



# Fungal pathogens occurring on *Orthoptera* in Thailand

D. Thanakitpipattana<sup>1</sup>, K. Tasanathai<sup>1</sup>, S. Mongkolsamrit<sup>1</sup>, A. Khonsanit<sup>1</sup>,  
S. Lamlerththong<sup>2</sup>, J.J. Luangsa-ard<sup>1</sup>

## Key words

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**Abstract** Two new fungal genera and six species occurring on insects in the orders Orthoptera and Phasmatodea (superorder Orthoptera) were discovered that are distributed across three families in the *Hypocreales*. Sixty-seven sequences generated in this study were used in a multi-locus phylogenetic study comprising SSU, LSU, *TEF*, *RPB1* and *RPB2* together with the nuclear intergenic region (IGR). These new taxa are introduced as *Metarhizium gryllidicola*, *M. phasmatodeae*, *Neotorrubiella chinghradicola*, *Ophiocordyceps kobayasii*, *O. krachonicola* and *Petchia siamensis*. *Petchia siamensis* shows resemblance to *Cordyceps mantidicola* by infecting egg cases (ootheca) of praying mantis (Mantidae) and having obovoid perithecial heads but differs in the size of its perithecia and ascospore shape. Two new species in the *Metarhizium* cluster belonging to the *M. anisopliae* complex are described that differ from known species with respect to phialide size, conidia and host. *Neotorrubiella chinghradicola* resembles *Torrubiella* in the absence of a stipe and can be distinguished by the production of whole ascospores, which are not commonly found in *Torrubiella* (except in *Torrubiella hemipterigena*, which produces multiseptate, whole ascospores). *Ophiocordyceps krachonicola* is pathogenic to mole crickets and shows resemblance to *O. nigrella*, *O. ravenelii* and *O. bamesii* in having darkly pigmented stromata. *Ophiocordyceps kobayasii* occurs on small crickets, and is the phylogenetic sister species of taxa in the 'sphecocephala' clade.

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## INTRODUCTION

The majority of the entomopathogenic fungi belong to the order *Hypocreales* in the *Ascomycota*. A reclassification of cordycipitoid fungi based on molecular phylogeny over a decade ago split *Cordyceps* s.lat. into three families: *Cordycipitaceae*, *Ophiocordycipitaceae* and *Clavicipitaceae* (Sung et al. 2007). However, many species still could not be confidently identified in the new classification system due to either a lack of ex-type cultures for molecular studies or specimens for morphological comparison, leaving these species without information relating to their taxonomy and phylogenetic position in *Cordyceps* s.lat. (Sung et al. 2007).

In a recent phylogenetic classification of the family *Cordycipitaceae* by Kepler et al. (2017), two new genera, *Hevansia* and *Blackwellomyces*, were described while nine genera were proposed to be protected including *Akanthomyces*, *Ascopolyporus*, *Beauveria*, *Cordyceps*, *Gibellula*, *Hyperdermium*, *Simplicillium*, and eight genera proposed to be rejected, including *Evlachovaea*, *Granulomanus*, *Isaria*, *Lecanicillium*, *Microhilum*, *Phytocordyceps*, *Synsterigmatocystis*, and *Torrubiella*, a sexually reproductive genus originally identified in *Cordycipitaceae* together with *Cordyceps*. Subsequently, a new genus *Samsoniella* was added to the family (Mongkolsamrit et al. 2018). The family is characterised by pallid to brightly coloured fleshy stromata possessing superficial to pseudo-immersed perithecia with filiform or bola-shaped whole ascospores or ascospores that disarticulate into part-spores.

The genus *Ophiocordyceps* belongs to *Ophiocordycipitaceae*, and is one of the most speciose genera in *Cordyceps* s.lat., with more than 260 species records (Index Fungorum continuously updated). The majority of the species possess darkly pigmented stromata with superficial to immersed perithecia that produce whole or non-disarticulating ascospores (Sung et al. 2007, Luangsa-ard et al. 2018). The type of *Ophiocordyceps* is *O. blattae* (Petch 1931). The asexual morphs linked with *Ophiocordyceps* are known as *Hirsutella*, *Hymenostilbe*, *Paraisaria*, *Stilbella* and *Syngliocladium* (Sung et al. 2007). *Ophiocordycipitaceae* fungi can be found on a broad range of substrates including insects of Coleoptera, Diptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Neuroptera, Odonata and Orthoptera species (Kobayasi 1941, Mains 1958, Kepler et al. 2013, Sanjuan et al. 2015), and even occur on the fungal genus *Elaphomyces* (Nees 1820).

The *Clavicipitaceae* is the most heterogeneous and diverse family in *Hypocreales* with species occurring on plants, insects and other invertebrates (Chaverri et al. 2005, Sung et al. 2007, Kepler et al. 2012, Luangsa-ard et al. 2017). It contains the genus *Metarhizium* that has a greenish appearance when sporulating on arthropod hosts or in culture. They can be pathogenic to plants (e.g., *Claviceps*, *Balansia*), to other fungi (e.g., *Verticillium epiphytum*, *Tyrannicordyceps*), and infect a broad range of insect orders (Spatafora et al. 2007). *Metarhizium anisopliae* is a generalist occurring on more than seven insect orders, whereas *M. acridum* is specific only to insects from the Acrididae family (Moon & Hue 2017), and plays an important role as controller of insect populations in nature (Mondal et al. 2016).

Out of the 31 insect orders, 20 are susceptible to infection by entomopathogenic fungi in all stages of the insects' life cycle - eggs, larvae, pupae, nymphs and adults (Araújo & Hughes 2016). The four major insect orders that are parasitized by

<sup>1</sup> Plant Microbe Interaction Research Team, Bioscience and Biotechnology for Agriculture (BBR) BIOTEC, NSTDA, 113 Thailand Science Park, Phahonyothin Rd., Khlong Nueng, Khlong Luang, Pathum Thani 12120, Thailand; corresponding author e-mail: jajen@biotec.or.th.

<sup>2</sup> Center of Excellence in Fungal Research, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand.

entomopathogenic fungi are the Coleoptera, Lepidoptera, Hemiptera and Hymenoptera (Shrestha et al. 2016). In Thailand, entomopathogenic fungi have been found on various invertebrate hosts including c. 11 insect orders and spiders. They occur on Blattodea, Coleoptera, Diptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Mantodea, Neuroptera, Odonata, Orthoptera and the spider order Araneae. There have been many recent reports of fungi occurring in the superorder Orthopterida (Orthoptera and Phasmatodea) from the three families of entomopathogenic fungi, especially on adult grasshoppers, locusts and stick insects. Most of them were reported from the New World (Mains 1959), such as *Beauveria acridophila*, *B. diapheromeriphila*, *B. locustiphila*, *Cordyceps grylli*, *C. gryllotalpae*, *C. monticola*, *C. parvula*, *C. trinidadensis*, *C. uleana*, *Metarhizium acridum*, *M. majus* and *M. robertsii*, and *Ophiocordyceps amazonica*, but recent findings also report them from the Old World, including *Beauveria loeiensis*, *B. gryllotalpicicola* (Ariyawansa et al. 2015), *B. bassiana*, *Cordyceps neogryllotalpae*, *C. mantidicola*, *Metarhizium acridum*, *M. anisopliae* and *M. majus*.

The aims of this study were:

- 1 to establish a species list of entomopathogenic fungi parasitizing Orthopterida, which are rarely found in Thailand; and
- 2 to clarify the taxonomic and phylogenetic positions of these fungi by using partial DNA sequences of multiple genetic loci: small and large subunits of the ribosomal DNA (SSU, LSU), elongation factor 1- $\alpha$  (*TEF*), the largest and second largest subunits of RNA polymerase II (*RPB1*, *RPB2*) and the nuclear intergenic regions (IGR).

## MATERIALS AND METHODS

### Specimens and isolations

Collection trips were done regularly throughout the year but intensively during the rainy season from June to September from 2011–2016. The forest floor, leaf litter and the underside of leaves were carefully scanned for fungi growing on invertebrates, especially insects and spiders. Specimens were collected from Khao Yai National Park in Nakhon Ratchasima province, Khao Luang National Park in Nakhon Si Thammarat province, Khlong Nakha Wildlife Sanctuary in Ranong province, Ban Hua Thung Community Forest in Chiang Mai province and Ban Phao Thai Community Forest in Phitsanulok province. Fungal collection and isolation followed the protocols described in previous studies (Luangsa-ard et al. 2017, Mongkolsamrit et al. 2018).

### Cultivation

Starter cultures were grown on Potato Dextrose Agar (PDA: potato 200 g, dextrose 20 g, agar 15 g) for 7 d at room temperature. Mycelial plugs were cut from an actively growing colony using 5-mm-diam cork borer and inoculated into three media used for comparative studies: PDA, Potato Sucrose Agar (PSA: potato 200 g, sucrose 20 g, calcium carbonate 5 g, agar 15 g) and quarter-strength Sabouraud dextrose agar with yeast extract (SDAY/4: dextrose 10 g, peptone 2.5 g, yeast extract 2.5 g, agar 15 g) (Bischoff et al. 2009). *Ophiocordyceps* were grown in the dark at 20 °C following Ban et al. (2015), while *Metarhizium*, *Petchia* and *Neotorribiella* specimens were incubated at room temperature under daylight conditions. Morphological observations were recorded at 7, 14, 21 and 30 d depending on the sporulation of each species.

### Morphology

Colours of fresh specimens and cultures on PDA, PSA and SDAY/4 after 7–30 d were characterised using the colour chart of Kornerup & Wanscher (1963), as well as the Online Auction

Colour Chart (abbreviated 'OAC' herein) and the Naturalist's Color Guide (Smithe 1975) due to the absence of some colour ranges in each colour chart. Photographs of fresh specimens were taken to document the colour of stromata and the hosts when possible (Nikon D5100). Fungal materials, such as the perithecia, asci, ascospores, synnemata, phialides and conidia were mounted in lactophenol cotton blue and measured using a compound microscope (Olympus SZ61). Measurements of important morphological characters such as length and width were made from 20–50 observations, and variability calculated using standard deviation (with absolute minima and maxima in brackets) and average  $\pm$  standard deviation values. Specimens were either air-dried or dried in an electric food dryer (50–55 °C) overnight and deposited in BIOTEC Bangkok Herbarium (BBH) for further study.

### DNA isolation, PCR and sequencing

Genomic DNA was extracted from 5–10-d-old fungal cultures grown on PDA plates by a modified CTAB method (Doyle & Doyle 1987). The fungal mycelium was scraped out from the agar using a sterile spatula and lysed in 600  $\mu$ L CTAB extraction buffer (1M Tris-HCl, 5M NaCl, 0.5M EDTA, CTAB and PVP-40). Mycelium was ground using a sterile pestle and incubated at 65 °C for 30 min. After incubation, 600  $\mu$ L of Chloroform:Isoamyl Alcohol (24:1) was added and mixed by inverting the tube. The samples were centrifuged at 12000 rpm for 15 min and the supernatant transferred to a new tube. To precipitate the DNA, 300  $\mu$ L of ice-cold Isopropanol was added and placed in –20 °C for 1 h. Samples were centrifuged at 4 °C at 12000 rpm for 20 min to precipitate the DNA. After centrifugation the DNA pellet was washed with 70 % ethanol and centrifuged at 12000 rpm for 20 min. The DNA pellets were air dried and dissolved in 1X TE buffer and stored at –20 °C.

PCR was conducted in 25 mL reaction volumes consisting of 1 $\times$  PCR buffer, 200  $\mu$ M of each of the four dNTPs, 2.5 mM MgCl<sub>2</sub>, 1 U Taq DNA Polymerase, recombinant (Thermo Scientific, US), 0.5  $\mu$ M of each primer and 50–100 ng DNA template. Sequences of the nuclear ribosomal small and large subunits (SSU and LSU), the largest and second largest subunits of RNA polymerase II (*RPB1* and *RPB2*), elongation factor 1- $\alpha$  (*TEF*) and 5' intron-rich region of elongation factor 1- $\alpha$  (5'*TEF*) were used for this analysis (White et al. 1990, Bischoff et al. 2009). In addition, to verify cryptic diversification within the *M. anisopliae* species complex, seven nuclear intergenic loci were sequenced as they have shown a good performance in separating species of the PARB and MGT clades (Kepler & Rehner 2013, Rehner & Kepler 2017). The PCR primers used to amplify the gene regions for this study were: NS1 and NS4 for SSU, LROR and LR7 for LSU (White et al. 1990), 983F and 2218R for *TEF*, CRPB1 and RPB1Cr for *RPB1*, fRPB2-5F2 and fRPB2-7cR for *RPB2* (Castlebury et al. 2004), EF1T and EF2T for 5' *TEF* (Bischoff et al. 2009), BTIGS, MzFG543, MzFG546, MzIGS2, MzIGS3, MzIGS5, MzIGS7 for IGR (Kepler & Rehner 2013). Sequencing primers were the same as for amplification, and conditions as set in Sung et al. (2007).

### Sequence alignment and phylogenetic analyses

Sequences from this study were assembled using BioEdit v. 7.2.3 (Hall 2004) and compared to sequences in GenBank via a BLAST search. Assembled sequences were aligned using MUSCLE (Edgar 2004) and manually refined. All alignments were analysed together with other fungi from previously published studies (Table 1). The dataset was analysed separately in each family within the order *Hypocreales*, using maximum parsimony (MP), bayesian inference (BI) and maximum likelihood (ML). The 5' *TEF* was analysed separately in a supplementary tree (see Appendix).

**Table 1** List of specimens and GenBank accession numbers of sequences used in this study. **Bold** accession numbers were generated from this study.

Species	Strains <sup>1</sup>	Genbank Accession no.				
		SSU	LSU	TEF	RPB1	RPB2
<i>Akanthomyces aculeatus</i>	HUA 186145	MF416572	MF416520	MF416465	–	–
<i>Akanthomyces attenuatus</i>	CBS 402.78	AF339614	AF339565	EF468782	EF468888	EF468935
<i>Akanthomyces confragosa</i>	spat 08-146	MF416581	MF416528	MF416472	MF416634	MF416436
<i>Akanthomyces kanyawimiae</i>	TBRC 7242	–	MF140718	MF140838	MF140784	MF140808
<i>Akanthomyces</i> sp.	BCC31657	MK652097	MK652098	MK652099	–	–
<i>Akanthomyces sulphureus</i>	TBRC 7248	–	MF140722	MF140843	MF140787	MF140812
<i>Akanthomyces thailandicus</i>	TBRC 7245	–	–	MF140839	–	MF140809
<i>Akanthomyces tuberculatus</i>	HUA 186131	MF416573	MF416521	MF416466	–	–
<i>Akanthomyces waltergamsii</i>	TBRC 7250	–	MF140715	MF140835	–	–
<i>Aschersonia badia</i>	BCC 8105	DQ522537	DQ518752	DQ522317	DQ522363	DQ522411
<i>Aschersonia placenta</i>	BCC 7869	EF469121	EF469074	EF469056	EF469085	EF469104
<i>Ascopolyporus polychrous</i>	P.C. 546	–	DQ118737	DQ118745	DQ127236	–
<i>Ascopolyporus villosus</i>	ARSEF 6355	–	AY886544	DQ118750	DQ127241	–
<i>Balansia henningsiana</i>	A.E.G. 96-27a	AY545723	AY545727	AY489610	AY489643	DQ522413
<i>Balansia pilulaeformis</i>	A.E.G. 94-2	AF543764	AF543788	DQ522319	DQ522365	DQ522414
<i>Beauveria acridophila</i>	HUA 179220	JQ895527	JQ895536	JQ958614	JX003852	JX003842
	HUA 179222	JQ895528	JQ895538	JQ958616	JX003849	–
<i>Beauveria asiatica</i>	ARSEF 4384	–	–	AY531935	HQ880857	HQ880929
<i>Beauveria australis</i>	ARSEF 4622	–	–	HQ880996	HQ880862	HQ880934
<i>Beauveria bassiana</i>	ARSEF 300	–	–	AY531924	HQ880831	HQ880903
<i>Beauveria brongniartii</i>	ARSEF 617	–	–	HQ880991	HQ880854	HQ880926
<i>Beauveria caledonica</i>	ARSEF 2567	AF339570	AF339520	EF469057	HQ880889	HQ880961
<i>Beauveria diapheromeriphila</i>	QCNE 186272	JQ895530	JQ895534	JQ958610	JX003848	–
	QCNE 186714	JQ895529	JQ895533	JQ958611	JX003850	–
<i>Beauveria gryllotalpidicola</i>	BCC19481	FJ584322	–	FJ584323	–	–
	BCC26300	FJ459791	MK632106	FJ459795	MK632184	MK632152
<i>Beauveria kipukae</i>	ARSEF 7032	–	–	HQ881005	HQ880875	HQ880947
<i>Beauveria locustiphila</i>	HUA 179217	JQ958597	–	JX003847	–	–
	HUA 179218	JQ895525	JQ895535	JQ958619	JX003846	JX003845
<i>Beauveria loeiensis</i>	BCC23107	–	MK632108	FJ459794	MK632183	MK632156
	BCC78420	MK632124	MK632101	MK632070	MK632180	MK632154
<i>Beauveria staphylinidicola</i>	ARSEF 5718	EF468981	EF468836	EF468776	EF468881	–
<i>Beauveria sungii</i>	ARSEF 5689	AF339574	AF339524	DQ522335	HQ880882	HQ880954
<i>Beauveria varroae</i>	ARSEF 2694	–	–	HQ881004	HQ880874	HQ880946
<i>Blackwellomyces cardinalis</i>	OSC 93609	AY184973	AY184962	DQ522325	DQ522370	DQ522422
<i>Blackwellomyces pseudomilitaris</i>	BCC 1919	MF416588	MF416534	MF416478	–	MF416440
<i>Claviceps fusiformis</i>	ATCC 26019	DQ522539	U17402	DQ522320	DQ522366	–
<i>Claviceps paspali</i>	ATCC 13892	U32401	U47826	DQ522321	DQ522367	DQ522416
<i>Claviceps purpurea</i>	SA cp11	EF469122	EF469075	EF469058	EF469087	EF469105
<i>Clonostachys rosea</i>	AFTOL-ID 187	DQ862044	DQ862027	DQ862029	–	–
	GJS90-227	AY489684	AY489716	AY489611	–	–
<i>Conoideocrella luteostrata</i>	NHJ 11343	EF468995	EF468850	EF468801	EF468906	–
	NHJ 12516	EF468994	EF468849	EF468800	EF468905	EF468946
<i>Conoideocrella tenuis</i>	NHJ 345.01	EU369111	EU369045	EU369030	–	EU369088
	NHJ 6293	EU369112	EU369044	EU369029	EU369068	EU369087
<i>Cordyceps amoenerosea</i>	CBS 107.73	AY526464	MF416550	MF416494	MF416651	MF416445
<i>Cordyceps bifusispora</i>	EFCC 5690	EF468952	EF468806	EF468746	EF468854	EF468909
<i>Cordyceps blackwelliae</i>	TBRC 7256	–	MF140702	MF140822	MF140771	MF140795
<i>Cordyceps exasperata</i>	MCA 2155	MF416596	MF416542	MF416486	MF416643	–
<i>Cordyceps farinosa</i>	CBS 111113	AY526474	MF416554	MF416499	MF416656	MF416450
<i>Cordyceps javanica</i>	TBRC 7259	–	MF140711	MF140831	MF140780	MF140804
<i>Cordyceps kintrischica</i>	ARSEF 7218	–	–	GU734751	–	–
<i>Cordyceps kysuyuensis</i>	EFCC 5886	EF468960	EF468813	EF468754	EF468863	EF468917
<i>Cordyceps lepidopterorum</i>	TBRC 7264	–	MF140700	MF140820	MF140769	MF140793
<i>Cordyceps militaris</i>	OSC 93623	AY184977	AY184966	DQ522332	DQ522377	AY545732
<i>Cordyceps ninchukispora</i>	EGS 38.166	EF468992	EF468847	EF468794	EF468901	–
<i>Cordyceps polyarthra</i>	MCA 1009	MF416598	MF416544	MF416488	MF416645	–
<i>Cordyceps pruinosa</i>	NHJ 10627	EF468967	EF468822	EF468763	EF468870	–
	ARSEF 5413	AY184979	AY184968	DQ522351	DQ522397	DQ522451
<i>Cordyceps tenuipes</i>	ARSEF 5135	MF416612	JF415980	JF416020	JN049896	JF416000
<i>Gibellula longispora</i>	NHJ 12014	EU369098	–	EU369017	EU369055	EU369075
<i>Gibellula pulchra</i>	NHJ 10808	EU369099	EU369035	EU369018	EU369056	EU369076
<i>Gibellula</i> sp.	NHJ 10788	EU369101	EU369036	EU369019	EU369058	EU369078
<i>Hevansia arachnophilus</i>	NHJ 10469	EU369090	EU369031	EU369008	EU369047	–
<i>Hevansia novoguineensis</i>	NHJ 11923	EU369095	EU369032	EU369013	EU369052	EU369072
<i>Hymenostilbe aurantiaca</i>	OSC 128578	DQ522556	DQ518770	DQ522345	DQ522391	DQ522445
<i>Hypocrella</i> sp.	GJS 89-104	–	DQ518772	DQ522347	DQ522393	DQ522448
<i>Lecanicillium psalliota</i>	CBS 363.86	AF339608	AF339559	EF468784	EF468890	–
	CBS 532.81	AF339609	AF339560	EF469067	EF469096	EF469112
<i>Metapochonia bulbillosa</i>	CBS 145.70	AF339591	AF339542	EF468796	EF468902	EF468943
<i>Metapochonia gonioides</i>	CBS 891.72	AF339599	AF339550	DQ522354	DQ522401	DQ522458
<i>Metapochonia rubescens</i>	CBS 464.88	AF339615	AF339566	EF468797	EF468903	EF468944
<i>Metarhizium acridum</i>	ARSEF 324	–	–	EU248844	EU248896	EU248924
	ARSEF 7486	–	–	EU248845	EU248897	EU248925
<i>Metarhizium album</i>	ARSEF 2082	DQ522560	DQ518775	DQ522352	DQ522398	DQ522452
<i>Metarhizium anisopliae</i>	ARSEF 7450	–	–	EU248852	EU248904	EU248932
	ARSEF 7487	–	–	DQ463996	DQ468355	DQ468370

Table 1 (cont.)

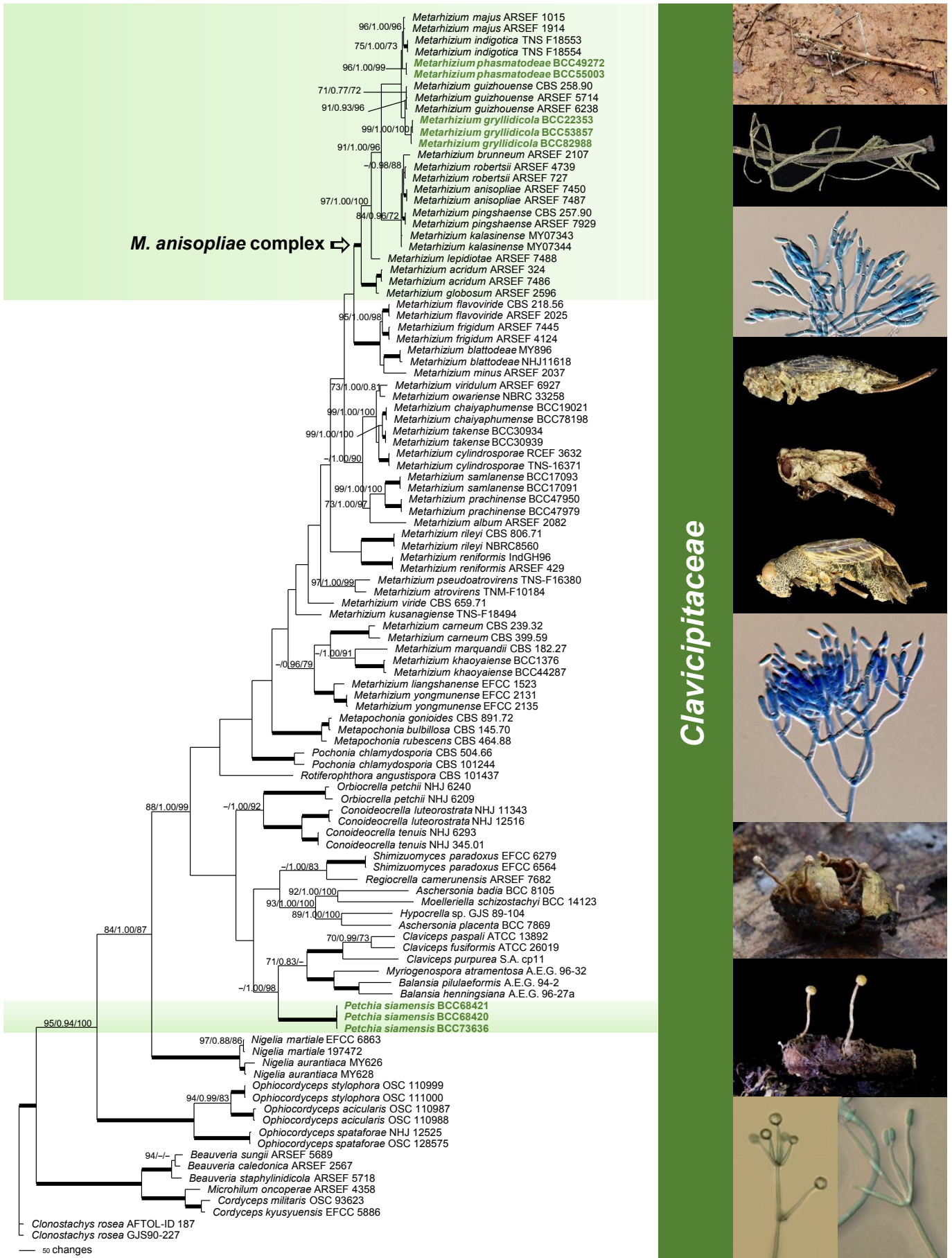
Species	Strains <sup>1</sup>	Genbank Accession no.				
		SSU	LSU	TEF	RPB1	RPB2
<i>Metarhizium atrovirens</i>	TNM-F10184	JF415950	JF415966	–	JN049884	–
<i>Metarhizium blattodeae</i>	MY00896	HQ165657	HQ165719	HQ165678	HQ165739	HQ165638
	NHJ11618	HQ165663	HQ165725	HQ165684	HQ165744	HQ165644
<i>Metarhizium brunneum</i>	ARSEF 2107	–	–	EU248855	EU248907	EU248935
<i>Metarhizium carneum</i>	CBS 239.32	EF468988	EF468843	EF468789	EF468894	EF468938
	CBS 399.59	EF468989	EF468842	EF468788	EF468895	EF468939
<i>Metarhizium chaiyaphumense</i>	BCC19021	HQ165655	HQ165717	HQ165676	HQ165738	HQ165636
	BCC78198	KX369596	KX369593	KX369592	KX369594	KX369595
<i>Metarhizium cylindrosporae</i>	RCEF 3632	JF415959	JF415982	JF416022	–	–
	TNS-16371	JF415964	JF415987	JF416027	JN049902	–
<i>Metarhizium flavoviride</i>	ARSEF 2025	–	–	DQ464000	DQ468359	DQ468374
	CBS 218.56	–	–	KJ398787	KJ398598	–
<i>Metarhizium frigidum</i>	ARSEF 4124	–	–	DQ464002	DQ468361	DQ468376
	ARSEF 7445	–	–	DQ464003	KJ398628	–
<i>Metarhizium globosum</i>	ARSEF 2596	–	–	EU248846	EU248898	EU248926
<i>Metarhizium gryllidicola</i>	<b>BCC22353</b>	<b>MK632116</b>	<b>MK632090</b>	<b>MK632061</b>	<b>MK632165</b>	<b>MK632142</b>
	<b>BCC53857</b>	<b>MK632118</b>	<b>MK632092</b>	<b>MK632063</b>	<b>MK632167</b>	<b>MK632144</b>
	<b>BCC82988<sup>T</sup></b>	<b>MK632117</b>	<b>MK632091</b>	<b>MK632062</b>	<b>MK632166</b>	<b>MK632143</b>
<i>Metarhizium guizhouense</i>	ARSEF 5714	AF543763	AF543787	AF543775	DQ522383	DQ522434
	ARSEF 6238	–	–	EU248857	EU248909	EU248937
	CBS 258.90	–	–	EU248862	EU248914	EU248942
<i>Metarhizium indigotica</i>	TNS-F18553	JF415952	JF415968	JF416010	JN049886	JF415992
	TNS-F18554	JF415953	JF415969	JF416011	JN049887	JF415993
<i>Metarhizium kalasinense</i>	BCC53581	KC011174	KC011182	KC011188	–	–
	BCC53582	KC011175	KC011183	KC011189	–	–
<i>Metarhizium khaoyaiense</i>	BCC1376	KX983468	KX983462	KX983457	–	KX983465
	BCC44287	KX983470	KX983464	KX983459	–	KX983467
<i>Metarhizium kusanagiense</i>	TNS-F18494	JF415954	JF415972	JF416014	JN049890	–
<i>Metarhizium lepidiotae</i>	ARSEF 7488	–	–	EU248865	EU248917	EU248945
<i>Metarhizium liangshanense</i>	EFCC 1523	EF468961	EF468814	EF468755	–	EF468918
<i>Metarhizium majus</i>	ARSEF 1015	–	–	EU248866	EU248918	EU248946
	ARSEF 1914	–	–	EU248868	EU248920	EU248948
<i>Metarhizium marquandii</i>	CBS 182.27	EF468990	EF468845	EF468793	EF468899	EF468942
<i>Metarhizium minus</i>	ARSEF 2037	AF339580	AF339531	DQ522353	DQ522400	DQ522454
<i>Metarhizium owariense</i>	NBRC 33258	HQ165669	HQ165730	HQ165689	HQ165747	–
<i>Metarhizium phasmatodeae</i>	<b>BCC49272<sup>T</sup></b>	<b>MK632119</b>	<b>MK632093</b>	<b>MK632064</b>	–	<b>MK632145</b>
	<b>BCC55003</b>	–	<b>MK632094</b>	<b>MK632065</b>	–	<b>MK632146</b>
<i>Metarhizium pingshaense</i>	ARSEF 7929	–	–	EU248847	EU248899	EU248927
	CBS 257.90	–	–	EU248850	EU248902	EU248930
<i>Metarhizium prachinense</i>	BCC47950	KC011172	KC011180	KC011186	KC011184	–
	BCC47979	KC011173	KC011181	KC011187	KC011185	–
<i>Metarhizium pseudoatrovirens</i>	TNS-F16380	–	JF415977	–	JN049893	JF415997
<i>Metarhizium reniforme</i>	ARSEF 429	HQ165671	HQ165733	HQ165690	–	HQ165650
	IndGH96	HQ165670	HQ165732	–	–	HQ165649
<i>Metarhizium rileyi</i>	CBS 806.71	AY526491	–	EF468787	EF468893	EF468937
	NBRC8560	HQ165667	HQ165729	HQ165688	–	–
<i>Metarhizium robertsii</i>	ARSEF 727	–	–	DQ463994	DQ468353	DQ468368
	ARSEF 4739	–	–	EU248848	EU248900	EU248928
<i>Metarhizium samlanense</i>	BCC17091	HQ165665	HQ165727	HQ165686	–	HQ165646
	BCC17093	HQ165666	HQ165728	HQ165687	HQ165746	HQ165647
<i>Metarhizium takense</i>	BCC30934	HQ165658	HQ165720	HQ165679	HQ165740	HQ165639
	BCC30939	HQ165659	HQ165721	–	HQ165741	HQ165640
<i>Metarhizium viride</i>	CBS 659.71	HQ165673	HQ165735	HQ165692	–	HQ165652
<i>Metarhizium viridulum</i>	ARSEF 6927	–	–	KJ398815	KJ398681	–
<i>Metarhizium yongmunense</i>	EFCC 2131	EF468977	EF468833	EF468770	EF468876	–
<i>Microhilum oncoperae</i>	EFCC 2135	EF468979	EF468834	EF468769	EF468877	–
	ARSEF 4358	AF339581	AF339532	EF468785	EF468891	EF468936
<i>Moelleriella schizostachyi</i>	BCC 14123	DQ522557	DQ518771	DQ522346	DQ522392	DQ522447
<i>Myriogenospora atramentosa</i>	A.E.G. 96-32	AY489701	AY489733	AY489628	AY489665	DQ522455
<i>Neotrorubiella chinghridicola</i>	<b>BCC39684</b>	<b>MK632122</b>	<b>MK632096</b>	<b>MK632071</b>	<b>MK632181</b>	<b>MK632148</b>
	<b>BCC80733<sup>T</sup></b>	<b>MK632121</b>	<b>MK632097</b>	<b>MK632072</b>	<b>MK632176</b>	<b>MK632149</b>
<i>Nigelia aurantiaca</i>	BCC19950	GU979934	GU979943	GU979952	GU979961	GU979968
	BCC19475	GU979935	GU979944	GU979953	GU979962	GU979969
<i>Nigelia martiale</i>	EFCC 6863	–	JF415975	JF416016	–	JF415995
	HMAS 197472	JF415955	JF415973	JF416015	JN049892	JF415994
<i>Ophiocordyceps acicularis</i>	OSC 110987	EF468950	EF468805	EF468744	EF468852	–
	OSC 110988	EF468951	EF468804	EF468745	EF468853	–
<i>Ophiocordyceps agriota</i>	ARSEF 5692	DQ522540	DQ518754	DQ522322	DQ522368	DQ522418
<i>Ophiocordyceps aphodii</i>	ARSEF 5498	DQ522541	DQ518755	DQ522323	–	DQ522419
<i>Ophiocordyceps appendiculata</i>	NBRC 106960	JN941728	JN941413	AB968577	JN992462	AB968539
<i>Ophiocordyceps arborescens</i>	NBRC 105890	AB968387	AB968415	AB968573	–	AB968535
<i>Ophiocordyceps bamesii</i>	BCC 28560	EU408776	–	–	EU408773	EU418599
	BCC 28561	EU408775	–	–	EU408774	EU408772
<i>Ophiocordyceps brunneinigra</i>	BCC69015	–	MF614653	MF614637	–	MF614680
	BCC69032	–	MF614654	MF614638	MF614668	MF614681
<i>Ophiocordyceps brunneipertheciata</i>	BCC64201	–	MF614658	MF614643	–	MF614685
	BCC66167	–	MF614659	MF614644	–	MF614684

Table 1 (cont.)

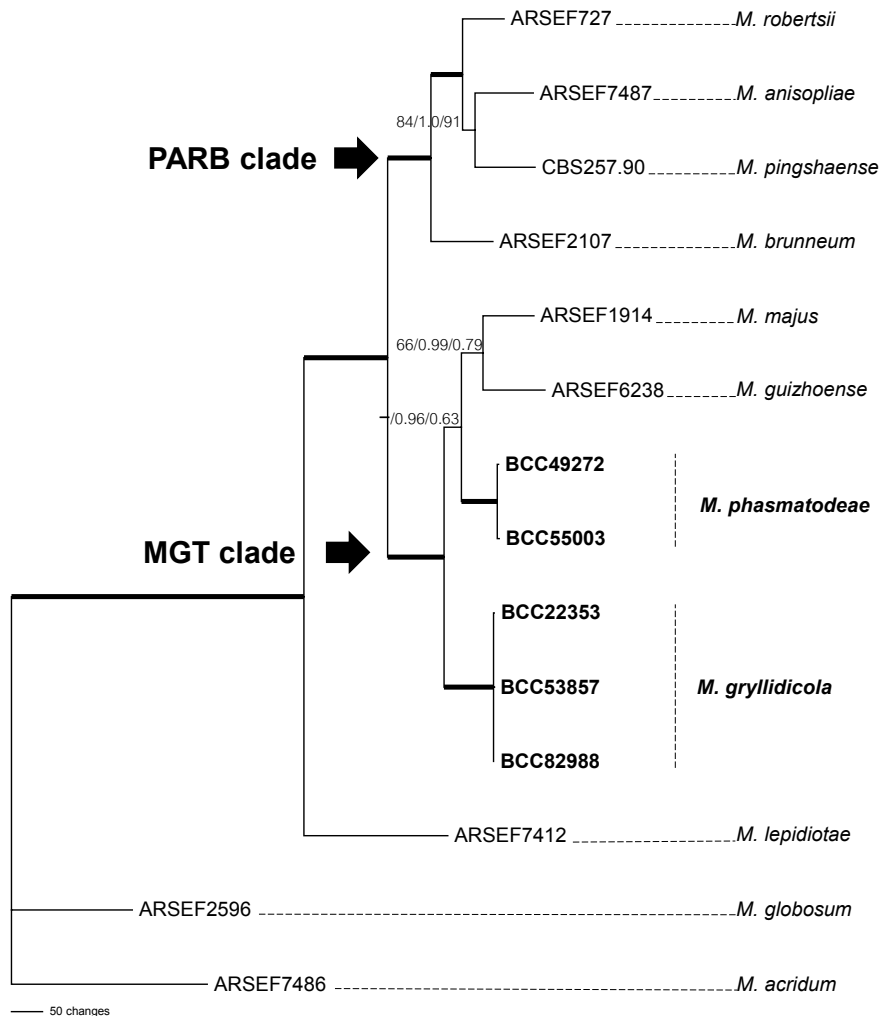
Species	Strains <sup>1</sup>	Genbank Accession no.				
		SSU	LSU	TEF	RPB1	RPB2
<i>Ophiocordyceps brunneipunctata</i>	OSC 128576	DQ522542	DQ518756	DQ522324	DQ522369	DQ522420
<i>Ophiocordyceps clavata</i>	NBRC 106961	JN941727	JN941414	AB968586	JN992461	AB968547
	NBRC 106962	JN941726	JN941415	AB968587	JN992460	AB968548
<i>Ophiocordyceps entomorrhiza</i>	KEW 53484	EF468954	EF468809	EF468749	EF468857	EF468911
<i>Ophiocordyceps geometridicola</i>	BCC79823	–	MF614648	MF614632	MF614663	MF614679
	BCC35947	–	MF614647	MF614631	MF614664	MF614678
<i>Ophiocordyceps gracillima</i>	HUA 186132	–	KC610768	KC610744	KF658666	–
<i>Ophiocordyceps halabalaensis</i>	MY5151	KM655826	–	GU797110	–	–
<i>Ophiocordyceps irangiensis</i>	OSC 128579	EF469123	EF469076	EF469060	EF469089	EF469107
<b><i>Ophiocordyceps kobayashii</i></b>	<b>BCC75694<sup>T</sup></b>	<b>–</b>	<b>–</b>	<b>MK632056</b>	<b>MK632172</b>	<b>MK632136</b>
	<b>BCC84222</b>	<b>–</b>	<b>–</b>	<b>MK632083</b>	<b>MK632173</b>	<b>MK632134</b>
	<b>BCC84223</b>	<b>MK632109</b>	<b>MK632084</b>	<b>MK632058</b>	<b>MK632174</b>	<b>MK632135</b>
<b><i>Ophiocordyceps krachonicola</i></b>	<b>BCC79667</b>	<b>–</b>	<b>MK632081</b>	<b>MK632055</b>	<b>MK632162</b>	<b>MK632133</b>
	<b>BCC79666<sup>T</sup></b>	<b>–</b>	<b>MK632080</b>	<b>MK632054</b>	<b>MK632161</b>	<b>MK632132</b>
<i>Ophiocordyceps longissima</i>	EFCC 6814	–	EF468817	EF468757	EF468865	–
<i>Ophiocordyceps macroacicularis</i>	BCC22918	–	MF614655	MF614639	MF614669	MF614675
	NBRC 100685	AB968388	AB968416	AB968574	–	AB968536
<i>Ophiocordyceps melolonthae</i>	OSC 110993	DQ522548	DQ518762	DQ522331	DQ522376	–
<i>Ophiocordyceps multiperitheciata</i>	BCC22861	–	MF614656	MF614640	MF614670	MF614683
	BCC69008	–	MF614657	MF614641	–	MF614682
<i>Ophiocordyceps nigrella</i>	EFCC 9247	EF468963	EF468818	EF468758	EF468866	EF468920
<i>Ophiocordyceps nutans</i>	OSC 110994	DQ522549	DQ518763	DQ522333	DQ522378	–
<i>Ophiocordyceps pauciovoperitheciata</i>	BCC39781	–	MF614650	MF614635	MF614667	MF614671
	BCC53615	–	MF614649	MF614636	MF614665	MF614672
<i>Ophiocordyceps pseudoacicularis</i>	BCC49256	–	MF614645	MF614629	MF614662	MF614676
	BCC53843	–	MF614646	MF614630	MF614661	MF614677
<i>Ophiocordyceps ramosissimum</i>	GZUHHN8	KJ028012	–	KJ028014	KJ028017	–
<i>Ophiocordyceps ravenelii</i>	OSC 110995	DQ522550	DQ518764	DQ522334	DQ522379	DQ522430
<i>Ophiocordyceps rhizoidea</i>	NHJ 12522	EF468970	EF468825	EF468764	EF468873	EF468923
	NHJ 12529	EF468969	EF468824	EF468765	EF468872	EF468922
<i>Ophiocordyceps sinensis</i>	EFCC 7287	EF468971	EF468827	EF468767	EF468874	EF468924
<i>Ophiocordyceps sobolifera</i>	KEW 78842	EF468972	EF468828	–	EF468875	EF468925
<i>Ophiocordyceps spatulifera</i>	NHJ 12525	EF469125	EF469078	EF469063	EF469092	EF469111
	OSC 128575	EF469126	EF469079	EF469064	EF469093	EF469110
<i>Ophiocordyceps sphecocephala</i>	OSC 110998	DQ522551	DQ518765	DQ522336	DQ522381	DQ522432
<i>Ophiocordyceps stylophora</i>	OSC 110999	EF468982	EF468837	EF468777	EF468882	EF468931
	OSC 111000	DQ522552	DQ518766	DQ522337	DQ522382	DQ522433
<i>Ophiocordyceps tricentri</i>	–	AB027330	AB027376	–	–	–
<i>Ophiocordyceps unilateralis</i>	KT3308	–	–	GU797112	–	–
<i>Ophiocordyceps variabilis</i>	ARSEF 5365	DQ522555	DQ518769	DQ522340	DQ522386	DQ522437
	OSC 111003	EF468985	EF468839	EF468779	EF468885	EF468933
<i>Ophiocordyceps xuefengensis</i>	GZUHHN13	KC631785	–	KC631790	KC631795	–
<i>Orbiocrella petchii</i>	NHJ 6209	EU369104	EU369039	EU369023	EU369061	EU369081
	NHJ 6240	EU369103	EU369038	EU369022	EU369060	EU369082
<i>Paraisaria amazonica</i>	HUA 186113	KJ917566	KJ917572	–	KP212903	KM411980
	HUA 186143	KJ917562	KJ917571	KM411989	KP212902	KM411982
<i>Paraisaria blattarioides</i>	HUA 186093	KJ917559	KJ917570	KM411992	–	–
	HUA 186108	KJ917558	KJ917569	–	–	KM411984
<i>Paraisaria graciloides</i>	HUA 186092	KJ917555	–	–	KP212915	–
	HUA 186095	KJ917556	–	KM411994	KP212914	–
<i>Paraisaria gracilis</i>	EFCC 3101	EF468955	EF468810	EF468750	EF468858	EF468913
	EFCC 8572	EF468956	EF468811	EF468751	EF468859	EF468912
<i>Paraisaria heteropoda</i>	EFCC 10125	EF468957	EF468812	EF468752	EF468860	EF468914
	OSC 106404	AY489690	AY489722	AY489617	AY489651	–
<b><i>Petchia siamensis</i></b>	<b>BCC68420</b>	<b>MK632113</b>	<b>MK632087</b>	<b>–</b>	<b>MK632163</b>	<b>MK632140</b>
	<b>BCC68421</b>	<b>MK632114</b>	<b>MK632088</b>	<b>MK632059</b>	<b>MK632164</b>	<b>MK632141</b>
	<b>BCC73636<sup>T</sup></b>	<b>MK632115</b>	<b>MK632089</b>	<b>MK632060</b>	<b>–</b>	<b>MK632138</b>
<i>Pochonia chlamydosporia</i>	CBS 101244	DQ522544	DQ518758	DQ522327	DQ522372	DQ522424
	CBS 504.66	AF339593	AF339544	EF469069	EF469098	EF469120
<i>Regiocrella camerunensis</i>	ARSEF 7682	–	DQ118735	DQ118743	DQ127234	–
<i>Rotiferophthora angustispora</i>	CBS 101437	AF339584	AF339535	AF543776	DQ522402	DQ522460
<i>Samsoniella aurantia</i>	TBRC 7272	–	MF140727	MF140845	–	MF140817
<i>Samsoniella inthanonensis</i>	TBRC 7916	–	MF140724	MF140848	MF140789	MF140814
<i>Shimizuomyces paradoxus</i>	EFCC 6279	EF469131	EF469084	EF469071	EF469100	EF469117
	EFCC 6564	EF469130	EF469083	EF469072	EF469101	EF469118
<i>Simplicillium lanosoniveum</i>	CBS 704.86	AF339602	AF339553	DQ522358	DQ522406	DQ522464
<i>Simplicillium obclavatum</i>	CBS 311.74	AF339567	AF339517	EF468798	–	–
<i>Torrubiella ratticaudata</i>	ARSEF 1915	DQ522562	DQ518777	DQ522360	DQ522408	DQ522467
<i>Torrubiella</i> sp.	NHJ 7859	EU369107	–	–	EU369064	EU369085
<i>Torrubiella wallacei</i>	CBS 101237	AY184978	AY184967	EF469073	EF469102	EF469119

<sup>T</sup> = culture ex-type

<sup>1</sup> Abbreviations for collections: A.E.G = A.E. Glenn personal collection, USA; AFTOL = Assembling the Fungal Tree of Life, USA; ARSEF = USDA-ARS Collection of Entomopathogenic Fungal Cultures, USA; ATCC = American Type Culture Collection, USA; BCC = BIOTEC Culture Collection, Thailand; CBS = Centraalbureau voor Schimmelcultures, The Netherlands; EFCC = Entomopathogenic Fungal Culture Collection, Korea; GJS = G.J. Samuels personal collection, USA; GZUH = Herbarium of Guizhou University, China; HMAS = Herbarium Mycologicum Academiae Sinicae, China; HUA = Herbarium Antioquia University, Colombia; IndGH = Indonesian isolate collected by USAID project; KEW = Royal Botanic Gardens, UK; KT = Kanoksri Tasanathai Collection, BIOTEC, Thailand; MCA = M. Catherine Aime personal collection, USA; MY = Mycology Laboratory in BIOTEC, Thailand; NBRC = NITE Biological Resource Center, Japan; NHJ = Nigel Hywel Jones Collection, BIOTEC, Thailand; OSC = Oregon State University Herbarium, USA; P.C. = Priscila Chaverri personal collection; QCNE = National Herbarium of Ecuador, Ecuador; RCEF = Research Center on Entomogenous Fungi, China; spat = Joseph W. Spatafora personal collection, USA; TBRC = Thailand Bioresource Research Center, Thailand; TNS = National Museum of Nature and Science, Tsukuba, Ibaraki, Japan.



**Fig. 1** Phylogenetic reconstruction of the *Clavicipitaceae* obtained from the combined SSU, LSU, *TEF*, *RPB1* and *RPB2* sequences based on Maximum Parsimony, Bayesian analysis and RAxML. Number on the nodes are MP bootstrap/Bayesian posterior probability/ML bootstrap values above 70 %. **Bold** lines mean support for the three analyses were 100 %.



**Fig. 2** Phylogenetic reconstruction of the nuclear intergenic region of *Metarhizium anisopliae* species complex obtained from the combined BTIGS, MzFG543, MzIGS2, MzIGS3, MzIGS5 and MzIGS7 loci based on Maximum Parsimony, Bayesian analysis and RAxML. Number on the nodes are MP bootstrap/Bayesian posterior probability/ML bootstrap values above 50 %. **Bold** lines mean support for the three analyses were 100 %.

A maximum parsimony analysis was conducted for SSU, LSU, *TEF*, *RPB1*, *RPB2* and *5'TEF* using parsimony via PAUP v. 4.0b10 (Swofford 2002). Heuristic MP bootstrap (MP BS) analyses (Felsenstein 1985) with TBR branch swapping option included 1000 replicates, and 10 random addition replicates were performed to provide bootstrap support values.

Bayesian analysis was conducted using MrBayes v. 3.2.6 (Ronquist et al. 2012) to determine posterior probabilities (BI PP), and analysis of the nucleotide substitution model was determined by using MrModeltest v. 2.2 (Nylander 2004) in each family. MrBayes was run with four independent Markov chains Monte Carlo (MCMC) for 20 000 000 generations with tree and parameter sampling occurring every 100 generations.

Maximum likelihood analyses were run using a randomised accelerated maximum likelihood model in RAxML v. 8.2.10 on XSEDE platform on CIPRES Science Gateway Portal (Stamatakis 2014). Relative support of internal nodes was assessed by a rapid bootstrap with 1000 replications (ML BS). Nodes were considered supported by bootstrap values greater than 70 %.

## RESULTS

### Fungal cultures

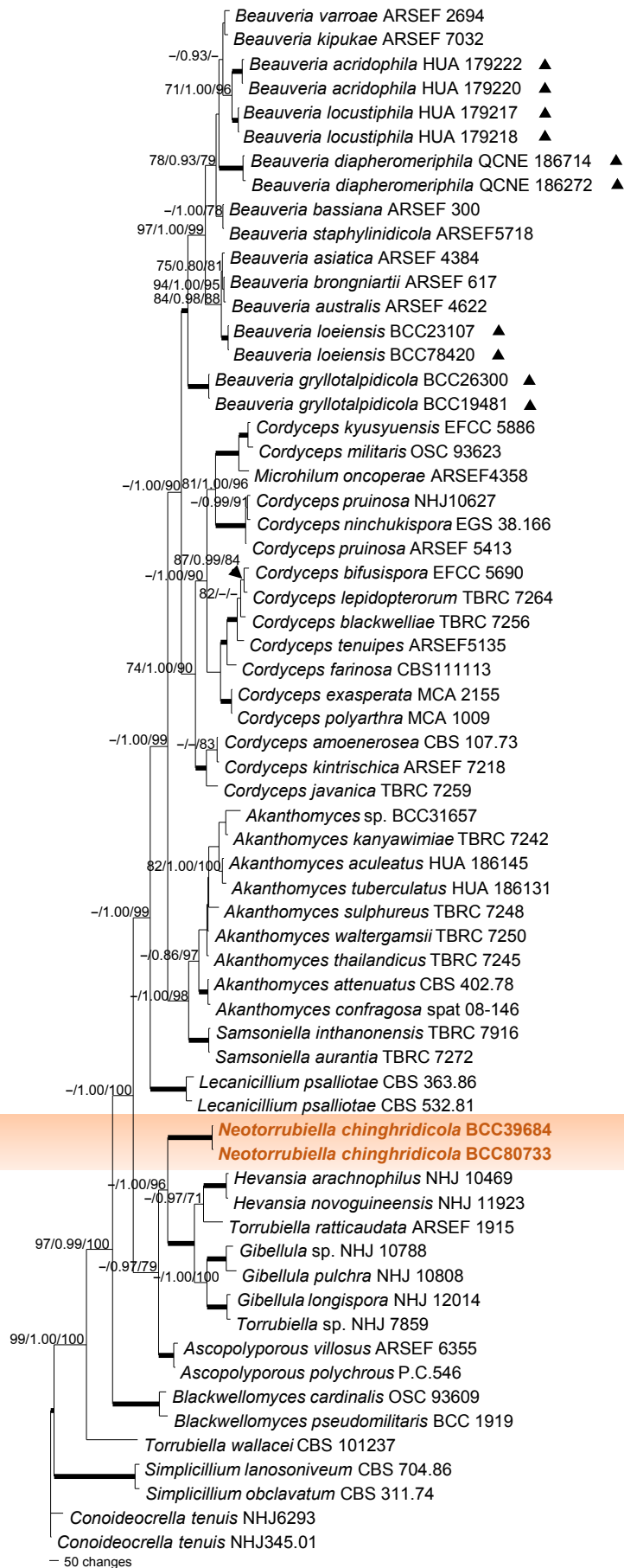
Growth rates on three kinds of media differed for each species. *Neotorribiella chinghradicola* (30 d, 0.8–1.4 cm) and *Ophiocordyceps kobayashii* (21 d, 0.8–1.2 cm) grew slower among the

fungi studied. In addition, the best growth rate and conidiation was observed using PSA medium for almost all species, except for *Neotorribiella*. Hyphal growth and mycelium formation of *Neotorribiella chinghradicola* was observed after prolonged cultivation (> 30 d). There was no significant difference between growth on PDA and SDAY/4 among the species studied.

### Phylogenetic analyses

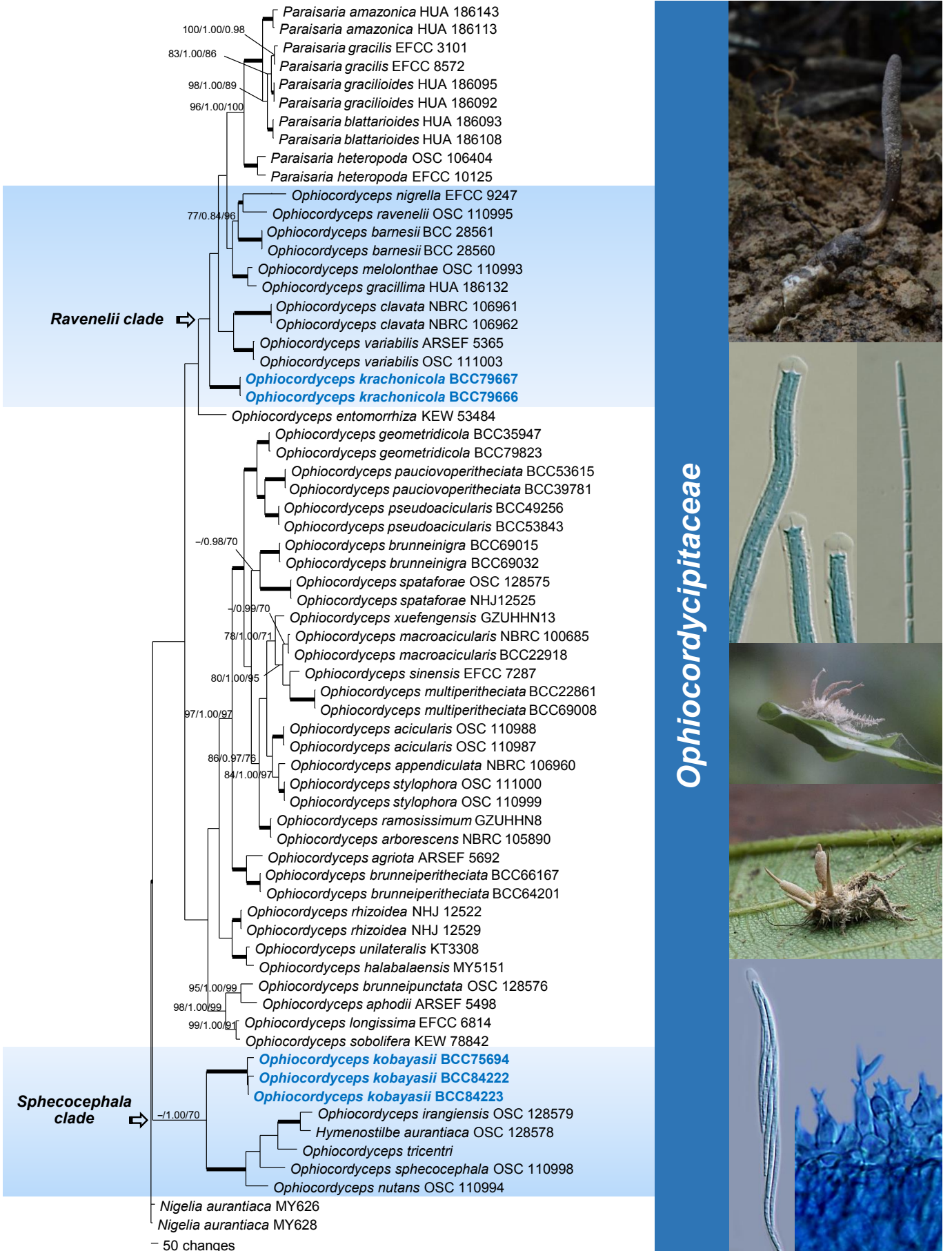
Sixty-seven sequences were generated from 15 specimens, which were obtained from *Cordycipitaceae* (10 sequences of two samples), *Clavicipitaceae* (35 sequences of eight samples) and *Ophiocordycipitaceae* (22 sequences of five samples). The six new species and two new genera were phylogenetically distinct from other known species previously reported from Orthoptera.

The first analysis of the family *Clavicipitaceae* (Fig. 1) was made to evaluate the preliminary identification of *Metarhizium* species complex and related genera selected from the dataset described by Luangsa-ard et al. (2017). *Cionostachys rosea* was designated as outgroup. The combined dataset of 107 taxa consisted of 4 292 bp (SSU 1 020 bp, LSU 872 bp, *TEF* 902 bp, *RPB1* 700 bp and *RPB2* 798 bp). Two species of *Metarhizium*, *M. gryllidicola* and *M. phasmatodeae*, clustered within the *M. anisopliae* species clade (Kepler & Rehner 2013, Kepler et al. 2014) are closely related to *M. majus* and *M. guizhouense* (MGT clade, Bischoff et al. 2009). One new genus, *Petchia*, which is resolved in these analyses, also nested within the



**Fig. 3** Phylogenetic reconstruction of the Cordycipitaceae obtained from the combined SSU, LSU, *TEF*, *RPB1* and *RPB2* sequences based on Maximum Parsimony, Bayesian analysis and RAxML. Number on the nodes are MP bootstrap/Bayesian posterior probability/ML bootstrap values above 70 %. **Bold** lines mean support for the three analyses were 100 %. Triangles represent taxa occurring on Orthoptera.



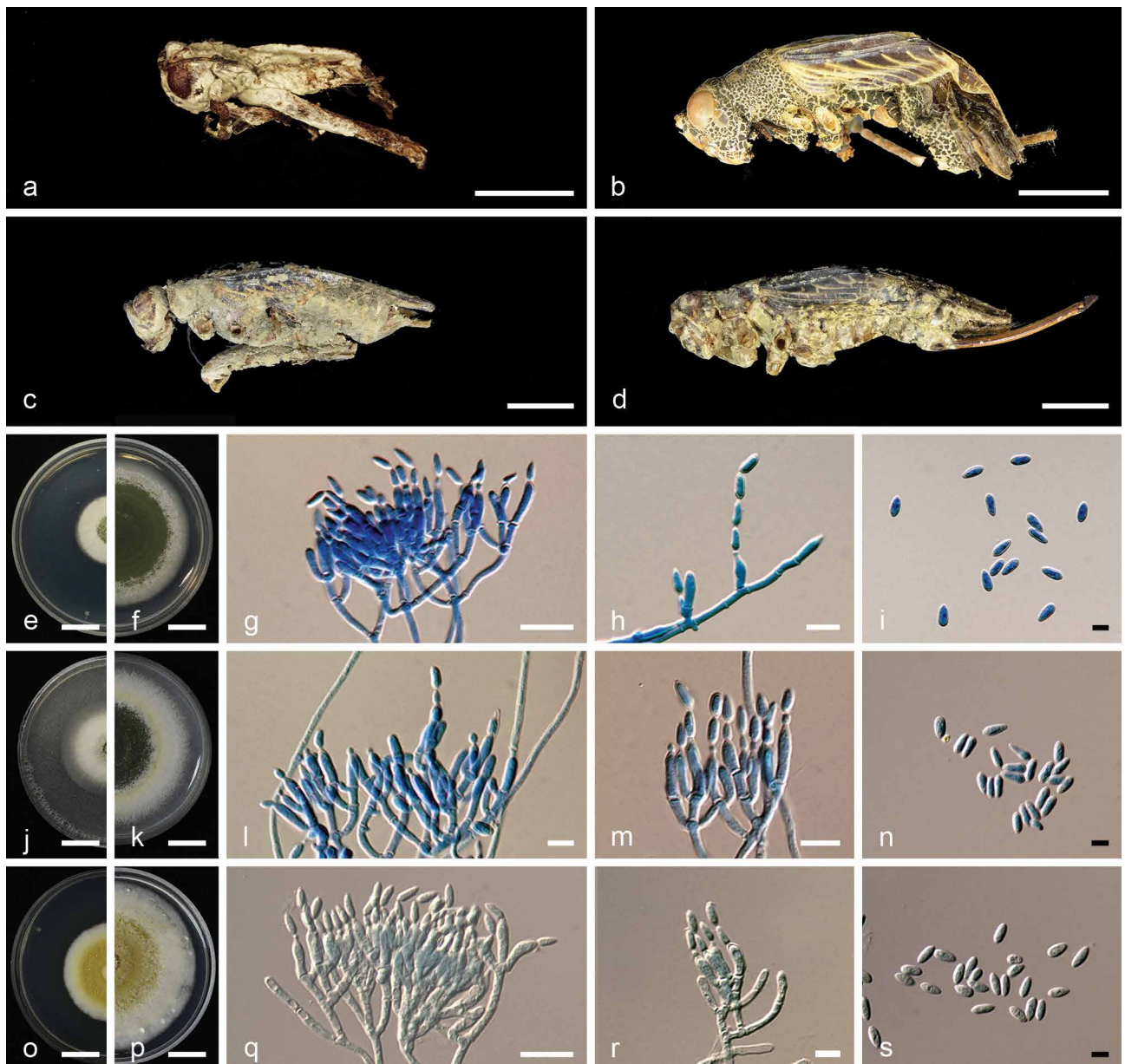


**Fig. 4** Phylogenetic reconstruction of the *Ophiocordycipitaceae* obtained from the combined SSU, LSU, *TEF*, *RPB1* and *RPB2* sequences based on Maximum Parsimony, Bayesian analysis and RAxML. Number on the nodes are MP bootstrap/Bayesian posterior probability/ML bootstrap values above 70 %. **Bold** lines mean support for the three analyses were 100 %.

*Clavicipitaceae*, closely related to the plant associated genera *Balansia*, *Claviceps*, *Myriogenospora* and *Shimizuomyces*, as well as the scale insect pathogens *Hypocrella*, *Moelleriella* and *Regiocrella*. It is separate from other known entomopathogenic genera. For this reason, *C. mantidicola* was transferred to *Clavicipitaceae*. This study also revealed that two isolates from stick insects, *M. phasmatodeae* (MP BS = 96 %, BI PP = 1.00, ML BS = 99 %), three isolates from crickets, *Metarhizium gryllidicola* (MP BS = 99 %, BI PP = 1.00, ML BS = 100 %) and three isolates occurring on egg cases (Ootheca) of praying mantis (Mantidae), *Petchia* (MP BS = 100 %, BI PP = 1.00, ML BS = 100 %) were strongly supported in *Clavicipitaceae* with high bootstrap values and posterior probabilities. Furthermore, in these analyses we evaluated the relationship within the *M. anisopliae* species complex by focusing on six nuclear intergenic loci (BTIGS, MzFG543, MzIGS2, MzIGS3, MzIGS5 and MzIGS7) of PARB and MGT clades, which includes *M. pingshaense*, *M. anisopliae*, *M. robertsii*, *M. brunneum*, *M. majus* and *M. guizhouense*. The result of this phylogenetic analysis (Fig. 2) showed that *M. phasmatodeae* (MP BS = 50 %, BI PP =

0.96, ML BS = 63 %) belongs to the MGT clade and is a sister taxon to *M. gryllidicola* which showed strong support as the basal species lineage to the MGT and *M. phasmatodeae* clade (MP BS = 100 %, BI PP = 1.00, ML BS = 100 %). Based on morphological and molecular studies, it is appropriate to describe a new genus and two new species in this family.

The second analysis of the family *Cordycipitaceae* (Fig. 3) was established to determine the taxonomic position of a new genus *Neotorrubiella* and a new species *N. chinghridicola*. The dataset used contained almost all genera belonging to this family (Kepler et al. 2017, Mongkolsamrit et al. 2018), and the analysis included sequences from related species occurring on Orthoptera: *Beauveria acridophila*, *B. diapheromeriphila*, *B. locustiphila*, *B. loeiensis* and *B. gryllotalpidicola* (Sanjuan et al. 2014, 2015, Ariyawansa et al. 2015) with other species of *Torrubiella*, especially *T. wallacei* (= *Lecanicillium wallacei*), which is one of the earliest diverging members of this family, and other known species. Sequences of *Conoideocrella tenuis* were used as outgroup. The alignment of 64 taxa is 4390 bp (SSU 1011 bp, LSU 870 bp, *TEF* 981 bp, *RPB1* 730 bp and



**Fig. 5** *Metarhizium gryllidicola* (BBH 44436, BCC82988). a–d. Fungus on adult cricket (Gryllidae) hosts; e–f. colony on PDA: (e) at 7 d (f) at 14 d; g–h. conidiophores bearing phialides and conidia; i. conidia; j–k. colony on PSA: (j) at 7 d (k) at 14 d; l–m. conidiophores bearing phialides and conidia; n. conidia; o–p. colony on SDAY/4: (o) at 14 d (p) at 21 d; q–r. conidiophores bearing phialides and conidia; s. conidia. — Scale bars: a, e–f, j–k, o–p = 10 mm; b–d = 5 mm; g, l, q = 10 µm; h–i, m–n, r–s = 5 µm.

*RPB2* 798 bp) long. The genus *Neotorrubiella* was strongly supported (MP BS = 100 %, BI PP = 1.00, ML BS = 100 %) in *Cordycipitaceae* as a separate genus from other genera in the phylogenetic reconstruction, closely related to the genera *Hevansia* and *Gibellula*. Therefore, a new species, *Neotorrubiella chinghridicola*, is proposed.

The analysis of the family *Ophiocordycipitaceae* (Fig. 4) was mainly targeted to elucidate the phylogenetic positions of putative new *Ophiocordyceps* species. The alignment consisted of 4349 bp (SSU 1012 bp, LSU 914 bp, *TEF* 904 bp, *RPB1* 725 bp and *RPB2* 794 bp) from 67 taxa including *Nigelia aurantiaca* as outgroup. The results of these analyses showed two well-supported clades in *Ophiocordycipitaceae* distinct from other known taxa. Two new species are identified: *Ophiocordyceps kobayashii*, which formed a sister clade to species producing a *Hymenostilbe* asexual morph in the sphecocephala clade, and *O. krachonicola*, which formed a separate clade basal to other species of *Ophiocordyceps* including *O. variabilis*, *O. barnesii*, *O. clavata*, *O. melolonthae*, *O. gracillima*, *O. nigrella* and *O. ravenelii* clades and *Paraisaria* (Mongkolsamrit et al. 2019), with credible bootstrap supports (MP BS = 100 %, BI PP = 1.00, ML BS = 100 %).

## TAXONOMY

### Clavicipitaceae

***Metarhizium gryllidicola*** Khons., Thanakitp. & Luangsa-ard, sp. nov. — MycoBank MB830169; Fig. 5

*Etymology.* In reference to the family of the host, namely a cricket (Gryllidae).

*Typus.* THAILAND, Nakhon Ratchasima Province, Khao Yai National Park, on adult cricket (Gryllidae), in leaf litter, 1 Nov. 2016, B. Sakolrak, D. Thanakitpipattana, N. Arnarnart, N. Kobmoo & R. Somnuk (holotype BBH 44436, ex-type culture BCC82988).

*Sexual morph.* Unknown.

Specimens found only on adult crickets (Gryllidae) in leaf litter on the forest floor. The host body is covered with greyish green (27C4, 29C6), pastel green (28A4) and dark green (28F8) conidia.

*Culture characteristics* — Colonies on PDA fast-growing, attaining 4.7 cm diam after 14 d at 25 °C, at first white with smooth and cottony mycelia. Conidiation starts in the middle of colony at 5 d, becoming lime green (No. 59), buff yellow (No. 53) and olive green (No. 46) with age. Colony reverse white-cream at the edges and sulphur yellow (No. 57) in the centre. Conidiophores

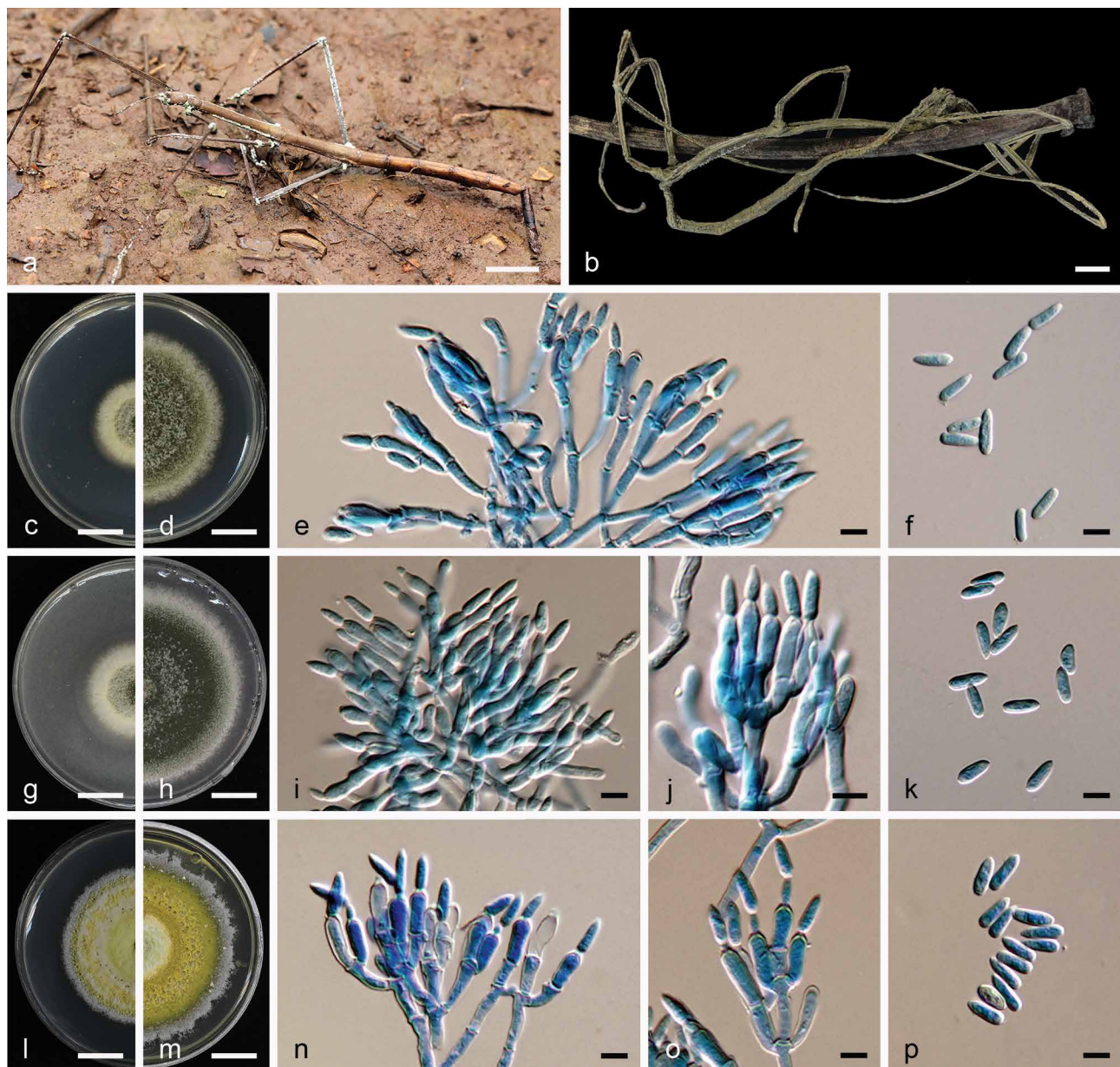
arising from aerial mycelium, smooth, cylindrical. Phialides smooth-walled, cylindrical to clavate, without a distinct neck, (5–)6–9(–12) × 2–3 µm. Conidia smooth-walled, cylindrical, ovoid, (5–)5.7–6.9(–7) × (2–)2.2–2.9(–3) µm. Colonies on PSA fast-growing, attaining 5 cm diam after 14 d at 25 °C, at first white with smooth and cottony mycelia turning cream (No. 54). Conidiation starts in the middle of colony at 5 d, turning to yellow to olive green (No. 50), lime green (No. 59) and olive green (No. 46) with age. Colony reverse white-cream at the edges and straw yellow (No. 56) in the centre. Conidiophores arising from aerial mycelium, smooth, cylindrical. Phialides smooth-walled, cylindrical, clavate, without a distinct neck, (6–)7–10(–13) × 2 µm. Conidia smooth-walled, cylindrical, ovoid, obclavate, (5–)5.5–6.4(–7) × 2.5–3 µm. Colonies on SDAY/4 slow-growing, attaining 3.5 cm diam after 14 d at 25 °C, at first white with smooth and cottony mycelia becoming sulphur yellow (No. 157), spectrum yellow (No. 55), straw yellow (No. 56) and pale horn (No. 92) with age. Conidiation starts after 30 d, turning to olive yellow (No. 52) to citrine (No. 51) and olive green (No. 46). Colony reverse white-cream at the edges and straw yellow (No. 56) in the centre. Conidiophores arising from aerial mycelium smooth, cylindrical. Phialides smooth-walled, cylindrical, clavate, without a distinct neck, (6–)7–9.5(–11) × 2–3 µm. Conidia smooth-walled, cylindrical, ovoid, obclavate, (4–)5–6(–7) × 2–3 µm.

*Additional materials examined.* THAILAND, Nakhon Ratchasima Province, Khao Yai National Park, on adult crickets (Gryllidae), in leaf litter, 11 July 2012, A. Khonsanit, K. Tسانathai, P. Srikitikulchai, S. Mongkolsamrit & W. Noisripoom (BBH 32733, BCC53857); *ibid.*, 14 Aug. 2009, K. Tسانathai, P. Srikitikulchai & S. Mongkolsamrit (BBH 26529, BCC37915), (BBH 26533, BCC37918); *ibid.*, 13 Sept. 2009, K. Tسانathai, P. Srikitikulchai & S. Mongkolsamrit (BBH 27261, BCC39045); *ibid.*, 18 June 2008, B. Thongnuch, J.J. Luangsa-ard, K. Tسانathai, P. Srikitikulchai, R. Promharn & S. Mongkolsamrit (BBH 23876, BCC30917); *ibid.*, 5 July 2006, B. Thongnuch, J.J. Luangsa-ard, K. Tسانathai, P. Srikitikulchai & S. Mongkolsamrit (BBH 18647, BCC22353).

*Notes* — *Metarhizium gryllidicola* is found only on insects in the family Gryllidae (Orthoptera). This species is in the *M. anisopliae* species complex, which is a sister species to *M. majus*, *M. indigotica*, *M. guizhouense* and *M. taii*, which is a later synonym of *M. guizhouense* (Bischoff et al. 2009) and *M. phasmatodeae* (Fig. 1, 2). It differs from these known species significantly in the size of its phialides and conidia. The conidial size of *M. gryllidicola* is the same as in *M. phasmatodeae* but smaller than in *M. majus*, the phialides of both species are shorter than those of *M. majus* and *M. guizhouense*. *Metarhizium phasmatodeae* can only be separated from *M. gryllidicola* in terms of host and genetic data (Table 2; Fig. 1, 2).

**Table 2** Morphological comparison of species (asexual morph).

Species	Host	Colony colour	Phialides (µm)	Conidia (µm)	References
<i>Metarhizium acridum</i>	Orthoptera, Soil	Greyish yellow to greyish green	4.5–13 × 2–4.5	5–7 × 2–4	Bischoff et al. (2009)
<i>Metarhizium anisopliae</i>	Coleoptera, Hemiptera, Orthoptera	Greyish green	8–11.5 × 2–3	5–7 × 2–3.5	Bischoff et al. (2009)
<i>Metarhizium brunneum</i>	Coleoptera, Hemiptera, Soil	Pale-yellow to olive	6–18 × 2–5	4.5–8 × 2–3.5	Bischoff et al. (2009)
<i>Metarhizium gryllidicola</i>	Orthoptera	Pale horn to sulfur yellow	6–11 × 2–3	4–7 × 2–3	This study
<i>Metarhizium guizhouense</i>	Coleoptera, Diptera, Lepidoptera, Soil	–	6–20 × 2–3.5	5–5.9 × 2–3.5	Bischoff et al. (2009)
<i>Metarhizium kalasinense</i>	Coleoptera	Greenish olive and spectrum yellow	8–12 × 2–3	6–8 × 2–3	Luangsa-ard et al. (2017)
<i>Metarhizium majus</i>	Coleoptera, Lepidoptera, Soil	Olive to dark green	9–23.5 × 2.5–4.5	8–14.5 × 2.5–5	Bischoff et al. (2009)
<i>Metarhizium phasmatodeae</i>	Phasmatodea	Spectrum to sulfur-yellow and lime green	5–11 × 2–3	5.5–8 × 2–3	This study
<i>Metarhizium pingshaense</i>	Coleoptera, Isoptera	Olive	7–17 × 2–3.5	4.5–8 × 2–3.5	Bischoff et al. (2009)
<i>Metarhizium robertsii</i>	Coleoptera, Orthoptera, Soil	Olive to greyish green	7–14.5 × 2–3.5	5–7.5 × 2–3.5	Bischoff et al. (2009)



**Fig. 6** *Metarhizium phasmatodeae* (BBH 32532, BCC49272). a–b. Fungus on stick insect hosts; c–d. colony on PDA: (c) at 7 d (d) at 14 d; e. conidiophores bearing phialides and conidia; f. conidia; g–h. colony on PSA: (g) at 7 d (h) at 14 d; i–j. conidiophores bearing phialides and conidia; k. conidia; l–m. colony on SDAY/4: (l) at 14 d (m) at 21 d; n–o. conidiophores bearing phialides and conidia; p. conidia. — Scale bars: a–d, g–h, l–m = 10 mm; e–f, i–k, n–p = 5  $\mu$ m.

***Metarhizium phasmatodeae*** Khons., Thanakitp. & Luangsaard, *sp. nov.* — MycoBank MB830170; Fig. 6

*Etymology.* In reference to the order of the insect host – Phasmatodea.

*Typus.* THAILAND, Chiang Mai Province, Ban Hua Thung Community Forest, on stick insect (Phasmatodea), in leaf litter, 16 Aug. 2011, A. Khonsanit, J. Kumsao, K. Tasanathai, P. Srikitikulchai & S. Mongkolsamrit (holotype BBH 32532, ex-type culture BCC49272).

*Sexual morph.* Unknown.

Specimens found only on stick insects (Phasmatodea) in leaf litter on the forest floor. The host body is covered with green (28B6, 29C6) and deep green (27E8) conidia.

*Culture characteristics* — Colonies on PDA fast-growing, attaining 4 cm diam after 14 d at 25 °C, at first white with smooth and cottony mycelium turning to leaf-green (No. 146), greenish olive (No. 49) due to conidiation at the middle of colony after 5 d. Colony reverse clay (No. 123B) to cinnamon (No. 123B). Conidiophores arising from aerial mycelium, smooth, cylindrical. Phialides smooth-walled, cylindrical to clavate, without a distinct neck, (6–)7.5–10.5(–12)  $\times$  2–3  $\mu$ m. Conidia smooth-walled, cylindrical to obclavate, (7–)7.5–9.5(–10)  $\times$  2–2.5(–3)  $\mu$ m.

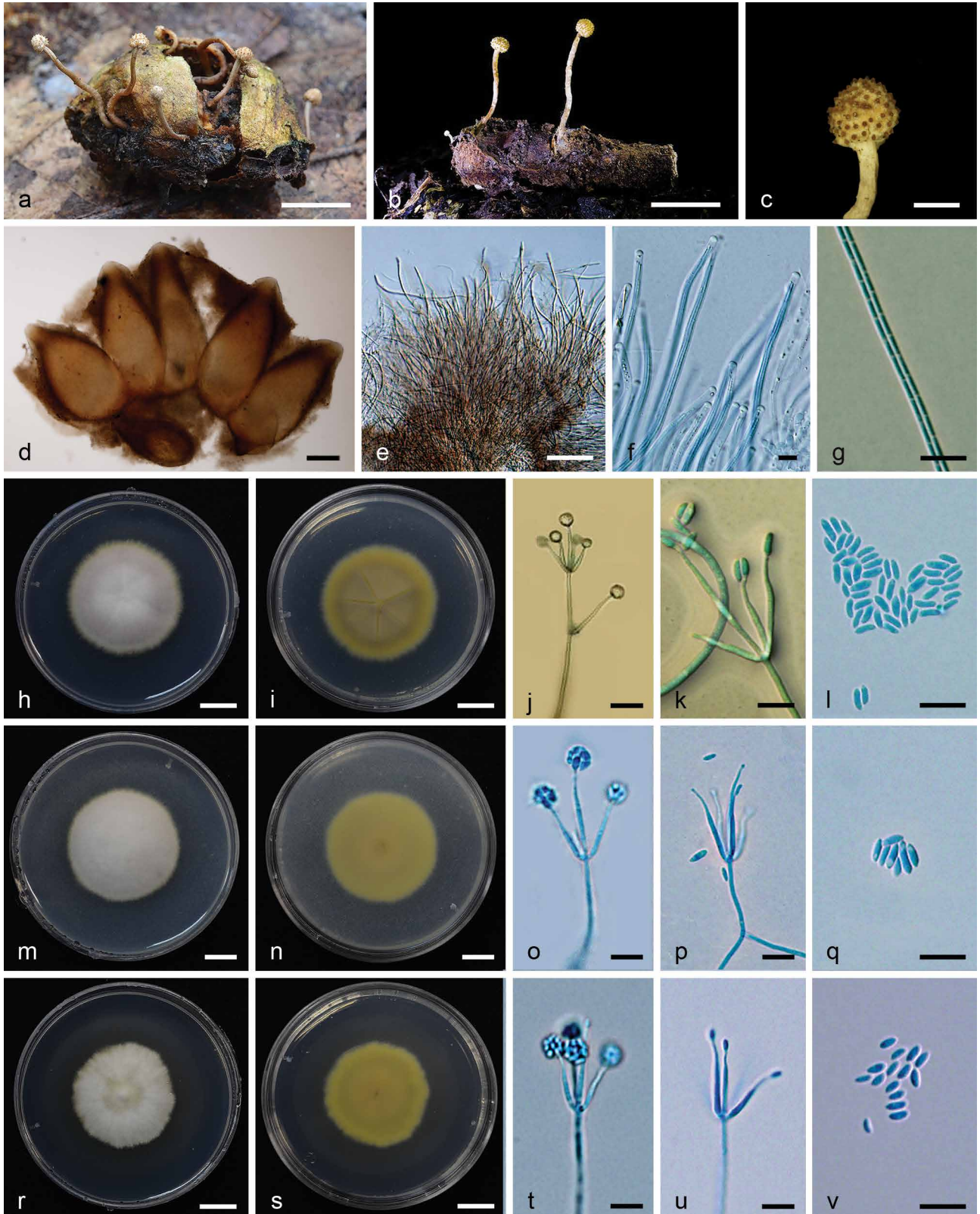
Colonies on PSA fast-growing, attaining 5.2 cm diam after 14 d at 25 °C, at first white with smooth and cottony mycelia, becoming yellowish to olive green (No. 50), lime green (No. 59) and greenish olive (No. 49) at 5 d due to the production of conidia. Colonies turn to light pink with age. Colony reverse beige (No. 219D). Conidiophores arising from aerial mycelium, smooth, cylindrical. Phialides smooth-walled, cylindrical to clavate, without a distinct neck, (7–)7.5–11(–12)  $\times$  (2–)2.5–3  $\mu$ m. Conidia smooth-walled, cylindrical to ovoid, (6–)7–8.5(–10)  $\times$  2–3  $\mu$ m. Colonies on SDAY/4 moderate-growing, attaining 3.5 cm diam after 14 d at 25 °C, at first white, smooth, turning spectrum yellow (No. 55), sulphur yellow (No. 157), apple green (No. 61), citrine (No. 51), olive yellow (No. 52) and olive green (No. 46), the medium becoming lime green (No. 59). Conidiation starts after 16 d, turning to olive yellow (No. 52) to citrine (No. 51) and olive green (No. 46). Colony reverse olive green in the centre (No. 47–48) and olive grey (No. 42) at the edges. Conidiophores arising from aerial mycelium, smooth, cylindrical. Phialides smooth-walled, cylindrical, without a distinct neck, (5–)6.5–9.5(–11)  $\times$  (2–)2.5–3  $\mu$ m. Conidia smooth-walled, cylindrical, ovoid, obclavate (5.5–)6.5–7.5(–8)  $\times$  2–3  $\mu$ m.

Table 3 Morphological comparison of species (sexual morph).

Species	Host	Stromata (cm)	Fertile part (mm)	Perithecia (µm)	Asci (µm)	Ascospores (µm)	References
<i>Beauveria locustiphila</i>	Adults and nymphs of Acrididae	Claviform, 1–1.5 long	Clavate, 0.8–1.2 × 2–2.5	Aggregated, immersed, 150 long	Cylindrical, clavate, 150–200 × 3–3.5	Filiform, breaking into part-spores, 0.6–0.9	Hennings (1904)
<i>Beauveria loeiensis</i>	Adult Orthoptera (Gryllacrididae)	Several, scattered, simple or branched, cylindrical to enlarged	–	Superficial, narrowly ovoid with acute apices, 650–710 × 280–320	Cylindrical, 370–450 × 5	Filiform, breaking into part-spores, 5–10 × 1	Ariyawansa et al. (2015)
<i>Cordyceps parvula</i>	Orthoptera	Yellowish cream, scattered	–	Superficial, ovoid, 500–650 × 250–300	Cylindrical, 400–500 × 5–6	Filiform, 8–10 × 1	Mains (1959)
<i>Neotortubella chinghricicola</i>	Adult Orthoptera (Gryllidae)	White mat of mycelium covering the host	–	Superficial, ovoid to obclavate, 220–280 × 130–170	Cylindrical, 125–165 × 10–15	Filiform, breaking into part-spores, 70–90 × 4–5	This study
<i>Ophiocordyceps amazonica</i>	Adult Orthoptera (Romaleidae, Acrididae)	Simple, gregarious, 2–4.5 long	Subglobose, spherical, reddish brown, 2.5–5.5	Immersed, ovoid-ellipsoid, 760–1100 × 220–400	Cylindrical, 325–450 × 5	9–17 × 0.5–2	Hennings (1904), Mains (1959), Sanjuan et al. (2015)
<i>Ophiocordyceps kobayasi</i>	Adult Gryllidae	Multiple, cylindrical, up to 0.8 long	Pale cream, densely packed in the middle of stromata, cylindrical to clavate, up to 3 long	Superficial, ovoid, 180–250 × 140–200	Cylindrical, up to 125 × 10–15	Whole ascospores, filiform, 45–72 × 2–2.5	This study
<i>Ophiocordyceps krachonicola</i>	Nymph of Gryllotalpa orientalis (Gryllidae)	Solitary, cylindrical, 3.7–4.0 long	Cylindrical to clavate, 25 × 2–3	Immersed, lanceolate, 460–580 × 180–300	Cylindrical, 250–400 × 4–5	Part spores, 4–10 × 1	This study
<i>Ophiocordyceps ravenelii</i>	Beetle larvae (Coleoptera)	Clavate, single or occasionally two, 1.5–4.5	Cylindrical, 2 × 0.8	Immersed, narrowly flask-shaped or ovoid, 240–420 × 144–240	Clavate-cylindrical, 170–220 × 8–10	Whole ascospores, multiseptate, 102–164 × 2–3	Berkeley (1856), Mains (1941)
<i>Petchia mantidicola</i>	Cocoon of Mantidae	Simple, usually solitary, 2.5–4 × 3–4 long	Spherical, pale rufous, 1.5–2.1	400–450 × 200–250	Cylindrical	Breaking into part-spores, 3–5 × 1	Kobayasi & Shimizu (1983)
<i>Petchia mantidicola</i>	Cocoon of Mantidae	Single or two caespitous stromata, 0.9–1.5	1.5–5	570–750 × 350–520	Cylindrical, 180–200 × 1.8–2.3	Breaking into part-spores, 3–5 × 1	Liu et al. (1997)
<i>Petchia siamensis</i>	Ootheca of praying mantis	Multiple, cylindrical, up to 2 long	Globose, pale cream, 1.5–1.8	Immersed, obpyriform, 600–870 × 320–400	Cylindrical, up to 320 × 2.5–3	Multiseptate, 200–300 × 1–1.5	This study
<i>Tortubella arancida</i>	On spider	Dispersed or aggregate on subcula	–	Elongated conoid, 0.5–0.7 × 0.3–0.4	Uniloculate, 350–400 × 4–6	Filiform, not breaking into part-spores, 350–400 × 1–1.5	Boudier (1885), Doi (1977)
<i>Tortubella gonylepticida</i>	On a large spider	White film of mycelium	–	Superficial, ovoid to conoid, 300–400 long	–	–	Möller (1901), Petch (1937)

**Additional materials examined.** THAILAND, Nong Khai Province, Amphoe Si Chiang Mai, on stick insect (Phasmatodea), in leaf litter, 30 Aug. 2009, K. Tasanathai, P. Srikitikulchai, T. Chohmee, N. Thanh & N. Toan (BBH 27078); Chiang Mai Province, Ban Hua Thung Community Forest, in leaf litter, 25 Oct. 2013, A. Khonsanit, D. Thanakitpipattana, K. Tasanathai, P. Srikitikulchai & W. Noisripoom (BBH 37785, BCC68409); Saraburi Province: Jedkod waterfall, in leaf litter, 27 Aug. 2012, A. Khonsanit, D. Thanakitpipattana, J.J. Luangsa-ard, S. Mongkolsamrit & W. Noisripoom (BBH 32525, BCC55003).

**Notes** — *Metarhizium phasmatodeae* is found only on stick insects (order Phasmatodea). It is a sister species of *M. majus*, *M. indigotica*, *M. guizhouense*, *M. taii* and *M. gryllicicola*. However, the conical size of *M. phasmatodeae* is in the same range as in *M. gryllicicola* and could only be distinguished based on the host, but is smaller than *M. majus*, and the phialides of both aforementioned species are shorter than those of *M. majus* and *M. guizhouense* (Table 2).



**Fig. 7** *Petchia siamensis* (BBH 39551, BCC73636). a–b. Fungus on egg case of praying mantis; c. ascomata; d. perithecia; e–f. asci; g. ascospores; h–i. colony on PDA at 21 d (h) obverse (i) reverse; j–k. phialides with conidia on PDA; l. conidia; m–n. colony on PSA at 21 d (m) obverse (n) reverse; o–p. phialides with conidia on PSA; q. conidia; r–s. colony on SDAY/4 at 21 d (r) obverse (s) reverse; t–u. phialides with conidia on SDAY/4; v. conidia. — Scale bars: a–b, h–i, m–n, r–s = 1 cm; c = 1 mm; d = 200  $\mu$ m; e = 50  $\mu$ m; f = 5  $\mu$ m; g, j–l, o–q, t–v = 10  $\mu$ m.

***Petchia*** Thanakitp., Mongkols. & Luangsa-ard, *gen. nov.* — MycoBank MB830167

*Etymology.* In honour of Tom Petch (1870–1948), renowned English mycologist and plant pathologist best remembered for his work on entomopathogenic fungi.

*Type species.* *Petchia siamensis* Thanakitp., Mongkols. & Luangsa-ard.

*Asexual morph.* Acremonium-like.

*Stromata* arising from the egg cases of praying mantis (Mantidae), multiple, mostly erect up to 2 cm high. Fertile part is at the terminal end of the stroma, pale cream (OAC900), globose, 1.5–1.8 cm diam. *Perithecia* narrowly ovoid, brown, immersed, with packed mycelium surrounding each perithecium. *Asci* cylindrical, 8-spored. Ascus cap rounded. *Ascospores* hyaline, filiform, multiseptate, whole. On PDA cultures hyphae branched, smooth, hyaline, sometimes fasciculate. *Conidiogenous structures* phialidic, conidiophores elongate, erect, non-septate, verticillately or irregularly branched, bearing phialides singly, or in whorls of one to five. *Conidia* ellipsoidal or reniform.

*Habitat* — Egg cases (Ootheca) of praying mantis (Mantidae) in leaf litter.

*Distribution* — Japan, China, Thailand.

***Petchia siamensis*** Thanakitp., Mongkols. & Luangsa-ard, *sp. nov.* — MycoBank MB830168; Fig. 7

*Etymology.* Referring to 'Siam', old name for Thailand, where the species was collected.

*Typus.* THAILAND, Nakhon Ratchasima Province, Khao Yai National Park, on ootheca of praying mantis (Mantidae), in leaf litter, 20 July 2014, K. Tasanathai, A. Khonsanit, W. Noisripoom & D. Thanakitpipattana (holotype BBH 39551, ex-type culture BCC73636).

*Asexual morph.* Acremonium-like.

*Stromata* erect, robust, multiple, arising from the egg case of insect host, pale cream (OAC900), cylindrical, up to 2 cm long, 0.8–1 cm wide. Fertile part occurs at the terminal end of stroma, globose, 1.5–1.8 mm diam. *Perithecia* pseudo-immersed, obpyriform, (600–)637–827(–870) × (320–)343–393(–400) µm. *Asci* cylindrical, 8-spored, up to 320 µm long, 2.5–3 µm wide. Ascus cap rounded, 2 × 2.5 µm. *Ascospores* whole, multiseptate, filiform, hyaline, septate, (200–)202–268(–300) × 1–1.5 µm.

*Culture characteristics* (from germinating ascospores) — Colonies on PDA moderate-growing, attaining c. 3 cm diam after 21 d at 20 °C. Colony purplish grey (OAC557), cottony, with high mycelial density. Colony reverse pale brown (OAC813). Vegetative hyphae smooth, septate, hyaline, c. 3 µm diam. Conidiogenous structures consisting of erect conidiophores arising from the vegetative hyphae and aerial hyphae. Conidiophores forming verticillate branches with phialides singly or in whorls of three to five. Phialides (10–)12–20(–28) × 1–2 µm, awl-shaped, acremonium-like. Conidia aggregated in slimy heads at the apex of phialides, hyaline, ellipsoidal or reniform, 1-celled, (3–)4–6 × 1–2 µm. Chlamydo spores not observed. Colonies on PSA moderate-growing, attaining c. 3 cm diam after 21 d at 20 °C. Colony purplish grey (OAC557), cottony with high mycelial density. Colony reverse pale yellow (OAC813). Vegetative hyphae smooth, septate, hyaline, c. 3 µm diam. Conidiogenous structures consisting of erect conidiophores arising from the vegetative hyphae and aerial hyphae. Conidiophores forming verticillate branches with phialides singly or in whorls of three to five. Phialides 10–20(–25) × 1–2 µm, awl-shaped, acremonium-like. Conidia aggregated in slimy heads at the apex of phialides, hyaline, ellipsoidal 1-celled, (3–)4–5(–6) × 1–2 µm. Chlamydo spores not observed. Colonies on SDAY/4 moderate-growing, attaining c. 2.5 cm diam after 21 d at 20 °C.

Colony white with irregular edges, velvety. Colony reverse pale yellow (OAC813). Vegetative hyphae smooth, septate, hyaline, c. 3 µm diam. Conidiogenous structures consisting of erect conidiophores arising from the vegetative hyphae and aerial hyphae. Conidiophores forming verticillate branches with phialides singly or in whorls of three to five. Phialides 12–20(–35) × 1–2 µm, awl-shaped, acremonium-like. Conidia aggregated in slimy heads at the apex of phialides, hyaline, ellipsoidal or reniform, 1-celled, (3–)3.5–5(–6) × 1–2 µm. Chlamydo spores not observed.

*Additional materials examined.* THAILAND, Nakhon Ratchasima Province, Khao Yai National Park, on ootheca of praying mantis (Mantidae), in leaf litter, 2 June 2011, K. Tasanathai, P. Srikitikulchai, S. Mongkolsamrit, A. Khonsanit, K. Sansatchanon & W. Noisripoom (BBH 37736, BCC48062); Chiang Mai Province, Ban Hua Thung community forest, on ootheca of praying mantis (Mantidae), 25 Oct. 2013, K. Tasanathai, P. Srikitikulchai, A. Khonsanit, W. Noisripoom, D. Thanakitpipattana & S. Watcharapayungkit (BBH 38368, BCC68420), (BBH 37855, BCC68421), (BBH 37793, BCC68417); Ban Hua Thung community forest, on ootheca of praying mantis (Mantidae), 29 Oct. 2014, K. Tasanathai, A. Khonsanit, W. Noisripoom, D. Thanakitpipattana, P. Srikitikulchai & S. Wongkanoun (BBH 39627, BCC75724), (BBH 39660, BCC75750); Nakhon Ratchasima Province, Khao Yai National Park, on ootheca of praying mantis (Mantidae), in leaf litter, 20 July 2014, K. Tasanathai, A. Khonsanit, W. Noisripoom & D. Thanakitpipattana (BBH 39552, BCC73637).

***Petchia mantidicola*** (Kobayasi & Shimizu) Thanakitp., Mongkols. & Luangsa-ard, *comb. nov.* — MycoBank MB833017

*Basionym.* *Cordyceps mantidicola* Kobayasi & Shimizu [as '*mantidicola*'], Bull. Natl. Sci. Mus., Tokyo, Ser. B 9: 12. 1983.

*Notes* — Both *P. siamensis* and *P. mantidicola* occur on ootheca of praying mantis and produce cream terminal obovoid perithecial heads. However, in *P. siamensis* the perithecia are distinctly larger (600–870 × 320–400 µm) than those of *P. mantidicola* (400–450 × 200–250 µm), and the ascospores in *P. siamensis* are whole while they disarticulate into part-spores in *P. mantidicola* (Table 3).

**Cordycipitaceae**

***Neotorrubiella*** Tasan., Thanakitp. & Luangsa-ard, *gen. nov.* — MycoBank MB830171

*Etymology.* Referring to the phenotypic similarity of the perithecial formation to *Torrubiella*.

*Type species.* *Neotorrubiella chinghradicola* Tasan., Thanakitp. & Luangsa-ard.

*Asexual morph.* Unknown.

*Scant mycelium* covering the host, flattened, scattered, white to cream. *Perithecia* superficial, pale cream, ovoid to obclavate arising all over the body and legs of the host. *Asci* cylindrical with thickened hyaline cap, 8-spored. Ascus cap rounded. *Ascospores* filiform, multiseptate, not breaking into part-spores. In culture the colonies on PDA, PSA, SDAY/4 are slow-growing, and do not produce conidiogenous structures, even after 1 mo.

*Habitat* — On Gryllidae, on underside of leaves.

*Distribution* — Thailand, known from Khlong Nakha Wildlife Sanctuary, Khao Luang and Khao Yai National Parks.

*Notes* — *Neotorrubiella* resembles the genus *Torrubiella* in its lack of a stipe, and the production of superficial perithecia directly on the body of the host. It differs from *Torrubiella* by its type of ascospores. Most species in *Torrubiella* have part-spores whereas *Neotorrubiella* produces whole ascospores. Species of *Torrubiella* occur mainly on spiders and hoppers while *Neotorrubiella* was only found on crickets.

***Neotorrubiella chinghridicola*** Tasan., Thanakitp. & Luangsa-ard, *sp. nov.* — MycoBank MB830172; Fig. 8

**Etymology.** Named after the host in Thai language, chringrid, meaning cricket.

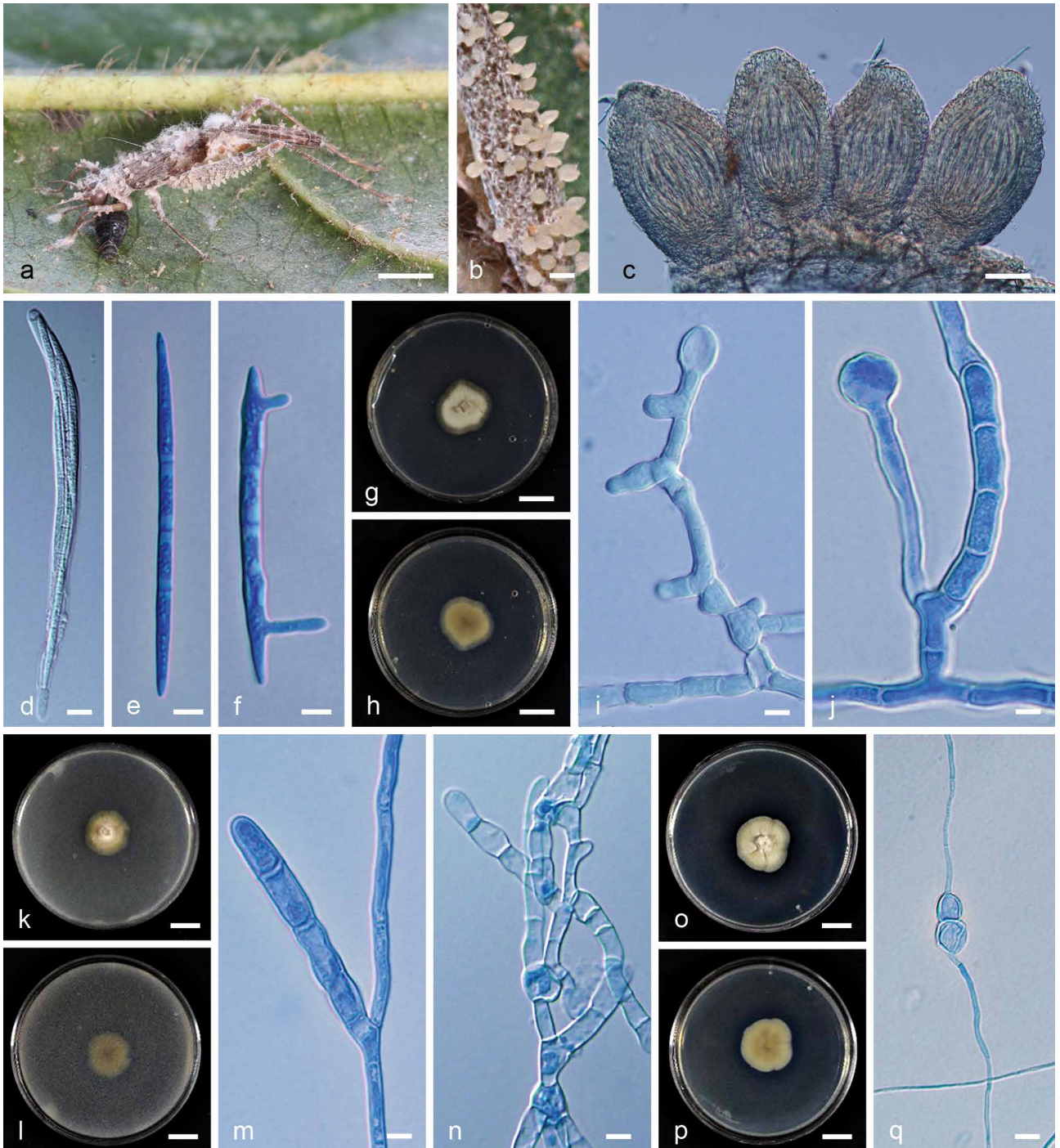
**Typus.** THAILAND, Nakhon Si Thammarat Province, Khao Luang National Park, on Gryllidae, on the underside of leaves, 26 Jan. 2016, K. Tasanathai, S. Mongkolsamrit, D. Thanakitpipattana, W. Noisripoom, R. Promharn, P. Srikitikulchai & S. Wongkanoun (holotype BBH 41240, ex-type culture BCC80733).

**Asexual morph.** Unknown.

Scant *mycelium* covering the host body, flattened, tomentose, white to cream. *Perithecia* superficial, pale cream (OAC900), ovoid to obclavate, (220–)226–261(–280) × (130–)145–167.5 (–170) µm. *Asci* cylindrical with thickened ascus apex, 8-spored,

(125–)134–162.5 × (9.5–)10–12.5(–15) µm. *Ascus* cap rounded. *Ascospores* filiform, multiseptate, not disarticulating into part-spores, (68–)70–83.5(–87.5) × 4–5 µm.

**Culture characteristics** (from germinating ascospores) — Colonies on PDA slow-growing, attaining c. 1.2 cm diam after 30 d at 20 °C. Colony white cream (OAC795), cottony with high mycelial density, pigmented around the colony. Colony reverse cream yellow (OAC813). Colony on PDA did not produce any conidiogenous structures after 1 mo. Chlamydo-spores observed on PDA after 1 mo. Colonies on PSA slow-growing, attaining c. 0.8 cm diam after 30 d at 20 °C. Colony white cream (OAC816) with a brown clear zone at the colony edges. Colony reverse yellow brown (OAC799). Colony on PSA did not produce any conidiogenous structures after 1 mo. Chlamydo-spores observed after 1 mo. Colonies on SDAY/4



**Fig. 8** *Neotorrubiella chinghridicola* (BBH 41240, BCC80733). a. Fungus on Gryllidae host; b. superficial perithecia on host; c. perithecia; d. asci with ascospores; e. ascospore; f. germination of ascospore; g–h. colony on PDA at 30 d: (g) obverse (h) reverse; i–j. swollen hyphae produced on PDA; k–l. colony on PSA at 30 d: (k) obverse (l) reverse; m–n. swollen hyphae produced on PSA; o–p. colony on SDAY/4 at 30 d (o) obverse (p) reverse; q. swollen hyphae produced on SDAY/4. — Scale bars: a = 3 mm; b = 280 µm; c = 70 µm; d = 10 µm; e–f = 7 µm; g–h = 7 mm; i–j, m–n, q = 100 µm; k–l = 6 mm; o–p = 8 mm.



slow-growing, attaining c. 1.4 cm diam after 30 d at 20 °C. Colony cream (OAC815), cottony with high mycelial density. Colony reverse yellow cream (OAC814) with a brown clear zone at the colony edges. Similar to the colonies on PDA and PSA, it did not produce any conidiogenous structures after 1 mo. Chlamydozoospores observed after 1 mo.

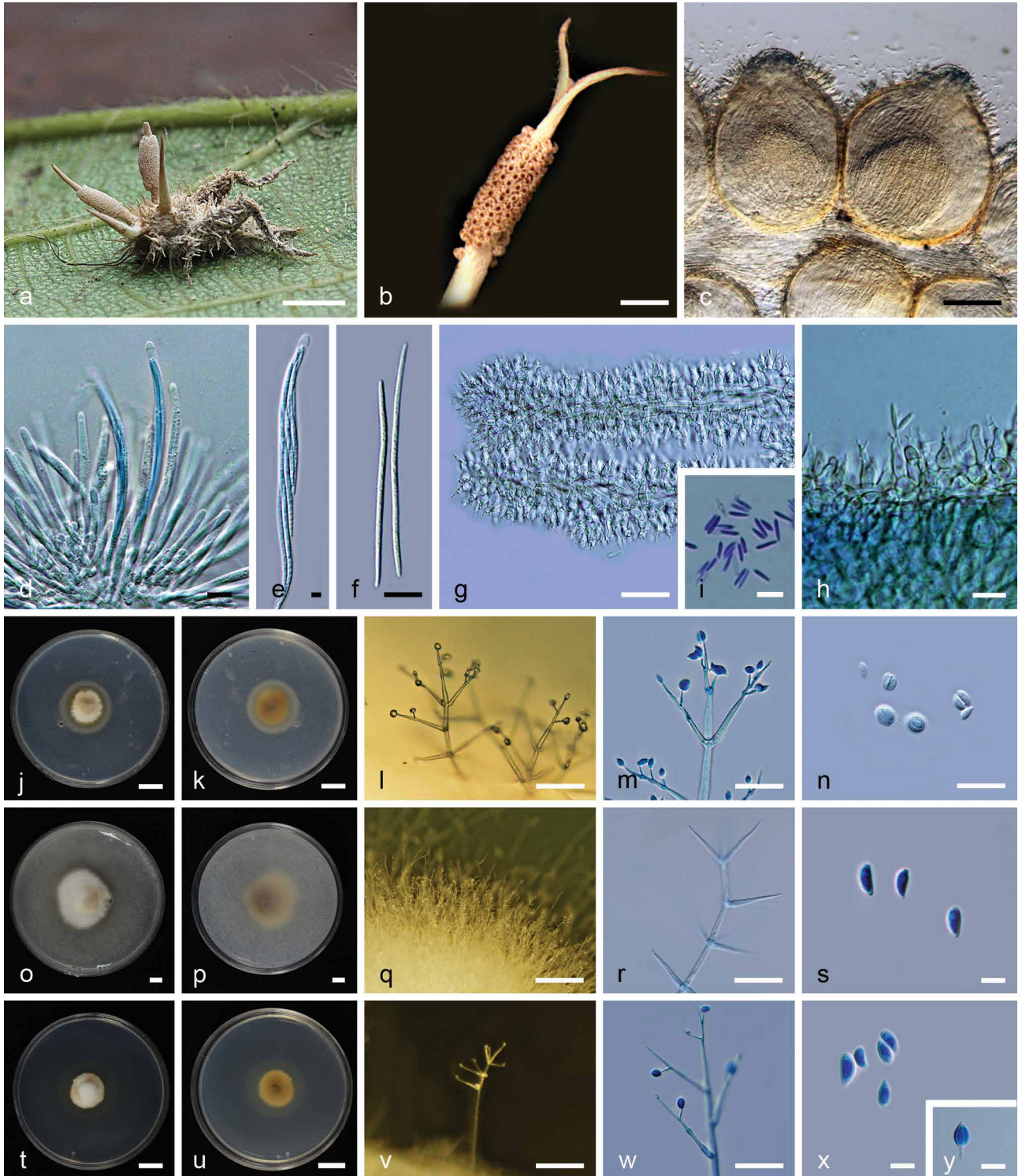
**Additional materials examined.** THAILAND, Ranong Province, Khlong Nakha Wildlife Sanctuary, on Gryllidae, on the underside of leaves, 11 Jan. 2006, K. Tسانathai, W. Chaygate, S. Mongkolsamrit, P. Srikitikulchai, B. Thongnuch, L. Hung & L. Yen (BBH 16541); Nakhon Ratchasima Province, Khao Yai National Park, on Gryllidae, on the underside of leaves, 14 Aug. 2009, K. Tسانathai, P. Srikitikulchai, S. Mongkolsamrit, T. Chohmee & R. Ridkaew

(BBH 30241, BCC39684); Nakhon Ratchasima Province, Khao Yai National Park, on Gryllidae, on the underside of leaves, 6 Oct. 2010, K. Tسانathai, P. Srikitikulchai, A. Khonsanit, R. Somnuk, K. Sansatchanon & W. Noisripoom (BBH 30006, BCC46588).

### *Ophiocordycipitaceae*

*Ophiocordyceps kobayasii* Mongkols., Thanakitp., Luangsaard & Hywel-Jones, *sp. nov.* — MycoBank MB830173; Fig. 9

**Etymology.** Named after Prof. Yosio Kobayasi (1907–1993), a Japanese mycologist best known for his work on *Cordyceps* and its allies.



**Fig. 9** *Ophiocordyceps kobayasii* (BBH 39608, BCC75694). a. Fungus on Gryllidae host; b. perithecia on stroma; c. perithecia; d–e. asci; f. ascospores; g–i. phialides with conidia on synnema; j–k. colony on PDA at 21 d: (j) obverse (k) reverse; l–m. phialides with conidia on PDA; n. conidia; o–p. colony on PSA at 21 d: (o) obverse (p) reverse; q–r. phialides with conidia on PSA; s. conidia; t–u. colony on SDAY/4 at 21 d: (t) obverse (u) reverse; v–w. phialides with conidia on SDAY/4; x–y. conidia. — Scale bars: a, o–p = 5 mm; b = 1 mm; c = 80 µm; d, n, s, x–y = 5 µm; e–i, m = 10 µm; j–k, t–u = 1 cm; l, q–r, w = 20 µm; v = 50 µm.

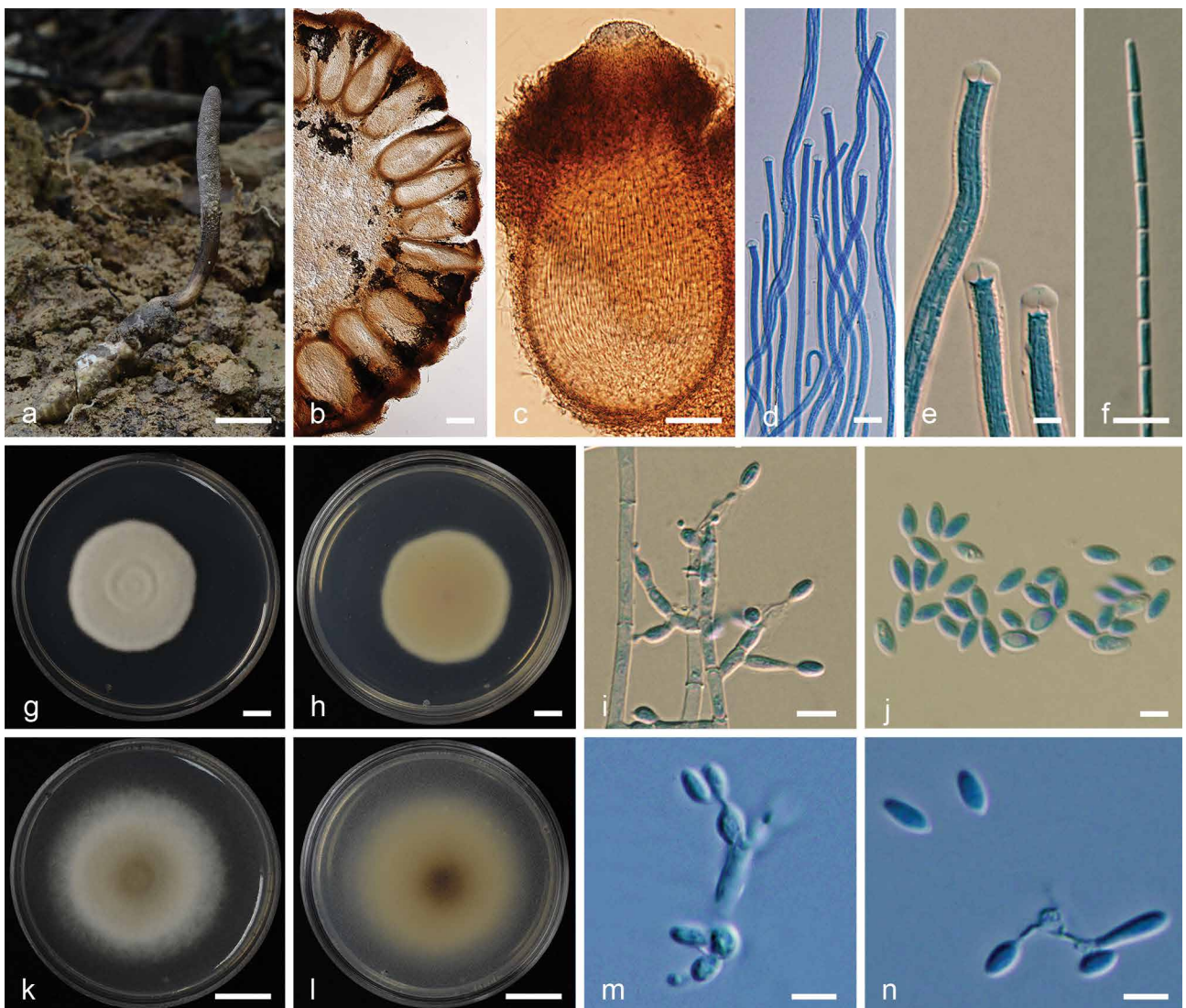
*Typus.* THAILAND, Nakhon Ratchasima Province, Khao Yai National Park, on Gryllidae, in leaf litter, 1 Oct. 2014, S. Mongkolsamrit, A. Khonsanit, W. Noisripoom, D. Thanakitpipattana & R. Somnuk (holotype BBH 39608, ex-type culture BCC75694).

**Asexual morph.** Hymenostilbe-like on the host and acremonium-like in culture.

**Stromata** multiple, arising from the head region and body of the insect host, pale cream (OAC900), cylindrical, up to 8 mm long. Densely packed perithecia occur in the middle of stromata, pale cream (OAC900), c. 3 mm long. **Perithecia** superficial, ovoid, (180–)190–230(–250) × (140–)150–175(–200) µm. **Asci** cylindrical, 8-spored, up to 125 µm long, 10–15 µm wide. Ascus cap rounded, 5 × 5 µm. **Ascospores** whole, filiform, hyaline, spirally arranged inside the ascus, aseptate, (45–)52–72(–90) × 2–2.5 µm. **Asexual morph:** Numerous synnemata growing from all over the host body. Occasionally hymenostilbe-like asexual morph is produced on the apex of the stroma and below the perithecial layer. Numerous synnemata arising from the body and legs of the host producing hymenostilbe-like asexual morph. The synnemata measured up to 1 cm long and c. 0.5 mm wide, powdery due to sporulation, pale cream (OAC900). **Conidiophores** arising from hyphae of synnemata, forming a hymenial layer. **Phialides** (6–)8–12(–15) × (3–)3.5–5 µm, hymenostilbe-like, clavate, cylindrical basal parts, bearing mini

cylindrical projections, c. 1 × 0.5 µm. **Conidia** hyaline, smooth-walled, narrow fusoid, 1-celled, (5–)6–8 × 1–2 µm.

**Culture characteristics** (from germinating ascospores) — Colonies on PDA slow-growing, attaining c. 0.8 cm diam after 21 d at 20 °C. Colony pink greyish (OAC550), cottony with high mycelial density, brown pigment around the colony. Colony reverse pale brown (OAC638). Vegetative hyphae smooth, septate, hyaline, 2–3 µm diam. Conidiogenous structures consisting of erect conidiophores arising from the vegetative hyphae. Conidiophores consist of verticillate phialides, singly or in whorls of two to five. Phialides awl-shaped, acremonium-like, smooth-walled, (18–)26–43(–50) × 2–3.5(–5) µm, bearing numerous lateral necks along the main phialide, (2–)2.5–8(–10) × 1 µm. Conidia hyaline, ellipsoidal or reniform, 1-celled, (4–)4.5–6.5(–8) × (1.5–)2–2.5(–3) µm, aggregated in slimy heads (Fig. 9l). Chlamydo spores not observed. Colonies on PSA slow-growing, attaining c. 1.2 cm diam after 21 d at 20 °C. Colony pinkish grey (OAC550), cottony with high mycelial density and brown pigment around the colony. Colony reverse pale brown (OAC638). Vegetative hyphae smooth, septate, hyaline, 2–3 µm diam. Conidiogenous structures consisting of erect conidiophores arising from the vegetative hyphae. Conidiophores consist of verticillate phialides, singly or in whorls of two to five. Phialides awl-shaped, acremonium-like, (25–)28–38(–42) ×



**Fig. 10** *Ophiocordyceps krachonicola* (BBH 40672, BCC79666). a. Stroma of fungus emerging from a mole cricket; b. cross section of stroma showing perithecia; c. perithecium; d. asci; e. part of asci with ascus tip; f. part-spores; g–h. colony on PDA: (g) obverse (h) reverse; i. conidiogenous cells with conidia; j. conidia; k–l. colony on PSA: (k) obverse (l) reverse; m. conidiogenous cells with conidia; n. conidia. — Scale bars: a = 10 mm; b = 150 µm; c = 80 µm; d = 10 µm; e–f = 5 µm; g–h = 5 mm; i–j, m–n = 5 µm; k–l = 10 mm.

(2–)2.5–4 µm. Conidia hyaline, ellipsoidal or reniform, 1-celled, 5–6 × (2–)2.5–3.5(–4) µm, aggregated in slimy heads at the apex of phialides. Chlamydoconidia not observed. Colonies on SDAY/4 slow-growing, attaining c. 8 mm diam after 21 d at 20 °C. Colony pinkish grey (OAC550), cottony with high mycelial density. Colony reverse pale brown (OAC638). Vegetative hyphae smooth, septate, hyaline, 2–3 µm diam. Conidiogenous structures consisting of erect conidiophores arising from the vegetative hyphae. Conidiophores consist of verticillate phialides, singly or in whorls of two to five. Phialides awl-shaped, acromonium-like, (20–)28–44(–50) × (2–)2.5–4 µm, bearing numerous necks, (3–)4–6(–8) × 1 µm. Conidia hyaline, ellipsoidal or reniform, 1-celled, (5–)5.5–7 × 2–3 µm, aggregated in slimy heads. Chlamydoconidia not observed.

**Additional materials examined.** THAILAND, Nakhon Ratchasima Province, Khao Yai National Park, on Gryllidae, on the underside of leaves, 8 Nov. 2012, S. Mongkolsamrit, A. Khonsanit, W. Noisriboom, P. Srikitikulchai & R. Somnuk (BBH 38353, BCC57812 and BCC58417), (BBH 35186); Saraburi Province, Khao Yai National Park, on the underside of leaves, 27 May 2015, K. Tasanathai, A. Khonsanit, W. Noisriboom, D. Thanakitpipattana, N. Kobmoo & J.J. Luangsa-ard (BBH 41178, BCC84222), (BBH 41180, BCC84223).

***Ophiocordyceps krachonicola*** Tasan., Thanakitp. & Luangsa-ard, sp. nov. — MycoBank MB830174; Fig. 10

**Etymology.** Named after the host in Thai, 'krachon'; meaning mole cricket.

**Typus.** THAILAND, Phitsanulok Province, Ban Phao Thai Community Forest, on Gryllotalpa orientalis nymph (Gryllidae), buried in the ground, 10 Oct. 2015, S. Mongkolsamrit, D. Thanakitpipattana, W. Noisriboom & A. Khonsanit (holotype BBH 40672, ex-type culture BCC79666).

**Asexual morph.** Hirsutella-like.

**Stroma** solitary, cylindrical, arising from the head of cricket nymph buried in the ground, 37–40 × 1–1.5 mm. Fertile part cylindrical to clavate, pale grey (OAC907), ostiole dark grey (OAC901), up to 25 mm long, 2–3 mm wide. **Perithecia** completely immersed, ordinal in arrangement, ovoid to obclavate, (460–)474.5–539(–580) × (180–)193.5–258(–300) µm. **Asci** cylindrical with thickened ascus apex, 8-spored, (250–)286–377(–400) × 4–5 µm. **Ascus** cap rounded. **Ascospores** divided into 64 part-spores after maturity, (300–)351–416.5(–450) × 1 µm, part-spores cylindrical, (4–)4.4–7(–10) × 1 µm.

**Culture characteristics** (from germinating ascospores) — Colonies on PDA fast growing, attaining c. 2.2 cm diam after 21 d at 20 °C, white to cream with distinct margin. Phialides flask-shaped with distinct neck, (3–)3.5–6(–7) × 1.5–2 µm. Conidia not in chains, hyaline, 1-celled, fusiform, (3–)4–5.5(–7) × 2–2.5(–3) µm. Colonies on PSA fast growing, attaining c. 3 cm diam after 21 d at 20 °C, white to pale yellow (OAC858) with packed mycelium. Colony reverse pale brown (OAC638). Phialides flask-shaped, 3–5 × 2–3 µm. Conidia hyaline, 1-celled, fusiform, 4–6 × 2–3 µm. Colonies on SDAY/4 fast growing, attaining c. 3 cm diam after 21 d at 20 °C. Colony pinkish grey (OAC550) with cottony and high mycelial density. Colony reverse pale brown (OAC638). Colony on SDAY/4 did not produce any conidiogenous structures after 1 mo.

**Additional materials examined.** THAILAND, Phitsanulok Province, Ban Phao Thai Community Forest, on Gryllotalpa orientalis nymph (Gryllidae), buried in the ground, 10 Oct. 2015, S. Mongkolsamrit, D. Thanakitpipattana, W. Noisriboom & A. Khonsanit (BBH 41219, BCC79667).

## DISCUSSION

### *Clavicipitaceae*

Two *Metarhizium* species that parasitize crickets and stick insects are nested in the core *Metarhizium* clade (Kepler et al. 2014, Luangsa-ard et al. 2017) within the *Metarhizium anisopliae* species complex (Kepler & Rehner 2013), and

separated from *Metarhizium acridum* that was previously reported to be specific to Orthoptera (Fig. 1). Nishi & Sato (2017) classified similar specimens on stick insects (Phasmatodea) and crickets (Orthoptera) as *Metarhizium majus* by using DNA sequence data of the 5' *TEF* (see Appendix). However, our multi-gene phylogenetic analyses showed our samples on stick insects and on crickets to be distinct from *M. majus*. These results were confirmed using nuclear intergenic sequence markers, which showed better phylogenetic informativeness than other legacy genes (Kepler & Rehner 2013). Two new species are thus introduced as *Metarhizium phasmatodeae* on stick insects and *Metarhizium gryllidicola* on crickets. Morphologically, both new species are difficult to distinguish from other species in the *M. anisopliae* species complex (MGT clade) but can be circumscribed based on multi-loci and IGR phylogenetic analyses and host affinity. Recent genomic studies on species of *Metarhizium* has shown that generalists evolved from specialists via transitional species with intermediate host ranges (Hu et al. 2014, Zhang et al. 2019). Based on the phylogeny presented here, the two new species in the MGT clade (Fig. 2) exhibited host-specific interactions, as opposed to the intermediate host ranges reported for *M. majus* and *M. guizhouense* (Hu et al. 2014). However, as the discovery of these entomopathogens in the field are mostly of serendipitous nature, additional data are necessary to resolve these discrepancies to place the new taxa in their proper context.

Specimens on egg cases (ootheca) of praying mantis previously identified as *Cordyceps mantidicola* from Japan (Kobayasi & Shimizu 1983) look morphologically similar to *Petchia siamensis* (Fig. 7). *Cordyceps mantidicola* was reported occurring on cocoons of Mantidae (Kobayasi & Shimizu 1983, Liu et al. 1997). However, mantis do not produce cocoons but egg cases. *Cordyceps mantidicola* from Japan and China produces part-spores while *Petchia siamensis* produces whole ascospores. We believe the Chinese '*C. mantidicola*' is different from the Thai and Japanese materials, but this can only be verified through molecular phylogenetic reconstruction using strains from Japan, China and Thailand. Multi-locus phylogenetic analyses clearly placed our new species in the *Clavicipitaceae* as a separate genus. We therefore proposed a new genus, *Petchia*, and transferred *C. mantidicola* (on ootheca of mantis from Japan) to this genus (Table 3).

### *Cordycipitaceae*

Specimens producing superficial perithecia on crickets exhibited an outer appearance (lack of stipe and perithecial characters) similar to *Torrubiella arancida* and *T. gonylepticida* occurring on spiders. However, our specimens showed a different type of ascospore morphology compared to the aforementioned species. It shares similarity with *T. wallacei* (*Lecanicillium wallacei* = *Simplicillium wallacei*) in producing whole ascospores, but differs in host as well as in phylogenetic position (Table 3). Many species of *Torrubiella* produce part-spores. In his monograph on *Paecilomyces* and related genera, Samson (1974) reported the asexual morph for both *T. arancida* and *T. gonylepticida* as *Cordyceps farinosa* (= *Paecilomyces farinosus* = *Isaria farinosa*). From our molecular phylogenetic analyses, we clearly showed that our specimens were nested in *Cordycipitaceae*, but distinct from *Cordyceps* (Fig. 3). The production of the asexual morph was not observed in any of the three culture media used. We therefore described a new genus *Neotorrubiella* to accommodate these isolates.

### *Ophiocordycipitaceae*

Known *Ophiocordyceps* species occurring on Orthoptera could be found on adult and nymph stages and have different

morphologies from the newly described species as follows: *O. amazonica* was first reported on adult Orthoptera by Hennings (1904), producing solitary or single stroma to multiple or gregarious stromata, reddish brown and rounded cylindrical to compressed fertile heads (Sanjuan et al. 2015; Table 3). The stroma of *O. krachonicola* that parasitize Orthoptera larvae is solitary, simple, dark brown, and produces a hirsutella-like asexual morph, while *O. kobayashii* has multiple, pale cream, cylindrical stromata and also parasitizes adult orthopterans (small crickets). Our molecular phylogeny and host affiliations suggest that both *Ophiocordyceps* species represent new species from Thailand (Fig. 4).

## Ecology

New species on Orthoptera (orders Orthoptera and Phasmatodea) were found buried in the ground, on the underside of leaves of dicotyledonous plants as well as lying loosely in leaf litter. These fungi occur on adults and nymphs, as well as on egg cases (ootheca) of praying mantis (Mantidae). All of the collections were derived from national parks and community forests where human disturbance is still low, with only a few individual collections. No specimens were found in agricultural ecosystems.

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**Appendix** Phylogenetic reconstruction of *Metarhizium* using 5' *TEF* sequences based on Maximum Parsimony, Bayesian analysis and RAXML. Number on the nodes are MP bootstrap/Bayesian posterior probability/ML bootstrap values above 70 %. **Bold** lines mean support for the three analyses were 100 %.

