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Beltrania-like taxa from Thailand

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Abstract – Four *Beltrania*-like taxa, *viz*., *Beltrania rhombica, Beltraniella fertilis, Beltraniopsis longiconidiophora sp. nov.* and *Hemibeltrania cinnamomi* were identified during a survey of hyphomycetes in Thailand. Each species is provided with a description and a molecular analysis. The new species is introduced based on morphological and molecular differences and compared with similar taxa. *Beltraniella fertilis* and *H. cinnamomi* are new records for Thailand.

***Beltrania*-complex / Beltraniaceae / Phylogeny / Taxonomy / Xylariomycetidae**

INTRODUCTION

The family Beltraniaceae Nann. was introduced by Nannizzi in 1934 to accommodate the genus *Beltrania* Penz. and some similar genera, and the tribe Beltranieae was treated as a synonym of this family (Pirozynski, 1963). Presently, eight genera, *viz*., *Beltrania*, *Beltraniella* Subram., *Beltraniopsis* Bat. & J.L. Bezerra, *Hemibeltrania* Piroz., *Parapleurotheciopsis* P.M. Kirk, *Porobeltraniella* Gusmão, *Pseudobeltrania* Henn. and *Subramaniomyces* Varghese & V.G. Rao, are accepted in the family (Crous *et al.*, 2015b; Maharachchikumbura *et al.*, 2015, 2016; Rajeshkumar *et al.*, 2016a). The conidia of these genera are very distinctive, often being biconic, with or without a hyaline equatorial, subequatorial or supraequatorial band, and with or without swollen separating cells. The unbranched or branched conidiophores and/or setae arise from radially lobed basal cells (Ellis, 1971, 1976; Seifert *et al.*, 2011).

The genus *Beltrania* was established by Penzig (1882) to accommodate *B. rhombica* Penzig. This genus contains 13 species (Morelet, 2001; Zhang & Zhang, 2003; Rambelli & Ciccarone, 2008; Crous *et al.*, 2015b), each characterized by

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having dark and mostly unbranched setae with radially lobed basal cells, unbranched conidiophores arising from basal cells of setae or from separate radially lobed basal cells, sympodial and denticulate conidiogenous cells, swollen separating cells and biconic, spicate or apiculate conidia with a hyaline transverse band (Seifert *et al.*, 2011). *Beltrania rhombica* was reported to have antibacterial activities against *Staphylococcus aureus* ATCC 25923 and antifungal activities against *Candida albicans* (Rukachaisirikul *et al.*, 2005).

Subramanian (1952) described a beltrania-like genus (*Beltraniella*) to accommodate *Be. odinae* Subram. Hodges & Barr (1971) obtained a *Beltraniella* asexual morph in pure cultures of *Pseudomassaria carolinensis* M.E. Barr & Hodges. Jaklitsch *et al.* (2016) synonymized *P. carolinensis* under the new combination *Be. carolinensis*, based on a phylogenetic analysis. Presently, 22 species are accepted in the genus *Beltraniella* (Fernando & Gusmao, 2004; Shirouzu *et al.*, 2010; Priya *et al.*, 2011; Crous *et al.*, 2014; Jaklitsch *et al.*, 2016).

The genus *Beltraniopsis* was established by Batista & Bezerra (1960) to accommodate *Bel. esenbeckiae* Bat. & J.L. Bezerra. Currently, there are nine species in this genus (Gusmão *et al.*, 2000; Ruiz *et al.*, 2006; Crous *et al.*, 2014), each having branched, setiform conidiophores arising from radially lobed basal cells, swollen separating cells, and biconic conidia with a median transverse hyaline band (Ellis, 1971; Seifert *et al.*, 2011).

Pirozynski (1963) introduced the genus *Hemibeltrania* Piroz. for *H. cinnamomi* (Deighton) Piroz. and *H. nectandrae* (Bat. & H. Maia) Piroz. Twelve species are accepted in this genus (Rajeshkumar *et al.*, 2016b), each characterized by having unbranched or sparingly branched conidiophores with radially lobed basal cells, monoblastic or polyblastic conidiogenous cells and broadly ellipsoidal, limoniform, ovoid, obovoid, cymbiform, navicular, biconic or fusiform conidia without a transverse band (Ellis, 1971; Seifert *et al.*, 2011).

The genus *Beltrania* and its allies are mostly found in litter and on submerged wood in freshwater (Pirozynski, 1963; Goh & Hyde, 1996; Sakayaroj *et al.*, 2005; Duong *et al.*, 2008). During a survey of hyphomycetes in Thailand, several beltrania-like species were collected. They were shown to belong to *Beltrania*, *Beltraniella*, *Beltraniopsis* and *Hemibeltrania* in Beltraniaceae based on both morphology and analyses of ITS and LSU sequence data.

MATERIALS AND METHODS

Collection and isolation of fungi

Dead stems, wood, and leaves from a variety of plants were collected from July 2015 to August 2016 in Chiang Rai, Chiang Mai and Phetchaburi provinces in Thailand. Samples were taken to the laboratory in Zip-lock plastic bags for examination. The specimens were incubated in sterile moist chambers and examined using a Motic SMZ 168 series microscope. Fungi were removed with a needle and placed in a drop of distilled water on a slide for morphological study. Photomicrographs of fungal structures were captured using a Nikon ECLIPSE Ni compound microscope with a Canon 600D digital camera. All measurements were made using the Tarosoft (R) Image FrameWork program. Photo-plates were made with Adobe Photoshop

CS6 Extended version 13.0.1 (Adobe Systems, USA). Isolation onto potato dextrose agar (PDA) or malt extract agar (MEA) was performed by the single spore isolation method (Chomnunti *et al.*, 2014; Dai *et al.*, 2017). The material is deposited in the Herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), Kunming, China. Cultures are deposited at Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand and Kunming Institute of Botany, Chinese Academy of Sciences (KUMCC), Kunming, China. Faces of Fungi and Index Fungorum numbers are registered (Jayasiri *et al.*, 2015; Index Fungorum, 2017).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fungal mycelium grown on PDA or MEA at room temperature with the Fungal gDNA Kit (BioMIGA, USA) according to the manufacturer's instructions. The internal transcribed spacer region of ribosomal DNA (ITS) and large subunit nuclear ribosomal DNA (LSU) genes were amplified via polymerase chain reaction (PCR) using the following primers: ITS5 and ITS4 (White *et al.*, 1990) for ITS, LROR and LR5 (Vilgalys & Hester, 1990) for LSU. The PCR products were sequenced with the same primers. The PCR amplification was performed in a 25 μL reaction volume containing 12.5 μL of 2 × Power Taq PCR MasterMix (a premix and ready to use solution, including 0.1 Units/μL Taq DNA Polymerase, 500 μM dNTP Mixture each (dATP, dCTP, dGTP, dTTP), 20 mM Tris-HCl pH 8.3, 100 Mm KCl, 3 mM $MgCl_2$, stabilizer and enhancer), 1 μL of each primer (10 μM), 1 μL genomic DNA extract and 9.5 μL deionised water. The PCR thermal cycle program of ITS and LSU were followed as: initially 94°C for 3 min., followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 50 s, elongation at 72°C for 1 min., and final extension at 72°C for 10 min.

Phylogenetic analyses

Original sequences were checked using BioEdit version 7.0.5.3 (Hall, 1999), and most reference sequences originated from previous publications. The remaining homogenous sequences were obtained by BLAST searches (Altschul *et al.*, 1990) from GenBank. All sequences used in this study are listed in Table 1. Alignments for each locus were done in MAFFT v7.307 online version (Katoh & Standley, 2016) and manually verified in MEGA v6.06 (Tamura *et al.*, 2013). Conserved blocks were selected from the initial alignments with Gblocks v0.91b (Castresana, 2000). The interleaved NEXUS files for Bayesian inference analyses were formatted with AliView v1.19-beta1k (Larsson, 2014). Bayesian inference (BI), maximum parsimony (MP) and maximum likelihood (ML) were used for phylogenetic analyses. For Bayesian inference analysis, the best model of evolution was determined using MrModeltest v2 (Nylander, 2004). Bayesian inference analysis was done with MrBayes v3.2.6 (Ronquist *et al.*, 2012). Maximum parsimony analysis was performed in PAUP*4.0b10 (Swofford, 2002). Maximum likelihood analysis was performed in raxmlGUI v1.3.1 (Silvestro & Michalak, 2012). Phylogenetic trees were drawn with TreeView v1.6.6 (Page, 1996) or FigTree v1.4.3 (Rambaut, 2017).

Table 1. GenBank accession numbers of isolates used in this study

Families	Species	Culture accession no.	Country	LSU	ITS	References
Amphisphaeriaceae	***Seimatosporium botan***	**NBRC 104200T**[a]	Japan	**AB593731**	**AB594799**	Tanaka *et al.* (2011)
	S. discosioides	NBRC 104201	Japan	AB593732	AB594800	Tanaka *et al.* (2011)
	S. lichenicola	NBRC 32625 = IMI 079706 = IFO 32625	UK	AB593726	AB594794	Tanaka *et al.* (2011)
Apiosporaceae	*Apiospora tintinnabula*	ICMP 7019	New Zealand	DQ810216	–[b]	Unknown
	Arthrinium aureum	**CBS 244.83 = IMI 238036**[a]	Spain	**KF144935**	**AB220251**	Crous & Groenewald (2013)
	A. gutiae	**CBS 135835**[a]	India	**KR149063**	–	Crous & Groenewald (2013)
	A. hydei	**CBS 114990 = HKUCC 3990**[a]	China	**KF144936**	**KF144890**	Crous & Groenewald (2013)
	A. kogelbergense	CBS 113332	South Africa	KF144937	KF144891	Crous & Groenewald (2013)
	A. kogelbergense	**CBS 113333**[a]	South Africa	**KF144938**	**KF144892**	Crous & Groenewald (2013)
Beltraniaceae	***Beltrania pseudorhombica***	**CBS 138003 = CPC 23656**[a]	China	**KJ869215**	**KJ869158**	Crous *et al.* (2014)
	B. querna	ICMP 15825	New Zealand	–	EF029240	Unknown
	B. querna	BCRC 34620	China	–	GU905994	Unknown
	B. rhombica	Strain 10353	Japan	AB496423	–	Shirouzu *et al.* (2010)
	B. rhombica	CBS 141507 = CPC 27482	Malaysia	KX519521	KX519515	Rajeshkumar *et al.* (2016a)
	B. rhombica	MFLUCC 15-0835	Thailand	MF580252	MF580245	This study
	Beltraniella botryospora	**TUFC 10083**[a]	Japan	**AB496426**	–	Shirouzu *et al.* (2010)
	Be. carolinensis	IFO 9502	Unknown	DQ810233	–	Unknown
	Be. endiandrae	**CBS 137976 = CPC 22193**[a]	Australia	**KJ869185**	**KJ869128**	Crous *et al.* (2014)
	Be. fertilis	MFLUCC 17-2136	Thailand	MF580253	MF580246	This study
	Be. fertilis	MFLUCC 17-2137	Thailand	MF580254	MF580247	This study
	Be. fertilis	MFLUCC 17-2138	Thailand	MF580255	MF580248	This study
	Be. portoricensis	BCRC 34590	China	–	GU905993	Unknown
	Be. portoricensis	NFCCI 3993	India	KX519522	KX519516	Rajeshkumar *et al.* (2016a)
	Beltraniopsis longiconidiophora	**MFLUCC 17-2139**	**Thailand**	**MF580256**	**MF580249**	**This study**
	Bel. longiconidiophora	MFLUCC 17-2140	Thailand	MF580257	MF580250	This study
	Bel. neolitseae	**CBS 137974 = CPC 22168**[a]	Australia	**KJ869183**	**KJ869126**	Crous *et al.* (2014)
	Beltraniopsis sp.	TUFC 10081	Japan	AB496424	–	Shirouzu *et al.* (2010)

Families	Species	Culture accession no.	Country	LSU	ITS	References
	Hemibeltrania cinnamomi	NFCCI 3695	India	KT119565	KT119564	Rajeshkumar *et al.* (2016b)
	H. cinnamomi	MFLUCC 17-2141	Thailand	MF580258	MF580251	This study
	H. cinnamomi	NFCCI 3997	India	KX519523	KX519517	Rajeshkumar *et al.* (2016a)
	Hemibeltrania sp.	CL12WA	Malaysia	–	JQ621881	Unknown
	Porobeltraniella porosa	NFCCI 3994	India	KX519524	KX519518	Rajeshkumar *et al.* (2016a)
	P. porosa	NFCCI 3995	India	KX519525	KX519519	Rajeshkumar *et al.* (2016a)
	P. porosa	NFCCI 3996	India	KX519526	KX519520	Rajeshkumar *et al.* (2016a)
	Pseudobeltrania ocoteae	**CBS 140664 = CPC 26219**[a]	France	**KT950870**	**KT950856**	Crous *et al.* (2015b)
	Subramaniomyces fusisaprophyticus	CBS 418.95 = INIFAT C94/134	Cuba	EU040241	EU040241	Crous *et al.* (2007)
	Subsessila turbinata	**MFLUCC 15-0831**[a]	Thailand	**KX762289**	**KX762288**	Lin *et al.* (2017)
Pestalotiopsidaceae	***Neopestalotiopsis aotearoa***	**CBS 367.54 = ATCC 11763 = QM 381**[a]	New Zealand	**KM116247**	**KM199369**	Maharachchikumbura *et al.* (2014)
	N. eucalypticola	**CBS 264.37 = BBA 5300**[a]	Unknown	**KM116256**	**KM199376**	Maharachchikumbura *et al.* (2014)
	Pestalotiopsis arceuthobii	**CBS 434.65 = ATCC 16339**[a]	USA	**KM116243**	**KM199341**	Maharachchikumbura *et al.* (2014)
	P. arengae	**CBS 331.92**[a]	Singapore	**KM116207**	**KM199340**	Maharachchikumbura *et al.* (2014)
	P. camelliae	CBS 443.62	Turkey	KM116225	KM199336	Maharachchikumbura *et al.* (2014)
	P. chamaeropis	**CBS 186.71**[a]	Italy	**KM116210**	**KM199326**	Maharachchikumbura *et al.* (2014)
	Pseudopestalotiopsis cocos	**CBS 272.29**[a]	Indonesia	**KM116276**	**KM199378**	Maharachchikumbura *et al.* (2014)
Robillardaceae	***Robillarda africana***	**CBS 122.75 = BCC 38220**[a]	South Africa	**KR873281**	**KR873253**	Crous *et al.* (2015a)
	R. sessilis	CBS 101440 = BCC 37544	USA	KR873283	KR873255	Crous *et al.* (2015a)
	R. sessilis	**CBS 114312**[a]	Germany	**KR873284**	**KR873256**	Crous *et al.* (2015a)
Outgroup	*Anthostomella leucospermi*	CBS 110126	South Africa	EU552100	EU552100	Marincowitz *et al.* (2008)

ATCC, American Type Culture Collection, Manassas, United States; **BCC**, BIOTEC Culture Collection, Thailand; **BCRC**, The Bioresource Collection and Research Center, Taiwan, China; **CBS**, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; **CPC**: Culture collection of Pedro Crous, housed at CBS; **HKUCC**, The University of Hong Kong Culture Collection, Department of Ecology and Biodiversity, Hong Kong, China; **ICMP**, The International Collection of Microorganisms from Plants, New Zealand; **IFO**, Institute for Fermentation Culture Collection, Osaka, Japan; **IMI**, International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; **INIFAT**, INIFAT Fungus Collection, Ministerio de Agricultura Habana; **MFLUCC**, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **NBRC**, Distribution and Deposit of Biological Resources, Japan; **NFCCI**, National Fungal Culture Collection of India, Agharkar Research Institute, Pune, India; **QM**, Quatermaster Research and Development Center, US Army, Natick, MA, United States; **TUFC**, Tottori University Fungal Culture Collection, Fungus/Mushroom Resource and Research Center, Tottori, Japan.

[a] Ex-type and ex-epitype cultures are in bold

[b] No data in GenBank.

RESULTS

Molecular phylogeny

The aligned sequence matrix comprises LSU (871 bp) and ITS (660 bp) sequence data for 42 taxa and one outgroup taxon for a total of 1531 characters, of which 283 are parsimony informative, 112 are parsimony-uninformative, and 1136 characters are constant. The maximum likelihood (ML) analysis, based on combined LSU and ITS sequence data, resulted in five families (Amphisphaeriaceae, Apiosporaceae, Beltraniaceae, Pestalotiopsidaceae and Robillardaceae) within the subclass Xylariomycetidae (Fig. 1).

Of the newly isolated strains from Thailand, *Beltrania rhombica* (MFLUCC 15-0835) grouped together with *B. rhombica* (strain 10353) with 69% ML bootstrap support, 50% MP bootstrap support and 53% Bayesian posterior probabilities. The strains of *Beltraniella fertilis* (MFLUCC 17-2136, MFLUCC 17-2137 and MFLUCC 17-2138) formed a separate clade with 99% ML bootstrap support, 59% MP bootstrap support and 98% Bayesian posterior probabilities within the genus *Beltraniella*. *Beltraniopsis longiconidiophora* (MFLUCC 17-2139 and MFLUCC 17-2140) clustered together in a well-supported clade (BSML = 97%, BSMP = 94%, BYPP = 1.00), sister to the ex-type strain of *Beltraniopsis neolitseae* (CBS 137974). *Hemibeltrania cinnamomi* (MFLUCC 17-2141) formed a clade with a strain of *H. cinnamomi* (NFCCI 3695) and an unidentified *Hemibeltrania* species (CL12WA) with 96% ML bootstrap support, 96% MP bootstrap support and 100% Bayesian posterior probabilities.

Taxonomy

Beltraniopsis longiconidiophora C.G. Lin & K.D. Hyde, **sp. nov.** **Figs 2, 3**

Index Fungorum number: IF 553841; *Facesoffungi number*: FoF 03633

Etymology: In reference to the long setiform conidiophores.

Holotype: MFLU 17-1265

Saprobic on decaying leaves. ***Asexual morph*: *Colonies*** on plant substrate effuse, velutinous, fuscous. *Mycelium* mostly immersed in the substratum. *Conidiophores* macronematous, single or in small groups, setiform, straight or flexuous, unbranched or mostly branched in the lower part, septate, smooth, thick-walled, pale brown to dark brown at the lower part, pale brown to hyaline at the upper part, 100-680 µm long, 4-6.7 µm wide at the base, tapering to a pointed apex, arising from a dark brown, swollen, radially lobed cell, 5.5-20 µm diam. *Conidiogenous cells* polyblastic, discrete, intercalary, located in the lower part of conidiophores near the base, sympodial, denticulate, smooth, ampulliform, flask-shaped, pale brown, 5-8 µm long, 4-10 µm wide at the base. *Separating cells* ellipsoidal to ovoid, thin-walled, smooth, hyaline, 5.5-9 µm ($\bar{x}$ = 7.2 µm, n = 30) long, 3.5-6 µm ($\bar{x}$ = 4.7 µm, n = 30) wide in the broadest part. *Conidia* arise directly from separating cells, simple, dry, straight, smooth, biconic, turbinate, rostrate, hyaline with a supraequatorial transverse band, sometimes deeply constriction at the supraequatorial zone, 18-29 µm ($\bar{x}$ = 24.4 µm, n = 60) long, 3-8.5 µm ($\bar{x}$ = 5.8 µm, n = 60) wide in the broadest part. ***Sexual morph*:** Undetermined.

Culture characteristics: Conidia germinating on PDA within 12 h. Colonies on PDA effuse, pale white from above, light yellow to dark brown from below, reaching a diam. of 4-5 cm in 3 days at 25°C.

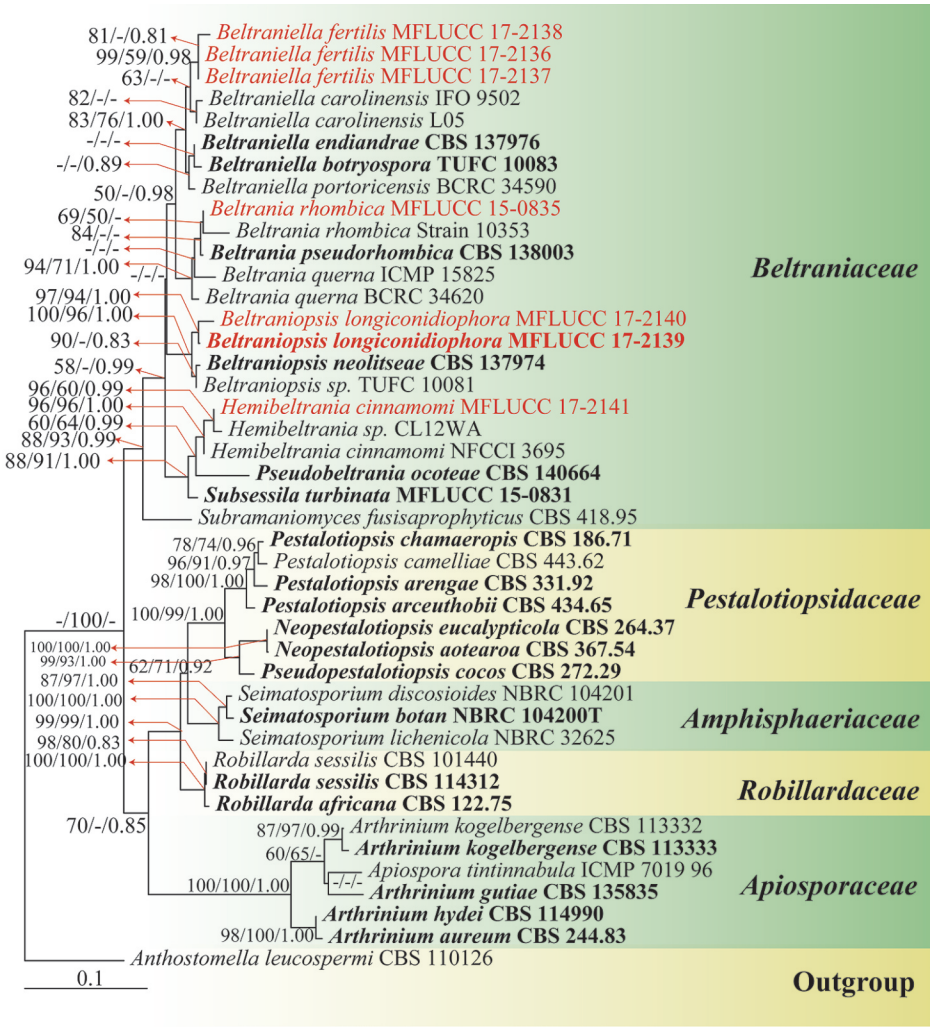

Fig. 1. Phylogenetic tree generated from maximum likelihood (ML) analysis based on combined LSU and ITS sequence data for selected families within subclass Xylariomycetidae. Bootstrap support values for maximum likelihood and maximum parsimony greater than 50% and Bayesian posterior probabilities (PP) greater than 0.8 are indicated above or below the nodes as ML/MP/PP. Ex-type strains are in bold, the new isolates are in bold and red. The tree is rooted with *Anthostomella leucospermi* (CBS 110126).

Material examined: THAILAND, Chiang Mai, on decaying leaf, 24 August 2016, Chuan-Gen Lin, MRC 6-1 (MFLU 17-1265, **holotype**; HKAS, **isotype**), ex-type living culture MFLUCC 17-2139, KUMCC; ibid, MRC 12-2 (MFLU 17-1266, **paratype**; HKAS), living culture MFLUCC 17-2140, KUMCC.

Notes: In the tree generated from maximum likelihood analysis based on combined ITS and LSU sequence data for the subclass Xylariomycetidae (Fig. 1), *Beltraniopsis longiconidiophora* (MFLUCC 17-2139 and MFLUCC 17-2140)

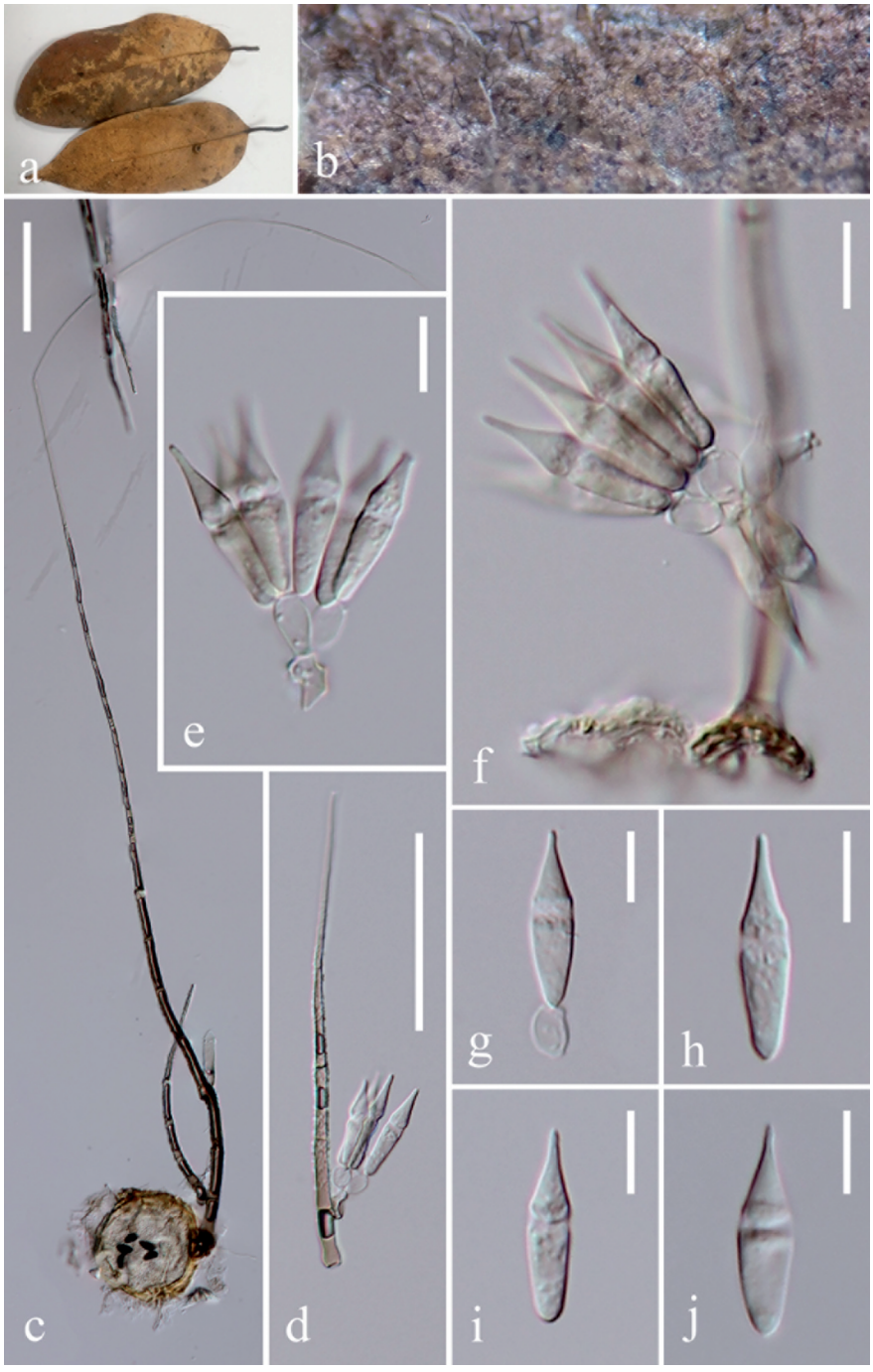

Fig. 2. ***Beltraniopsis longiconidiophora*** (MFLU 17-1265, **holotype**). **a.** Host material, **b.** Conidiophores on leaf surface, **c-d.** Conidiophores, conidiogenous cells and conidia, **e-f.** Conidiogenous cells and conidia, **g-j.** Separating cell and conidia. **Scale bars**: c-d = 50 µm, e-j = 10 µm.

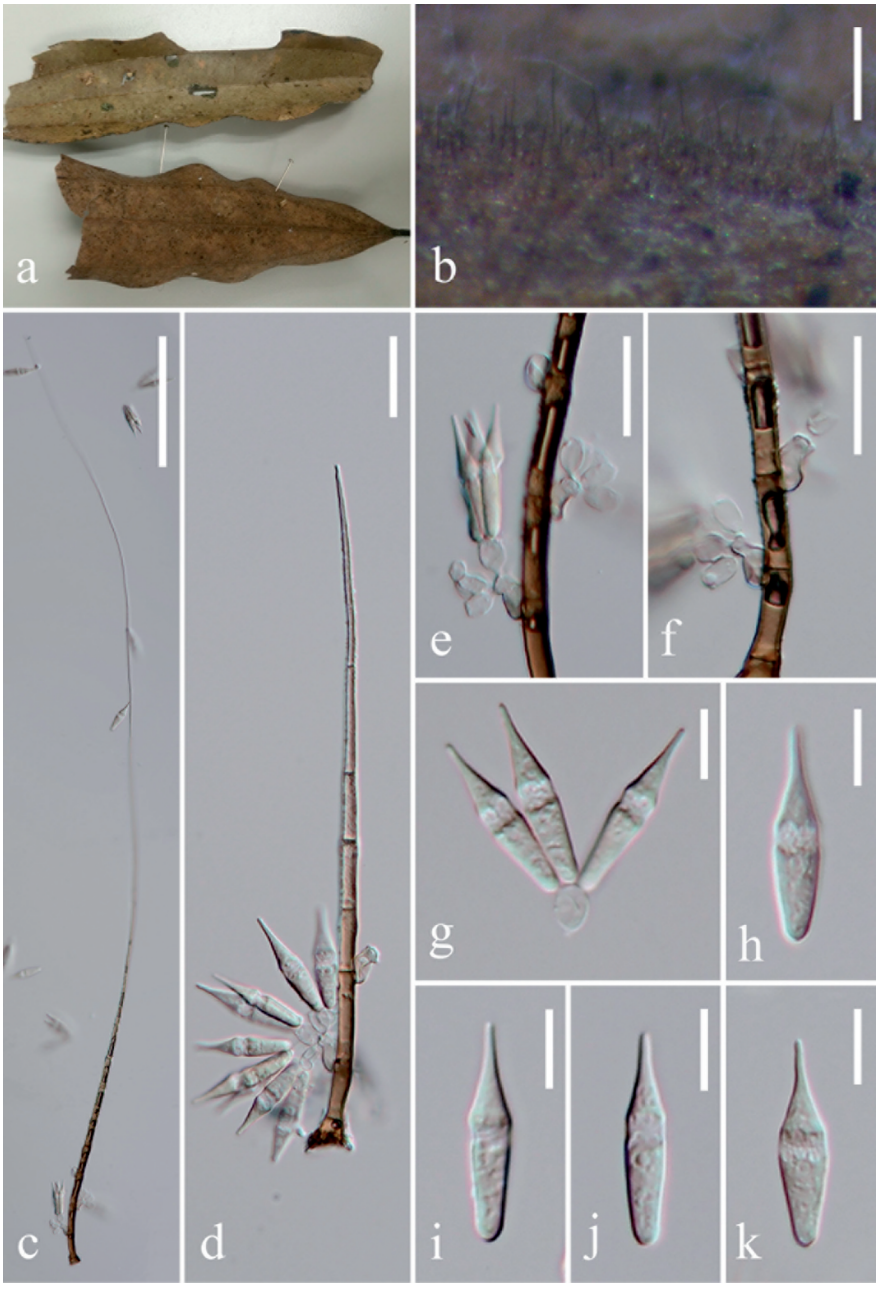

Fig. 3. ***Beltraniopsis longiconidiophora*** (MFLU 17-1266, **paratype**). **a.** Host material, **b.** Conidiophores on leaf surface, **c-d.** Conidiophores, conidiogenous cells and conidia, **e-f.** Conidiogenous cells and conidia, **g-k.** Separating cell and conidia. **Scale bars**: b = 200 µm, c = 100 µm, d-f = 20 µm, g-k = 10 µm.

grouped together with *Bel. neolitseae* (CBS 137974) and *Beltraniopsis* sp. (NFCCI 3695) with 100% ML bootstrap support, 96% MP bootstrap support and 100% Bayesian posterior probabilities within the family Beltraniaceae. *Beltraniopsis longiconidiophora* can be distinguished from *Bel. neolitseae* by its setiform conidiophores.

Morphologically, *Bel. longiconidiophora* is similar to several species within the genus *Beltraniopsis*. Based on the synopsis and key to *Beltraniopsis* species (Gusmão *et al.*, 2000), *Bel. longiconidiophora* is most similar to *Bel. ramosa* R.F. Castañeda, *Bel. esenbeckiae* Bat. & J.L. Bezerra and *Bel. tanzaniensis* Piroz. *Beltraniopsis longiconidiophora* can be distinguished from *Bel. esenbeckiae* and *Bel. tanzaniensis* by its setiform branched conidiophores without a fertile apex. *Beltraniopsis longiconidiophora* differs from *Bel. ramosa* in having longer setiform conidiophores and wider conidia.

Beltrania rhombica Penz., Michelia 2(no. 8): 474 (1882) **Fig. 4**

Facesoffungi number: FoF 03631

Saprobic on decaying leaves. ***Asexual morph*****:** *Colonies* on plant substrate effuse, dark brown, velutinous. *Mycelium* mostly immersed in the substratum. *Setae* numerous, erect, arising from radially lobed basal cells, flexuous, unbranched, single, thick-walled, smooth, pale brown at the base, pale brown to dark brown at the middle, paler at the apex, 103-167 µm long, 4-7.5 µm wide at the base, tapering to a pointed apex, arising from a dark brown, swollen, radially lobed cell, 10-16 µm diam. *Conidiophores* macronematous, single or in small groups, straight or flexuous, septate, smooth, thick-walled, mostly geniculate at the apical region, cylindrical or clavate, pale brown, 22.5-46 µm long, 2-4.5 µm wide at the base, arising from basal cells of setae or from separate dark brown, swollen, radially lobed cells, 9-18 µm diam. *Conidiogenous cells* polyblastic, integrated, terminal, sympodial, denticulate, cylindrical, clavate, pale brown, smooth, 7.5-23 µm ($\bar{x}$ = 13.3 µm, n = 20) long, 4-6.5 µm ($\bar{x}$ = 4.8 µm, n = 20) wide at the base. *Separating cells* ellipsoidal, obovoid, thin-walled, smooth, hyaline, 8.8-11.5 µm ($\bar{x}$ = 10.0 µm, n = 22) long, 4-5.7 µm ($\bar{x}$ = 5.0 µm, n = 22) wide in the broadest part. *Conidia* arise directly from conidiogenous cells or from separating cells, acrogenous, simple, dry, straight, smooth, biconic, appendiculate, rostrate, pale brown with a hyaline to subhyaline equatorial transverse band, 21-32 µm ($\bar{x}$ = 26.1 µm, n = 35) long including appendage, 6-8.5 µm ($\bar{x}$ = 7.3 µm, n = 35) wide in the broadest part. ***Sexual morph*****:** Undetermined.

Culture characteristics: Conidia germinating on PDA within 12 h. Colonies on PDA effuse, pale white from above, light yellow to dark brown from below, reaching a diam. of 4-6 cm in 3 days at 25°C.

Material examined: THAILAND, Phetchaburi, Cha-am District, Kao Yai, Khao Nang Panthurat, on decaying leaf, 28 July 2015, Chuan-Gen Lin, KNP 4-5 (MFLU 17-1261, HKAS), living culture MFLUCC 15-0835, KUMCC.

Notes: *Beltrania rhombica*, the type species of the genus *Beltrania*, was reported by Penzig (1882). It is characterized by setae and conidiophores arising from radially lobed basal cells, polyblastic and sympodial conidiogenous cells, swollen separating cells and biconic, appendiculate conidia.

Beltraniella fertilis Heredia, R.M. Arias, M. Reyes & R.F. Castañeda, Fungal Diversity 11: 100 (2002) **Fig. 5**

Facesoffungi number: FoF 03632

Saprobic on decaying leaves. ***Asexual morph*****:** *Colonies* on plant substrate effuse, thin, pale brown. *Mycelium* mostly immersed in the substratum. *Setae*

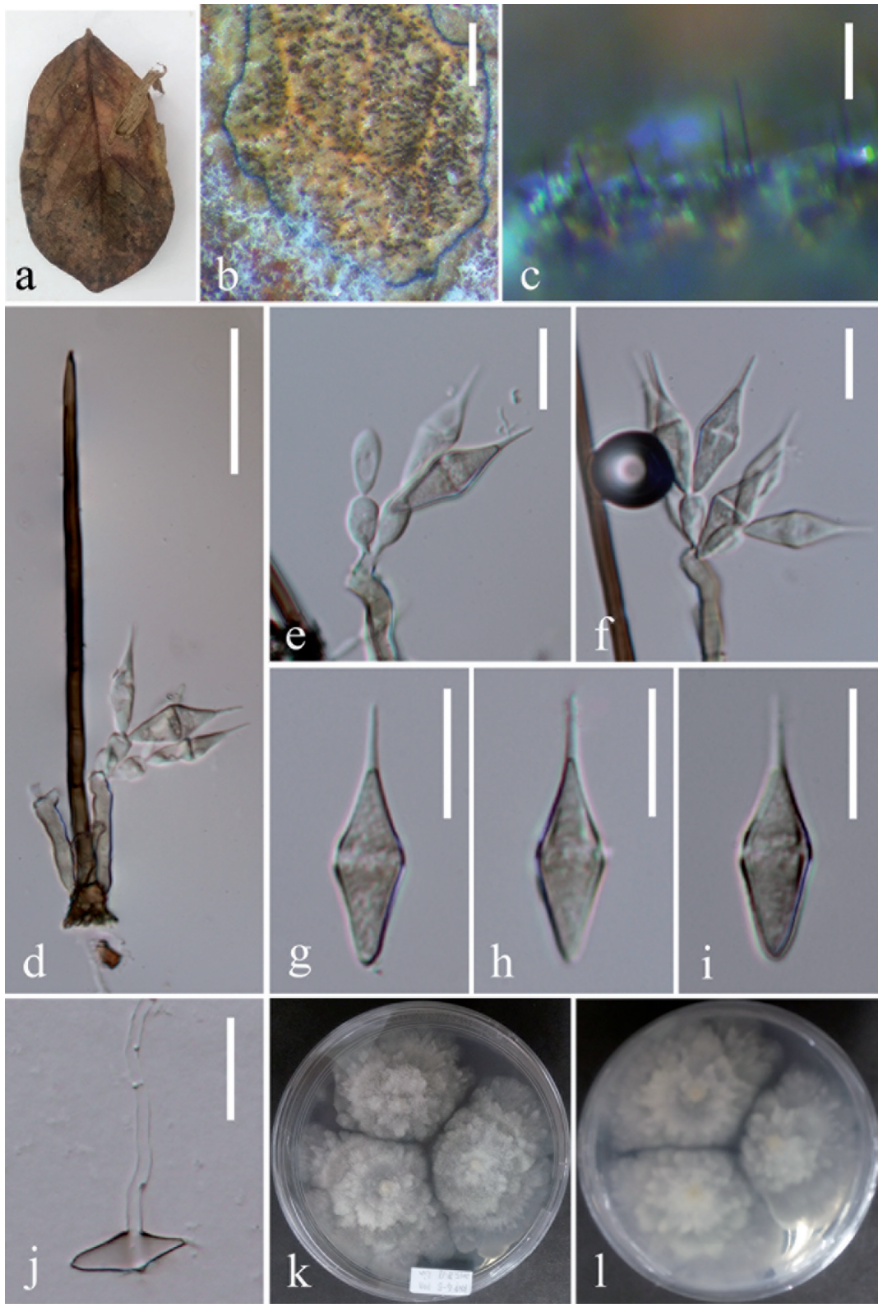

Fig. 4. ***Beltrania rhombica*** (MFLU 17-1261). **a.** Host material, **b-c.** Conidiophores on leaf surface, **d.** Conidiophores, conidiogenous cells and conidia, **e-f.** Conidiogenous cells and conidia, **g-i** Conidia, **j.** Germinating conidium, **k-l.** Colonies on PDA from above and below. **Scale bars**: b = 500 µm, c = 100 µm, d = 50 µm, e-j = 20 µm.

numerous, erect, arising from radially lobed basal cells, straight or flexuous, unbranched, single or in small groups, thick-walled, verrucose, dark brown at the base, paler at the apex, 90-220 µm long, 3.5-13 µm wide at the base, tapering to a pointed apex, arising from a dark brown, swollen, radially lobed cell, 5.5-22 µm

Fig. 5. ***Beltraniella fertilis***. **a.** Host material. **b.** Conidiophores on host surface. **c.** Setae with short conidiophores. **d.** Long conidiophore with conidiogenous cell and conidium. **e.** Short conidiophores with conidiogenous cells and conidia. **f.** Conidiogenous cells on a long conidiophore. **g-l.** Separating cells and conidia. **Scale bars**: c-d = 50 µm, e-f = 20 µm, g-l = 10 µm.

diam. *Conidiophores* macronematous, long setiform and short; long conidiophores single, straight, septate, verrucose, thick-walled, sometimes branched at the apical region, dark brown at the base and paler at the apex, 145-380 µm long, swollen at the base and 8.5-22.5 µm wide, 4-9 µm wide just above the swollen base, slightly tapering to a pointed apex; short conidiophores simple or branched, septate, smooth-walled, subhyaline to pale brown, thin-walled, 9-37.5 µm long, swollen at the base and 4.5-9.3 µm wide, 2-6.3 µm wide just above the swollen base. *Conidiogenous cells* polyblastic, integrated, determinate, terminal, cylindrical, oblong, hyaline to subhyaline, smooth, 5-22 µm ($\bar{x}$ = 11.4 µm, n = 25) long, 3-7.5 µm ($\bar{x}$ = 4.3 µm, n = 25) wide at the base. *Separating cells* ovoid or obovoid, fusiform, thin-walled, smooth, hyaline to subhyaline, 1-denticulate at each end, 7.5-11.5 µm ($\bar{x}$ = 9.6 µm, n = 30) long, 3.5-4.6 µm ($\bar{x}$ = 4.1 µm, n = 30) wide in the broadest part. *Conidia* arise directly from conidiogenous cells or from separating cells, aggregated, acrogenous, simple, dry, straight, smooth, thin-walled, biconic, turbinate to pyriform, rostrate to pointed at proximal end, truncate at distal end, hyaline to subhyaline with a hyaline supraequatorial transverse band, 19-25 µm ($\bar{x}$ = 21.2 µm, n = 30) long, 5.5-8.5 µm ($\bar{x}$ = 6.4 µm, n = 30) wide in the broadest part. ***Sexual morph*:** Undetermined.

Culture characteristics: Conidia germinating on PDA within 12 h. Colonies on PDA effuse, pale white from above, light yellow to dark brown from below, reaching a diam. of 4-5 cm in 3 days at 25°C.

Material examined: THAILAND, Chiang Mai, on decaying leaf, 24 August 2016, Chuan-Gen Lin, MRC 3BEL (MFLU 17-1263, HKAS), living culture MFLUCC 17-2137, KUMCC; ibid, MRC 4-1 (MFLU 17-1264, HKAS), living culture MFLUCC 17-2138, KUMCC; ibid, MRC 2-1 (MFLU 17-1262, HKAS), living culture MFLUCC 17-2136, KUMCC.

Notes: A key to the genus *Beltraniella* was provided by Castañeda Ruiz *et al.* (1996), while Shirouzu *et al.* (2010) provided a synopsis of all the accepted species. Twenty-two species are presently accepted in the genus *Beltraniella* (Fernando & Gusmao, 2004; Shirouzu *et al.*, 2010; Priya *et al.*, 2011; Crous *et al.*, 2014; Jaklitsch *et al.*, 2016). *Beltraniella fertilis* is similar to *Be. botryospora* Shirouzu & Tokum. as both have two types of conidiophores (long setiform and short non-setiform), polyblastic conidiogenesis, separating cells and turbinate conidia. However, *Be. fertilis* can be clearly distinguished from *Be. botryospora* by having short setae and narrower conidia. This study is the first report of *Be. fertilis* in Thailand and also the first study to produce sequence data for this species.

Hemibeltrania cinnamomi (Deighton) Piroz., Mycol. Pap. 90: 32 (1963) **Fig. 6**

≡ *Hansfordia cinnamomi* Deighton, Mycol. Pap. 78: 14 (1960)

Facesoffungi number: FoF 03634

Saprobic on decaying leaves. ***Asexual morph*:** *Colonies* on plant substrate effuse, olivaceous, velvety. *Mycelium* partly superficial, partly immersed. *Setae* absent. *Conidiophores* macronematous, single or in small groups, unbranched or occasionally with one branch near the apex, flexuous, septate, smooth, cylindrical, medium brown at the lower part, pale brown to hyaline at the upper part, 170-390 µm ($\bar{x}$ = 276 µm, n = 30) long, 3-8 µm ($\bar{x}$ = 4.7 µm, n = 25) wide at the base, arising from a dark brown, swollen, radially lobed cell, 6.9-11.8 µm ($\bar{x}$ = 9.5 µm, n = 25) diam. *Conidiogenous cells* polyblastic, integrated, terminal becoming intercalary, sympodial, cylindrical, smooth, straight or flexuous, denticulate, hyaline to pale brown, 19.5-52 µm ($\bar{x}$ = 32.7 µm, n = 20) long, 3-6.6 µm ($\bar{x}$ = 4.6 µm, n = 20) wide at the broadest part; denticles cylindrical. *Conidia* solitary,

acropleurogenous, simple, smooth, aseptate, obovoid or broadly ellipsoidal, hyaline, 15.5-21 µm ($\bar{x}$ = 18.4 µm, n = 23) long, 11-13 µm ($\bar{x}$ = 11.9 µm, n = 23) wide in the broadest part. ***Sexual morph*:** Undetermined.

Culture characteristics: Conidia germinating on PDA within 12 h. Colonies on PDA effuse, pale white from above, light yellow to dark brown from below, reaching a diam. of 4-5cm in 3 days at 25°C.

Material examined: THAILAND, Chiang Mai, on decaying leaf, 24 August 2016, Chuan-Gen Lin, MRC 12-4 (MFLU 17-1267; HKAS), living culture MFLUCC 17-2141, KUMCC.

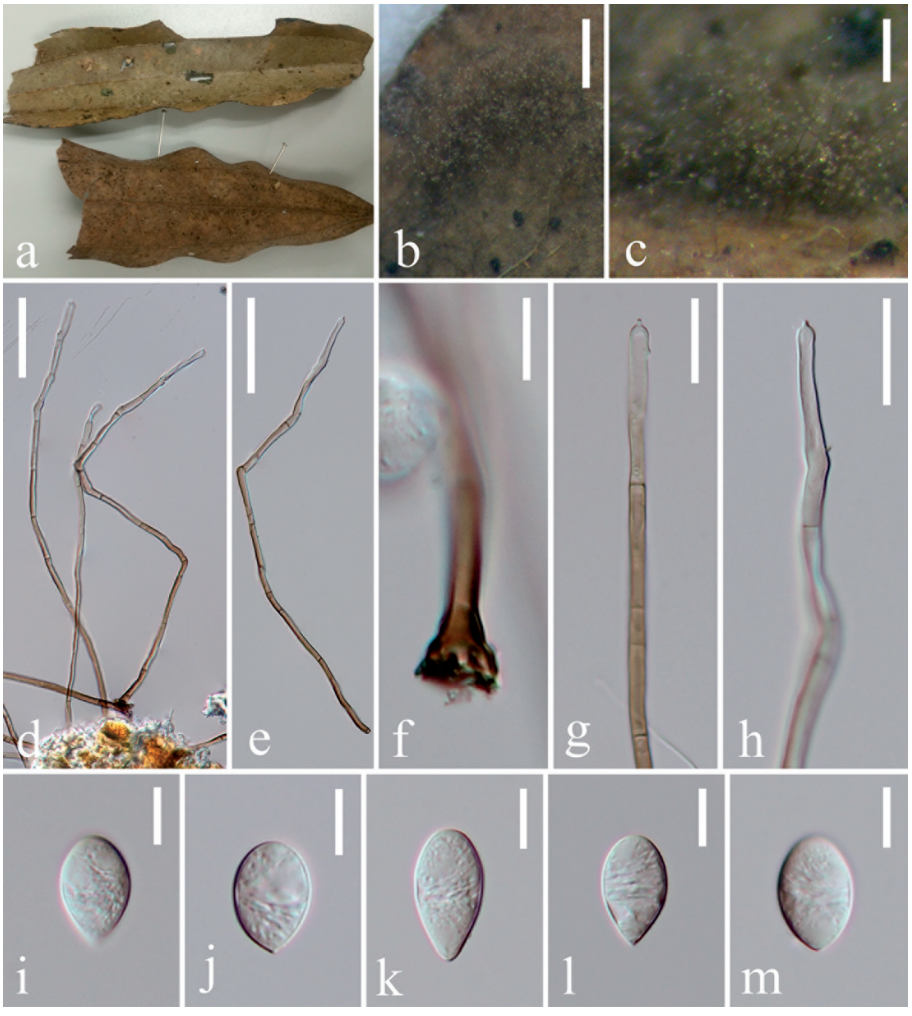

Fig. 6. ***Hemibeltrania cinnamomi*** (MFLU 17-1267). **a.** Host material. **b-c.** Conidiophores on the host surface. **d-e.** Conidiophores. **f.** Radially lobed basal cell. **g-h.** Conidiogenous cells. **i-m.** Conidia. **Scale bars**: b = 500 µm, c = 200 µm, d-e = 50 µm, g-h = 20 µm, f, i-m = 10 µm.

Notes: Deighton (1960) initially described this species as *Hansfordia cinnamomi*. Pirozynski (1963) transferred the species to *Hemibeltrania*. It is characterized by conidiophores arising from radially lobed basal cells, polyblastic and sympodial conidiogenous cells and obovoid, ellipsoidal conidia. Today there are about ten species in the genus; this is the first report of *H. cinnamomi* in Thailand.

DISCUSSION

Xylariales and Amphisphaeriales were accommodated in the subclass Xylariomycetidae (Smith *et al.*, 2003; Senanayake *et al.*, 2015; Samarakoon *et al.*, 2016). Maharachchikumbura *et al.* (2016) synonymized Amphisphaeriales under Xylariales, based on a phylogenetic analysis, and 22 families were accepted, including the family Beltraniaceae. Crous *et al.* (2015b) emended the family Beltraniaceae within the order Xylariales and accepted *Beltrania*, *Beltraniella*, *Beltraniopsis*, *Parapleurotheciopsis* and *Pseudobeltrania*. Three other genera, *Hemibeltrania*, *Porobeltraniella* and *Subramaniomyces* were accepted within the family Beltraniaceae based on published phylogenetic analyses (Maharachchikumbura *et al.*, 2016; Rajeshkumar *et al.*, 2016a). Samarakoon *et al.* (2016) recommend that Amphisphaeriales should be retained as a well-supported order, based on the application of divergence times. Hongsanan *et al.* (2017) updated the phylogeny of Sordariomycetes based on phylogenetic and molecular clock evidence; Amphisphaeriales was supported as a distinct order, and 12 families were accepted, *viz*., Amphisphaeriaceae, Apiosporaceae, Beltraniaceae, Clypeophysalosporaceae, Coniocessiaceae, Hyponectriaceae, Melogrammataceae, Oxydothidaceae, Phlogicylindriaceae, Pseudomassariaceae, Sporodaceae and Vialaeaceae.

Presently, 14 genera have beltrania-like characters, *viz.*, *Beltramono* Dubey, Pandey & Manohar., *Beltrania*, *Beltraniella*, *Beltraniomyces* Manohar., D.K. Agarwal & Rao, *Beltraniopsis*, *Beltramono* Dubey, Pandey & Manohar., *Hemibeltrania*, *Kiliophora* Kuthub. & Nawawi, *Maxibeltrania* Rambelli, *Parabeltrania* Rambelli, *Porobeltraniella*, *Pseudobeltrania*, *Rhombostilbella* Zimm., *Scolecobeltrania* Iturr., R.F. Castañeda & R. Fernández and *Subsessila* C.G. Lin & K.D. Hyde (Lin *et al.*, 2017). Ten beltrania-like species were previously reported in Thailand *viz*., *Beltrania rhombica* (Sakayaroj *et al.*, 2005; Duong *et al.*, 2008; Kodsueb *et al.*, 2008; Osono *et al.*, 2009; Monkai *et al.*, 2013), *B. mangiferae* (Duong *et al.*, 2008), *B. querna* (Wang *et al.*, 2008), *Beltraniella nilgirica* (Wang *et al.*, 2008), *Be. odinae* (Duong *et al.*, 2008), *Be. pini* (Tokumasu *et al.*, 1990), *Be. portoricensis* (Duong *et al.*, 2008; Wang *et al.*, 2008; Osono *et al.*, 2009), *Ellisiopsis occulta* (Monkai *et al.*, 2013), *Rhombostilbella rosea* (Chomnunti *et al.*, 2014) and *Subsessila turbinata* (Lin *et al.*, 2017). This number has now increased to twelve following the present study.

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