



A multiple gene genealogy reveals the phylogenetic placement of *Iodosphaeria tongrenensis* sp. nov. in Iodosphaeriaceae (Xylariales)

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Abstract

During collections of Sordariomycetes in Guizhou Province, China, we collected *Iodosphaeria tongrenensis* sp. nov.. It is unique in the genus because of its ellipsoidal ascospores (18.5–22.5 × 6.5–8.5 μm) with a slimy sheath covering the whole spore, and J+, wedge-shaped, ascus apical apparatus. *Iodosphaeria* is a monotypic genus in *Iodosphaeriaceae* and phylogenetic analyses of combined LSU, ITS and SSU sequence data indicate that it is a distinct family in the order *Xylariales*.

Key words: phylogeny, Sordariomycetes, taxonomy

Introduction

Samuels *et al.* (1987) introduced the generic name *Iodosphaeria* in the family *Amphisphaeriaceae* with type species, *I. phyllophila* (Mouton) Samuels, E. Müll. & Petrini. *Iodosphaeria* species are characterised by their superficial, black, non-papillate ascomata, with unbranched, brown flexuous hairs radiating from the peridium surface. The peridium is composed of two distinct regions; an outer region of angular, pigmented cells, and an inner region of flattened hyaline cells. Asci are 8-spored, unitunicate, cylindrical to narrowly clavate, and usually have amyloid, discoid apical apparatuses staining in Melzer's reagent. Ascospores are allantoid to ellipsoidal, aseptate, hyaline, with or without sheaths (Samuels *et al.* 1987). Selenosporella-like and ceratosporium-like asexual morphs have been observed, both on the surface of host and in culture. There are eight records in the genus *Iodosphaeria* (Index Fungorum 2015), which have been reported worldwide, from Argentina, Europe, Hong Kong, New Zealand South America and USA (Samuels *et al.* 1987, Barr 1993, Hyde 1995, Candoussau *et al.* 1996, Hsieh *et al.* 1997, Taylor & Hyde 1999, Catania & Romero 2012).

Ellis & Ellis (1985) described *Lasiosphaeria phyllophila* Mouton from fallen dead twigs and debris of *Acer*, *Populus* and *Salix*. Because *Lasiosphaeria phyllophila* combined characters of selenosporella-like and ceratosporium-like asexual morphs and forming an amyloid apical apparatus in the sacus, Samuels *et al.* (1987) placed it in a new genus *Iodosphaeria*. *Iodosphaeria* was considered similar to *Phaeotrichosphaeria* Sivan. (Sivanesan 1983) and *Endophragiella* B. Sutton (Hughes 1979). *Iodosphaeria* was included in *Amphisphaeriaceae* by Samuels *et al.* (1987). Barr (1993) transferred *Trichosphaeria arundinariae* Ellis and Everh. to *Iodosphaeria arundinariae* (Ellis and Everh.) M.E. Barr based on its peridium and centrum structure. Barr (1990, 1994) suggested that *Iodosphaeria* was better placed in the family *Lasiosphaeriaceae* (*Sordariales*). Hyde (1995) added a new species, *I. aquatica*, to the genus, and noted that *I. aquatica* is close to *Pseudohalonectria* Minoura & T. Muroi and some species in *Lasiosphaeriaceae*. Hsieh *et al.* (1997) excluded *I. aquatica* from *Iodosphaeria*, while describing another new species. *Iodosphaeria* was excluded from *Amphisphaeriaceae* by Kang *et al.* (1998, 1999). Réblová (1999) placed the genus in the family *Trichosphaeriaceae* and Eriksson *et al.* (2001) placed it in the *Amphisphaeriaceae*. *Iodosphaeriaceae* was introduced as a new family by Hilber & Hilber (2002) to accommodate *Iodosphaeria*. Based on phylogenetic analyses of 28S rDNA, *Iodosphaeria* was shown to be similar to *Phomatospora* Sacc., and excluded from the *Amphisphaeriaceae* by

Jeewon *et al.* (2003). The taxonomic position of *Iodosphaeria* is unclear as it lacks molecular data (Senanayake *et al.* 2015).

An interesting species characteristic of *Iodosphaeria* was found in China. Sequence data showed that its phylogenetic placement is in the family *Iodosphaeriaceae* (*Xylariales*, *Sordariomycetes*).

Materials and Methods

Morphological studies

Samples were collected in Guizhou Province in China. Specimens were taken to the laboratory in plastic bags. Observations, photography and measurements of ascomata, asci and ascospores were made from squash-mounts in distilled water and Melzer's reagent for optical microscopy (Kang *et al.* 1999). Asci and ascospores were examined by light microscopy (BX41, Olympus Optical Co. Ltd., Tokyo, Japan). Materials were deposited in the herbarium of Guizhou University (GZUH) and MFLU (Mae Fah Luang University). Faces of fungi numbers and Index Fungorum numbers are as explained in Jayasiri *et al.* (2015) and Index Fungorum (2015).

DNA extraction, PCR and sequencing

Three to five ascomata were collected to extract DNA from the host under the stereomicroscope. Genomic DNA was extracted from ascomata by using an OMEGA Micro Elute Genomic DNA Kit (D3096-01). The DNA preparations were stored at -20 °C until used for PCR.

Sequences alignment and phylogenetic analyses

The amplifications of rDNA regions of internal transcribed spacers (ITS) and large subunit ribosomal RNA gene (LSU) were carried out using ITS5 and ITS4 (White *et al.* 1990), LROR and LR5 (Vilgalys & Hester 1990) primers. Small subunit ribosomal RNA gene (SSU) was amplified with primer pairs NS1 and NS4 (Tanaka *et al.* 2009, Hsieh *et al.* 2010). The amplification conditions were carried out according to Liu *et al.* (2010). Amplified PCR fragments were then sent to SinoGenoMax, Beijing, China for DNA sequencing. The sequence data obtained were deposited in GenBank (Table 1). Combined sequence data were manually adjusted using BioEdit 7.0.1 (Hall 1999), and further implemented with Clustal X v1.83 (Thompson *et al.* 1997) to allow maximum alignment and maximum sequence similarity. Maximum parsimony (MP) analyses were performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). All characters were assessed to be unordered and equally weighed. 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. Taxa included in the present study.

Species	Culture	GenBank accession numbers			Reference
		LSU	ITS	SSU	
<i>Amphisphaeria umbrina</i>	CBS 172.96	FJ176863	AF009805	FJ176809	McTaggart <i>et al.</i> 2013
<i>Annulatascus triseptatus</i>	SMH 2359, CBS 128831	AY346257		JQ429242	Miller & Huhndorf 2005
<i>Annulatascus velatispora</i>	HKUCC 3701	AF132320	AF177150		Miller & Huhndorf 2005
<i>Anthostomella formosa</i>	MFLUCC:14-0170	KP340544	KP297403		Kowalski & Andruch 2012
<i>Apiosordaria backusii</i>	ATCC 34568	AY780051	GQ922520		Miller & Huhndorf 2005
<i>Apiosordaria verruculosa</i>	F1 52, 365	AY346258			Cai <i>et al.</i> 2005
<i>Apiospora bambusae</i>	ICMP 6889	DQ368630		DQ368662	Tang <i>et al.</i> 2007
<i>Apiospora montagnei</i>	AFTOL-ID 951	DQ471018		FJ190614	Spatafora <i>et al.</i> 2006
<i>Apiospora setosa</i>	ICMP 4207	DQ368631		DQ368661	Tang <i>et al.</i> 2007
<i>Arecophila bambusae</i>	HKUCC 4794	AF452038		AY083802	Maharachchikumbura <i>et al.</i> 2015

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TABLE 1. (Continued)

Species	Culture	GenBank accession numbers			Reference
		LSU	ITS	SSU	
<i>Arthrinium hydei</i>	CBS 114990	KF144936	KF144890		Maharachchikumbura <i>et al.</i> 2015
<i>Arthrinium phaeospermum</i>	CBS: 114317	KF144953	KF144906	AY083816	Jaklitsch & Voglmayr 2012
<i>Arthrotrrys foliicola</i>	CBS 242.90	AY261182	U51954		Jaklitsch & Voglmayr 2012
<i>Astrocystis cocoes</i>	HKUCC 3441	AY083823			Maharachchikumbura <i>et al.</i> 2015
<i>Barrina polyspora</i>	AWR 9560A	AY346261			Miller & Huhndorf 2005
<i>Beltrania pseudorhombica</i>	CBS: 138003	KJ869215	KJ869158		Crous <i>et al.</i> 2012
<i>Beltraniella endiandrae</i>	CBS: 137976	KJ869185	KJ869128		Crous <i>et al.</i> 2012
<i>Bertia moriformis</i>	SMH 4320	AY695260			Miller & Huhndorf 2005
<i>Bombardia bombardia</i>	SMH 3391	NG_027572		NG_013187	Stchigel <i>et al.</i> 2006
<i>Bombardioidea anartia</i>	HHB 99-1	AY346264			Stchigel <i>et al.</i> 2006
<i>Botryosphaeria ribis</i>	CMW 7773, CBS 115475	DQ246263	AY236936	DQ678000	Barber <i>et al.</i> 2005
<i>Cainia graminis</i>	CBS 136.62	AF431949		AF431952	Lumbsch <i>et al.</i> 2005
<i>Camarops amorpha</i>	SMH 1450	AY780054			Tang <i>et al.</i> 2009
<i>Camarops tubulina</i>	SMH 4614	AY346266			Tang <i>et al.</i> 2009
<i>Cercophora caudate</i>	CBS 606.72	AY999113	AY999135	DQ368659	Cai <i>et al.</i> 2005
<i>Cercophora newfieldiana</i>	SMH 3303	AY780062			Miller & Huhndorf 2005
<i>Chaetomium funicola</i>	CBS 179.84	JX280679	JX280785		Miller & Huhndorf 2005
<i>Chaetomium microascoides</i>	F 153, 395	AY346273			Miller & Huhndorf 2005
<i>Chaetosphaerella phaeostroma</i>	SMH 4257	AY695264			Fan <i>et al.</i> 2012
<i>Chaetosphaeria innumera</i>	SMH 2748	AY017375	AY906956		Huhndorf & Fernández 2005
<i>Chaetosphaeria ovoidea</i>	SMH 2605	AF064641			Tang <i>et al.</i> 2007
<i>Clypeosphaeria uniseptata</i>	6349 (HKUCC)	DQ810219		DQ810255	Dai <i>et al.</i> 2014
<i>Coniocessia anandra</i>	Iran 1468C	GU553349			Maharachchikumbura <i>et al.</i> 2015
<i>Coniocessia maxima</i>	CBS 593.74	GU553344			Asgari & Zare 2011
<i>Coniocessia nodulisporioides</i>	CBS 281.77	GU553352	GU553333	AJ875185	Maharachchikumbura <i>et al.</i> 2015
<i>Coniochaeta discoidea</i>	CBS 158.80	AY346297		AJ875179	Tang <i>et al.</i> 2007
<i>Coniochaeta ligniaria</i>	C 8	AY198388	AY198390	AY198389	Hujšlová <i>et al.</i> 2014
<i>Coniochaetidium savoryi</i>	TRTC 51980	AY346276			Tang <i>et al.</i> 2007
<i>Creosphaeria sassafras</i>	CM AT-018	DQ840056			Tang <i>et al.</i> 2009
<i>Diamantinia citrina</i>	Buck 26886	AY346278			Shenoy <i>et al.</i> 2006
<i>Diaporthe eres</i>	AR 3519	AF362565	KJ210523		Farr <i>et al.</i> 2002
<i>Diatrype disciformis</i>	CBS 197.49	DQ470964	AJ302423	DQ471012	Raja <i>et al.</i> 2010
<i>Discostroma botan</i>		DQ368629		DQ368660	Tang <i>et al.</i> 2009
<i>Ellisemia calyptrata</i>	HKUCC 10821	DQ408564			Jaklitsch & Voglmayr 2012
<i>Eutypa lata</i>	CBS 208.87	DQ836903	JQ041700	DQ836896	Raja <i>et al.</i> 2010
<i>Graphostroma platystoma</i>	AFTOL-ID 1249	DQ836906	JX658535	DQ836900	Zhang <i>et al.</i> 2006
<i>Heleococcum japonense</i>	CBS: 397.67	JX158442	JX158420	JX158485	Rehner & Samuels 1995

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TABLE 1. (Continued)

Species	Culture	GenBank accession numbers			Reference
		LSU	ITS	SSU	
<i>Hypomyces luteovirens</i>	Genbank	AF160237	EU652350		Miller <i>et al.</i> 2005
<i>Hyponectria buxi</i>	UME 31430	AY083834		AF130976	Tang <i>et al.</i> 2007
<i>Iodosphaeria tongrenensis</i>	GZUH 0109	KR095283	KR095282	KR095284	This study
<i>Lepteutypa cupressi</i>	IMI 052255	AF382379	AF009817	AY083813	Jaklitsch & Voglmayr 2012
<i>Lopadostoma turgidum</i>	LT 3	KC774618	KC774619		Maharachchikumbura <i>et al.</i> 2015
<i>Magnaporthe oryzae</i>	CBS: 659.66	KM485005	KM484895		Klaubauf <i>et al.</i> 2014
<i>Melanochaeta hemipsila</i>	SMH 2125	AF466083			Zelski <i>et al.</i> 2014
<i>Melogramma campylosporium</i>	MBU	JF440978	JF440978		Jaklitsch & Voglmayr 2012
<i>Microdochium bolleyi</i>	SRC1dJ1a	HM216199	HM216206	HM216190	Jaklitsch & Voglmayr 2012
<i>Monographella nivalis</i>	UPSC 3273, ATCC MYA-3967	AF452030		AF064049	Tang <i>et al.</i> 2007
<i>Monosporascus cannonballus</i>	FMR 6682			AF340016	Maharachchikumbura <i>et al.</i> 2015
<i>Nectriopsis exigua</i>	GJS 98-32	GQ505986	HM484865		Chaverri <i>et al.</i> 2011
<i>Nectriopsis violacea</i>	CBS: 424.64	AY489719		AY489687	Pinruan <i>et al.</i> 2010
<i>Nitschkia grevillei</i>	SMH 4663	AY346294			Tang <i>et al.</i> 2007
<i>Ochronectria calami</i>	CBS: 125.87	AY489717		AY489685	Pinruan <i>et al.</i> 2010
<i>Phlogicylindrium eucalypti</i>	CBS H-19749	DQ923534	DQ923534		Jaklitsch & Voglmayr 2012
<i>Phlogicylindrium eucalyptorum</i>	CBS: 111680	KF251707	KF251204		Quaedvlieg <i>et al.</i> 2013
<i>Phomatospora bellaminuta</i>	AFTOL-ID 766	FJ176857		FJ176803	Hujslóvá <i>et al.</i> 2014
<i>Podospora fimiseda</i>	CBS 990.96	AY346296	AY515361		Cai <i>et al.</i> 2005
<i>Pseudohalonectria lignicola</i>	M95, SMH 2440	JX134691	JX134679	JX134665	Luo <i>et al.</i> 2004
<i>Pseudomassaria carolinensis</i>	9502 (IFO)	DQ810233		DQ810262	Jaklitsch & Voglmayr 2012
<i>Pseudonectria rousseliana</i>	CBS 114049, AR 2716	JF937575	JF937565	AF543767	Zeng & Zhuang 2014
<i>Robillarda sessilis</i>	BCC 13393	FJ825378	FJ825373	FJ825368	Jaklitsch & Voglmayr 2012
<i>Rosellinia necatrix</i>	HKUCC 9037	AY083824			Maharachchikumbura <i>et al.</i> 2015
<i>Roumegueriella rufula</i>	CBS 346.85	DQ518776		DQ52256	Spatafora <i>et al.</i> 2007
<i>Seimatosporium eucalypti</i>	CBS: 114876	JN871212	JN871202		Jaklitsch & Voglmayr 2012
<i>Seimatosporium grevilleae</i>	ICMP 10981	AF382372	AF405304		Jaklitsch & Voglmayr 2012
<i>Seimatosporium vaccinii</i>	ICMP 7003	AF382374			Jaklitsch & Voglmayr 2012
<i>Seiridium cardinale</i>	ICMP 7323, CMW 18607, ATCC52521	AF382377	AF405305	AF346556	Tang <i>et al.</i> 2009
<i>Seiridium phylicae</i>	CPC: 19962	KC005807	KC005785		Crous <i>et al.</i> 2012
<i>Selinia pulchra</i>	AR 2812	GQ505992	HM484859		Chaverri <i>et al.</i> 2011
<i>Seynesia erumpensnew</i>	SMH 1291	AF279410		AF279409	Maharachchikumbura <i>et al.</i> 2015
<i>Sinosphaeria bambusicola</i>	SMH 1999	AY780077			Miller & Huhndorf 2005
<i>Sordaria fimicola</i>	HKUCC 3714	AF132330	AY681188	AY545724	Checa <i>et al.</i> 2008
<i>Sordaria macrospora</i>	Buck s.n	AY346301	AF246293		Zare <i>et al.</i> 2004

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TABLE 1. (Continued)

Species	Culture	GenBank accession numbers			Reference
		LSU	ITS	SSU	
<i>Sporidesmina malabarica</i>	NN 43118	DQ408580			Shenoy <i>et al.</i> 2006
<i>Sporidesmium knawiae</i>	CBS: 123529	FJ349610	FJ349609		Santa <i>et al.</i> 2013
<i>Stanjehughesia polypora</i>	NN 47796	DQ408569			Shenoy <i>et al.</i> 2006
<i>Stephanonectria keithii</i>	GJS 92-133	AY489727		AY489695	Doveri <i>et al.</i> 2010
<i>Stilbocrea macrostoma</i>	GJS 73-26	AY489725		AY489693	Doveri <i>et al.</i> 2010
<i>Striatosphaeria codinaeaphora</i>	SMH 1524	AF466088	AF178546		Miller & Huhndorf 2005
<i>Triangularia tanzaniensis</i>	TRTC 51981	AY780081			Miller & Huhndorf 2005
<i>Truncatella angustata</i>	ICMP 7062	AF382383	AF405306		Jeewon <i>et al.</i> 2003
<i>Valsa nivea</i>	CBS: 109489	NG_027590	JX438624	NG_013203	Fan <i>et al.</i> 2015
<i>Valsa sordida</i>	HMBF 15	KF225627	KF225613		Fan <i>et al.</i> 2015
<i>Valsonectria pulchella</i>	SMH 1193	AY346304			Tang <i>et al.</i> 2007
<i>Vialaea mangifia</i>	MFLUCC 12-0808	KF724975	KF724974		Maharachchikumbura <i>et al.</i> 2015
<i>Vialaea minutella</i>	BRIP 56959	KC181924	KC181926		Maharachchikumbura <i>et al.</i> 2015
<i>Xylaria curta</i>	BCC 1007	AB376681	GU322444	U32417	Ramesh <i>et al.</i> 2012
<i>Xylaria hypoxylon</i>	ATCC 42768, CBS 122620	AY327480	AY327477		Nonaka <i>et al.</i> 2013

Note: **AFTOL:** Assembling the Fungal Tree of Life; **ATCC:** American Type Culture Collection; **BCC:** Universitat de Barcelona; **CBS:** Centraalbureau voor Schimmelcultures; **GZUH:** Herbarium of Guizhou University; **HKUCC:** Hong Kong University Culture Collection; **ICMP:** International Collection of Microorganisms from Plants; **IFO:** Institute for Fermentation; **IMI:** CABI Bioscience UK Centre; **MFLUCC:** Mae Fah Luang University Culture Collection; **MRC:** Rocky Mountain Research Station; **MUCL:** University Catholique de Louvain; **SMH:** Sabine M. Huhndorf; **TRTC:** Royal Ontario Museum; **Others:** information not available.

Results

Combined analysis of LSU, ITS and SSU sequence data

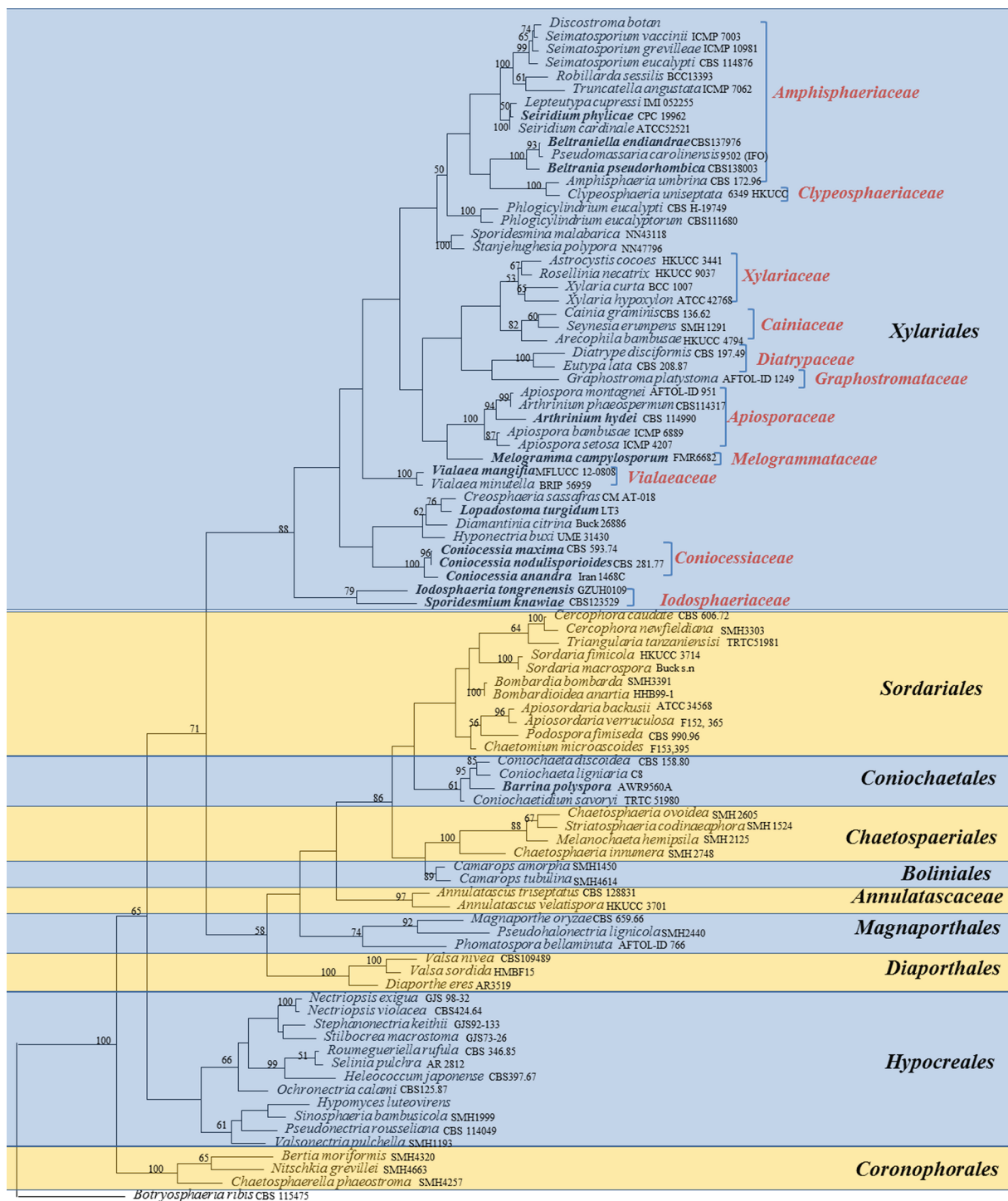
The combined LSU, ITS and SSU dataset comprised 90 sequences from 89 taxa with *Botryosphaeria ribis* Grossenb. & Duggar (Huhndorf *et al.* 2004) as the outgroup taxon. The dataset comprised 2,858 characters (LSU 934 characters, ITS 577 characters, SSU 1347 characters) after alignment, of which 1,793 are conserved, 360 are variable and 705 are parsimony informative. 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= 4,741, CI= 0.367, RI= 0.629, RC= 0.231, HI= 0.633). Xylariales grouped a single clade. Sordariales, Coniochaetales, Chaetosphaeriales, Boliniales, Annulatasaceae, Magnaporthales and Diaporthales clustered together and showed a far phylogenetic distance from Xylariales. *Iodosphaeria tongrenensis* clustered with families in Xylariales with high bootstrap support (88%).

Taxonomy

Iodosphaeria tongrenensis Q.R. Li, J.C. Kang & K.D. Hyde, *sp. nov.* (Fig. 2) MycoBank no.: MB 812255; FoF 00642.

Differs from other species in the genus by its ellipsoidal ascospores (18.5–22.5 × 6.5–8.5 µm) with a slimy sheath covering the entire spore, and a J+, wedge-shaped ascus apical apparatus.

Type—CHINA. Guizhou Province: Guiyang, saprobic on dead twigs of unidentified host, March 2014, Q.R. Li (GZUH0109, holotype!)



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FIGURE 1. MP tree based on dataset of LSU, ITS and SSU sequences. Bootstrap support values for maximum parsimony (MP) greater than 50% are given above the nodes. The strains numbers are given after the species names. The tree is rooted to *Botryosphaeria ribis*. All sequences from type strains are shown in bold face.

Saprobic on dead twigs of unidentified host. Sexual morph: *Ascomata* 250–350 µm high × 260–370 µm diam., globose to subglobose, superficial, black, solitary to gregarious, with numerous long, flexuous, unbranched brown hairs, arising from cells at the peridium surface, apex flattened, non-papillate, ostiole pore-like, periphysate. *Peridium* up to 50 µm wide, comprising two strata, an outer layer of angular, dark brown to black thick-walled cells, and an inner layer of flattened, hyaline cells. *Paraphyses* 3–4 µm wide, hypha-like, septate, tips rounded, not tapering, of the same

length as the asci. *Asci* 150–210 × 12–16 μm (mean 165.8 × 14.3 μm, n = 30), 8-spored, unitunicate, cylindrical, short-pedicellate, apex rounded, with a J+, wedge-shaped apical apparatus, 1 μm high × 1.5 μm wide. *Ascospores* 18.5–22.5 × 6.5–8.5 μm (mean 21.2 × 7.2 μm, n = 30), overlapping uniseriate, ellipsoidal to fusiform, unicellular, hyaline, smooth, slightly narrower at the center, with slimy sheath covering entire spore. Asexual morph: ceratosporium-like conidia were observed on the surface of ascomata but may not be related.

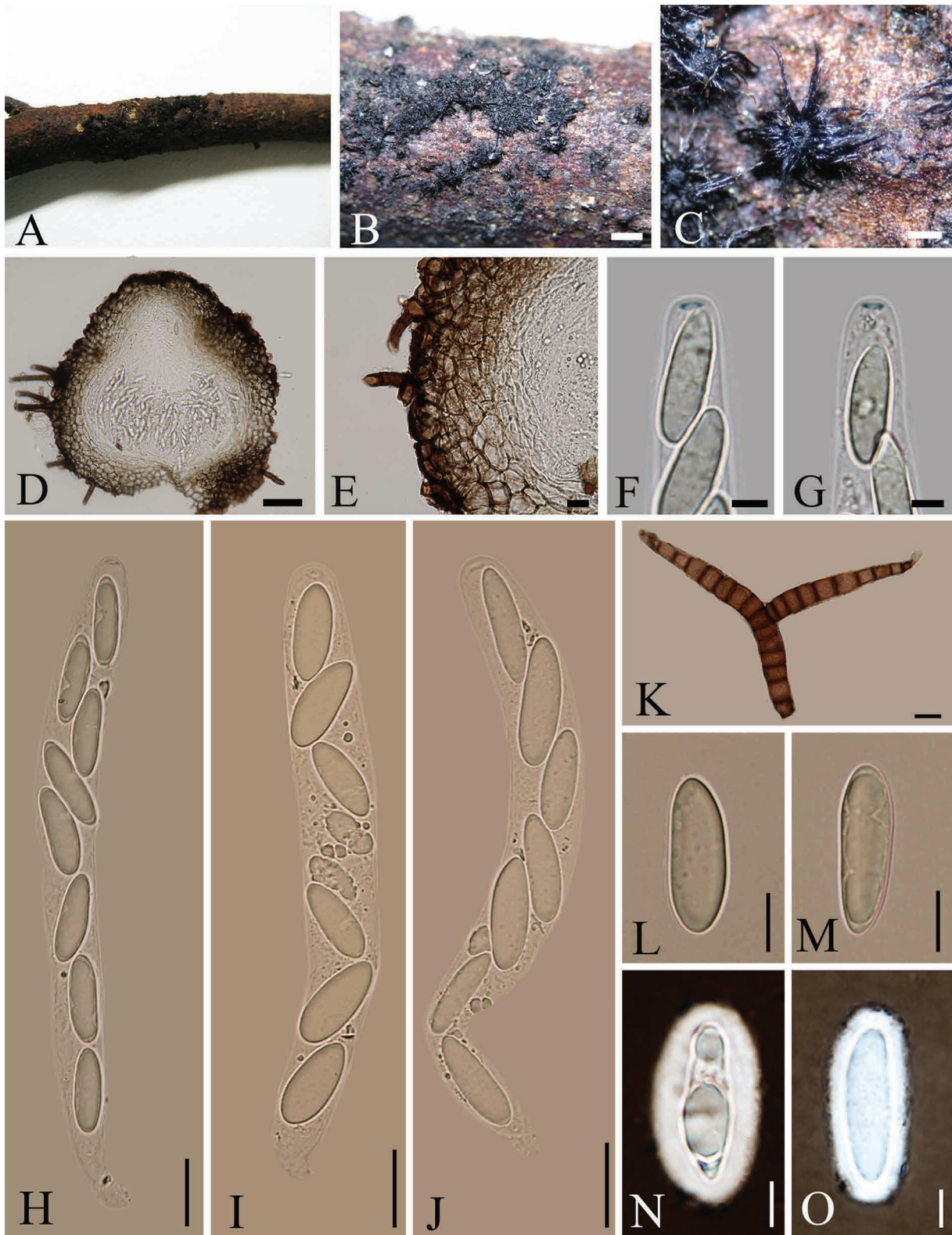


FIGURE 2. *Iodosphaeria tongrenensis* (holotype). A. Herbarium material. B, C. Ascomata on the surface of host. D. Section of ascoma. E. Peridium. F, G. Ascus apical apparatus (stained in Melzer's reagent). H–J. Mature asci with ascospores. K. Ceratosporium-like conidia. L–O. Ascospores (N, O stained in India ink). Scale bars: B=1 mm, C=300 μm, D=50 μm, E=10 μm, F, G=5 μm, H–K=10 μm, L–O=5 μm.

Habitat/Distribution:—Known to inhabit dead twigs of unknown plant, Guizhou Province, China.

Etymology:—In reference to collection location (Tongren city).

Material examined: China, Guizhou Province, Tongren City, Fanjingshan Nature Reserve, on dead twigs of unidentified host, March 2014, Qirui Li (GZUH0109, holotype!; *Ibid.*, MFLU 15-0393, Isotype!).

Discussion

In this paper we use morphology and sequence data to describe one new species of *Iodosphaeria* from China and introduce the placement of *Iodosphaeria*.

Iodosphaeria tongrenensis is most similar to *I. polygoni* W.H. Hsieh, Chi Y. Chen & Sivan, but differs in having sheathed ascospores (Hsieh *et al.* 1997).

Key to *Iodosphaeria* species

1.	Asci with a distinct apical apparatus	2
1.	Asci lacking a distinct apical apparatus.....	6
2.	Apical apparatus staining blue in Melzer's reagent	3
2.	Apical apparatus not staining blue in Melzer's reagent	5
3.	Asci longer than 150 μm	4
3.	Asci shorter than 150 μm long	5
4.	Ascospores ellipsoidal, with a mucilaginous sheath	<i>I. tongrenensis</i>
4.	Ascospores ellipsoidal, lacking a mucilaginous sheath.....	<i>I. polygoni</i>
5.	Ascospores allantoid.....	<i>I. phyllophila</i>
5.	Ascospores inequilaterally ellipsoid to navicular.....	<i>I. podocarpi</i>
6.	Ascospore wall verruculose.....	<i>I. arundinariae</i>
6.	Ascospore wall smooth.....	<i>I. tarda</i>
7.	Ascospores ellipsoidal, with a mucilaginous sheath	<i>I. ripogoni</i>
7.	Ascospores ellipsoidal to fusiform, lacking a mucilaginous sheath.....	<i>I. hongkongensis</i>

The taxonomic position of *Iodosphaeria* is still unclear and it has been placed in various families by different authors. In our study, phylogenetic analyses of LSU-ITS-SSU sequence data showed that *Iodosphaeria tongrenensis* clustered in Xylariales, forming a distinct branch close to the genera *Sporidesmium*. *Iodosphaeria tongrenensis* has unitunicate asci, with a J⁺, apical apparatus which is similar to species of *Amphisphaeriaceae*. However, phylogenetic analyses indicated *Iodosphaeria tongrenensis* differs from the genera of *Amphisphaeriaceae* and that *Iodosphaeria* belongs in *Iodosphaeriaceae* in Xylariales, agreeing with Hilber & Hilber (2002) and Senanayake *et al.* (2015). Based on ribosomal and RPB2 DNA sequence data, the phylogenies showed that *Sporidesmium* is not monophyletic, and species are phylogenetically distributed in Dothideomycetes and Sordariomycetes (Shenoy *et al.* 2006). *Sporidesmium macrurum* within the Apiosporaceae/*Amphisphaeriaceae* clustered in the Xylariales clade (Shenoy *et al.* 2006). *Sporidesmium* has conidia with 3–5-transverse septa borne singly at the ends of short conidiophores (Ellis 1958). Phylogenetic analysis showed that *Iodosphaeria tongrenensis* is related to *Sporidesmium knawiae* Crous. *Sporidesmium knawiae* groups in the *Iodosphaeriaceae* with 79 % bootstrap value. However, no conidia of *Sporidesmium* were observed around the ascomata.

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