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Vamsapriya (*Xylariaceae*) re-described, with two new species and molecular sequence data

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Abstract – *Vamsapriya* comprises two species from bamboo and is characterized by erect, rigid, dark brown, synnematous conidiophores, monotretic conidiogenous cells and brown to dark brown, septate, conidia in chains. *Vamsapriya indica*, the generic type of *Vamsapriya*, was recollected and isolated from bamboo culms in Chiang Rai Province, Thailand and is described, illustrated and epitypified in this paper. Two new species in the genus were also discovered and are introduced as *V. khunkonensis* and *V. bambusicola*. The new species differs from the type and the other known species, *V. mahabaleshwarensis*, in the shape and size of the conidia. Maximum-parsimony (MP) analysis of combined LSU, SSU and RPB2 sequence data and Bayesian analysis based on multi-gene data set of beta-tubulin, ITS, LSU, and RPB2 show *Vamsapriya* belongs in *Xylariaceae, Xylariales*.

Asexual morphs / conidial fungi / hyphomycetes / phylogeny / taxonomy

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INTRODUCTION

The general term "Hyphomycetes" is used for asexual fungi which produce conidia on conidiophores arising directly from the substrate (Goettel & Inglis 1997). Approximately, 1,480 genera have so far been described and about 1,420 genera are synonyms or names with uncertain taxonomic affinities (Seifert *et al.*, 2011).

We are studying the fungi on bamboo in northern Thailand (Dai *et al.*, 2012; 2014a; 2014b). In studies on giant bamboo (*Dendrocalamus giganteus* (F); *Poaceae*) in northern Thailand, we encountered three similar, interesting synnematous conidial fungi which belong to the genus *Vamsapriya*. The genus is typified by *V. indica* Gawas & Bhat and is characterized by erect, rigid, dark brown and velvety synnematous conidiophores, monotretic, polytretic and enteroblastic conidiogenous cells and brown to dark brown, septate, conidia in chains (Gawas & Bhat 2005). A second species, *V. mahabaleshwarensis* Pratibha & Bhat, was described by Pratibha & Bhat (2008). These two species differ in the shape, structure and dimensions of conidia, and the latter species differs from the type by its branched conidial chains.

In this paper we carried out morphological, cultural and molecular studies on the three taxa. Sequence data reveal placement in the order *Xylariales*, while we redescribe and epitypify the type species and introduce two new species from Thailand.

MATERIALS AND METHODS

Collection and isolation of fungi. Fallen and decomposing bamboo culms were collected from various localities in Chiang Rai Province, Thailand (Dai *et al.*, 2012; 2014a; 2014b). The samples were placed in plastic Zip lock bags and brought to laboratory for examination. The specimens were incubated in sterile moist chambers and examined at regular intervals until the resident fungi attained maturity and sporulated. The fungi were examined under dissecting and compound microscopes to establish if they required further study. Specimens were isolated from single spores following the method of Chomnunti *et al.* (2011; 2014). The colonies were transferred to 1.5 ml. microcentrifuge tube with 2% potato-dextrose agar (PDA) to deposit at 6°C and suspended in 2 ml screw cap microcentrifuge tubes with 10% glycerol to deposit at – 20°C. Microscopic observations and photomicrographs were made as described in Liu *et al.* (2012) and Boonmee *et al.* (2011). Herbarium materials are deposited at the MFLU herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU) with duplicates in KUM. The cultures are maintained at Mae Fah Luang University Culture Collection (MFLUCC) and Research Institute of Resource Insects, Chinese Academy of Forestry (IFRD) or Landcare Research, New Zealand (ICPM).

DNA extraction, PCR amplification and sequencing. Fungal isolates were grown on PDA for 30 d at 27°C and genomic DNA was extracted from fresh mycelia, following the specification of Biospin Fungus Genomic DNA Extraction Kit (BioFlux®). ITS5 and ITS4, NS1 and NS4 (White *et al*. 1990) and LROR and LR5 (Vilgalys & Hester 1990) primers were used for the amplification of internal transcribed spacers (ITS), small subunit rDNA (SSU) and large subunit rDNA (LSU) respectively. β-tubulin gene region was amplified by using T12 and T22 primers (O'Donnell & Cigelnik 1997). Polymerase chain reaction (PCR) amplification was carried out following the methods of Liu *et al*. (2012). Amplified PCR fragments were sequenced at Kunming Shuo Yang Technology Company, P.R. China. Generated new sequences of beta-tubulin, ITS, LSU, SSU and RPB2 regions are deposited in GenBank (Table 1).

DNA sequence analyses. Blast reaches at GenBank were carried out in order to reveal the closest taxa to our strains. To reveal the phylogenetic position of *Vamsapriya* within *Xylariales*, multi-gene analyses were performed with a combined matrix of three genes (SSU, LSU and RPB2). Sequence data of closest taxa in *Xylariaceae* were downloaded (Table 1). Furthermore, we have included other families in *Xylariales* i.e. *Amphisphaeriaceae, Apiosporaceae, Clypeosphaeriaceae, Diatrypaceae, Graphostromataceae, Hyponectriaceae* and *Melogrammataceae* (Lumbsch & Huhndorf 2010; Jaklitsch & Voglmayr 2012).

Combined multi-gene (beta-tubulin, ITS, LSU, and RPB2) analyses are used to determine placement of *Vamsapriya* within *Xylariaceae*. Sequences in *Xylariaceae* were selected from Hsieh *et al*. (2010), Jaklitsch & Voglmayr (2012), Pažoutová *et al*. (2010) and Stadler *et al*. (2013); in addition, sequences of *Amphisphaeria umbrina*, *Bartalinia robillardoides* and *Diatrype disciformis* were added (Jeewon *et al.*, 2002; 2003; Spatafora *et al.*, 2006).

Sequences were aligned using Bioedit (Hall 2004) and ClustalX (Kohli & Bachhawat 2003). Alignments were checked and manual adjustments were made wherever necessary. The whole ambiguously aligned regions within each dataset were excluded from the analyses (Begoude *et al*., 2010). In the analyses, gaps were treated as missing data, and all characters were unordered and of equal weight. All characters were unordered and of equal weight and gaps were treated as missing data (Liu *et al.*, 2011).

Maximum-parsimony analysis was carried out using PAUP v. 4.0b10 (Swofford 2003) and performed using the heuristic search option with 1,000 random taxa addition and tree bisection and reconnection (TBR) as the branch swapping algorithm. Max trees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1,000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis & Bull 1993).

Bayesian analyses were performed by using PAUP v. 4.0b10 (Swofford, 2003) and MrBayes v. 3.0b4 (Ronquist & Huelsenbeck, 2003). The model of evolution was estimated by using MrModeltest 2.2 (Nylander, 2004). Posterior probabilities (PP) (Rannala & Yang, 1996) were performed by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4 (Liu *et al.*, 2012). Six simultaneous Markov chains were run for 1 m generations and trees were sampled every 100th generations (resulting 10 000 total trees) (Cai *et al.*, 2006). The first 2000 trees, representing the burn-in phase of the analyses, were discarded and the remaining 8000 (post-burning) trees used for calculating posterior probabilities (PP) in the majority rule consensus tree (Cai *et al.*, 2006; Liu *et al.,* 2012). Trees were visualized with TreeView (Page 1996).

Table 1. DNA sequence data used in the phylogenetic trees in Figs 1-2. The ex-types and authentic strains are highlighted in bold

Species	*Strain*	*Type status/Reference*	*GenBank accession numbers*				
			ITS	*LSU*	*SSU*	*RPB2*	*beta-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 *et al.*, 2012; Schoch *et al.*, 2009)	AF009805	AF452029		FJ238348	
Annulohypoxylon moriforme var. microdiscus	**CBS123834**	Authentic (Tang *et al.*, 2009)	DQ631935	DQ840061		DQ631960	DQ840095
Anthostomella brabeji	CBS 110128	(Jaklitsch *et al.*, 2012; Stadler *et al.*, 2013)	EU552098	EU552098			
Apiospora montagnei	AFTOL 951, H3_83	(Jaklitsch *et al.*, 2012)	JN688916	DQ471018	JN546134	DQ470921	
Arthrinium phaeospermum	CBS 114317, HKUCC 3395	(Jaklitsch *et al.*, 2012)		KF144953	JN634086		
Arthrinium sacchari	CBS 664.74, CBS 334.86	(Jaklitsch *et al.*, 2012)		KF144965	AB220206		
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 *et al.*, 2012)	AF405301	AF382366		DQ368653	
Bionectria ochroleuca	CCFC226708, CBS 406.95, CBS 114056	(Seifert *et al.*, 2003; Spatafora *et al.*, 2007)		AY283558	AY489684	DQ522415	
Biscogniauxia arima	**WSP 122**	Ex-type (Hsieh *et al.*, 2010)	EF026150			GQ304736	AY951672
Biscogniauxia nummularia	BCC 1101, H86	(Jaklitsch *et al.*, 2012)		AB376691	AF346563	FR715504	
Clypeosphaeria uniseptata	HKUCC6349, Mt28	(Jaklitsch *et al.*, 2012)	AF009808	DQ810219	DQ810255	DQ810238	
Collodiscula japonica	**CBS 124266**	Authentic (Jaklitsch *et al.*, 2012)	JF440974	JF440974			
Creosphaeria sassafras	CM AT-018	(Tang *et al.*, 2009)	AJ390425	DQ840056			DQ840094
Daldinia concentrica	CBS 113277, ATCC 36659	(Kuhnert *et al.*, 2013; Spatafora & Blackwe 1993)	AY616683	U47828	U32402	FR715506	KC977274
Diatrype disciformis	AFTOL 927	(Trouillas *et al.*, 2001)	AJ302437	DQ470964	DQ471012	DQ470915	

Table 1. DNA sequence data used in the phylogenetic trees in Figs 1-2. The ex-types and authentic strains are highlighted in bold *(continued)*

Species	*Strain*	*Type status/Reference*	*GenBank accession numbers*				
			ITS	*LSU*	*SSU*	*RPB2*	*beta-tubulin*
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
Fasciatispora nypae	**MFLUCC 11-0382**	Ex-type (Hyde *et al.*, 2015)	(Hyde *et al.*, 2015)	(Hyde *et al.*, 2015)	(Hyde *et al.*, 2015)		
Graphostroma platystoma	CBS 270.87, AFTOL-ID 1249	(Jaklitsch *et al.*, 2012)	JX658535	DQ836906	AY083808		
Hypocrea rufa	CBS 438.95, GJS89-127, ATCC 208838	(Jaklitsch *et al.*, 2012)	DQ315438	AY489726	AY489694	EU341806	
Hyponectria buxi	UME 31430	(Jaklitsch *et al.*, 2012)		AY083834	AF130976		
Hypoxylon fragiforme	**MUCL 51264, STMA07069, HKUCC 1022**	Authentic (Seifert *et al.*, 2003)	KM186294	KM186295	AY083810	KM186296	KM186301
Kretzschmaria guyanensi	HAST 89062903	(Hsieh *et al.*, 2010)	GU300079			GQ844792	GQ478214
Lopadostoma dryophilum	CBS 133213	Ex-epitype (Jaklitsch *et al.*, 2014)	KC774570	KC774570		KC774526	
Lopadostoma insulare	CBS 133214	Ex-type (Jaklitsch *et al.*, 2014)	KC774589	KC774589		KC774542	
Melogramma campylosporum	MBU	(Jaklitsch *et al.*, 2012)	JF440978	JF440978			
Muscodor albus	**MSU 2081**	Ex-type (Seifert *et al.*, 2003)	AF324336	HM034864		FJ480345	
Nectria cinnabarina	CBS 256.47, CBS 114055	(Jaklitsch *et al.*, 2012)	HM484692	HM484755	AB003949	DQ522456	
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
Obolarina dryophila	UME30209	(Pažoutová *et al.*, 2010)			Z49784	FR715505	
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

Table 1. DNA sequence data used in the phylogenetic trees in Figs 1-2. The ex-types and authentic strains are highlighted in bold *(continued)*

Species	*Strain*	*Type status/Reference*	*GenBank accession numbers*				
			ITS	*LSU*	*SSU*	*RPB2*	*beta-tubulin*
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
Roumegueriella rufula	CBS 346.85	(Jaklitsch *et al.*, 2012)		DQ518776	DQ522561	DQ522461	
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	AF132330	AY545724	DQ368647	DQ840087
Stilbohypoxylon elaeicola	JDR 173	(Hsieh *et al.*, 2010)	EF026148			GQ844826	EF025616
Thamnomyces camerunensis	**MUCL 51396**	Ex-type (Stadler *et al.*, 2010a)	FN428828				
Vamsapriya bambusicola	**MFLUCC 11-0477**	Ex-type (this study)	KM462835	KM462836	KM462837	KM462834	KM462833
Vamsapriya indica	**MFLUCC 12-0544**	Ex-epitype (this study)	KM462839	KM462840	KM462842	KM462841	KM462838
Vamsapriya khunkonensis	**MFLUCC 11-0475**	Ex-type (this study)	KM462830	KM462831	KM462832	KM462829	KM462828
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	U20378	KM186302	KM186300

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; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **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

Phylogenetic analyses

New sequences including beta-tubulin, ITS, LSU, SSU and RPB2 regions are deposited in GenBank (Table 1). The combined data set of LSU, SSU and RPB2, contained 27 sequences of 27 taxa including *Sordaria fimicola* (CBS 723.96) as the outgroup taxon. Of the 2,236 characters used in the phylogenetic analysis, 1,586 are constant, and 342 variable characters are parsimony-uninformative. The best tree generated from maximum-parsimony analysis shows that *Vamsapriya* clusters within the family *Xylariaceae, Xylariales.* As most species of *Xylariaceae* in GenBank lack SSU and RPB2 genes, only few nodes received significant support in the phylogenetic analyses of the datasets (Fig 1). Bootstrap support (BS) values of MP are shown in Fig 1. Partial nucleotide sequences of the beta-tubulin, ITS, LSU, SSU and RPB2 ribosomal DNA determined the family placement for three isolates. The data set contained 40 sequences of 40 taxa including one outgroup taxon. Species of *Vamsapriya* are well-supported in *Xylariaceae,* and are closely related to *Fasciatispora nypae* (Hyde *et al.*, 2015). Bootstrap support (BS) values of the Bayesian posterior probabilities (PP) from MCMC analyses are shown in Fig 2.

Taxonomy

Vamsapriya Gawas & Bhat, Mycotaxon 94: 150 (2006)

Index Fungorum: IF 29041

Facesoffungi number: FoF 00372

Saprobic on bamboo culms, carbonaceous, formed on host surface. *Mycelium* immersed in the substrate, composed of septate, branched, brown hyphae. Sexual morph: Unknown. Asexual morph: *Conidiophores* macronematous, synnematous, brown to dark brown, septate, branched. *Synnemata* erect, rigid, dark brown, velvety, with apical fertile part globose to subglobose, smooth, composed of compact, parallel, adpressed conidiophores wide at the apical fertile region, with basal portion immersed in the host tissue. *Conidiogenous cells* polytretic terminal or intercalary, monotretic, enteroblastic, ellipsoidal, wider at the apex, brown to dark brown, smooth. *Conidia* catenate, cylindrical, ovoid, ellipsoidal or oblong, fusiform, wide in the middle, straight to flexuous, initially pale brown to dark brown, 0-20-septate (on incubated substrate conidia up to 20-septate), smooth to verrucose, with terminal cell occasionally pale brown and rounded, middle cells dark brown, basal cell dark brown and truncate, constricted at the septa, developing in acropetal chains.

Notes: Vamsapriya was introduced by Gawas & Bhat (2005). This genus was originally collected from bamboo. *Vamsapriya mahabaleshwarensis* (Pratibha & Bhat 2008) differs from the type species, *V. indica* in the shape and size of conidia and its branched conidial chains (Pratibha & Bhat 2008). *Vamsapriya khunkonensis* differs from other species by its long synnemata, and short and minutely verrucose conidia. *Vamsapriya bambusicola* is distinguished from other species in the genus by its long synnemata and cylindrical, smooth conidia.

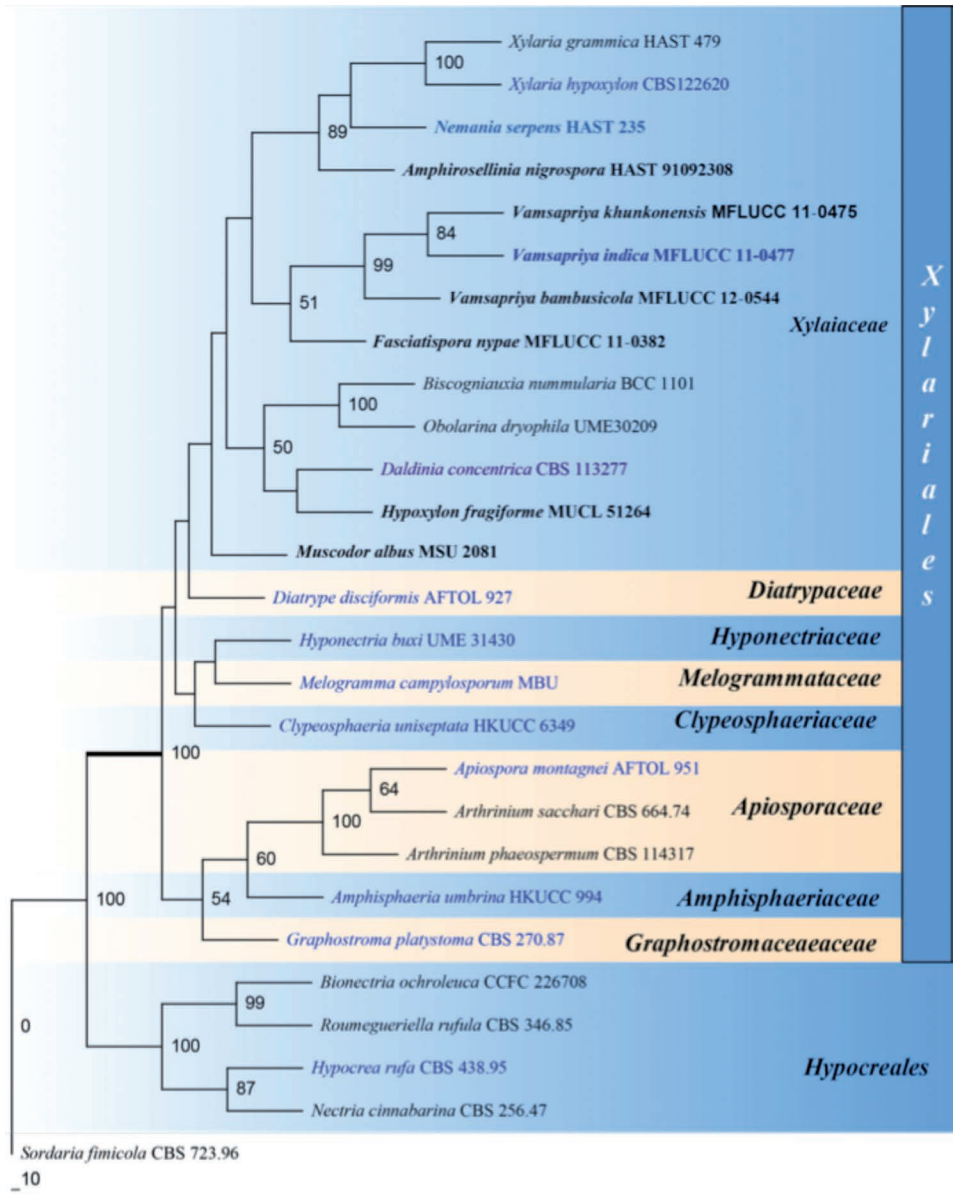

Fig. 1. Phylogenetic tree of *Xylariales* order level generated from maximum-parsimony (MP) analysis based on combined data set of LSU, SSU and RPB2 sequence data set. Bootstrap support (BS) values above 50% are shown at the nodes. The original isolate numbers or GenBank codes are noted after the species names. Ex-type and authentic strains are in bold and the type species are indicated in blue. The tree is rooted with *Sordaria fimicola* (CBS 723.96).

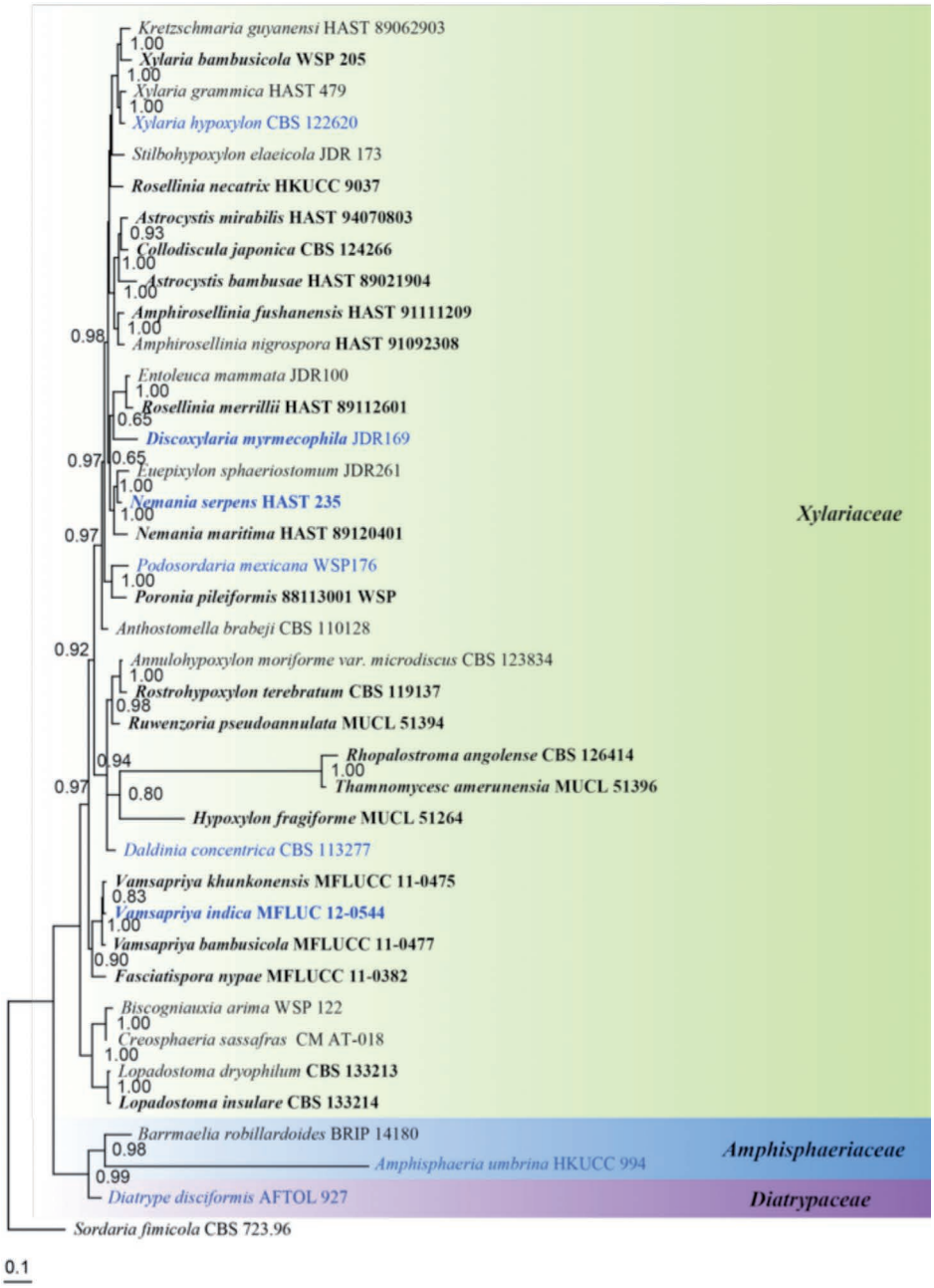

Fig. 2. Bayesian analysis based on combined data set of beta-tubulin, ITS, LSU, and RPB2 and sequence data sets. Bootstrap support (BS) values above 0.80 are shown at the nodes. The original isolate numbers or GenBank codes are noted after the species names. Ex-type and authentic strains are in bold and the type species are indicated in blue. The tree is rooted with *Sordaria fimicola* (CBS 723.96).

Key to species of *Vamsapriya*

1. Synnemata, length: 600-1100 µm **2**
1. Synnemata, length: 1100-1400 µm **3**
   2. Conidia developing in acropetal chains . . . . . ***V. indica***
   2. Conidia developing in branched chains . . . . . ***V. mahabaleshwarensis***
3. Conidia fusiform, wide in the centre, minutely verrucose . . . . ***V. khunkonensis***
3. Conidia cylindrical, smooth-walled . . . . . ***V. bambusicola***

Table 2. Comparison of species in the genus *Vamsapriya*

No, Name and reference	*Synnemata*	*Conidiogenous cell*	*Conidium*	*Conidial chain*
Vamsapriya indica Gawas & Bhat (Gawas & Bhat 2005)	Length: – 700-1100 µm; Width: – Base: 60-160 µm; Centre: 30-60 µm; Apex: 30-80 µm.	4-9 × 2.5-4.5 µm; Terminal Monotretic, enteroblastic.	10-65 × 3.5-6 µm; Cylindrical; 0-10-septate (On incubated substrate conidia up to 20-septate); Slightly verrucose.	35-290 × 4-6.5 µm; Always in acropetal chains.
V. mahabaleshwarensis Pratibha& Bhat (Pratibha & Bhat, 2008)	Length: 600-1100 µm; Width: – Base: 100-160 µm; Centre: 15-45 µm; Apex: 30-80 µm.	6-23 × 3-5 µm; Polytretic Terminal or intercalary.	5-25 × -9 µm; Ovoid, ellipsoidal or oblong; 0-4-sepate; Smooth to minutely verrucose.	Always in branched chains.
V. khunkonensis	Length: – 1100-1400 µm; Width: – Base: 70-200 µm; Middle: 35-65 µm; Apex: 20-35 µm.	5-17 × 2-4 µm; Terminal Monotretic, enteroblastic.	17.5-35 × 6-10 µm; Fusiform; wide in the middle; 1-5-septate; Minutely verrucose.	Rarely in chains.
V. bambusicola	Length: – 1100-1400 µm; Width: – Base: 80-200 µm; Centre: 25-35 µm; Apex: 55-125 µm.	6.5-12.5 × 3-4.5 µm; Terminal Monotretic, enteroblastic.	8-45 × 4.5-9.5 µm; Cylindrical; 1-5-septate; Smooth.	Rarely in chains.

Vamsapriya indica Puja & Bhat, 2006. *Mycotaxon* 94: 150 **Figs 3-4**

Index Fungorum number: IF 550801
Facesoffungi number: FoF 00374
Epitypus hic designatus: MFLU 13-0370

Saprobic on bamboo culms, carbonaceous, formed mostly at the nodal region on host surface. *Mycelium* immersed in the substrate, composed of septate, branched, brown hyphae. Sexual morph: Unknown. Asexual morph: *Conidiophores* macronematous, synnematous, brown to dark brown, septate, branched. *Synnemata* erect, rigid, dark brown, velvety, with apical fertile part globose to subglobose, smooth, composed of compact, parallel, adpressed conidiophores, 700-1100 µm long, 60-160 µm wide at the base, 30-60 µm wide in the middle, 30-80 µm wide at the apical fertile region, with basal portion immersed

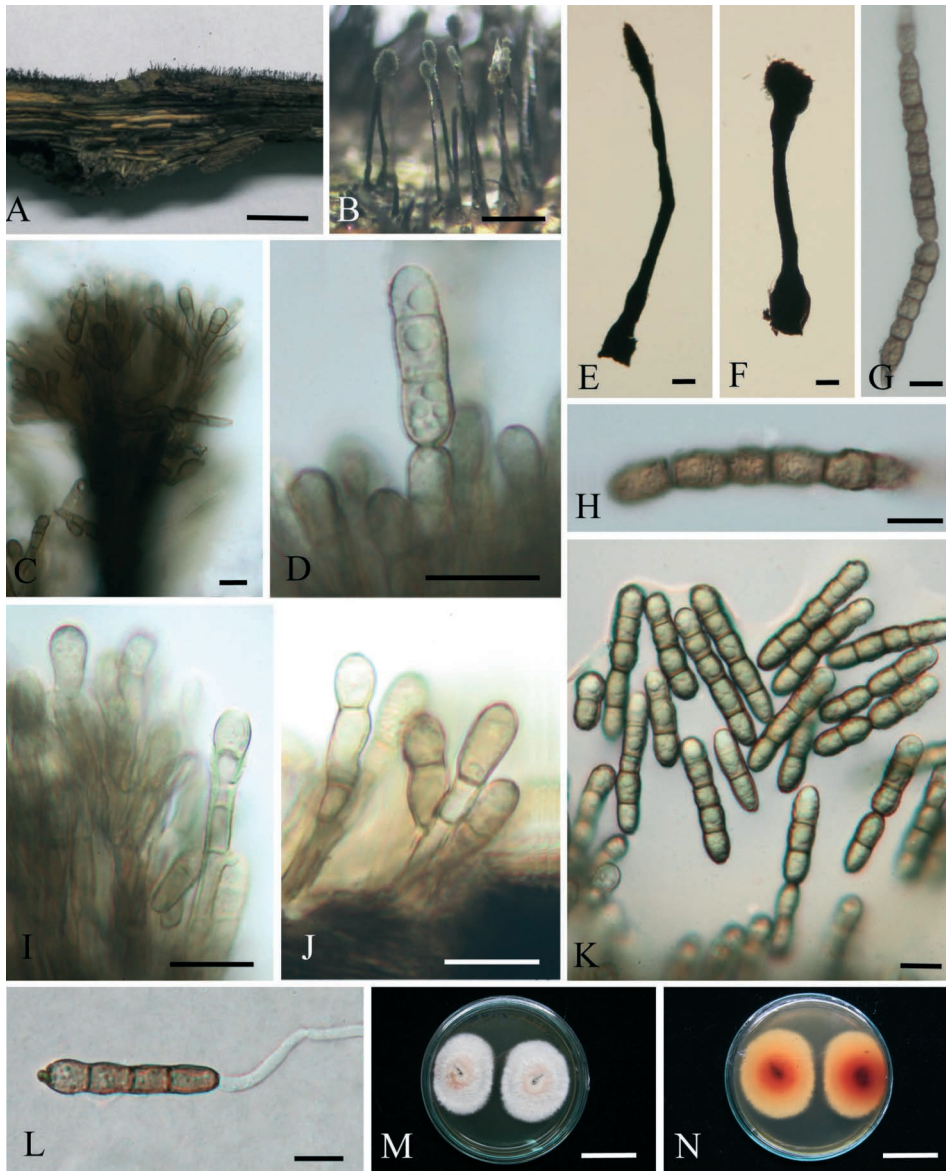

Fig. 3. ***Vamsapriya indica*** (epitype, MFLU 13-0370). **A, B.** Conidiomata on bamboo host. **C.** The apical part of synnema. **E, F.** Synnemata. **D, I, J.** Conidiophores and conidiogenous cells producing conidia. **G, H, K.** Dark brown conidia in chains. **L.** Geminating spore. **M, N.** Colonies on PDA after 30 d. Scale bars: A = 20 mm, B = 500 µm, E, F = 100 µm, C-L = 10 µm, M, N = 25 mm.

in the host tissue. *Conidiogenous cells* 4-9 × 2.5-4.5 µm ($\bar{x}$ = 6.5 × 3.7 µm, n = 20), monotretic, enteroblastic, non-cicatrized at the pore, terminal or discrete, ellipsoidal, wider at the apex, brown to dark brown, smooth. *Conidia* catenate, 35-290 × 4-6.5 µm ($\bar{x}$ = 66.6 × 5.6 µm, n = 20) µm, cylindrical, straight to flexuous,

Fig. 4. ***Vamsapriya indica*** (epitype, MFLU 13-0370, after incubation in a moist chamber). **A-F.** Conidia under incubated condition. **A.** Conidia with more than 20 septa. **B-F.** Conidia in chains. **D.** Conidia with slightly verrucose surface. Scale bars: A-F = 5 μm.

initially pale brown and 1-3-septate when young, later becoming moderately to dark brown and more than 20-septate at maturity, smooth to slightly verrucose, with terminal cell occasionally pale brown and rounded, middle cells dark brown, basal cell dark brown and truncate, constricted at the septa, developing in acropetal chains.

Culture characteristic: Colonies on PDA, fast growing, 4.7 cm diam. after 30 d at 27°C, circular, white and flat, becoming cottony and reddish-orange at the centre after 30 d. No sporulate in culture.

Material examined: INDIA, Karnataka, Uttara Kannada, Yellapur, on dead and decaying bamboo culms, Puja Gawas, 27 September 2005 (K, IMI 393674, holotype); THAILAND, Chiang Rai Province, Mae Fah Luang University, saprobic on bamboo culms, 21 June 2012, D. Jayarama Bhat,

DDQ00238 (MFLU 13-0370, epitype of *Vamsapriya indica* designated here; isoepitype in KUN under the code of HKAS 83859), ex-epitype living culture = MFLUCC 12-0544 = ICPM.

Note: Vamsapriya indica, the type species of *Vamsapriya*, is characterized by formation of cylindrical, phragmoseptate, minutely verrucose dark brown, catenate conidia developing in monotretic, enteroblastic conidiogenous cells on synnematous conidiophores (Gawas & Bhat 2005). The holotype of *Vamsapriya indica* was collected on bamboo culms from India in 2005. Gawas & Bhat (2005) described and illustrated the morphology of this specimen in detail. However, no culture characters and DNA phylogeny data were provided. In this paper we epitype a new collection based on a collection with the same characters as the holotype and collected and identified by the original describing author. It is, however, from a different country (Thailand), but from the same region (Asia) and also on bamboo. *Vamsapriya* appears to be a genus confined to grasses and bamboo and the type species is likely to comprise cryptic species as collections shown in Figures 3 and 4 show quite different conidia, after incubating the same specimen in a moist chamber. Therefore to stabilize the species we feel there is a need for epitypification as outlined in (Ariyawansa *et al.* 2014). The epitypification allows placement of *Vamsapriya* in Xylariaceae and resolution of species within the genus.

Vamsapriya khunkonensis D.Q. Dai, D.J. Bhat & K.D. Hyde, **sp. nov.** **Fig. 5**

Index Fungorum number: IF 550738

Facesoffungi number: FoF 00375

Etymology: Based on its collection location.

Holotype: MFLU 13-0367

Saprobic on bamboo culms, formed in small circular colonies, mostly at the nodal region on host surface. *Mycelium* immersed on the substrate, composed of septate, branched, brown-coloured hyphae. Sexual morph: Unknown. Asexual morph: *Conidiophores* macronematous, synnematous, brown to dark brown, septate, branched. *Synnemata* erect, rigid, dark brown, velvety, with apical fertile part sub-globose, smooth, composed of compact, parallel, adpressed conidiophores, 1100-1400 µm long, 70-200 µm wide at the base, 35-65 µm wide in the middle, 20-35 µm wide at the apical fertile region, with basal portion immersed in the host tissue and arising from the periphery of circular colonies. *Conidiogenous cells* 5-17 × 2-4 µm ($\bar{x}$ = 7.3 × 2.9 µm, n = 20), monotretic, enteroblastic, slightly cicatrized at the pore, terminal, discrete, ellipsoidal, wider at the apex, brown to dark brown, smooth. *Conidia* catenate, 17.5-35 × 6-10 µm ($\bar{x}$ = 23.4 × 7.6 µm, n = 20), initially pale brown to brown and 1-2-septate, becoming brown to dark brown and up to 5-septate at maturity, minutely verrucose, broadly fusiform, straight or curved, with terminal cell occasionally smallest and pale brown, middle cells dark brown and broad, basal cell dark brown, smaller and truncate, constricted at the septa, developing in acropetal chains.

Culture characteristic: Colonies on (PDA), fast growing, 4.6 cm diam. after 30d at 25-32°C, circular, white after 7 d, becoming cottony and dark coloured at the centre after 30 d. No sporulate in culture.

Material examined: THAILAND, Chiang Rai Province, Khunkorn Waterfall, on dead *Dendrocalamus giganteus* culms, 30 June 2011, Dong-Qin Dai, DDQ0063 (MFLU 13-0367, **holotype**; **isotype** in KUM under the code of HKAS 83859), ex-type living culture = MFLUCC 11-0475 = IFRDCC 2533 = ICPM.

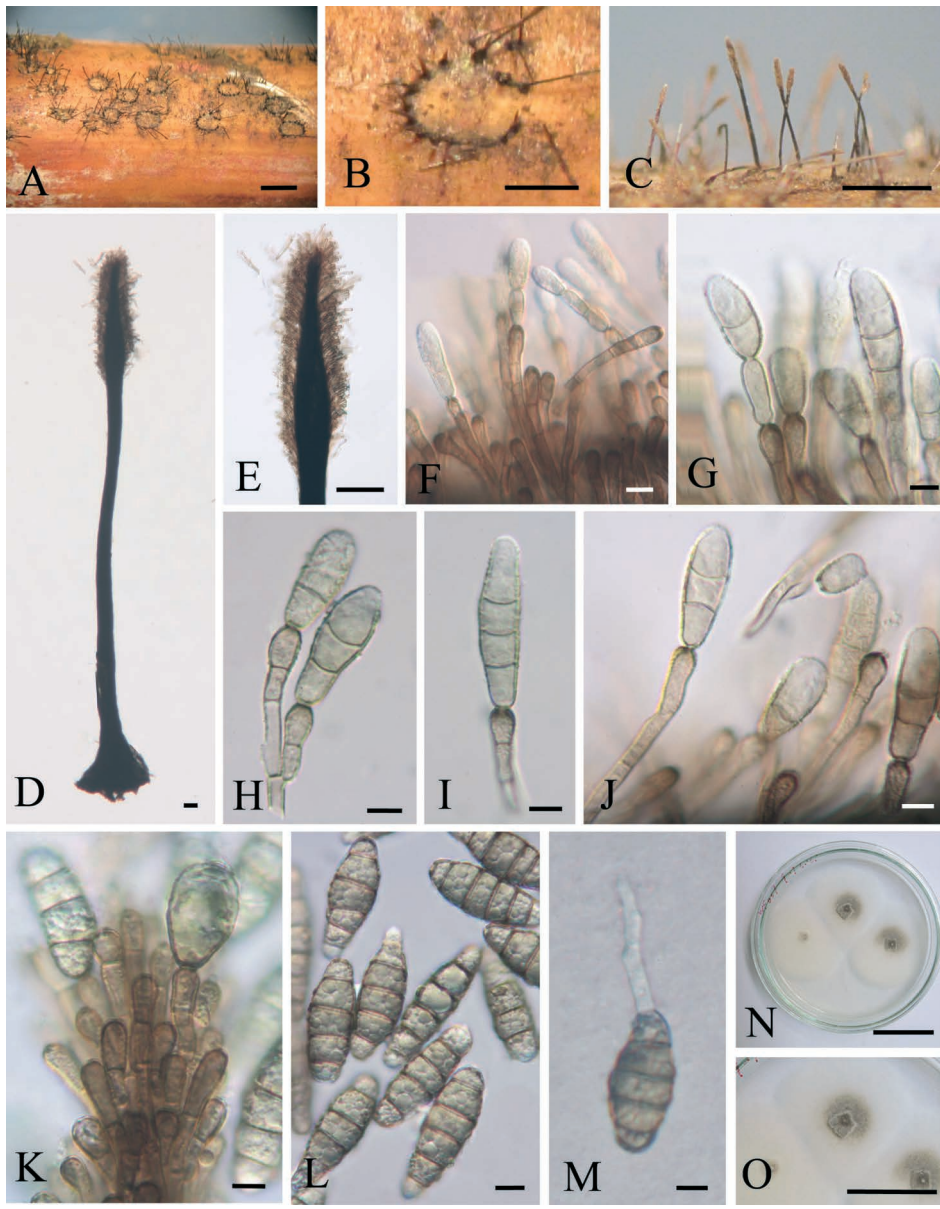

Fig. 5. ***Vamsapriya khunkonensis*** (holotype, MFLU 13-0367) **A-C.** Conidiomata on bamboo host. **D.** Black to brown synnema. **E.** The apical part of synnema. **F-J.** Conidiophores and conidiogenous cells producing conidia. **K-L.** From incubated substrate under humidity after 3 d. **K.** Conidiogenous cells and conidia. **L.** Dark brown conidia. **M.** Germinating spore. **N, O.** Colonies on PDA after 30 d. Scale bars: A = 3 mm, B, C = 1 mm, D, E = 50 µm, F-M = 5 µm, N, O = 30 mm.

Note: Vamsapriya khunkonensis is characterized by long synnematous conidiophores and fusiform, 1-5-septate, minutely verrucose conidia, which are wide in the centre. This differs from the other three species of *Vamsapriya* which have cylindrical conidia. The separation of species is also supported by molecular data.

Vamsapriya bambusicola D.Q. Dai, D.J. Bhat & K.D. Hyde, **sp. nov.** **Fig. 6**

Indexfungorum number: IF 550739
Facesoffungi number: FoF 00376
Etymology: With reference to its occurrence on *Bambusa* sp.

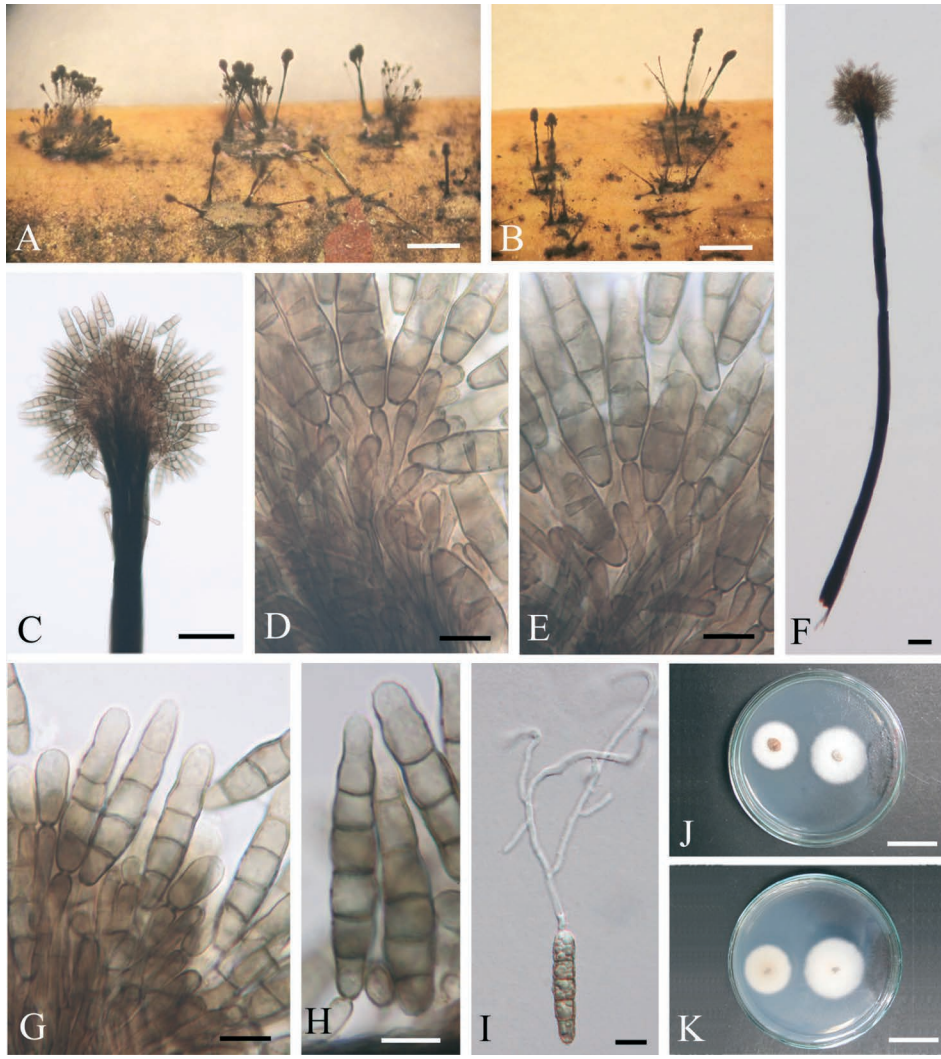

Fig. 6. ***Vamsapriya bambusicola*** (holotype, MFLU 13-0368). **A, B.** Synnemata on bamboo host. **C.** The apical part of synnema. **D, E, G.** Conidiophores and conidiogenous cells producing conidia. **F.** Synnema. **H.** Dark brown conidia. **I.** Geminating spore. **M, N.** Colonies on PDA after 30 d. Scale bars: A, B = 1 mm, C, F = 50 µm, D, E, G-I = 10 µm, J, K = 25 mm.

Holotype: MFLU 13-0368

Saprobic on bamboo culms, carbonaceous, formed in small circular colonies, mostly at the nodal region on host surface. *Mycelium* immersed on the substrate, composed of septate, branched, brown hyphae. Sexual morph: Unknown. Asexual morph: *Conidiophores* macronematous, synnematous brown to dark brown, septate. *Synnemata* erect, rigid, dark brown, velvety, with globose apical part, smooth, composed of compact, parallel, adpressed, branched conidiophores, 1100-1400 µm long, 80-200 µm wide at the base, 25-35 µm wide in the middle, 55-125 µm wide at the apical fertile region, with basal portion immersed in the host tissue and developing from the periphery of circular colonies. *Conidiogenous cells* 6.5-12.5 × 3-4.5 µm ($\bar{x}$ = 9.8 × 3.6 µm, n = 20), monotretic, enteroblastic, non-cicatrized at the pore, terminal, discrete, ellipsoidal, wider at the apex, brown to dark brown, smooth. *Conidia* 8-45 × 4.5-9.5 µm ($\bar{x}$ = 30.2 × 7.7 µm, n = 20), initially pale brown to brown and 1-2-septate, becoming brown to dark brown and up to 3-5-septate at maturity, smooth, cylindrical, straight, top cell occasionally pale brown and rounded or narrow, middle cells dark brown and expanded, basal cell dark brown, smaller, and truncate, constricted at the septa.

Culture characteristic: Colonies on (PDA), fast growing, 4.7 cm diam. after 30 d at 27°C, circular, white after 7 d, becoming cottony and light-coloured at the centre after 30 d. No sporulate in culture.

Material examined: THAILAND, Chiang Rai Province, Khunkorn Waterfall, on dead *Dendrocalamus giganteus* Munro (*Gramineae*) culms, 30 June 2011, Dong-Qin Dai, DDQ0068 (MFLU 13-0368, **holotype**; **isotype** in KUM under the code of HKAS 83860), ex-type living culture = MFLUCC 11-0477 = ICPM.

Note: Vamsapriya bambusicola is established herein for its 1100-1400 µm long synnemata and cylindrical, smooth conidia. The separation of species is also supported by molecular data.

DISCUSSION

The order *Xylariales* was introduced by Nannfeldt (1932) with six families (*viz. Diatrypaceae*, *Hypocreaceae*, *Hyponectriaceae*, *Lasiosphaeriaceae*, *Polystigmataceae* (as *Phyllachoraceae*) and *Xylariaceae* with the latter as the type family. Barr (1990) provided a broad concept of the *Xylariales*, accepting *Acrospermaceae*, *Amphisphaeriaceae*, *Boliniaceae*, *Clypeosphaeriaceae*, *Diatrypaceae*, *Hyponectriaceae*, *Melogrammataceae*, *Phyllachoraceae*, *Thyridiaceae*, *Trichosphaeriacae* and *Xylariaceae*. Lumbsch and Huhndorf (2010) however included six families i.e. *Amphisphaeriaceae, Clypeosphaeriaceae, Diatrypaceae, Graphostromataceae, Hyponectriaceae* and *Xylariaceae*. However, *Apiosporaceae* and *Melogrammataceae* were shown to nest in *Xylariales* (Hyde 1998; Jaklitsch & Voglmayr 2012).

Single gene phylogenetic analyses are not sufficiently informative to resolve the taxa of Xylariales (Jaklitsch *et al.* 2012; Stadler *et al.* 2013). The first attempt to resolve the *Xylariaceae* with multigene analysis was that of Tang *et al.* (2009) and more recently by Hsieh *et al.* (2010) and Pažoutová *et al.* (2010). However, due to lack of sequences from ex-type materials, the phylogenetic relationships and placement of various lineages of *Xylariales* are still unresolved

(Jaklitsch *et al.* 2012). Many important genera in *Xylariaceae* still lack phylogenetic studies (Stadler *et al.* 2013). Hence, in this paper we redescribe one genus of Xylariaceae with molecular data and introduce two new species.

In the phylogenetic analysis (Figure 1), we have included all the families belonging to *Xylariales*. The combined data set of LSU, SSU and RPB2 genes were used in the phylogenetic analysis to determine the generic placement of *Vamsapriya,* which is well-resolved in *Xylariales (*100% MPBS support (Figure 1). However, as most of the genera in this order lack SSU and RPB2 sequences in GenBank, most of the bootstrap supports values are low.

Asexual species of *Vamsapriya* are embedded within *Xylariaceae* (0.97 BYPP support (Figure 2) and in a same clade with *Fasciatispora* (Hyde *et al.* 2015) (0.90 BYPP support (Figure 2), according to the analyses of combined genes (beta-tubulin, ITS, LSU, and RPB2). Further species are needed in the phylogenetic analysis to clarify the relationship between *Fasciatispora* and *Vamsapriya*.

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