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New species in *Aciculosporium*, *Shimizuomyces* and a new genus *Morakotia* associated with plants in *Clavicipitaceae* from Thailand

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Abstract: Three new fungal species in the *Clavicipitaceae* (*Hypocreales*, *Ascomycota*) associated with plants were collected in Thailand. Morphological characterisation and phylogenetic analyses based on multi-locus sequences of LSU, *RPB1* and *TEF1* showed that two species belong to *Aciculosporium* and *Shimizuomyces. Morakotia* occupies a unique clade and is proposed as a novel genus in *Clavicipitaceae*. *Shimizuomyces cinereus* and *Morakotia fusca* share the morphological characteristic of having cylindrical to clavate stromata arising from seeds. *Aciculosporium siamense* produces perithecial plates and occurs on a leaf sheath of an unknown panicoid grass.

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INTRODUCTION

Clavicipitaceae is one of the most heterogeneous fungal families in the order *Hypocreales* (*Ascomycota*) that is associated with insects, plants, fungi and invertebrates (Gams & Zare 2003, Spatafora *et al*. 2007, Sung *et al*. 2007, Steiner *et al*. 2011, Kepler *et al*. 2012b). For instance, *Metarhizium* species are well-known entomopathogens in the *Clavicipitaceae* and are associated with both insects and plants. Furthermore, they play roles as endophytes and rhizosphere-inhabiting fungi (Greenfield *et al*. 2016, Nishi & Sato 2019, Mongkolsamrit *et al.* 2020). *Tyrannicordyceps* was proposed as a new genus associated with fungi, producing yellow or bright red stromata attacking the sclerotia of *Claviceps* (Kepler *et al*. 2012b). Clavicipitaceous fungi associated with plants have been described for species in *Aciculosporium*, *Atkinsonella*, *Balansia*, *Claviceps*, *Epichloë*, *Heteroepichloë*, *Myriogenospora*, *Periglandula*, *Shimizuomyces* and *Ustilaginoidea.* Species in *Aciculosporium*, *Atkinsonella*, *Balansia*, *Epichloë, Myriogenospora* and *Parepichloë* have been documented as fungal endophytes of grasses, and in their life cycle they can form ascomata (sexual morph) on these host plants (Cheplick & Faeth 2009, Torres & White 2009). Macro-morphology and habitats of several species associated with plants within these genera are epibiotic and produce ascomata on stems, leaves or culms, and inflorescences of plants. Most species of these genera produce pale brown to black coloured stromata such as *B. aristidae, C. purpurea*, *M. atramentosa*, *P. cinerea,* except for *Epichloë typhina* which produces white to yellow stromata (Bischoff & White 2003, Górzyńska *et al*. 2017).

The genus *Aciculosporium* was established by Miyake (1908) with *A. take* as type species. Almost a century later, a second species, *A. sasicola,* was reported from Japan (Oguchi 2001). *Aciculosporium take* and *A. sasicola* were documented as causative agents of the economically important witches' broom disease of bamboo in Japan, China, and Taiwan (Tsuda *et al*. 1997, Oguchi 2001, Tanaka *et al*. 2003). Recently, Píchová *et al*. (2018) combined *Cepsiclava phalaridis* and *Neoclaviceps monostipa* in *Aciculosporium.* To date, this genus comprises only four fungal species *viz*. *A. take, A. monostipum, A. phalaridis,* and *A. sasicola*. *Aciculosporium phalaridis* (= *Cepsiclava phalaridis*) produces stromata on sclerotia of commercial phalaris (*Phalaris aquatica*) seeds in southern New South Wales and Victoria (Walker 2004). *Neoclaviceps monostipum* was discovered in Costa Rica from unknown panicoid grasses (Sullivan *et al*. 2001).

Kobayasi (1981) established the genus *Shimizuomyces* from Japanese collections, comprising two species, namely *S. paradoxus* (type species) growing on *Smilax sieboldii* fruits and *S. kibianus* growing on *Smilax china* seeds (Kobayasi 1984). Besides Japan, *S. paradoxus* was also reported from Korea by Sung *et al.* (2010). Based on the morphological characters in the natural specimens, *Shimizuomyces* resembles *Cordyceps* in possessing brightly coloured, fleshy stromata with cylindrical stipes and enlarged apical heads (Sung *et al*. 2007).

During field surveys for arthropod-pathogenic fungi in central and western regions of Thailand, we collected two unidentified species producing brown cylindrical to clavate stromata and another species with grey stromata occurring on seeds. Based on the macro- and micro-morphological characteristics of all

collected strains, they were preliminarily identified as members of *Shimizuomyces.* Additionally, we also found one species that morphologically resembles *Aciculosporium* by producing brown ascomata on the leaf sheath of an unknown panicoid grass. The aims of this study were to clarify the placement and name these collections through molecular phylogenetic studies combined with observations of diagnostic micro-morphological characters.

MATERIALS AND METHODS

Collection and isolation

Fungal specimens occurring on seeds of dicot plants and the leaf sheath of an unknown panicoid grass were collected from Ban Phaothai community forest and Khao Yai National Park, Thailand. The specimens were collected carefully so as not to damage either host or stipe, and were placed in small plastic boxes before returning to the laboratory for isolation. The protocol for the isolation from stromata containing mature perithecia followed previous studies (Luangsa-ard *et al*. 2018, Mongkolsamrit *et al*. 2018). Ascospores were discharged on potato dextrose agar (PDA; freshly diced potato 200 g, dextrose 20 g, agar 15 g, in 1 L distilled water) and placed in a plastic box with moist tissue paper overnight to create a humid chamber with 99 % humidity at 25 °C. The following morning, plates were examined with an Olympus SZ61 dissecting microscope to observe discharged ascospores that were then transferred to fresh PDA plates. Pure cultures were deposited at the BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology, Thailand. Specimens were dried in an electric food dryer (50–55 °C) overnight and stored in plastic boxes before storage at the BIOTEC Bangkok Fungarium (BBH), National Biobank of Thailand.

Morphological study

Fungal structures, such as perithecia, asci and ascospores were mounted in lactophenol cotton blue solution and measured using a compound microscope. Twenty to fifty perithecia, asci, ascospores, phialides and conidia were measured and the range and standard deviation calculated. Morphological characters of these structures were photographed using an Olympus DP70 Digital Camera mounted on an Olympus BX51 compound microscope and SZX12 (Olympus) dissecting microscope. Colour changes of stromata were monitored in 3 % potassium hydroxide (KOH). For detailed morphological comparisons of conidia, phialides and colony colours, cultures were grown on PDA agar plates and 2 % malt extract agar (2 % MEA Difco; malt extract, 20 g; agar, 15 g in 1 L distilled water) at 25 °C under a zeitgeber 14:10 light : dark cycle for 21 to 30 d, depending on fungal sporulation. Protocols for culture observations and comparisons followed Mongkolsamrit *et al*. (2018). Colours of fresh specimens and cultures incubated on PDA and MEA were described following the Sixth Royal Horticultural Society (R.H.S.) Colour Chart.

DNA extraction, sequencing and phylogenetic analysis

Genomic DNA was harvested from mycelia on PDA plates and small pieces of fresh stromatal tissue using a modified cetyltrimethyl-ammonium bromide (CTAB) method as described previously (Mongkolsamrit *et al*. 2009). Nuclear loci, including nuc 28 rDNA (Large Subunit Ribosomal DNA: LSU), the partial gene regions of the RNA polymerase II largest subunit (*RPB1*) and the translation elongation factor-1α gene (*TEF1*), were sequenced. The primer pairs and thermocycler conditions for PCR amplifications used in this study followed the method described in Mongkolsamrit *et al*. (2019). The purified PCR products were sequenced with PCR amplification primers for Sanger dideoxy sequencing. The PCR amplicon sequences were examined for ambiguous base calls using BioEdit v. 7.2.5 (Hall 2004). Verified sequences were submitted to GenBank. Multi-locus sequences of closely-related taxa for analyses were taken from previous studies as shown in Table 1. The final alignment was deposited in TreeBASE (www.treebase.org) under accession number ID 26949. Phylogenetic analysis was performed using RAxML-VI-HPC2 v. 8.2.12 (Stamatakis 2014) on XSEDE (http://www.phylo.org/), with 1 000 bootstrap iterations. Bayesian inference (BI) analysis was performed in MrBayes v. 3.2.7a (Ronquist *et al*. 2012) on XSEDE, with the GTR + I + G model (General Time Reversible model with a proportion of invariable sites and a gamma-shaped distribution of rates across sites). Markov chain Monte Carlo (MCMC) simulations were run for 5 000 000 generations, sampling every 1 000, and discarding the first 10 % as burn-in. RAxML output was imported into TreeView v. 1.6.6 to view the phylogenetic tree (Page 1996; http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

RESULTS

Molecular phylogeny

We generated seven LSU, five *RPB1* and six *TEF1* sequences in this study from living cultures and fresh stromata (Table 1). The combined dataset of 55 taxa with multi-locus sequences had a total alignment length of 2 286 characters. Sequences of *Simplicillium lanosoniveum* CBS 704.86 and *Simplicillium lanosoniveum* CBS 101267 in the *Cordycipitaceae* were used as outgroups. The RAxML analysis resulted in a single tree which is shown in Fig. 1. The phylogenetic tree strongly supports *Aciculosporium, Morakotia* and *Shimizuomyces* as monophyletic clades. The descriptions based on morphological characters of two new species belong to *Aciculosporium* and *Shimizuomyces*, and a new genus *Morakotia* are provided below.

Taxonomy

Aciculosporium siamense Mongkolsamrit, Noisripoom & Luangsa-ard, ***sp. nov.*** MycoBank MB 838347. Fig. 2A–S.

Etymology: The specific epithet refers to the old name of the Kingdom of Thailand*, Siam*.

Typus: **Thailand**, Saraburi Province, Chet Kot Waterfall, Khao Yai National Park**,** on leaf sheath (*Poaceae*), 8 Jan. 2017, *U. Pinruan (UP), S. Mongkolsamrit (SM)* & *P. Srikitikulchai (PS)*, SM 2081 (holotype BBH 43077, ex-type culture BCC 85382).

Ascomata hemispherical perithecial plates, singly or composed of multiple perithecial plates, pale brownish orange (N167A), 2–8 mm in diam, 2–3 mm high. *Perithecia* immersed, obovate, 420–550(–600) × (160–)180–220(–230) µm, with dark brown ostioles. *Asci* cylindrical, (165–)203–347(–400) × 4–(4.5–5) µm with caps 4–5 µm thick. *Ascospores* hyaline, filiform with one end blunt and

Table 1. List of species and GenBank accession numbers of sequences used in this study. The novelties described here are in bold font.

Species	Strain	Host/Substratum	GenBank Accession no.			References
			LSU	*RPB1*	*TEF1*	
Aciculosporium monostipum	INBio 6 141^{T}	*Poaceae*	AF245293	DQ000353	AY986983	Sullivan *et al.* (2001), Chaverri *et al*. (2005b)
Aciculosporium phalaridis	CCC 293	*Poaceae*	–	–	LT216524	Píchová *et al*. (2018)
Aciculosporium siamense	**BCC 85382^{T}**	***Poaceae***	**MT743002**	–	**MT762147**	This study
	BCC 85384	***Poaceae***	**MT743003**	**MT762149**	**MT762148**	This study
Aciculosporium take	MAFF 241224	Plants	–	KC113319	KP689550	Schardl *et al*. (2013)
Aschersonia samoensis	BCC 2097	*Hemiptera*	AF327381	DQ000346	AY986945	Artjariyasripong *et al*. (2001), Chaverri *et al*. (2005b)
Atkinsonella hypoxylon	B4728	Plants	–	–	KP689546	Young *et al.* (2015)
Balansia henningsiana	GAM 16112	*Poaceae*	AY545727	AY489643	AY489610	Castlebury *et al*. (2004)
Balansia pilulaeformis	A.E.G. 94-2	*Poaceae*	AF543788	DQ522365	DQ522319	Currie *et al*. (2003), Spatafora *et al*. (2007)
Claviceps purpurea	GAM 12885	*Poaceae*	AF543789	AY489648	AF543778	Currie *et al.* (2003), Castlebury *et al*. (2004)
	S.A. cp11	*Poaceae*	EF469075	EF469087	EF469058	Sung *et al*. (2007)
Conoideocrella luteorostrata	NHJ 12516	*Hemiptera*	EF468849	EF468905	EF468800	Sung *et al*. (2007)
	NHJ 11343	*Hemiptera*	EF468850	EF468906	EF468801	Sung *et al*. (2007)
Conoideocrella tenuis	NHJ 6293	*Hemiptera*	EU369044	EU369068	EU369029	Johnson *et al*. (2009)
	NHJ 6791	*Hemiptera*	EU369046	EU369069	EU369028	Johnson *et al*. (2009)
Corallocytostroma ornithocopreoides	WAC 8705	Plants	–	–	LT216546	Píchová *et al*. (2018)
Dussiella tuberiformis	J.F. White	*Hemiptera*	–	JQ257015	JQ257027	Kepler *et al*. (2012b)
Epichloë elymi	C. Schardl760	–	AY986924	DQ000352	AY986951	Chaverri *et al*. (2005b)
Epichloë typhina	ATCC 56429	*Poaceae*	U17396	AY489653	AF543777	Rehner & Samuels (1995), Currie *et al*. (2003), Castlebury *et al.* (2004)
Helicocollum surathaniense	BCC 34463	*Hemiptera*	KT222328	–	KT222336	Luangsa-ard *et al*. (2017a)
	BCC 34464^{T}	*Hemiptera*	KT222329	–	KT222337	Luangsa-ard *et al*. (2017a)
Hypocrella discoidea	I93-901D	*Hemiptera*	EU392567	EU392700	EU392646	Chaverri *et al*. (2008)
Metapochonia goniodes	CBS 891.72^{T}	*Nematoda*	AF339550	DQ522401	DQ522354	Sung *et al*. (2001), Spatafora *et al*. (2007)
Metarhizium anisopliae	CBS 130.71^{T}	*Avena sativa*	MT078853	MT07886	MT078845	Mongkolsamrit *et al*. (2020)
Metarhizium minus	ARSEF 1099	*Hemiptera*	–	KJ398608	KJ398799	Kepler *et al*. (2014)
	ARSEF 2037^{T}	*Hemiptera*	AF339531	DQ522400	DQ522353	Spatafora *et al*. (2007)
Morakotia fusca	**BCC 64125**	**Plant**	**KY794862**	–	**KY794857**	This study
	BCC 79272^{T}	**Plant**	**KY794861**	**KY794865**	**KY794856**	This study
	BCC 79273	**Plant**	**KY794860**	**KY794866**	–	This study
Moelleriella phyllogena	P.C.555	*Hemiptera*	EU392610	EU392726	EU392674	Chaverri *et al*. (2008)

Table 1. (Continued).

Species	Strain	Host/Substratum	GenBank Accession no.			References
			LSU	*RPB1*	*TEF1*	
	J.B.130	*Hemiptera*	EU392610	EU392726	EU392674	Chaverri *et al*. (2008)
Myriogenospora atramentosa	A.E.G.96-32	*Poaceae*	AY489733	AY489665	AY489628	Castlebury *et al*. (2004)
Nigelia aurantiaca	BCC 37621	*Lepidoptera*	GU979946	GU979964	GU979955	Luangsa-ard *et al*. (2017b)
	BCC 37627	*Lepidoptera*	GU979947	GU979965	GU979956	Luangsa-ard *et al*. (2017b)
Orbiocrella petchii	NHJ 6240	*Hemiptera*	EU369038	EU369060	EU369022	Johnson *et al.* (2009)
	NHJ 6209	*Hemiptera*	EU369039	EU369061	EU369023	Johnson *et al.* (2009)
Periglandula ipomoeae	IasaF 13	Plant	–	JN587270	–	Schardl *et al.* (2013)
Purpureomyces khaoyaiensis	BCC 1376[T]	*Lepidoptera*	KX983462	–	KX983457	Luangsa-ard *et al*. (2017b)
	BCC 14290	*Lepidoptera*	JF415970	JN049888	JF416012	Kepler *et al.* (2012a)
Regiocrella camerunensis	CUP 67512[T]	*Hemiptera*	DQ118735	DQ127234	DQ118743	Chaverri *et al*. (2005a)
Regiocrella sinensis	CUP CH-2640[T]	*Hemiptera*	DQ118736	DQ127235	DQ118744	Chaverri *et al*. (2005a)
Rotiferophthora angustispora	CBS 101437	*Rotifera*	AF339535	DQ522402	AF543776	Chaverri *et al*. (2005a), Currie *et al*. (2003), Spatafora *et al*. (2007)
Samuelsia chalalensis	P.C. 560	*Hemiptera*	EU392637	EU392743	EU392691	Chaverri *et al*. (2008)
Samuelsia rufobrunnea	P.C. 613	*Hemiptera*	AY986918	DQ000345	AY986944	Chaverri *et al*. (2005b)
Shimizuomyces cinereus	**BBH 41714[T]**	***Smilacaceae* (Plant)**	**KY794864**	**KY794867**	**KY794859**	This study
	BBH 41715	***Smilacaceae* (Plant)**	**KY794863**	**KY794868**	**KY794858**	This study
Shimizuomyces paradoxus	EFCC 6279	*Smilacaceae* (Plant)	EF469084	EF469100	EF469071	Sung *et al*. (2007)
	EFCC 6564	*Smilacaceae* (Plant)	EF469083	EF469101	EF469072	Sung *et al*. (2007)
Simplicillium lanosoniveum	CBS 704.86	*Hemileia vastatrix* (*Uredinales*)	AF339553	DQ522406	DQ522358	Sung *et al.* 2001, Spatafora *et al*. (2007)
	CBS 101267	*Hemileia vastatrix* (*Uredinales*)	–	DQ522405	DQ522357	Spatafora *et al*. (2007)
Tyrannicordyceps fratricida	TNS 19011	Fungi	JQ257023	JQ257016	JQ257028	Kepler *et al*. (2012b)
Ustilaginoidea virens	ATCC 16180	Plant	–	JQ257014	JQ257026	Kepler *et al*. (2012b)
	MAFF 240421	Plant	JQ257011	–	JQ257024	Kepler *et al*. (2012b)
Verticillium epiphytum	CBS 154.61[T]	*Hemileia vastatrix* (*Uredinales*)	AF339548	–	EF468802	Sung *et al*. (2001), Sung *et al*. (2007)
	CBS 384.81	*Hemileia vastatrix* (*Uredinales*)	AF339547	DQ522409	DQ522361	Sung *et al*. (2001), Spatafora *et al.* (2007)

[T] = Type species.

narrow at the other end, 3-septate, (60–)71.5–95.5(–125) × (1–)1.5–2 µm. Colour change of stromata in 3 % KOH not observed.

Culture characteristics: Colonies on PDA attaining 8–10 mm diam in 14 d, yellowish white (N158C), reverse uncoloured. Dimorphic with mass of conidia, yeast-like and vegetative hyphae smooth, wet, 3–4 µm diam. *Conidia* holoblastic, hyaline, cylindrical to filiform, narrower at one end than the other, 1–3-septate, (22–)33–57.5(–70) × 1.5–2 µm, with dichotomously branched appendages, usually appearing at the narrow end of conidia, 1–5 × 0.5 µm.

Colonies on 2 % MEA attaining 4–5 mm diam in 14 d, light yellow (163D), reverse uncoloured. Colonies dimorphic, producing a cerebriform yeast-like mass of conidia at the centre

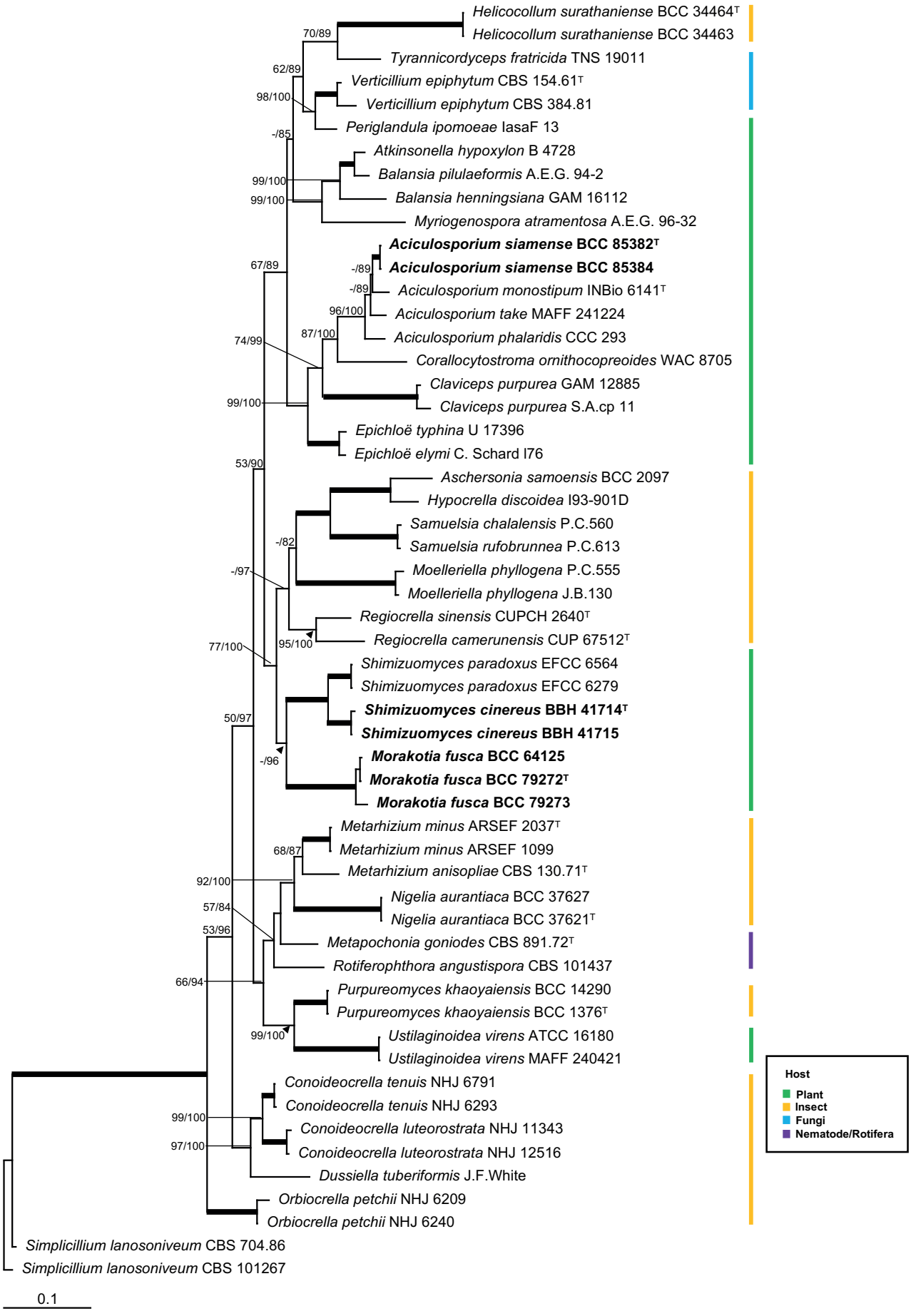

Fig. 1. RAxML tree of *Aciculosporium siamense*, *Morakotia fusca* and *Shimizuomyces cinereus* with other genera in the *Clavicipitaceae* from a combined LSU, *RPB1* and *TEF1* dataset. Numbers at the major nodes represent maximum likelihood bootstrap values (MLBP) and Bayesian posterior probabilities (BPP) multiplied by 100. Fully-supported (MLBP/BPP = 100/100) branches are thickened.

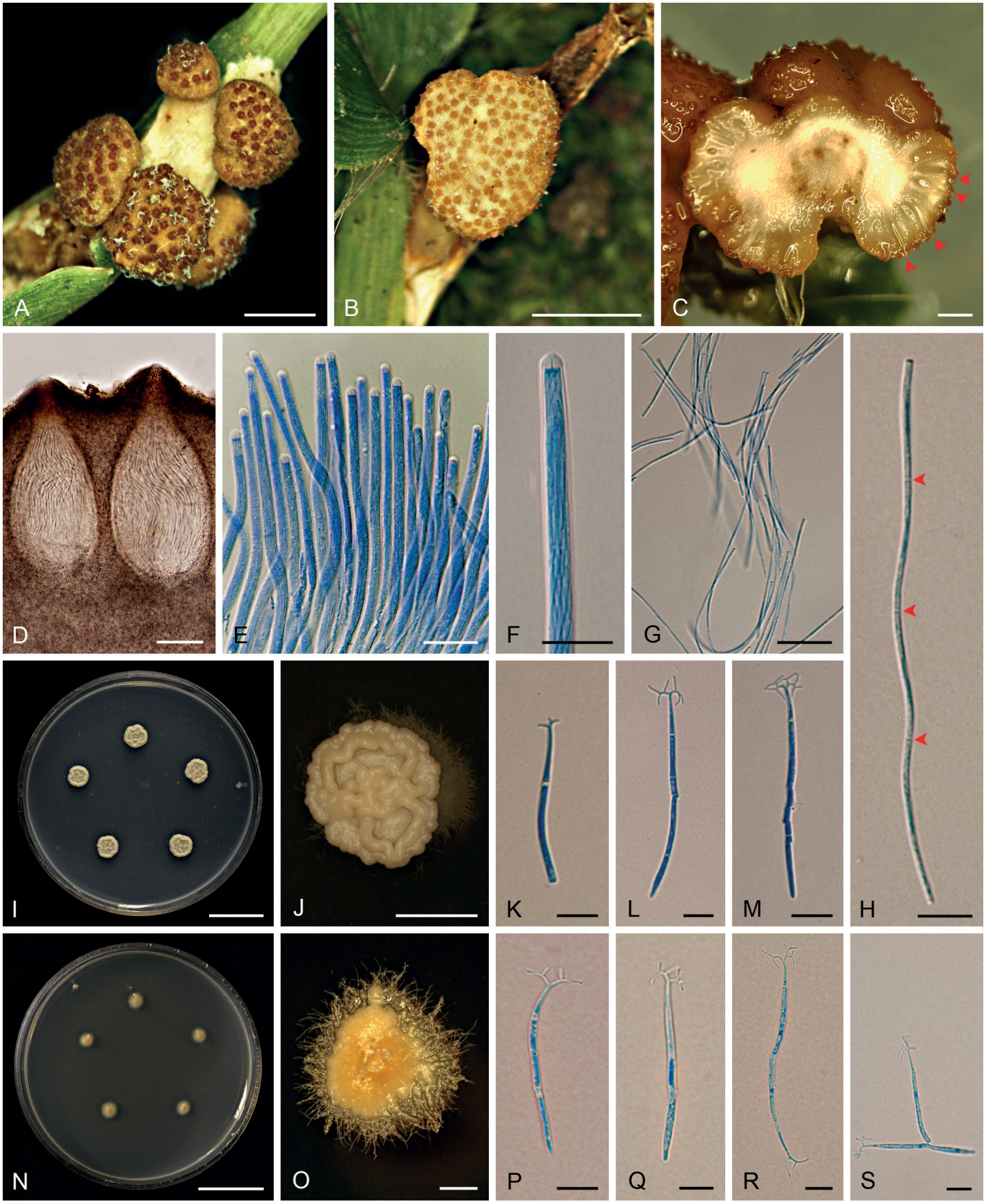

Fig. 2. *Aciculosporium siamense* (BBH 43077, BCC 85382). **A–C.** Ascomata on leaf sheaths of grass. **D.** Perithecia. **E.** Asci. **F.** Ascus tip. **G.** Whole ascospores. **H.** Whole ascospore with septations. **I, J.** Colonies on PDA. **K–M.** Holoblastic conidia on PDA. **N, O.** Colonies on 2 % MEA. **P–S.** Holoblastic conidia on 2 % MEA. Scale bars: I, N = 20 mm; A, J = 5 mm; B, C, O = 1 mm; D = 100 µm; E, G = 20 µm; F, H, P–S = 10 µm; K–M = 5 µm.

and smooth vegetative hyphae on the edges of the colony, wet, 3–4 µm diam. *Conidia* holoblastic, hyaline, cylindrical to filiform, narrower at one end than the other, 1–2-septate, (24–)30–50(–60) × 1.5–2 µm, with dichotomously branched appendages, usually appearing at the narrow end of conidia, 1–6 × 0.5 µm.

Distribution: Found in the central and western regions of Thailand.

Additional materials examined: **Thailand**, Chet Kot Waterfall, Khao Yai National Park**,** on leaf sheath (*Poaceae*), 8 Jan. 2017, *UP*, *SM & PS*, SM 2080 (BBH 43076 paratype), ex-paratype culture BCC 85381, SM 2082 (BBH 43078), culture BCC 85383, SM 2083 (BBH 43079), culture BCC 85384; Pi Tu Kro Waterfall, Umphang Wildlife Sanctuary, on leaf sheath (*Poaceae*), 26 Jun. 2008, *SM*, *K. Tasanathai (KT)*, *B. Thongnuch (BT)*, *PS*, *AK & J.J. Luangsa-ard (JJL)*, SM 517 (BBH 24722), culture BCC 32351.

Notes: *Aciculosporium siamense* is a rare species in Thailand, found only in Chet Kot Waterfall and Pi Tu Kro Waterfall. This species produces single to multiple hemispherical perithecial plates similar to the sexual morph of *Aschersonia luteola* and *A. badia* by producing crowded perithecia immersed in stromata (Mongkolsamrit *et al.* 2009). However, *Aciculosporium siamense* differs from *Aschersonia luteola* and *A. badia* on the basis of their hosts. *Aciculosporium siamense* occurs on leaf sheaths (*Poaceae*), while *Aschersonia luteola* and *A. badia* occur on scale insects (*Hemiptera*) and are found on the underside of leaves.

Morakotia Mongkolsamrit, Noisripoom, Khonsanit, Thanakitpipattana & Luangsa-ard, ***gen. nov.*** MycoBank MB 838348.

Etymology: In honour of Prof. Dr Morakot Tanticharoen, for her support of invertebrate-pathogenic fungi research in BIOTEC, Thailand.

Stromata solitary or multiple, unbranched, tough, arising from seed plant, cylindrical to clavate, moderate orange yellow to brown orange (164A–164B). *Fertile part* clavate. *Perithecia* crowded, densely packed, ovoid to long ovoid, ordinal in arrangement, completely immersed, with a reddish brown ostioles. *Asci* cylindrical. *Ascospores* hyaline, whole, filiform, elongate clavate with septations.

Type species: *Morakotia fusca* Mongkolsamrit, Noisripoom, Khonsanit, Thanakitpipattana & Luangsa-ard

Morakotia fusca Mongkolsamrit, Noisripoom, Khonsanit, Thanakitpipattana & Luangsa-ard, ***sp. nov.*** MycoBank MB 838349. Fig. 3A–P.

Etymology: The specific epithet is from the Latin "*fuscus*", referring to brown colour of fresh stromata.

Typus: **Thailand**, Phitsanulok Province, Ban Phaothai community forest, on seed (*Smilacaceae*), in leaf litter, 10 Oct. 2015, *A. Khonsanit (AK)*, *D. Thanakitpipattana (DT)*, *S. Lamlertthon (SL)*, *SM* & *W. Noisripoom (WN)*, MY 10972 (holotype BBH 41710, ex-type culture BCC 79272).

Stromata solitary or multiple, unbranched, tough, 20–75 mm long, 0.5–2 mm broad, cylindrical to enlarging apically, arising from the seed buried approximately 5–10 mm underground. *Fertile part* moderate orange yellow to brown orange (164A–164B), cylindrical to clavate, 5–20 mm long, 1.5–2.5 mm broad. *Perithecia* completely immersed, ordinal in arrangement, narrow flask-shaped, (320–)380–510(–570) × (120–)130–165(–180) µm, ostioles darker reddish orange (175B). *Asci* cylindrical, 8-spored, (105–)160–240(–245) × (7–)8–9(–10) µm with caps 4–5 µm thick. *Ascospores* hyaline, whole, filiform, 7–8-septate, (70–)80–95(–105) × 2–4 µm. Colour change of stromata in 3 % KOH not observed.

Culture characteristics: Colonies on PDA attaining 30–35 mm diam in 30 d, cottony with high mycelial density, pale yellow (11C–D) to strong orange yellow (163B), reverse pale brown. *Synnemata* deep orange yellow (163A), 5–20 × 2–2.5 mm. *Conidiogenous* structures consisting of erect conidiophores arising from the vegetative hyphae. *Conidiophores* consist of verticillate phialides, singly or in whorls of two. *Phialides* awl-shaped, (20–)22–35(–40) × 1.5–2.5 µm. *Conidia* hyaline, globose, not in chains, (4–)4.5–5.5(–6) µm diam. *Chlamydospores* not observed.

Colonies on 2 % MEA attaining 20–25 mm diam in 30 d, cottony, white, scarce mycelial density, reverse brownish orange (N167B). *Conidiogenous* structures consisting of erect conidiophores arising from the vegetative hyphae or monophialidic arising along the hyphae. *Conidiophores* consist of verticillate phialides, singly or in whorls of two. *Phialides* awl-shaped, (20–)25–35(–40) × (1–)1.5–2(–2.5) µm. *Conidia* hyaline, globose, singly not in chains, (4–)4.5–6 µm diam. *Chlamydospores* not observed.

Distribution: Found in the central and northeastern regions of Thailand.

Additional materials examined: **Thailand**, Phitsanulok Province, Ban Phaothai community forest, on seed (*Smilacaceae*), in the leaf litter, 10 Oct. 2015, *AK*, *DT*, *SL*, *SM & W. Noisripoom (WN)*; MY 10973 (BBH 41711 paratype), ex-paratype culture BCC 79273, MY 10974 (BBH 41712), culture BCC 79274; *idem.,* 4 Sep. 2016, *SM*, *WN*, *R. Somnuk* (*RS*), *PS*, *KT*, *DT*, *S. Wongkanoun* (*SW*), MY 11425 (BBH 41790), culture BCC 82798; Nakhon Ratchasima, Khao Yai National Park, on seed (*Palmae*), in the leaf litter, 24 Jun. 2012, *AK*, *SM*, *WN*, *RS*, *PS & KT*, MY 8554 (BBH 37585), culture BCC 64124, MY 8555 (BBH 37740) culture BCC 64125, *idem.,* 26 Jun. 2012, *AK*, *SM*, *WN*, *RS*, *PS & KT*, MY 8624 (BBH 37745), culture BCC 64172.

Notes: Based on the natural specimen*, Morakotia fusca* is similar to *Tolypocladium ophioglossoides* in the colour and shape of its stromata. Both *Morakotia fusca* and *T. ophioglossoides* have brown orange and cylindrical stromata. However, *Morakotia fusca* differs from *T. ophioglossoides* on the basis of their hosts. *Morakotia fusca* occurs on seeds, whereas *T. ophioglossoides* occurs on truffles.

Shimizuomyces cinereus Mongkolsamrit, Noisripoom, Khonsanit, Thanakitpipattana & Luangsa-ard, ***sp. nov.*** MycoBank MB 838350. Fig. 4A–H.

Etymology: The specific epithet is from the Latin "*cinereus*", referring to the grey colour of the stroma.

Typus: **Thailand,** Phitsanulok Province, Ban Phaothai community forest, on seed (*Smilacaceae*), in the leaf litter, 10 Oct. 2015, *AK*, *DT*, *SL*, *SM* & *WN*, MY 10976 (holotype BBH 41714).

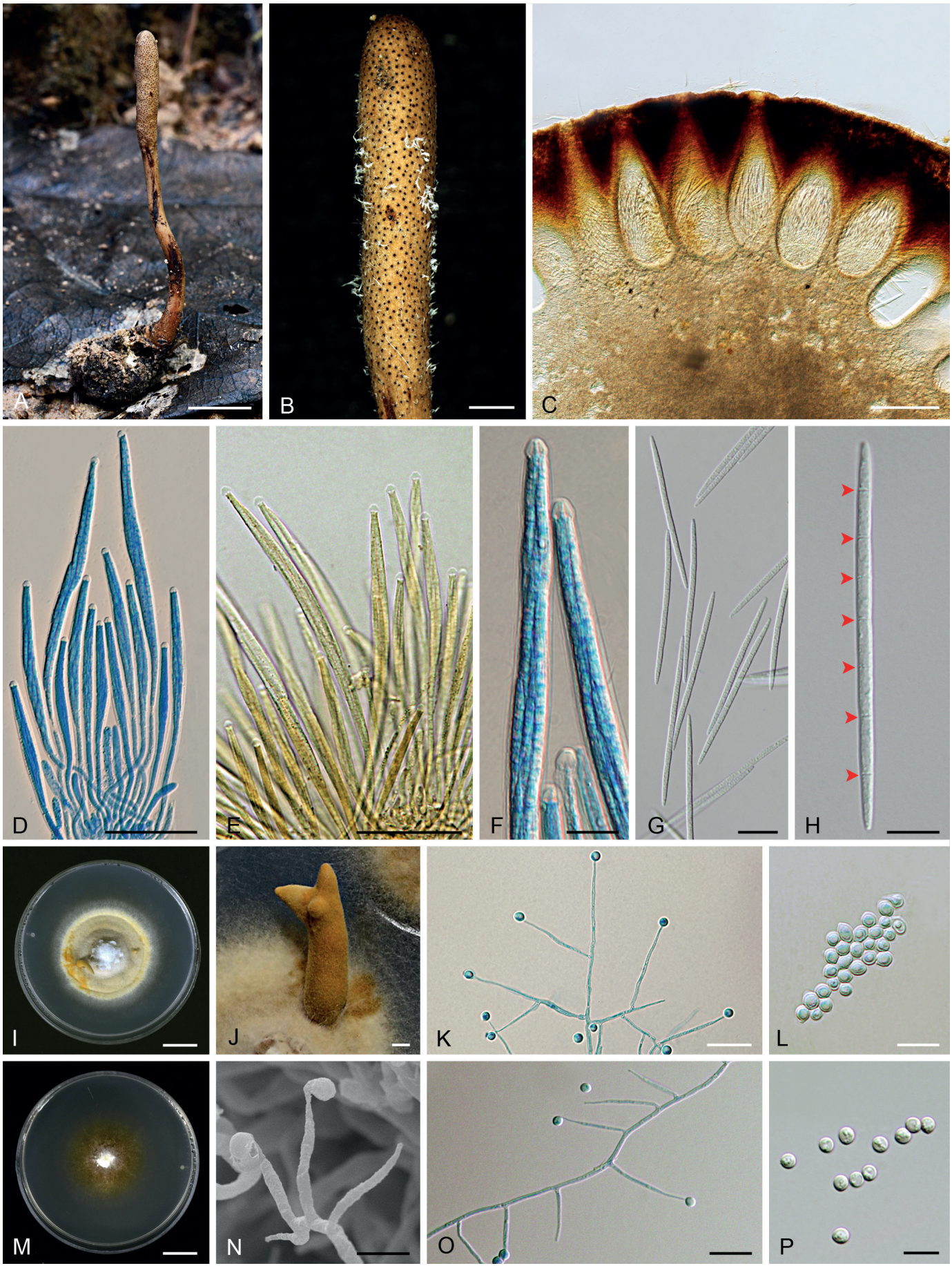

Fig. 3. *Morakotia fusca* (BBH 41710, BCC 79272). **A.** Stroma on seed. **B.** Fertile part of stroma. **C.** Immersed perithecia. **D–F.** Asci with asci caps. **G, H.** Whole ascospores with septations (arrows). **I, J.** Colony with synnema on PDA. **K.** Conidiophores consisting of verticillate phialides on PDA. **L.** Conidia on PDA. **M.** Colony on 2 % MEA. **N, O.** Conidiophores consisting of verticillate phialides on 2 % MEA. **P.** Conidia on 2 % MEA. Scale bars: I, M = 10 mm; A = 5 mm; B, J = 1 mm; C = 200 µm; D, E = 50 µm; G, K, O = 20 µm; F, H, L, N, P = 10 µm.

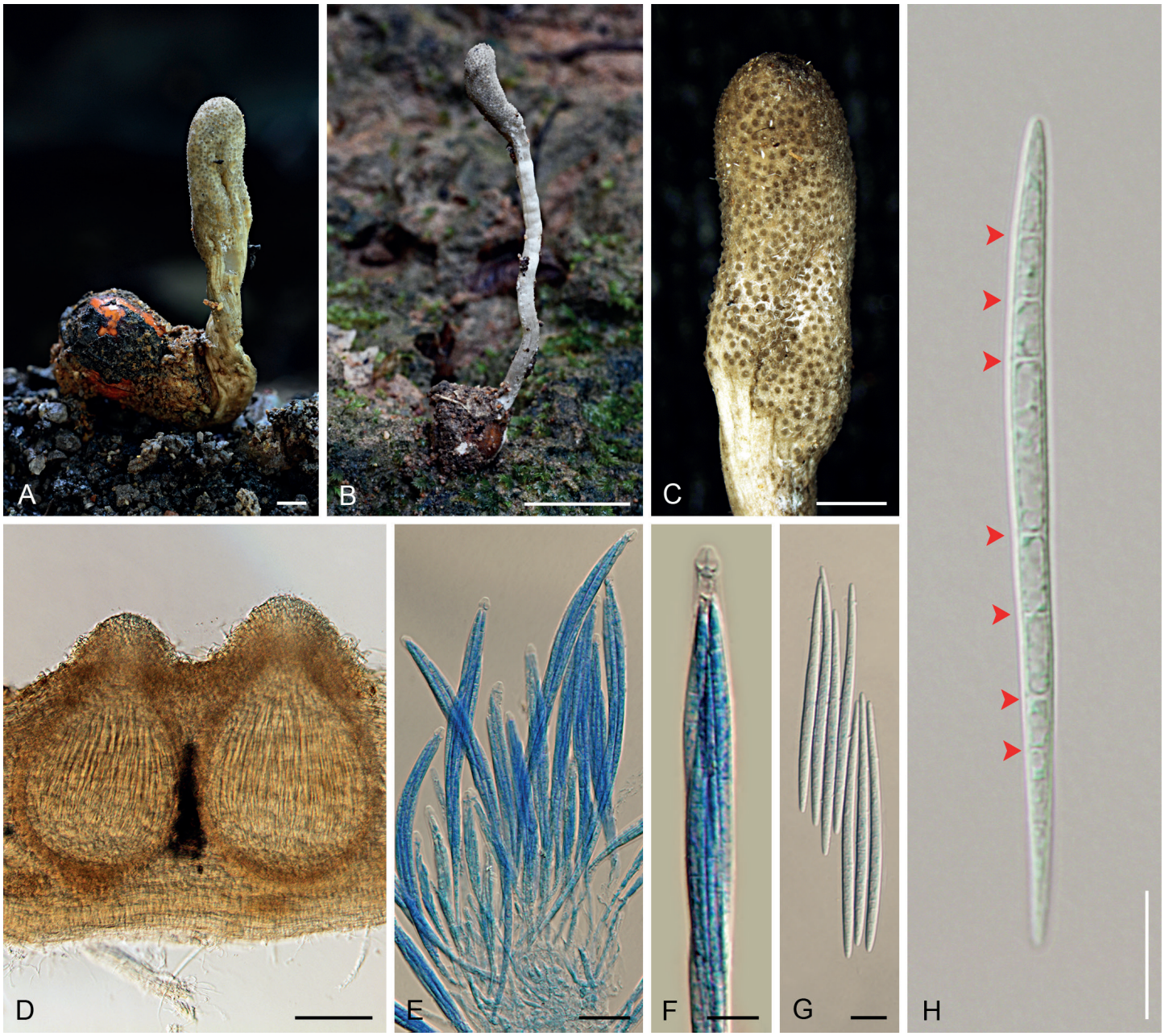

Fig. 4. *Shimizuomyces cinereus* (BBH 41714). **A, B.** Stroma on seed. **C.** Fertile part of stroma. **D.** Perithecia. **E, F.** Asci and asci caps. **G, H.** Whole ascospores with septations (arrows). Scale bars: B = 5 mm; A, C = 1 mm; D = 100 µm; E = 20 µm; F–H = 10 µm.

Stroma solitary, unbranched, 10–28 mm in long and 0.5–2 mm broad, cylindrical to enlarging apically, arising from the seed buried approximately 5–10 mm in the leaf litter. *Fertile part* yellowish grey (A–B), 3–8 × 1.5–3 mm, cylindrical to clavate. *Perithecia* immersed, with ostioles slightly projecting, ordinal in arrangement, pyriform, (310–)320–370(–380) × (150–)190–250 µm, ostioles greyish yellow green (197D), *ca*. 100 µm diam. *Asci* cylindrical, 8-spored, (125–)160–235(–250) × (8–)7–9(–10) µm, with caps 4–5 µm thick. *Ascospores* hyaline, whole, filiform, 7–8 septate, (65–)70–85 × (3–)3.5–4 µm. Colour change of stromata in 3 % KOH not observed.

Distribution: Found in the central region of Thailand.

Additional materials examined: **Thailand**, Phitsanulok Province, Ban Phaothai community forest, on seed (*Smilacaceae*), in the leaf litter, 10 Oct. 2015, *AK*, *DT*, *SL*, *SM*, *WN*, MY 10979 (**paratype** BBH 41715), MY 10959 (BBH 41709), MY 10975 (BBH 41713).

Notes: *Shimizuomyces cinereus* is only recorded from Ban Phaothai community forest in Phitsanulok Province. This species is easy to find in the leaf litter or on the ground due to the abundance of natural specimens located around the area producing bright grey stromata. *Shimizuomyces cinereus* and *Morakotia fusca* occur on seeds (*Smilacaceae*) and these two species can be found at the same site in Ban Phaothai community forest. *Shimizuomyces cinereus* differs from *Morakotia fusca* in having pale grey stromata meanwhile *M. fusca* has an orange and tough stroma.

DISCUSSION

Phylogenetic analyses combined with morphology classified Thai specimens associated with plants as new species in *Aciculosporium* and *Shimizuomyces,* and a novel genus,

Table 2. Morphological comparisons of *Morakotia fusca* and species in *Shimizuomyces.*

Species	Stroma (mm)	Fertile part (mm)	Perithecia (µm)	Asci (µm)	Ascospores (µm)	References
Morakotia fusca	20–75 × 0.5–2	5–20 × 1.5–2.5	narrow flask-shaped, 320–570 × 120–180	105–245 × 7–10	70–105 × 2–4, with septation	This study
Shimizuomyces cinereus	10–28 × 0.5–2	3–8 × 1.5–3	pyriform, 310–380 × 150–250	125–250 × 8–10	65–85 × 3–4, with septation	This study
S. kibianus	15–18 mm long	2.5 × 2	pyriform, 300–320 × 230–240	140–160 × 6	30–80 × 1.5, with septation	Kobayasi (1984), Shimizu (1994)
S. paradoxus	10–30 × 0.5–1.2	5–15 × 1–2	pyriform, 350–400 × 200–250	100–130 × 6–7	60–75 × 2–2.5, with septation	Kobayasi (1981)

Morakotia. This study has contributed to our knowledge on the taxonomy, morphology and geographical distribution of fungi in *Clavicipitaceae* (*Hypocreales*).

Aciculosporium siamense from Thailand can be easily recognised by its host – an unknown panicoid grass found in the rainforest that was also reported for *A. monostipum* from Costa Rica (South America) and *A. phalaridis* from *Phalaris aquatica* in Australia and New Zealand. *Aciculosporium take* and *A. sasicola* can be found on several genera of bambusoid grasses in Japan (Tsuda *et al*. 1997). We compared the morphological characters of *A. siamense* with known species in *Aciculosporium* and found that *A. siamense* is morphologically similar to *A. take* and *A. sasicola* in the formation of astipitate ascomatal stromata (Tsuda *et al*. 1997). *Aciculosporium monostipum* produces stipitate ascomatal stromata arising directly from parasitised plant ovaries (Sullivan *et al*. 2001), whereas *A. phalaridis* produces a discrete sclerotium with stalked ascostromata (Walker 2004) on seeds. Although the asexual morph of *Aciculosporium siamense* was not seen in the natural habitat, cream-coloured, yeast-like masses of conidia were produced on PDA and 2 % MEA. From our microscopic observation of the conidia on cultures, we found that *Aciculosporium siamense* produces holoblastic appendaged conidia on both media. Our results reveal that species in *Aciculosporium* share this unique character (apomorphies) in having a holoblastic appendaged conidia, which was also reported from *A. monostipum, A. phalaridis, A. take* and *A. sasicola* (Oguchi 2001, Sullivan *et al*. 2001, Walker 2004, Píchová *et al*. 2018).

Multi-gene phylogenetic analyses presented in Fig. 1 fully support (MLBP/BPP = 100/100) *Morakotia* as a distinct clade from *Shimizuomyces.* So far, only one species, *Morakotia fusca*, has been proposed in *Morakotia*. Considering the morphology of natural specimens and ecology, these two genera share similarity of having cylindrical stroma arising directly from seeds, the fertile parts are cylindrical to clavate, and can be found on the ground. Microscopic observation between species in the two genera showed the synapomorphic character of producing filiform ascospores with distinct septations. However, *Morakotia fusca* differs from *Shimizuomyces* spp. (Table 2) in that the perithecia in *M. fusca* are completely immersed and narrow flask-shaped, whereas the perithecia in all species of *Shimizuomyces* are immersed with slightly projecting ostioles and are pyriform in shape. The *Shimizuomyces* clade itself is fully supported (MLBP/BPP = 100/100) with the Thai specimens as a new member in *Shimizuomyces. Shimizuomyces cinereus* differs from *S. paradoxus* in having grey stroma, whereas stroma in *S. paradoxus* is bright yellow. *Shimizuomyces kibianus* was not included in the phylogenetic analysis because no sequence data was available for this taxon. According to the description and illustration given by Kobayasi (1984) and Shimizu (1994), *S. kibianus* also has grey stroma similar to *S. cinereus.* However, *S. cinereus* possesses a larger fertile part and perithecia, and longer asci than reported for *S. kibianus*. *Shimizuomyces* is thus distributed in Japan, Korea and Thailand.

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