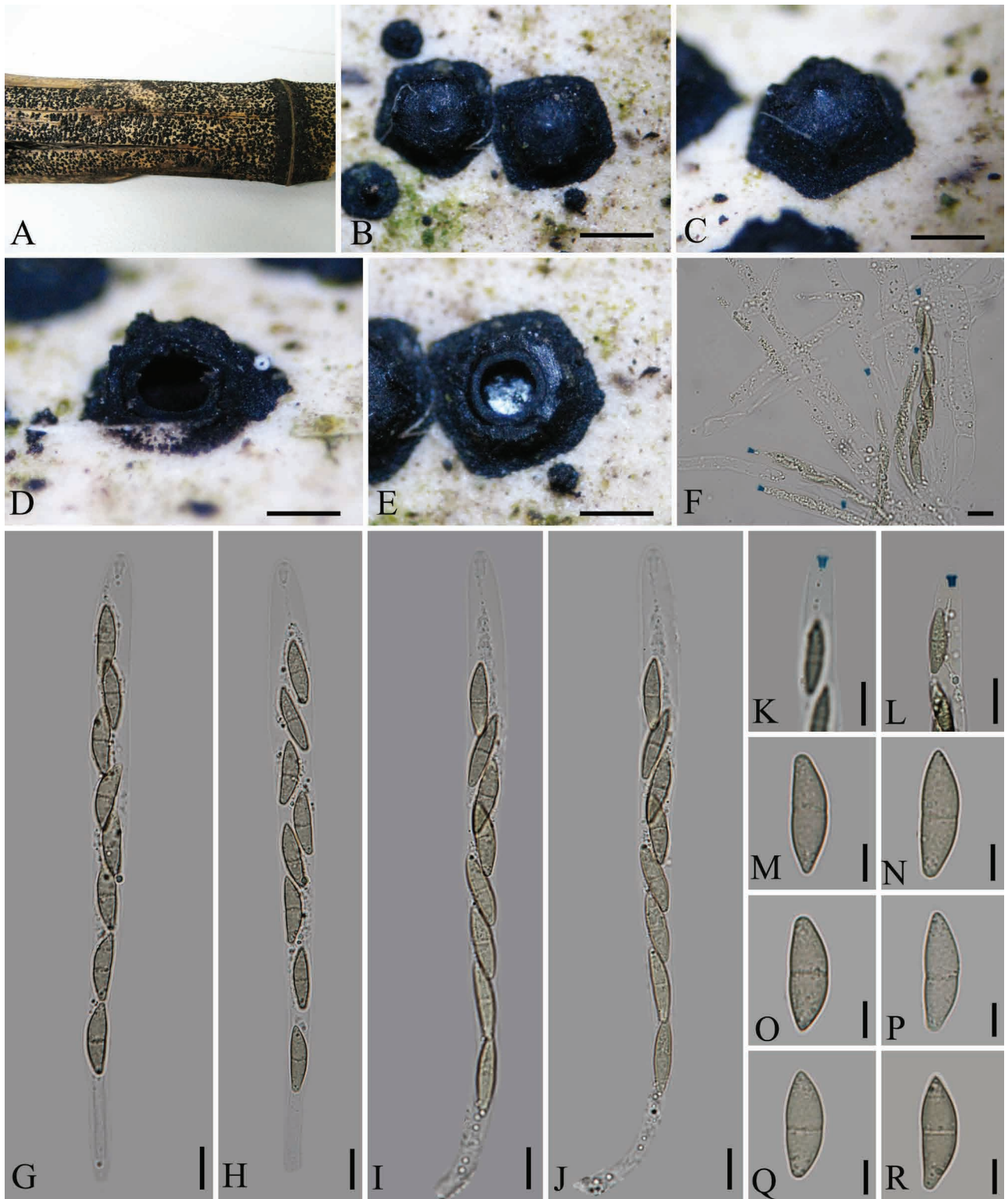


FIGURE 3. *Collodiscula bambusae*. A. Fresh material. B, C. Ascomata on the surface of host. D, E. Section of ascoma. F. Paraphyses. G–J. Mature asci with ascospores. K, L. Ascus apical apparatus (stained in Melzer's reagent). M–R. Ascospores. Scale bars: B–E=200 μ m, F–L=10 μ m, M–R=5 μ m.

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 $18\text{--}24 \times 4.5\text{--}5.5 \mu\text{m}$ 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 ($15\text{--}17.5 \times 4.5\text{--}5.5 \mu\text{m}$) 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*.

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