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Diversity, molecular dating and ancestral characters state reconstruction of entomopathogenic fungi in Hypocreales

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Abstract

Hypocreales (Sordariomycetes, Ascomycota) is a highly diversified order, with more than 1000 entomopathogenic species being reported in Clavicipitaceae, Cordycipitaceae, Ophiocordycipitaceae and Polycephalomycetaceae. Taxa in these families form intimate associations with members of up to 13 orders of Insecta and other arthropods. Their variable morphological characteristics along with the host affiliations have played important roles in the classification of entomopathogenic species. However, it is still unclear whether these morphological characteristics are informative at the family, genus or species level in a phylogenetic context. This study focuses on entomopathogenic taxa collected from Thailand and Southwest China. Thirty-six species belonging to the above four families were identified using morphology and phylogeny inferred from combined data of LSU-SSU-5.8S-*tef*1-*rpb*1-*rpb*2 sequences. Among them, *Pleurocordyceps ophiocordycipiticola* is a new species. Divergence time estimates indicated the crown age of Hypocreales at 200 Mya, while that of Clavicipitaceae was at 107 Mya, Cordycipitaceae at 129 Mya, Ophiocordycipitacae at 121 Mya and Polycephalomycetaceae at 74 Mya. Based on ancestral character state reconstruction, the ancestral ecologies of Clavicipitaceae, Ophiocordycipitaceae and Polycephalomycetaceae were animal-based, while that of Cordycipitacae was fungi-based. Multiple interkingdom jumps occurred in all entomopathogenic families. Mapping of morphological characters on the phylogeny identified cases of association of perithecial arrangement and stromal texture. Ascospore and secondary ascospores morphologies were not informative at genus and family level classifications. Expanding collections to additional hosts and environments will assist towards further understanding the diversity and ecology of hypocrealean entomopathogens.

Key word – 1 new species – China – Evolution – Phylogeny – Taxonomy – Thailand

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Introduction

Entomopathogenic fungi refer to a group of unicellular or multicellular, heterotrophic, eukaryotic microorganisms that can cause fatal diseases of insects. Thus, these fungi can be used as biopesticides in ecological farming as a safe alternative to toxic chemical insecticides (Litwin et al. 2020). In addtion to their action against insects, fungal entomopathogens have a broad range of activities. For instance, *Beauveria bassiana* and species of *Akanthomyces*, *Lecanicillium* and *Metarhizium* have been reported as plant endophytes exhibiting antagonistic activity against phytopathogens (Ownley et al. 2010, Gurulingappa et al. 2011, Nicoletti & Becchimanzi 2020). *Pochonia chlamydosporia* and *Metarhizium anisopliae* are rhizosphere colonizers that promote plant growth (Kerry 2000, Kabaluk & Ericsson 2007). Moreover, entomopathogenic species have been historically used in traditional Chinese medicine as they contain various bioactive components that are beneficial for enhancing immunity, improving kidney function, and treating cancer (Zha et al. 2018, Hyde et al. 2019).

Entomopathogens are taxonomically diverse belonging to various fungal phyla such as Ascomycota, Basidiomycota, Chytridiomycota, Entomophthoromycota, Microsporidia and Zygomycota (Wei et al. 2021a). Most insect pathogens are known from three families in Hypocreales (Sordariomycetes, Ascomycota) *viz.* Clavicipitaceae, Cordycipitaceae, and Ophiocordycipitaceae (Araújo & Hughes 2016). Hypocreales was introduced by Engler and Prantl (1897) and typified with *Hypocrea* (Hypocreaceae). Hyde et al. (2020) and Wijayawardene et al. (2020) listed 14 families within Hypocreales. Polycephalomycetaceae, a family containing pathogens of insects and fungi, was recently segregated from Ophiocordycipitaceae by Xiao et al. (2023). Hypocrealean entomopathogenic fungi mainly infect arthropod hosts including insects and spiders with which they form hemibiotrophic associations (Kaya & Vega 2012, Araújo & Hughes 2016). Evidence has increasingly supported that members of the above four families are more versatile than previously thought in terms of the hosts that they associate with (Vega et al. 2009, Xiao et al. 2023). In phylogenetic analyses, species isolated from microinvertebrates, other fungi, plant materials, soil and environmental resources are intermixed with entomopathogenic fungi. This can be seen even at the genus level. For example, species of *Tolypocladium* (Ophiocordycipitaceae) infect hosts across three kingdoms (animals, plants and fungi) (Yu et al. 2021). This is also the case for genera of Clavicipitaceae and Polycephalomycetaceae.

Jumping onto distantly related hosts to exploit additional nutritional resources is an effective strategy used by hypocrealean entomopathogens. This phenomenon can be seen in *Dussiella tuberiformis* and *Hypocrella macrostroma*, whose fruiting bodies are much larger than that of their insect hosts (Koroch et al. 2004). Once the insect host has degraded, the fungi continue absorbing nutrition from the plant sap leaking through the stylet of the insect cadavers. In trying to explain host jumping two hypotheses can be evoked *viz.* host habitat and host relatedness. The former hypothesis posits that new hosts are acquired due to their proximity in the environmental niches of pathogens, whereas under the latter, pathogens are more likely to jump to hosts that are more closely related to the original ones. The host habitat hypothesis could reasonably explain parasitic fungi jumping between cicada nymphs and false truffles owing to their co-occurrence of nearby plant roots (Nikoh & Fukatsu 2000, Spatafora et al. 2007). Host jumping not only facilitates lifestyle differentiation but also is a major driver of diversity and ultimately speciation (Thines 2019).

The polarity of host jumps is a mystery that has gained extensive attention by mycologists over the past three decades. Nutrition of the ancestor of Hypocreales was inferred as plant-based in molecular dating analysis using a fossilized scale insect parasite that was dated to the early Cretaceous (Sung et al. 2008). Phylogenetic and character reconstruction analysis revealed the occurrence of numerous interkingdom host jumps in clavicipitoid fungi. More specifically, the common ancestor of the grass symbionts of Clavicipitaceae seems to have originated through a series of interkingdom host jumps from an ancestor that was a pathogen of animals (Spatafora et al. 2007). In practice, it is difficult to determine the orientation of host jumping as the acquisition of a new host happens in nature over time and cannot be tested in laboratory conditions. Based on

phylogeny and reconstruction of ancient character states, it has been hypothesized that the ancestors of Clavicipitaceae and Ophiocordycipitaceae were animal pathogens, while the lifestyle of the ancestor of Cordycipitaceae remained ambiguous (Sung et al. 2008). The plant clade within Clavicipitaceae (e.g. *Balansia*, *Claviceps*, *Epichloe*, *Myriogenospora*, *Shimizuomyces*), a family that is primarily comprised of insect pathogenic genera (e.g. *Aschersonia*, *Conoideocrella*, *Dussiella*, *Hypocrella*, *Moelleriella*, *Orbiocrella*, *Regiocrella Samuelsia*) and fungicolous fungi (e.g. *Tyrannicordyceps fratricida* and *Verticillium epiphytum*) presents a striking case of host jumping (Kepler et al. 2012). While multiple host switching events have occurred during the evolutionary history of entomopathogenic Hypocreales, the timing and route of these host jumps remains unclear. Increasing molecular data and taxonomic sampling would provide further insights into the lifestyle trajectories of entomopathogenic Hypocreales.

In response to host jumping, various types of stromata, fertile parts, perithecia, asci, ascospores, secondary ascospores, and conidiophores evolved accordingly. Species with similar teleomorphic and anamorphic characteristics are generally restricted to a certain lineage or have associations with specific hosts. For example, species in the *Ophiocordyceps unilateralis* complex obligately infect ants and produce stalked stromata on which intercalary ascomata are born (Araújo et al. 2018). Pathogens infecting scale insects commonly form pulvinate, sessile stromata, which completely cover the cadavers and contain immersed conidiomata (Chaverri et al. 2008). The phylogeny of the four emtomopathogenic families has been studied extensively (Sung et al. 2007a, b, Quandt et al. 2014, Kepler et al. 2017, Mongkolsamrit et al. 2020a, Wang et al. 2020, Xiao et al. 2023). However, the morphology-phylogeny relationships have not been comprehensively investigated for these families yet.

This study aimed to 1) assess diversity of entomopathogenic fungi from Southwest China and Thailand; 2) estimate the divergence times of the main lineages of Hypocreales; 3) understand the distribution and evolutionary relationships of fungal, arthropod, microinvertebrate and plant associated fungi within clavicipitoids; and 4) examine whether specific morphological features are phylogenetically informative.

Methods & materials

Collection, isolation and morphological studies

Surveys of fresh specimens were conducted in Southwest China and Thailand during 2016–2021. A total of 37 specimens were collected from various localities including eight locations in China (*viz.* Kunming botanical garden, Yeyahu Park, Songhuaba national forest, West hill park, Xishuangbanna, Amushan natural reserve in Yunnan Province; Ceheng County and Guiyang City in Guizhou Province) and five locations in Thailand (viz. Mushroom research centre and Chiang Dao District in Chiang Mai Province; Thoeng District and Wiang Chai District in Chiang Rai Province; Lan Saka District in NaKhon Si Thammarat Province). Fungal specimens were examined from under and upper side of living leaves (monocotyledonous and dicotyledonous), leaf litter and on the ground in evergreen or deciduous forests. Shady slopes with less sunlight and humid conditions usually favour the growth of entomopathogenic fungi. The fresh fruiting bodies were recorded and documented with XS Max iPhone camera in the field. External examinations and free-hand sections were made using Nikon SMZ 745T dissecting microscope. Micro-morphological characteristics such as perithecia/conidiomata, peridium, asci, paraphyese, ascospores, secondary ascospores, conidiophores, conidiogenous cells/phialides and conidia were investigated. Hand-sections of fruiting bodies were mounted on slides with sterile water for light microscopy examination using a compound microscope (Nikon ECLIPSE Ni) and the micromorphological images were captured using a DS-Ri2 camera attached to the compound microscope. Congo red solution and cotton blue solution were added to the water mount to show clear structures of ascospores, asci, conidia and conidiophores. Axenic cultures were obtained from germinating ascospores/conidia or tissues of fungal stroma by following the methods described in Senanayake et al. (2020). Cultures were grown on PDA (potato dextrose agar) and incubated at 20–30 ◦C. The

dried herbaria collected from China and Thailand were respectively deposited in the Herbarium of Cryptogamic Kunming Institute of Botany Academia Sinica (HKAS), Chinese Academy of Sciences, Kunming, China and the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand. The living cultures from China and Thailand specimens were also respectively deposited in the Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany Culture Collection (KUNCC). Facesoffungi and Index Fungorum numbers were registered following protocol described in Jayasiri et al. (2015) and Index Fungorum (2022), respectively.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh mycelia or fungal tissue on dried specimens using Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, Hangzhou, China) following the instructions of the manufacturer. Amplifications of the internal transcribed spacer (ITS1-5.8S-ITS2, ITS), large subunit ribosomal RNA (LSU rRNA), small subunit ribosomal RNA (SSU rRNA), translation elongation factor 1-alpha gene (*tef1*) and RNA polymerase II largest subunit (*rpb1*) and RNA polymerase II second largest subunit (*rpb2*) were performed in 25μL volumes consisting of: 21 μL PCR mixture (TSINGKE TSE102 Mix Ver.2), 1 μL of each primer (10 μM stock concentration) and 3 μL DNA template using the primers and PCR conditions described in Wei et al. (2021b). The translation elongation factor 1-alpha gene (*tefA*) and beta-tubulin (tubB) were sequenced using primers and PCR condition outlined by Song and Nan (2015). The PCR products were sent to TSINGKE company for purification and sequencing. The newly generated sequences were submitted to GenBank for assignment of accession numbers.

Phylogenetic analysis

The newly generated forward and reverse sequences were assembled with Sequencing Project Management (SeqMan) software (Clewley 1995). All the sequences generated in this study were analyzed using the BLASTn searches in the GenBank. Reference sequences were obtained from GenBank referring to recently published relevant phylogenies (Supplementary Table 1) (Chaverri et al. 2008, Khonsanit et al. 2019, 2021, Mongkolsamrit et al. 2020a, Wang et al. 2020). To present the morphological and host/substrates diversity of entomopathogenic fungi in Hypocreales, taxon sampling was concentrated to Clavicipitaceae, Cordycipitaceae, Ophiocordycipitaceae and Polycephalomycetaceae. The data matrix comprised 299 taxa representing 286 species from Hypocreales (Hypocreomycetidae), nine species from four subclasses (Diaporthomycetidae, Sordariomycetidae and Xylariomycetidae) in Sordariomycetes and four species in Leotiomycetes. Majority (84%) of the taxa were from 12 families of Hypocreales: Bionectriaceae (five taxa), Calcarisporiaceae (three taxa), Clavicipitaceae (72 taxa), Cordycipitaceae (92 taxa), Flammocladiellaceae (two taxa), Hypocreaceae (five taxa), Nectriaceae (six taxa), Niessliaceae (three taxa), Ophiocordycipitaceae (76 taxa), Polycephalomycetacea (11 taxa), Stachybotryaceae (nine taxa) and Tilachlidiaceae (two taxa). Individual data sets of LSU, SSU, ITS, *tef*1, *rpb*1 and *rpb*2 were aligned using the default settings of MAFFT online platform. The alignments of the non-protein coding genes (LSU and SSU) and protein-coding genes (*tef*1 and *rpb*2) were trimmed with TrimAl v1.2 using default setting. The ITS1 and ITS2 regions and the intron regions of the protein-coding genes *rpb*1 (68 bp introns), were excluded from further analyses. Hence, only 5.8S and exons were used for further analyses. Four species (*Coccomyces dentatus*, *Mollisia ventosa*, *M. cinerea* and *Thelebolus globosus*) in Leotiomycetes were used as the outgroup taxa. jModel (v.2.2.10) under the Akaike Information Criterion (AIC) was used to determine the best suited model of evolution for each alignment individually. The concatenated LSU-SSU-5.8S-*tef*1-*rpb*1-*rpb*2 data matrix was subjected to maximum likelihood (ML) and Bayesian inference (BI) analyses. Maximum likelihood trees were inferred using IQ-TREE v.1.6.12 (Nguyen et al. 2015) in PhyloSuite (Zhang et al. 2020). The models were automatically selected by IQ-TREE ('Auto' option in IQ-TREE). Branch support was estimated from 5000 ultrafast bootstraps (UFB) (Minh et al. 2013) and the Shimodaira–Hasegawa–like approximate likelihood-ratio test (SH-aLRT) (Anisimova et al. 2011). In the ML analyses, bootstrap values were considered as: SH-aLRT ≥ 80

and UFB ≥ 95 has strong support; either SH-aLRT < 80 or UFB < 95 has moderate support; SH-aLRT < 80 and UFB < 95 has poor support (Yu et al. 2021).

Bayesian inference analysis was executed by employing MCMC sampling in MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001, Charleston & Robertson 2002) for 10,000,000 MCMC generations using four chains and trees sampled every 1000th generation. The first 25% of trees were considered as the burn-in phase and were discarded. The remaining 75% trees were used to calculate the posterior probabilities (PP). Nodes with posterior probability (BIPP) > 0.90 were considered to be well supported.

Divergence time estimation

The dataset used for divergence time estimation is the same as the one for ML and BI analyses. One fossil and a secondary data point were selected to calibrate the node age, following the methodology described in Samarakoon et al. (2016). The fossil *Paleoophiocordyceps coccophagus* (Sung et al. 2008) was used to calibrate the crown node of *Hirsutella*, *Ophiocordyceps* and *Paraisaria* (exponential distribution, offset = 100, mean = 27.5, with 95% credibility interval of 182.4 Mya). The rootHeight parameter was calibrated from the split of Leotiomycetes and Sordariomycetes (normal distribution, mean = 258 and SD = 35 with 95% credibility interval) (Samarakoon et al. 2016). The divergence time analysis was performed using BEAST v. 1.8.0 (Drummond et al. 2012). Aligned sequence matrices of the six genes were partitioned and exported to BEAUti v. 1.8.0 for preparing an XML file. Substitution models were set to be unlinked, while clock and the tree prior parameters were set as linked across partitions. The best-fit substitution model for each gene was selected from 88 substitution schemes using jModelTest v. 2.1.10 and determined by the AIC. The nucleotide substitution model was set to GTR+I+G for LSU, SSU, 5.8S and *rpb*2 gene, HKY+I+G for *tef*1 and *rpb*1 gene. An uncorrelated relaxed clock model (Drummond et al. 2006) with a lognormal distribution of rates was employed for each partition. We used the Yule process to model speciation, with uniform priors on probability of splits and extinctions. BEAST analyses were run for 400,000,000 generations and sampling parameters were recorded every 1000 generations in four parallel runs. Tracer v. 1.6 was used to assess ESS values for convergence across replicate analyses, where an ESS of 200 or greater for combined independent runs for each scenario was considered as sufficient posterior sampling. After removing a 10 % proportion of states of each run as burn-in, the remaining trees were combined in LogCombiner v. 1.8.0. The maximum clade credibility (MCC) tree was constructed with TreeAnnotator v. 1.8.0, and then visualized using FigTree v. 1.4.0 (Rambaut 2012). The PP equal to or greater than 0.95 was treated as significant support.

Ancestral character state reconstruction of host affiliations and morphological character mapping

The associated insect hosts play important roles in traditional and modern classification of entomopathogenic species in Hypocreales. In this study, we employed the software RASP v. 3.2.1 to reconstruct the ancestral states of the host affiliations. The time-calibrated MCC tree was input to RASP v. 3.2.1 to analyze using Bayesian Binary MCMC (BBM). The parameters were as follows: maximum number of areas 4; number of cycles 5000; number of chains 10; frequency of samples 100; discard samples 100; temperature 0.1; state frequencies fixed (JC); among-site rate variation equal. The states of the host affiliations used in this study are as follows: (a) arthropods (b) microinvertebrates (c) plants (d) fungi (e) others. The stromatal types, perithecial orientation, ascospores morphologies, secondary ascospores shape and conidiophores structures were redrawn from Shimizu (1997) and mapped to the RASP tree using Adobe Illustrator® CS3 (Version 15.0.0, Adobe®, San Jose, CA, USA).

Results

Phylogenetic analysis

Alignment had 4589 characters with 3155 distinct patterns, 2232 parsimony-informative, 415 singleton sites and 1942 constant sites. The data matrix contained six partitions including LSU (833 bp), SSU (1018 bp), 5.8S (155 bp), *tef*1 (897 bp), *rpb*1 (640 bp) and *rpb*2 (1046 bp). The optional model selected by Model were as follow: LSU: TrN+I+G, 5.8S: GTR+I+G, SSU: TrNef+I+G, *tef1*: HKY+I+G, *rpb1*: K80+G, *rpb2*: TVM+I+G. The likelihood of the best scoring ML tree was -171354.695391 (Fig. 1). The generic level relationships of the ML and BI trees were similar in topology.

Each of the families in Hypocreales was monophyletic with moderate to strong statistical support. Niessliaceae and Stachybotryaceae appeared as the early-diverging lineages within Hypocreales. Bionectriaceae, Flammocladiellaceae, and Tilachlidiaceae grouped together and this relationship had maximum statistical support (SH-aLRT 100%/ UFB 100 %/ PP 1.00, Fig. 1). Hypocreaceae and Calcarisporiaceae form a clade which is sister to Cordycipitaceae with moderate support from ML analysis and strong support from Bayesian analysis (SH-aLRT 72%/ 81 %/ PP 1.00, Fig. 1). Ophiocordycipitaceae is monophyletic with maximum support and branches off the clade consisting of Clavicipitaceae and Polycephalomycetaceae. This relationship is strongly supported. Thirty-seven newly generated specimens were included in the phylogenetic analysis. Nine of them claded in Clavicipitaceae. Among them, the specimens HKAS 102459, HKAS 102457 and HKAS 102454 grouped with *Metarhizium pemphigi*, *M. purpureonigrum* and *M. rileyi*, respectively. Specimen MFLU 22-0260 formed a monophyletic clade with *Orbiocrella petchii*. Specimens MFLU 22-0264 grouped with *Aschersonia samoensis*, MFLU 22-0263 with *Moelleriella chiangmaiensis*, while MFLU 22-0261 and MFLU 22-0262 grouped with *Moelleriella raciborskii*. Specimen HKAS 106462 cladded with *Epichloë.* Two fungicolous specimens MFLU 22-0265 and HKAS 102450 respectively clustered with *Pleurocordyceps marginaliradians* and *Perennicordyceps elaphomyceticola*, in Polycephalomycetaceae. Ten specimens grouped in Ophiocordycipitaceae, among them, eight specimens namely HKAS 102447, MFLU 22-0268, MFLU 22-0270, HKAS 102442, MFLU 22-0266, HKAS 102449, MFLU 22-0267 and MFLU 22-0271 were resolved as known species of *Ophiocordyceps crinalis*, *O. blattae*, *O. sporangifera*, *O. thanathonensis*, *O. pseudolloydii*, *O. vespulae*, *O. globiceps* and *Purpureocillium takamizusanense*, respectively. Specimen MFLU 22-0269 placed in the *Ophiocordyceps unilateralis* complex, while specimen HKAS 102461 formed a sister clade to *O. nutans*. Sixteen specimens nested in Cordycipitaceae. Fifteen of them belonged to known species as follows: specimens HKAS 102444 was identified as *Cordyceps qingchengensis*, HKAS 102445 as *C. tenuipes*, HKAS 102455 as *C. fumosorosea*, HKAS 102451 as *C. cateniannulata*, HKAS 102453 as *C. neopruinosa*, HKAS 102448 as *C. inthanonensis*, HKAS 102458 as *C. catenioliqua*, HKAS 102446 as *Beauveria bassiana*, HKAS 102452 as *B. medogensis*, MFLU 22-0272 as *B. majiangensis*, HKAS 102456 as *B. pseudobassiana*, MFLU 22-0273 as *Akanthomyces kanyawimiae*, HKAS 102443 as *Samsoniella hepiali*, MFLU 22-0274 as *Blackwellomyces roseostromatus*, and MFLU 22-0275 as *Gibellula gamsii*. Specimen HKAS 102460 grouped with *Cordyceps lepidopterorum* and *C. jakajanicola* without branch length.

All genera in the four entomopathogenic families were monophyletic, with the exception of *Lecanicillium, Metarhizium* and *Ophiocordyceps.* Sequences designated as *Lecanicillium* were nested as distantly related clades within Cordycipitaceae.

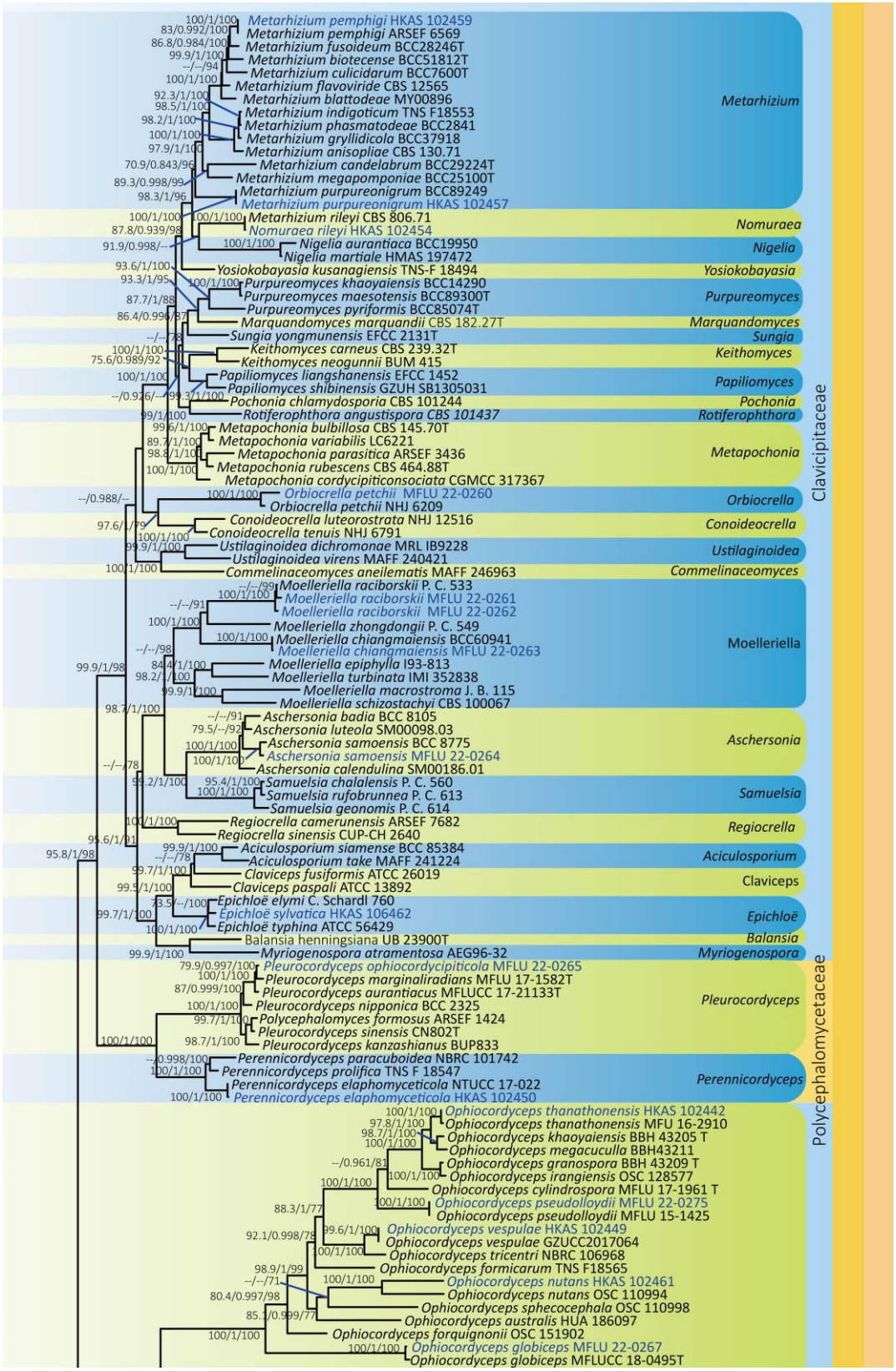

Figure 1 – Phylogram generated from maximum likelihood analysis based on combined LSU-SSU-5.8S-*tef1*-*rpb*1-*rpb*2 sequence data of 299 taxa and 4589 sites. The UFB value (left) and SH-aLRT value (right) equal to or greater than 80 and posterior probabilities (middle) equal to or higher than 0.95 are given near to the nodes. Genus, family and subclass, class names are indicated on the left side of the tree. The newly generated sequences are in blue.

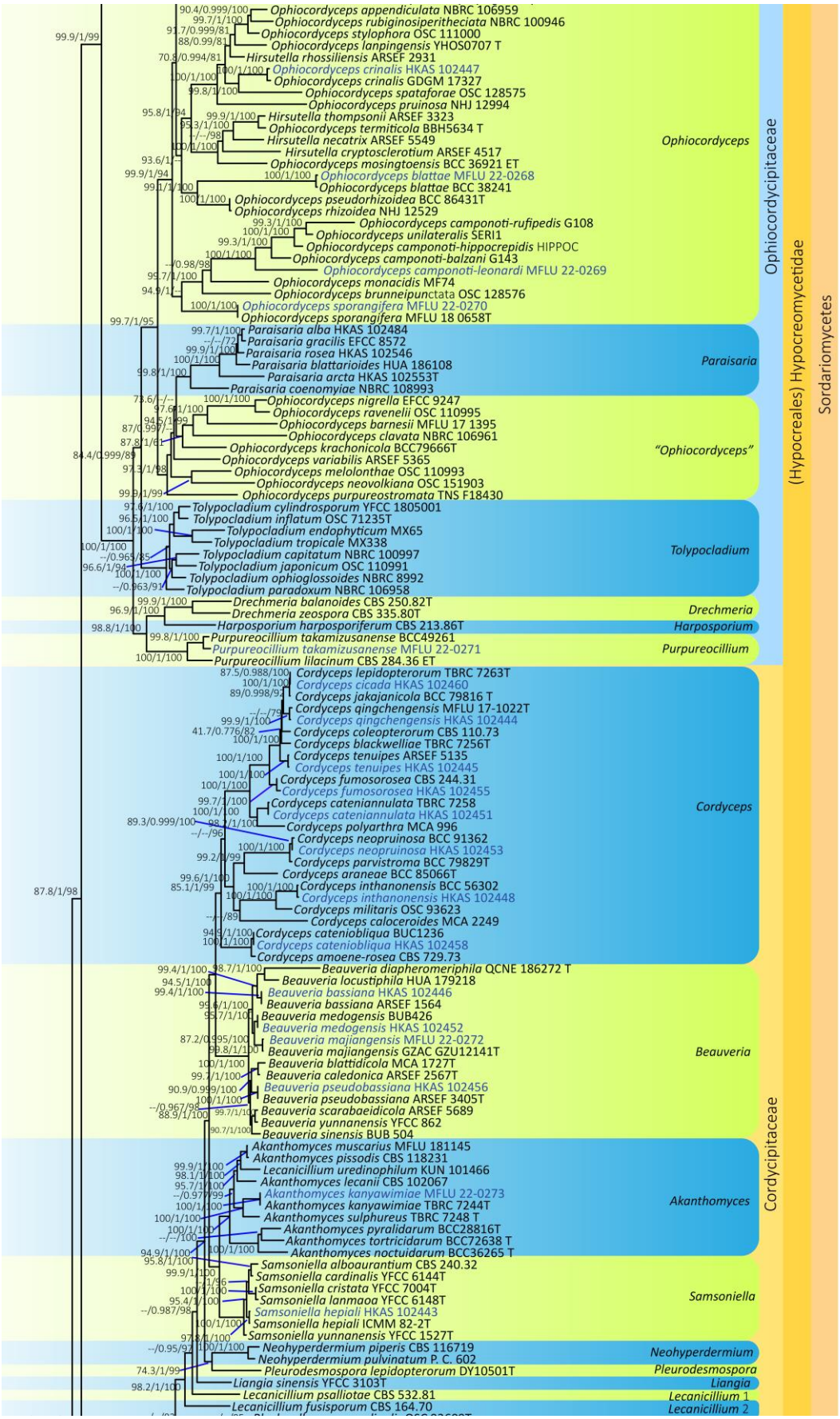

Figure 1 – Continued.

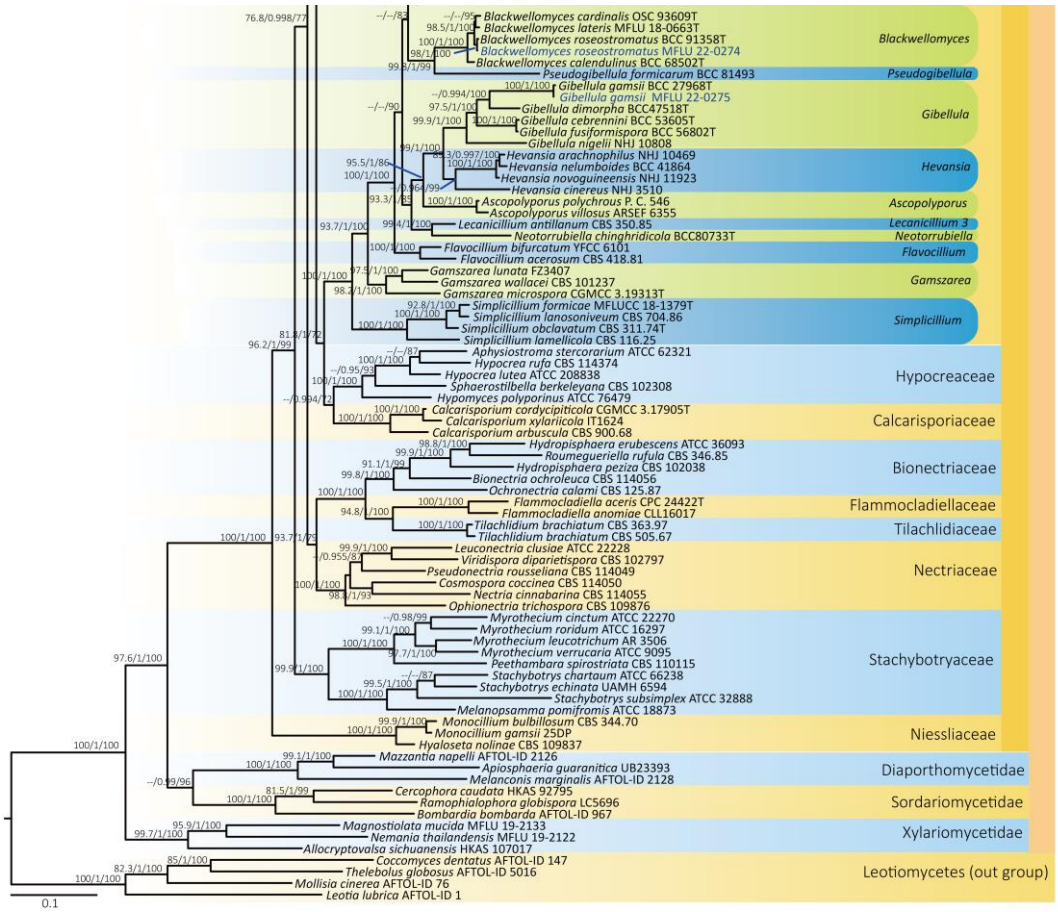

Figure 1 – Continued.

Divergence time estimation

The maximum clade credibility (MCC) tree with divergence estimates is shown in Fig. 2 with bars representing 95% confidence intervals for each node. The topology of MCC tree was largely consistent with that of ML and BI trees regarding the major lineages within Hypocreales with one notable exception. Calcarisporiaceae was a sister clade to Hypocreaceae in ML and BI trees, while it branched off the clade consisting of Clavicipitaceae, Ophiocordycipitaceae and Polycephalomycetaceae in the MCC tree. The detailed divergence times (stem and crown age) of families in Hypocreales are listed in Table 2. The divergence date between Leotiomycetes and Sordariomycetes was estimated many times in previous studies, with its median age being at 290–280 Mya in Lucking et al. (2009), 340 Mya in Gueidan et al. (2011), 247 Mya in Prieto and Wedin (2013), 315 Mya in Beimforde et al. (2014), 290 Mya in Pérez-Ortega et al. (2016) and 314 Mya in Hongsanan et al. (2016). The MCC tree in this study shows that Leotiomycetes diverged from Sordariomycetes in the Carboniferous 305 Ma (247–370Mya, 95% HPD interval), which agreed with the result in previous studies. The crown age of Hypocreales was estimated as 193 Mya in Sung et al. (2008) and 106 Mya or 167 Mya in Samarakoon et al. (2016). Similarly, in the present study the crown age of Hypocreales was estimated at 200 Mya (174–232). Based on the geological timescale, the fossil age of *Paleoophiocordyceps coccophagus* has been estimated at around 99–105 Mya (Cruickshank & Ko 2003). This fossil taxon was used to calibrate the crown age of *Ophiocordyceps sensu lato* in this study. Herein, the crown age of *Ophiocordycpes s.l.* was in the Cretaceous at 105 Mya (100–120). In our dataset, Tilachlidiaceae was represented by a single species, *Tilachlidium brachiatum*, thus its crown and stem age were not informative for family-level delimitation. The crown ages of most families (10 out of 11, 66 %) in Hypocreales were between 29 to 151 Mya and their stem ages were between 81 to 200 Mya (Table 2).

Table 2 Bayesian estimates of divergence times (Mya), including 95% credibility intervals (CI), for families of Hypocreales.

Node	Taxa	Stem age (CI)	Geological period	Crown age (CI)	Geological period
1	Bionectriaceae	119 (90–142)	Cretaceous	92 (69–114)	Cretaceous
2	Clavicipitaceae	129 (108–151)	Cretaceous	107 (90–126)	Cretaceous
3	Cordycipitaceae	151 (129–177)	Jurassic	129 (109–151)	Cretaceous
4	Flammocladiellaceae	81 (60–116)	Cretaceous	29 (12–48)	Genozoic
5	Hypocreaceae	151 (129–177)	Jurassic	93 (66–124)	Cretaceous
6	Nectriaceae	158 (133–186)	Jurassic	100 (75–128)	Cretaceous
7	Niessliaceae	200 (174–232)	Jurassic	33 (18–55)	Genozoic
8	Ophiocordycipitaceae	146 (130–167)	Jurassic	121 (108–139)	Cretaceous
9	Polycephalomycetaceae	129 (108–151)	Cretaceous	74 (45–100)	Cretaceous
10	Stachybotryaceae	188 (159–210)	Jurassic	151 (121–177)	Jurassic
11	Tilachlidiaceae	87 (60–116)	Cretaceous	6.5 (2.5–11)	Genozoic

Ancestral character state reconstruction analysis and morphological character mapping

Our analysis showed that the ancestral state of the nutritional mode of hypocrealean taxa was plant-based (node 1, Fig. 3) and followed by switches towards fungal and animal hosts (nodes 2 and 3, Fig. 3). This result agreed with the study conducted by Sung et al. (2008). The polarity of the host shifts in hypocrealean entomopathogenic fungi was plant-fungus-animal. The crown age of Cordycipitaceae is estimated at the Jurassic, ca. 129 Mya with the ancestral state being fungi-based (node 4 Fig. 3). Species of *Simplicillium*, *Lecanicillium*, *Liangia* and *Samsoniella* have retained the fungal-based state. Switches to arthropod and plant hosts are noted. An animal pathogen represents the ancestral ecology of Ophiocordycipitaceae and the crown age of this family was at the Cretaceous, ca. 121 Mya (node 5, Fig. 3). *Ophiocordyceps* contains species infecting a wide range of arthropod hosts including Coleoptera, Blattodea, Dictyoptera, Diptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Nematoda and Orthoptera. Interestingly, *Ophiocordyceps unilateralis sensu lato*, *O. sphecocephala sensu lato* and *O. nutans sensu lato* show high specificity to adults of ants, wasps and stinkbugs, respectively. Microinvertebrate, fungal and plant hosts are present in this family. The crown node of Polycephalomycetaceae (74 Mya) was indicated as animal-based (node 6, Fig. 3). This family comprises species that infect other *Cordyceps* species, *Elaphomyces* and arthropods. The most common ancestor of Clavicipitaceae was estimated at the Cretaceous, ca. 107 Mya, with animal pathogens as the ancestral ecology (node 7, Fig. 3). Species of this family also have developed the ability to parasitize diverse orders of arthropods. A clade consisting of *Aschersonia*, *Moelleriella*, *Regiocrella* and *Samuelsia* form a monophyletic group and specifically infect scale insects and whiteflies. Two distant clades contain species with plant hosts (nodes 8, 9, Fig. 3). Microinvertebrate and fungal hosts are sparsely noted in this family. Details of the host can be found in column 1, Fig. 3

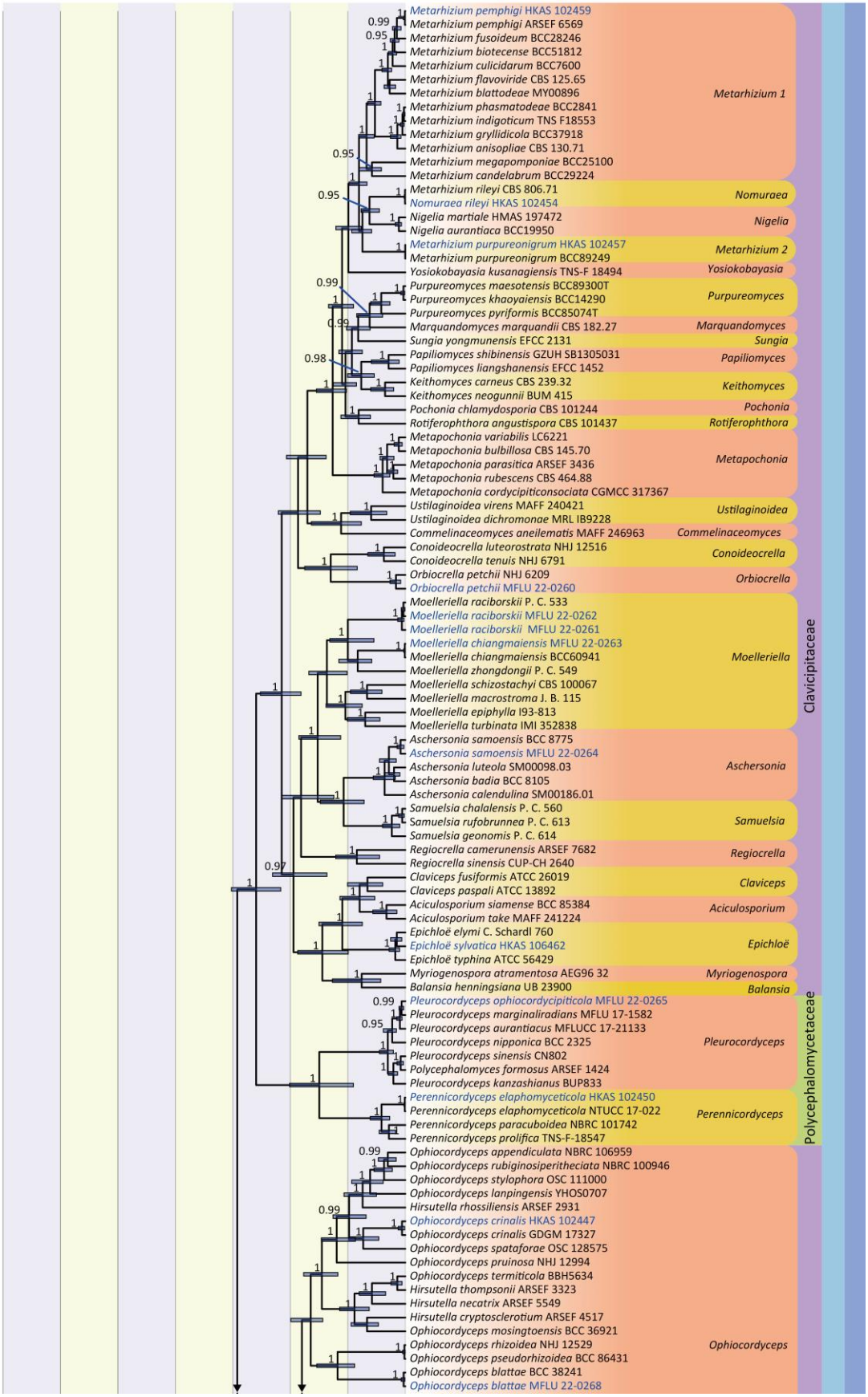

Figure 2 – Maximum clade credibility (MCC) tree with divergence times estimates for main groups of the Hypocreales obtained from a Bayesian approach (BEAST) using one fossil minimum age constraints and a secondary data. Numbers at nodes indicate posterior probabilities for node support. Bars correspond to the 95% highest posterior density intervals. For estimated median ages

of hypocrealean families, see Table 2. Assignments in the tree of the fossil minimum age constraints and secondary data are marked with red and yellow circles, respectively.

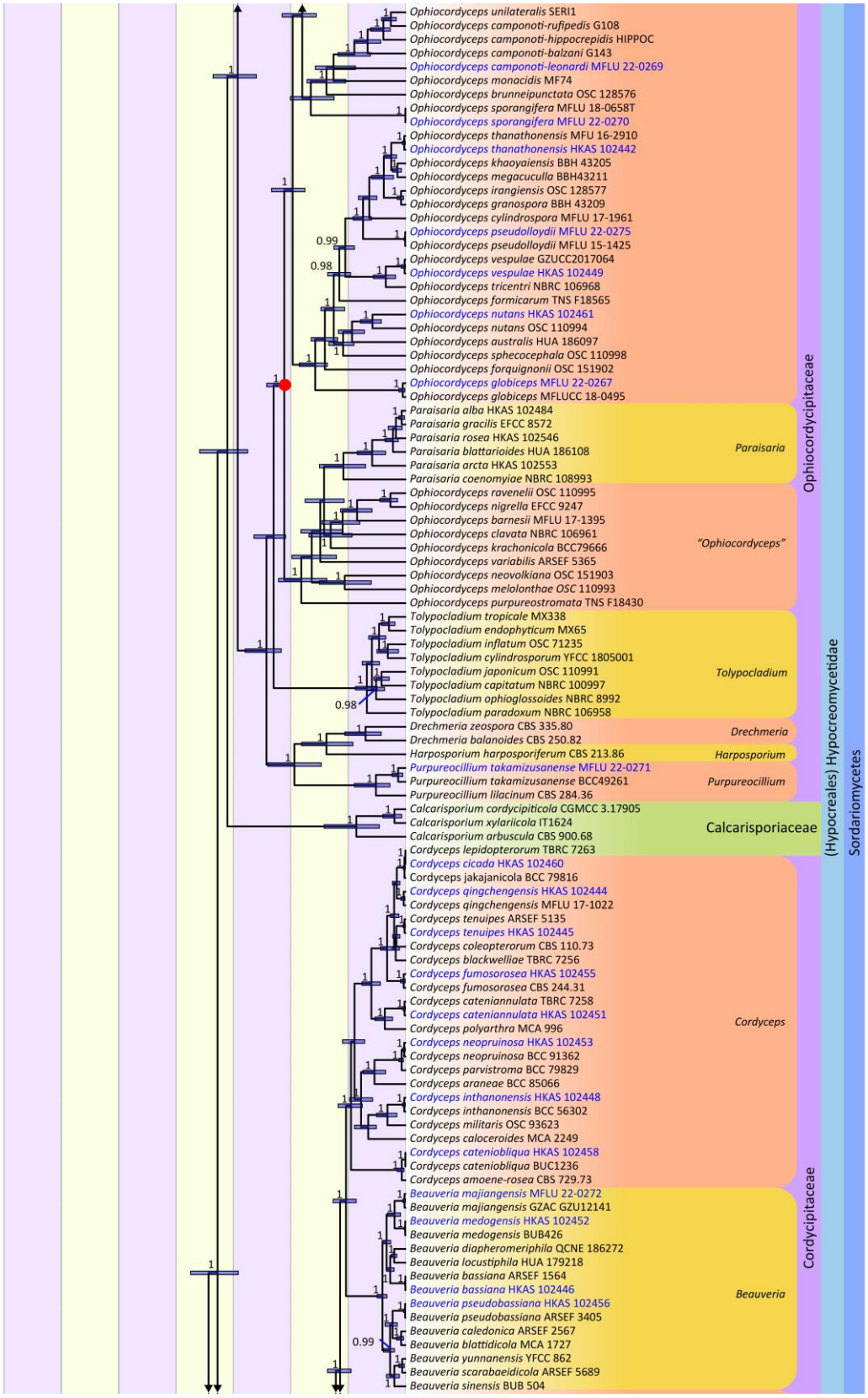

Figure 2 – Continued.

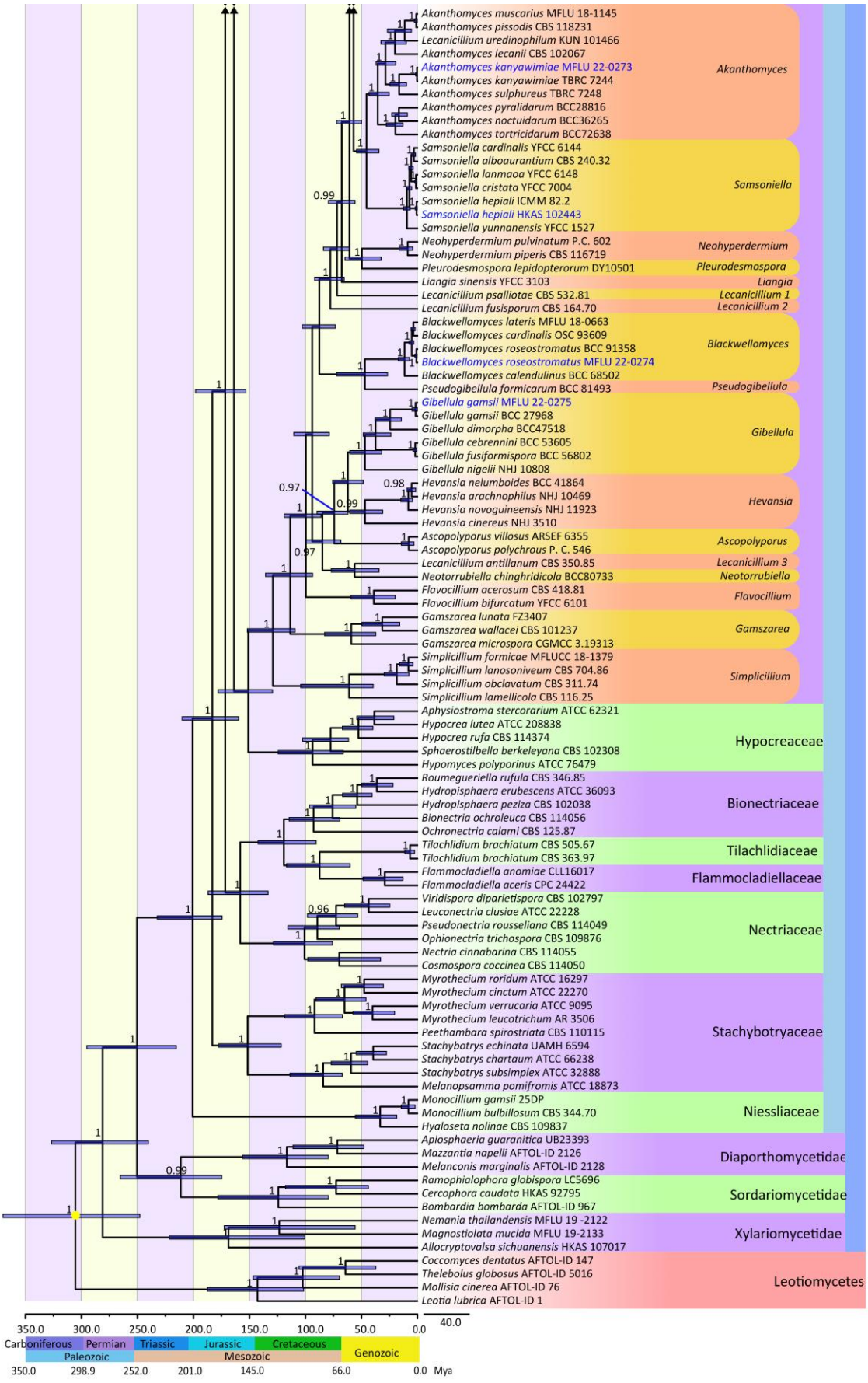

Figure 2 – Continued.

Stroma type (column 2, Fig. 3), perithecia arrangement (column 3, Fig. 3), ascospores (column 4, Fig. 3), secondary ascospores (column 5, Fig. 3) and conidiophores (column 6, Fig. 3) serve as important diagnostic characters in traditional classification of entomopathogenic species in Hypocrelaes. Even now, evolution of these characters has still rarely been investigated in part because for many taxa only the asexual morph is known. Our study shows that the early-diverging hypocrealean families (171–200 Mya, Jurassic period) have astromatic fruiting bodies with few-celled ascospores. Stromatic fruiting bodies are widely distributed in Clavicipitaceae (ca. 107 Mya), Cordycipitaceae (ca. 129 Mya), Ophiocordycipitaceae (ca. 121 Mya) and Polycephalomycetaceae (ca. 74 Mya). *Simplicillium* and *Gamszarea* are early diverging clades in Cordycipitaceae and their sexual morphs are linked to *Torrubiella* spp., which has subicular stromata (Zare & Gams 2001, Zhang et al. 2021). This stromatal form is noted in certain genera of Cordycipitaceae (e.g. *Neotorrubiella*, *Hevansia*, *Gibellula*) and Clavicipitaceae (e. g. *Myriogenospora*, *Balansia*, *Epichloë* and *Regiocrella*). Pulvinate stromata were mainly found in genera of Clavicipitaceae (e. g. *Aschersonia, Conoideocrella, Moelleriella, Orbiocrella* and *Samuelsia*). However, this stromatal form is rarely reported from genera in the rest of entomopathogenic families. Stroma with a terminal fertile part was frequently observed from genera in Ophiocordycipitaceae, while it is less reported in other entomopathogenic families. Stroma with a lateral fertile part is particularly specific to species in *Ophiocordyceps unilateralis* complex and *Pleurocordyceps* (Polycephalomycetaceae). Stroma with a subterminal fertile part is mainly found in genera of Cordycipitaceae and Clavicipitaceae, while this form has rarely been described from Ophiocordycipitaceae and Polycephalomycetaceae. Stroma with an intercalary fertile part can be seen in species of *Ophiocordyceps* and *Perennicordyceps* (Polycephalomycetaceae), while it is hardly observed in species of Cordycipitaceae and Clavicipitaceae. Certain genera commonly develop similar perithecial arrangements. *Ophiocordyceps* is an exceptional genus, which contains a large number of species with various types of perithecia. Species of the basal families of Hypocreales usually produce short, few-celled, non-disarticulating ascospores, while toward the crown lineages of this order, elongated fusiform to filiform, multiseptate, disarticulating ascospores are frequently observed. A certain genus could have various shapes of secondary ascospores. Most genera in the entomopathogenic families have specific conidiophore morphologies, except for isaria-like and verticillium-like forms, which have been observed in multiple genera.

Taxonomy

Clavicipitaceae Rogerson, Mycologia 62(5): 900 (1970)

Notes – We described nine species in Clavicipitaceae belonging to *Aschersonia, Epichloë, Metarhizium, Moelleriella, Nomuraea* and *Orbiocrella.* Species of *Aschersonia, Moelleriella* and *Orbiocrella* infect scale insects or white flies and produce sessile pulvinate to tuberculate stromata, immersed conidiomata and aschersonia-like conidiophores. *Epichloë* species are symbiotic on plants and have sessile subicular stroma, immersed perithecia and filiform, multiseptate, non-disarticulating ascospores. Species in *Metarhizium* and *Nomuraea* infect Coleoptera, Hemiptera, and Lepidoptera hosts and produce metarhizium-like and nomuraea-like conidiophores, respectively.

Aschersonia Mont., Annls Sci. Nat., Bot., sér. 3 10: 121 (1848)

Aschersonia samoensis Henn., Bot. Jb. 23: 289 (1896) Fig. 5

Index Fungorum number: IF 246679; Facesoffungi number: FoF 13944

Parasitic on nymphs of scale insects. Sexual morph: undetermined. Asexual morph: *Stromata* 1.4–2.75 ($\bar{x}$ = 2, n = 5) mm in diam., up to 680 μm in high sessile, orange yellow, pulvinate, scattered. *Conidiomata* 350–550 × 270–470 ($\bar{x}$ = 442 × 382, n = 5) μm, subglobose, immersed, ostiolate. *Conidiophores* reduced to phialides. *Paraphyses* 0.7–2 ($\bar{x}$ = 1.4, n = 20) μm, filiform, aseptate, unbranched. *Phialides* 10–35 × 1.9–2.6 ($\bar{x}$ = 19 × 2.3, n = 20) μm, monophialidic,

cylindrical, straight, aseptate, smooth-walled. *Conidia* 8–12 × 1.5–2.5 ($\bar{x}$ = 11 × 2, n = 30) μm, fusiform, hyaline, guttulate, aseptate.

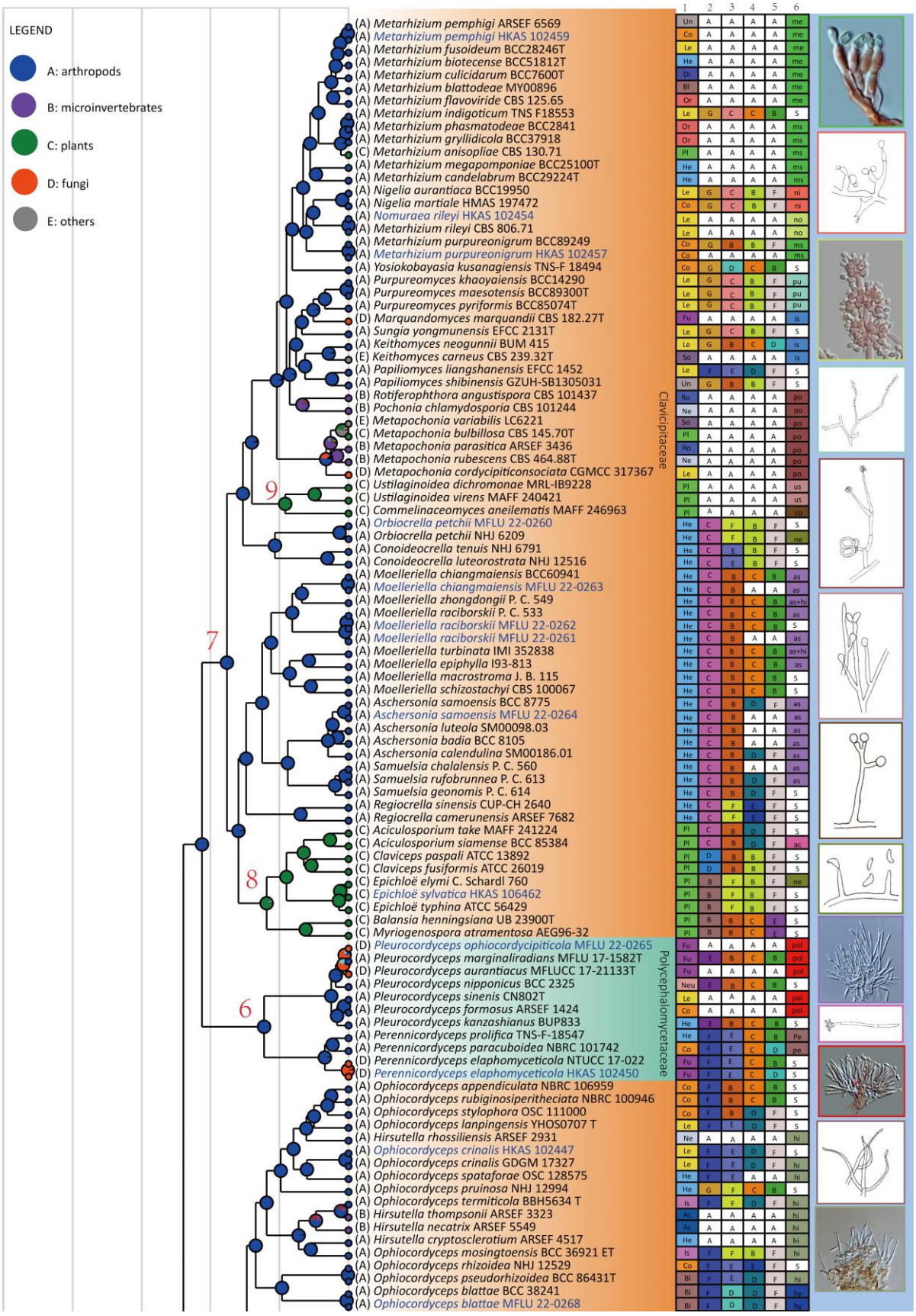

Figure 3 – Estimation of ancestral character states for hosts. Character states are given on the top-right corner and are mentioned in front of each taxon. Pie charts indicate the marginal likelihoods

of the character reconstruction at that node. Important events are denoted with red Arabic numerals. The newly generated sequences are in blue. More definitive host information (1), stromata types (2), perithecia arrangements (3), ascospore characteristics (4), secondary ascospores shapes (5) and conidiophore morphologies (6) were mapped in the table at right side of the tree. The representative of conidiophores morphologies is given for each genus in entomopathogenic families. The definitive hosts were indicated with two to three codes as follows: Ac, Acari; Ar, Araneae; Bl, Blattodea; Co, Coleoptera; Di, Diptera; Dic, Dictyoptera; Fu, Fungi; He, Hemiptera, Hy, Hymenoptera; Is, Isoptera; Le, Lepidoptera; Ne, nematodes; Neu, Neuroptera; Or, Orthoptera; Ph, Phasmatodea; Pl, Plant; Ro, rotifers; So, soil; un, indetermined insect. The sexual characters stated in columns 2 to 5 are presented in Fig. 4 and the asexual taxa are indicated with the letter "A". In column 2 the meaning of the letters is as follows: "B" = Subicular, "C" = Pulvinate, "D" = Terminal, "E" = Lateral, "F" = Intercalary, "G" = Subterminal, "H" = Astromatic. In column 3 the meaning of the letters is as follows: "B" = Immersed, "C" = Obliquely immersed, "D" = Subimmersed, "E" = Superficial, "F" = Pesudo-immersed. In column 4 the meaning of the letters is as follow: "B" = Filiform, septate or not, non-disarticulating, "C" = Filiform, multiseptate, disarticulating, "D" = Needle-like or elongated fusiform, whole, "E" = Clavate, short fusiform to ellipsoidal, few-celled, non-disarticulating. In column 5 the meaning of the letters is as follow: "B" = Cylindrical, "C" = Cubic, "D" = Allantoid, "E" = Fusiform. The letter "S" and blank in column 6 signify sexual taxa and species that do not belong to entomopathogenic families, respectively. The conidiophore types of each species in the entomopathogenic families are denoted with two to three initial letters of the corresponding genera, except for the code "ev" (evlachovaea-like), "is" (isaria-like), "hy" (hymenostilbe-like), "ma" (mariannaea-like) and "ve" (verticillium-like).

Culture characteristics – Colonies on PDA reaching 1.5 cm after 30 at 30 °C, yellow, circular, cottony, raised, with dense mycelia, conidial mass abundant, yellow.

Material examined – Thailand, Chiang Rai Province, Thoeng District, on whiteflies attached to the lower side of living dicotyledonous leaves, 23 February 2018, De-Ping Wei, Thoeng18022302 (MFLU 22-0264), living culture MFLUCC 18-1386.

Notes – *Aschersonia samoensis* is a commonly encountered species in natural forests. This species is obligately pathogenic on homopteran hosts with ubiquitous distribution spanning Australia, the Philippines, Sri Lanka and Thailand (Hywel-Jones & Evans 1993). Detailed morphological description of *A. samoensis* and its associated sexual morphs have been provided by Hywel-Jones and Evans (1993). Our collection (MFLU 22-0264) is sister to *A. samoensis* (BCC 8775) with maximum statistical support (Fig. 1). This collection was characterized by sessile, pulvinate, pliant stromata, immersed conidiomata and aschersonia-like conidiophores.

Epichloë (Fr.) Tul. & C. Tul. [as 'Epichloë'], Select. fung. carpol. (Paris) 3: 24 (1865)
Epichloë sylvatica Leuchtm. & Schardl, Mycol. Res. 102(10): 1178 (1998) Fig. 6

Index Fungorum number: IF 627492; Facesoffungi number: FoF 13076

Symbiotic with *Microstegium* sp. Sexual morph: subiculum covering the stem of host, initially white, compact, waxy, gradually turning bright yellow, tuberculate. *Perithecia* 350–500 × 110–230 ($\bar{x}$ = 438 × 171, n = 15) μm, partly immersed in the subiculum, pyriform, yellow, aggregated with periphyses ostiole. *Peridium* 20–40 ($\bar{x}$ = 29, n = 25) μm wide, composed of thick-walled, hyaline cells of *textura angularis*. *Asci* 140–350 × 6–9 ($\bar{x}$ = 215 × 7.2, n = 25) μm, eight-spored, cylindrical, with a thickened apex. *Ascus cap* 4.7–6.6 × 3.1–3.8 ($\bar{x}$ = 5.3 × 3.4, n = 15) μm, hemispherical. *Ascospores* 150–230 × 1.3–1.8 ($\bar{x}$ = 198 × 1.6, n = 10) μm, hyaline, filiform, smooth-walled, transversely multiseptate, non-fragmented with septa at intervals of 10–20 μm. Asexual morph: Undetermined.

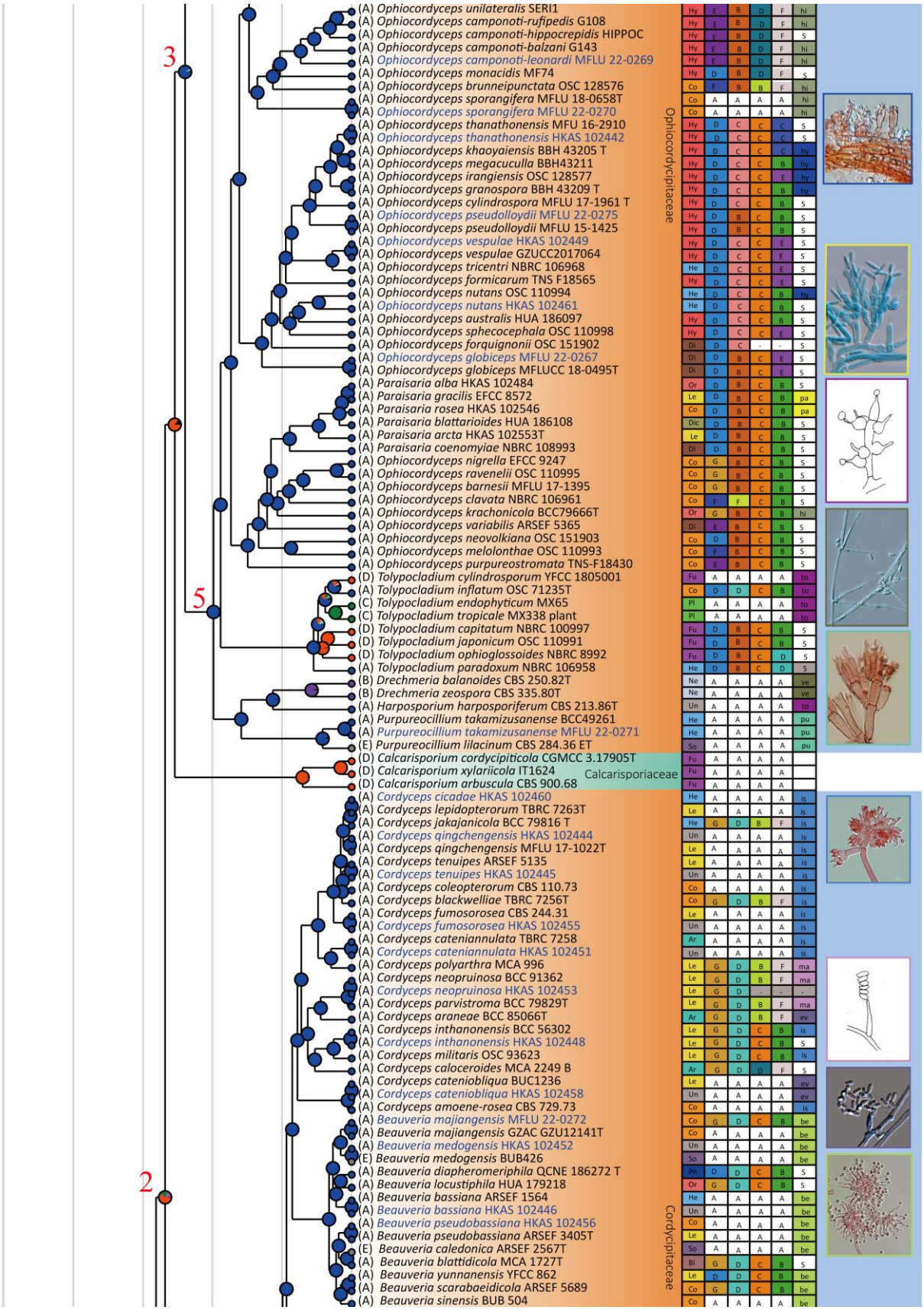

Figure 3 – Continued.

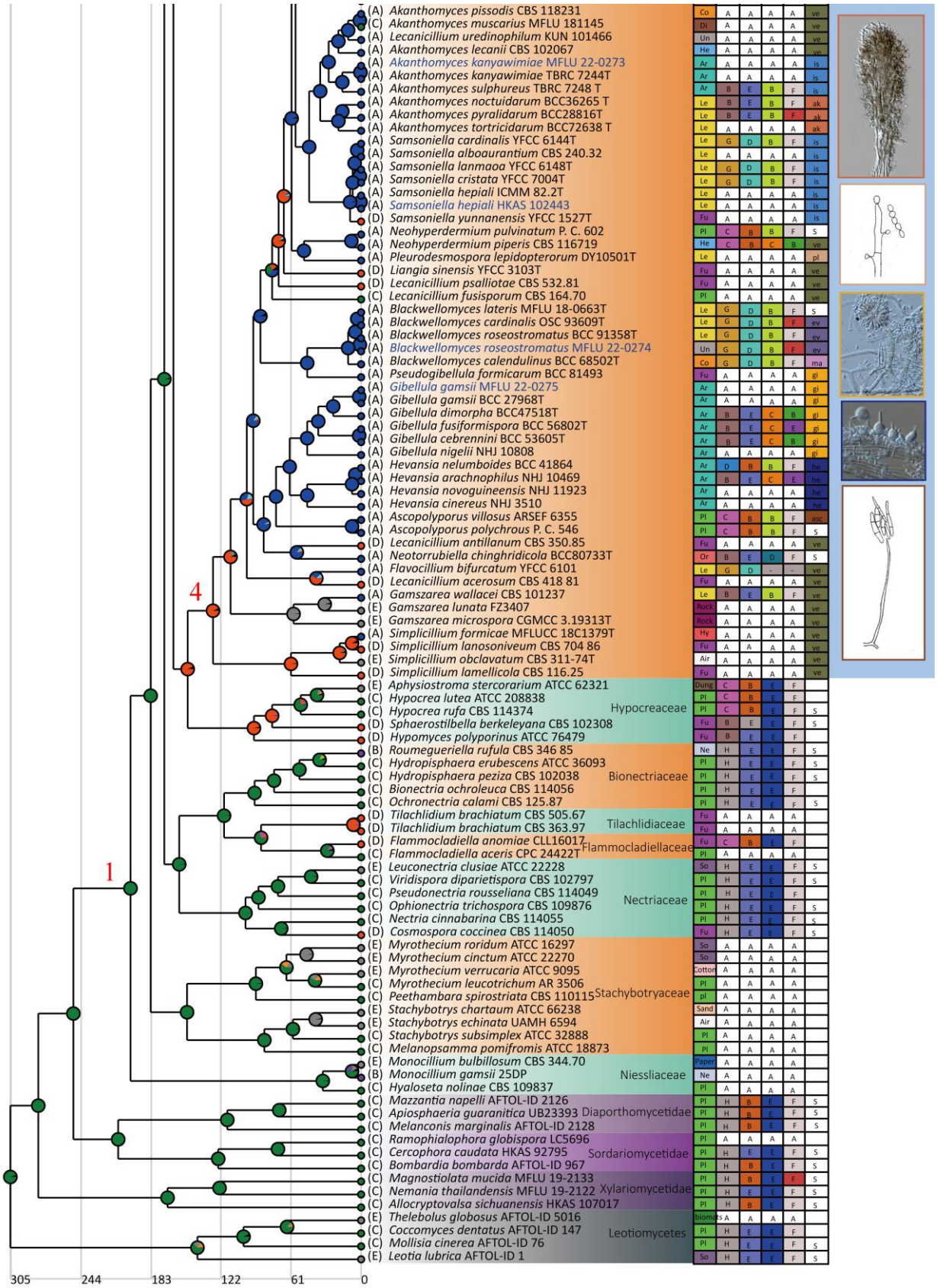

Figure 3 – Continued.

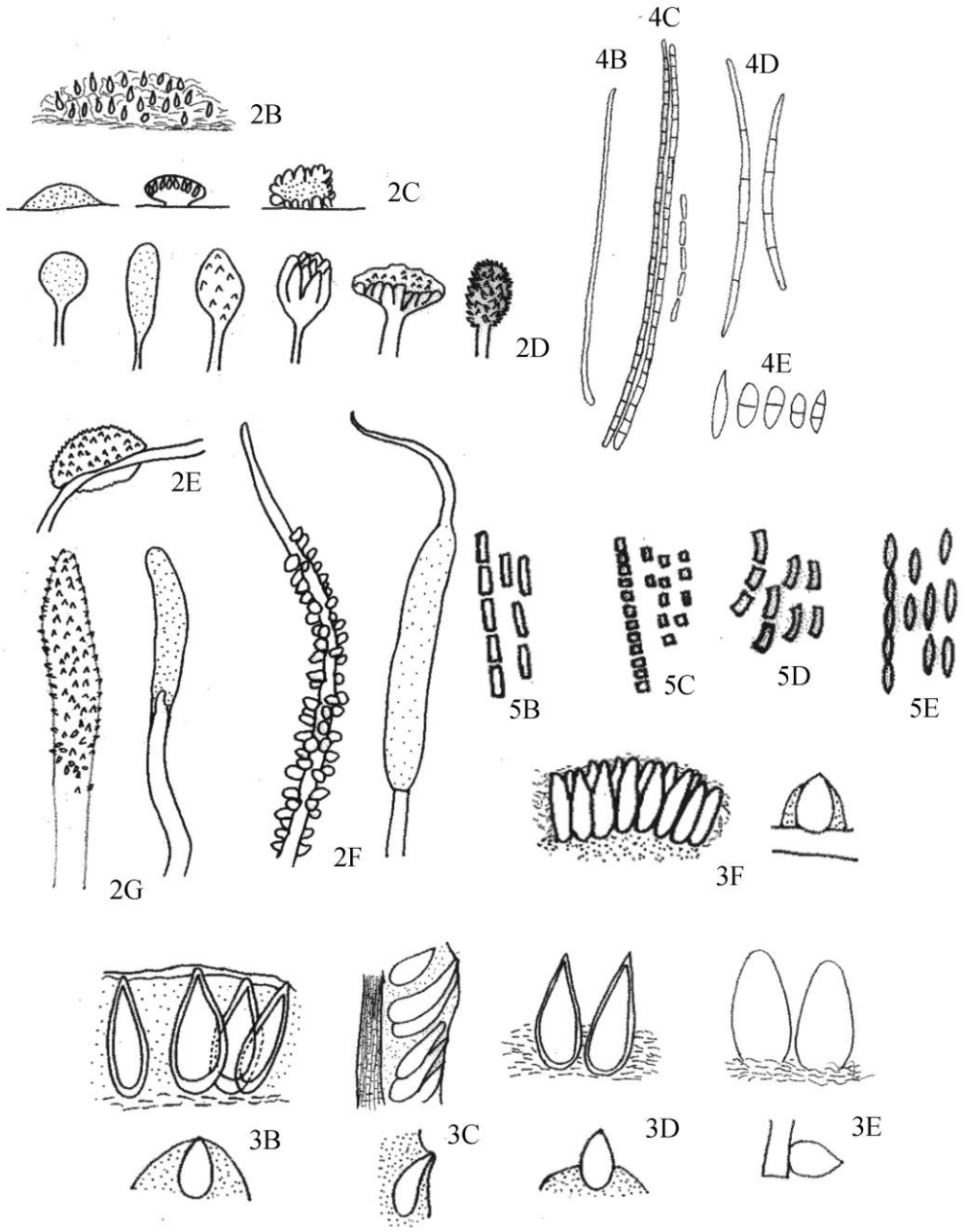

Figure 4 – The sexual characters stated in columns 2 to 5 of the Fig. 3. 2B–2F Stromata types. 3B–3F Perithecia arrangements. 4B–4E Ascospore shapes. 5B–5E Secondary ascospores shapes. 2B Subicular, 2C Pulvinate, 2D Terminal, 2E Lateral, 2F Intercalary, 2G Subterminal. 3B Immersed, 3C Obliquely immersed, 3D Subimmersed, 3E Superficial, 3F Pesudoimmersed. 4B Filiform, whole, 4C Filiform, multiseptate, disarticulating, 4D Needle-like or elongated fusiform, whole. 5E Clavate, short fusiform to ellipsoidal, few-celled. 5B Cylindrical, 5C Cubic, 5D Allantoid, 5E Fusiform.

Material examined – China, Guizhou Province, Guiyang City, Huaxi District, Tongxin Village, on living stem of *Microstegium* sp., 15 June 2019, De-Ping Wei, TXC16 (HKAS 106462).

Notes – *Epichloë* species are well-known for production of alkaloids, which cause toxicosis in herbivorous invertebrates and grazing animals (Leuchtmann et al. 2014). The occurrence of the ectophytic stroma on the grass column prevents emergence of the inflorescence and this is commonly known as choke disease (White Jr & Bultman 1987). The asexual morphs of *Epichloë* have been linked to *Neotyphodium* whose members commonly were isolated from grass as endophytes (Leuchtmann et al. 2014). Species of *Epichloë* share similar sexual morphology in

forming subicular stroma, immersed perithecia, filiform non-disarticulating ascospores and the expression as endophytes of grass (Yan et al. 2011, Komaki et al. 2017, Bisson & Liu 2021). It is difficult to identify species in this genus solely based on morphological characteristics. In the phylogenetic analysis of concatenated LSU, SSU, 5.8S, *tef*1, *rpb*1 and *rpb*2 sequences our collection (HKAS 106462) grouped with species of *Epichloë* with strong support (Fig. 1). The The BLASTn results of the *tubB* gene revealed our specimen have 99% similarity with *Epichloe sylvatica* within 899 bp nucleotides.

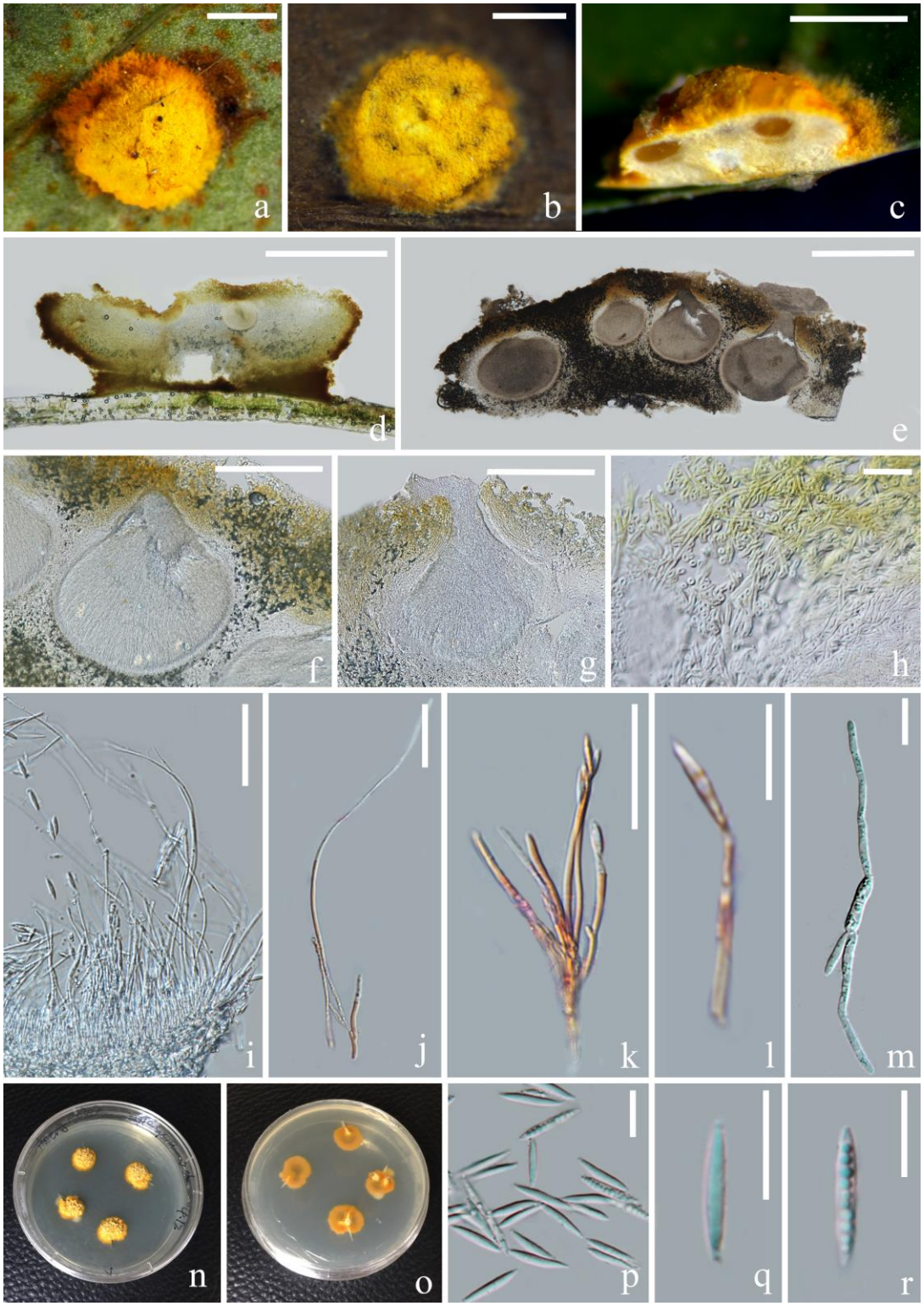

Figure 5 – *Aschersonia samoensis* (MFLU 22-0264). a, b Conidiomata. c–e Vertical section of conidiomata. f, g Perithecia. h Peridium. i, j Paraphyses. k, l Phialides. m Germinating conidium.

n, o Upper and lower view of culture on PAD. p–r Conidia. Scale bars: a–d = 1000 µm, e = 500 µm, f, g = 200 µm, i–k = 30 µm, l, m, p–r = 10 µm (j–l stained with Congo red solution).

Figure 6 – *Epichloë sylvatica* (HKAS 106462). a Fungus associated with host (*Microstegium* sp.). b, c Perithecia on mycelial mat. d–f Transverse section of perithecia. g Peridium. h Ascus cap. i, j Asci. k Part of ascus. l Ascospores. Scale bar: b = 1500 µm, c–f = 300 µm, g = 30 µm, h = 10 µm, i–l = 50 µm.

Metarhizium Sorokīn, Veg. Parasitenk. Mensch Tieren 2: 268 (1879)

Metarhizium pemphigi (Driver & Milner) Kepler, Humber & S.A. Rehner, Mycologia 106(4): 824 (2014) Fig. 7a–g

Index Fungorum number: IF 807860; Facesoffungi number: FoF 13945

Parasitic on adults of Coleoptera. *Colony* white, slightly green when producing sporulation. *Mycelium* 1.6–2.8 ($\bar{x}$ = 2.1, n = 40) μm in wide, hyaline, smooth-walled. *Conidiophores* micronematous, closely packed together but not forming synnmata, irregularly branched, septate. *Phialides* 10–20 × 2–3 ($\bar{x}$ = 13 × 2.6, n = 20) μm, monophialidic, cylindrical, hyaline, aseptate. *Conidia* 5–8 × 1.8–2.5 ($\bar{x}$ = 6.1 × 2.1, n = 30) μm, cylindrical-ellipsoidal, hyaline, aseptate, smooth-walled, aggregating in chains.

Culture characteristics – Colonies on PDA reaching 5.5 cm after 30 days at 25 °C, green, circular, velvety, with sparse mycelia, sporulation.

Material examined – China, Yunnan Province, Kunming City, Yeyahu Park, on adult of Coleoptera lying on ground, 4 July 2021, De-Ping Wei, YYH07 (HKAS 102459), living culture KUNCC 21-10511.

Notes – *Metarhizium pemphigi* was initially introduced as *Metarhizium flavoviride* var. *pemphigum* by Driver et al. (2000) and raised to species level by Kepler et al. (2014). This species was found to infect *Pemphigus bursarius* and *Pemphigus trehernei*, root aphids from the UK (Driver et al. 2000). The collection (HKAS 102459) produces typical metarhizium-like conidiophores and hyaline ovoid to enlongated conidia. The colonies on PDA are light green due to formation of abundant sporulation. These characters were also mentioned in *M. pemphigi* (Driver et al. 2000). In the multi-locus phylogeny our collection groups with *M. pemphigi* (ARSEF 6569) with maximum support (Fig. 1).

Metarhizium purpureonigrum Luangsa-ard, Tasan., Thanakitp. & Samson, Stud. Mycol. 95: 233 (2020) Fig. 7h–o

Index Fungorum number: IF 834913; Facesoffungi number: FoF 13946

Parasitic on larvae of *Campsosternus auratus* or *C. fruhstorfi* (Coleoptera). *Stroma* up to 8.5 cm long, 3.5 mm wide when dry, merging from head of the host, stipitate. *Stipe* up to 5 cm long, yellow, straight, unramified. *Fertile part* ramified, caespitous, giving rise to green, powdery hymenium, with purple-grey sterile tips. *Conidiophores* consisting of verticillate branches with whorls of phialides. *Phialides* 5–10 × 2–3 ($\bar{x}$ = 6.7 × 2.5, n = 30) μm, hyaline, smooth, cylindrical, slightly attenuated at the apices, phialidic. *Conidia* 4–6.5 × 1.6–3 ($\bar{x}$ = 4.6 × 2.1, n = 50) μm, hyaline, oblong, guttulate, aseptate, smooth-walled, aggregating in chains.

Material examined – China, Yunnan Province, Xishuangbanna, on larva of *Campsosternus auratus* (Coleoptera) in soil, 28 July 2016, Samantha C. Karunarathna, E-C-001 (HKAS 102457).

Notes – Our collection (HKAS 102457) is morphologically similar to *Metarhizium purpureonigrum* and *Ophiocordyceps jiangxiensis* in producing green, powdery hymenium on ramified, caespitous fertile parts and commonly infecting larvae of *Campsosternus* sp. (Coleoptera). *Ophiocordyceps jiangxiensis* was initially introduced as a *Cordyceps* based on its sexual morph, which was collected from Jiangxi Province, China (Liang et al. 2001). Liang (2007) linked *O. jiangxiensis* to the asexual species *Penicillum jiangxienses*. Molecular data of *O. jiangxiensis* and *P. jiangxienses* is limited the in NCBI database so their phylogenetic position has not been studied yet. Mongkolsamrit et al. (2020a) introduced *M. purpureonigrum* from Coleoptera larvae in Thailand based on morphological and phylogenetic analysis, without mention of *O. jiangxiensis*. In this study, we propose that *M. purpureonigrum* and *O. jiangxiensis* are conspecific due to their similarity in macro- and micro-characteristics (see Table 3. Although *O. jiangxiensis* was established prior to *M. purpureonigrum*, we assign the latter name to our collection. *M. purpureonigrum* was identified based on sufficient evidence, while the *O. jiangxiensis* was introduced based solely on morphological observation. This is the first time that the asexual morph for *M. purpureonigrum* based on Chinese specimen is described and clear illustrations and molecular data are provided.

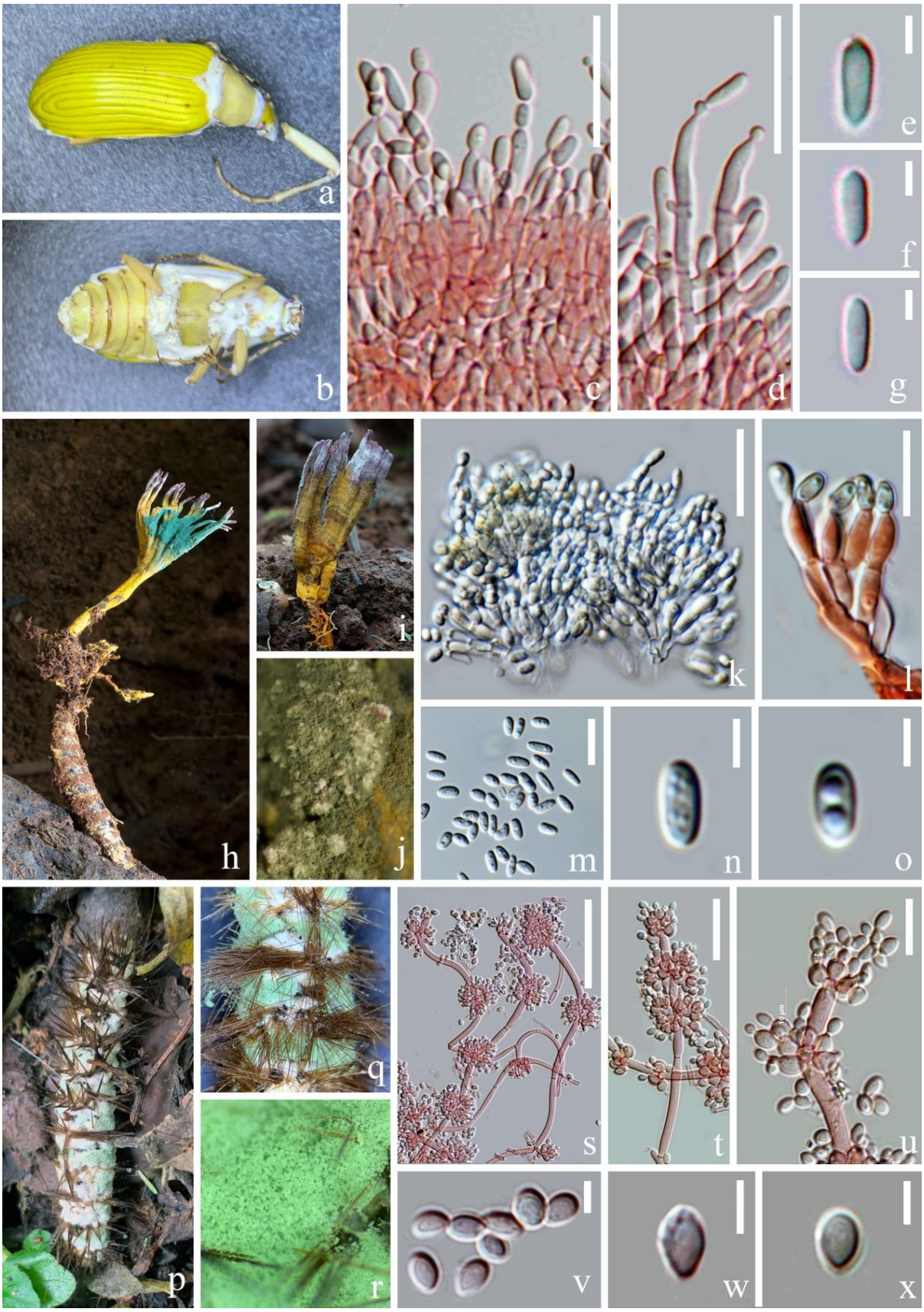

Figure 7 – *Metarhizium pemphigi* (HKAS 102459, a–g), *Metarhizium purpureonigrum* (HKAS 102457, h–o), *Metarhizium rileyi* (HKAS 102454, p–x). a, b, p, q, r Fungal colony on hosts. h, i Stroma on host. j Enlargement of hymenium. c, d, k, l, s–u Conidiophores and phialides. e–g, m–o, v–x Conidia. Scale bars: s, t = 50 μm, c, d, k = 20 μm, l, m, u = 10 μm, e–g, n, o, v–x = 3 μm (c, d, l, s–u stained with Congo red solution).

Table 3 Morphological comparison of *M. purpureonigrum* and *O. jiangxiensis*.

Species	Strain no.	Characteristics	Reference
Metarhizium purpureonigrum	BBH 47504	Parasitic on larva of *Campsosternus* sp. from Thailand, stroma 10–15 cm long, up to 10 mm wide, perithecia elongate ovoid, (600–)685–846(–870) × (250–)275–420(–500) μm, asci (245–)250–275(–280) × 6–8 μm, ascospores filiform, multiseptate (200–)228–270(–275) × 1.5–2 μm, phialides cylindrical, 5–7.2(–8) × 2–3 μm on specimen, 10–12 × 3–4 μm on OA medium, conidia cylindrical, (4–)5.4–7.7(–10) × 2–3 μm on specimen, (6–)7–9.2(–10) × 2–3 μm on OA medium.	Mongkolsamrit et al. (2020a)
Metarhizium purpureonigrum	HKAS 102457	Parasitic on larva of *Campsosternus* sp. from China, phialides cylindrical, 5.3–10 × 2–3.1 on specimen, conidia cylindrical, 4–6.5 × 1.6–3 μm on specimen.	This study
Ophiocordyceps jiangxiensis	LFRGU20-710	Parasitic on larva of *Campsosternus* sp. from China, stroma 4–9 × 0.5 mm, perithecia elongated-ovate, 520–600 × 300 μm, asci 6 μm in with, ascospores long cylindric, multiseptate,1.0–1.2 μm thick.	Liang et al. (2001)
Penicilium jiangxiensis	-	Parasitic on larva of *Campsosternus* sp. from China, phialides cylindrical to clavete, 8–9.5 × 1.8–2.5 um on Czapek-DoxAgar, conidia cylindrica, 1.8–2.5 × 1.2–2 μm.	Liang et al. (2001)

Nomuraea Maubl., Bull. Soc. mycol. Fr. 19(3): 295 (1903)
Nomuraea rileyi (Farl.) Samson, Stud. Mycol. 6: 81 (1974) Fig. 7p–x

Index Fungorum number: IF 318728; Facesoffungi number: FoF 13947

Parasitic on larvae of Lepidoptera. Colony covering the whole body of host, powdery, green. *Mycelium* 1.6–3.2 ($\bar{x}$ = 2.5, n = 30) μm in wide, hyaline, septate, smooth-walled. *Conidiophores* micronematous, developing from procumbent mycelia, bearing clusters of metulae or phialides. *Metulae* 3.5–7 × 3–4 ($\bar{x}$ = 5 × 3.6, n = 20) μm, oblong to subglobose, carrying a group of phialides. *Phialides* 3.5–5 × 2–4 ($\bar{x}$ = 4 × 2.7, n = 20) μm, monophialidic, ellipsoidal to subglobose, hyaline, smooth-walled, aseptate. *Conidia* 3.5–4.5 × 1.5–3 ($\bar{x}$ = 4 × 2.3, n = 30) μm, oval to subglobose, hyaline, aseptate, adhering in chains.

Material examined – China, Yunnan Province, Kunming City, Panlong District, forest near Songhuaba reservoir, on dead lepidopteran larva lying on grass land, 30 October 2021, De-Ping Wei, SHB04 (HKAS 102454).

Notes – Samson (1974) revisited a series of herbaria and illustrated *Nomuraea rileyi*, by providing line-drawings of the conidiophores and conidia. This species was reported to infect a wide range of hosts in Europe, including *Plusia brassicae*, *Anticarsia gemmatalis*, soybean loopers, *Trichoplusia ni* (USA), caterpillar (Vadosta), grassworn (Romano), *Prodenia ornitbogali* (Orlando), insect larvae (Auburndale), spiders (Great Britain), *Heliotbis armigera* (France) and *Spodoptera littoralis* (Israel). *Nomuraea rileyi* was later synonymized under *Metarhizium* based on three strains (namely AF36850, CBS 806.7 and NBRC 8560) (Mongkolsamrit et al. 2020a). The specimen (HKAS 102454) was found to infect a lepidopteran larva on grass land. It produces typical nomuraea-like conidiophores. We are determined to revive the species name *Nomuraea rileyi* to accommodate our specimens with the reason given in the discussion.

Moelleriella Bres., Boll. Soc. bot. ital., 1897: 292 (1897)
Moelleriella chiangmaiensis Khons., Noisrip. & Luangsa-ard, Mycol. Progr. 20(7): 852 (2021)
Fig. 8

Index Fungorum number: IF 835903; Facesoffungi number: FoF 13948

Parasitic on nymphs of scale insects (Hemiptera). Sexual morph: see Khonsanit et al. (2021). Asexual morph: *Stromata* 1.5–3.6 ($\bar{x}$ = 2.3, n = 10) mm, sessile, scattered, superficial, circular, pulvinate, with thin white hypothallus. *Conidiomata* 190–390 × 90–250 ($\bar{x}$ = 264 × 133, n = 5) μm, cupulate, with wide opening. *Paraphyses* 20–40 × 1.2–1.5 ($\bar{x}$ = 30 × 1.4, n = 5) μm, linear, unbranched, aseptate, with round ends. *Phialides* 8–13 × 1.1–1.6 ($\bar{x}$ = 10 × 1.3, n = 15) μm, monophialidic, cylindrical, smooth-walled, aseptate. *Conidia* 6.7–10 × 1–1.6 ($\bar{x}$ = 7.9 × 1.3, n = 50) μm, hyaline, fusiform, unicellular, guttulate.

Culture characteristics – Colonies on PDA reaching 2 cm after 30 days at 30 °C, white, circular, cottony, raised, with dense mycelia, reverse creamy yellow.

Material examined – Thailand, NaKhon Si Thammarat, Lan Saka District, Kam Lone (99°46'37"E, 8°27'6"N, elevation 180 m), on scale insect nymphs, 27 April 2019, De-Ping Wei, NK01 (MFLU 22-0263), living culture MFLUCC 22-0185.

Notes – The phylogenetic analysis shows that our collection (MFLU 22-0263) clusters with *Moelleriella chiangmaiensis* (BCC60941) with maximum statistical support (Fig. 1). Morphologically, our collection resembles *M. chiangmaiensis* in producing sessile white pulvinate stromata, wide-opening conidiomata, yellow-orange conidial mass and fusiform conidia (Khonsanit et al. 2021). This species commonly infects scale insects attached to the underside of leaves and produces aschersonia-like asexual morphs.

Moelleriella raciborskii (Zimm.) P. Chaverri, M. Liu & K.T. Hodge, Stud. Mycol. 60: 3 (2008)

Fig. 9

Index Fungorum number: IF 539558; Facesoffungi number: FoF 13949

Parasitic on scale insects or whiteflies. Sexual morph: *Stromata* 2–3.6 ($\bar{x}$ = 2.6 × 5, n = 5) mm, sessile, yellow, tubercular, superficial, scattered. *Perithecia* 240–560 × 180–300 ($\bar{x}$ = 360 × 246, n = 10) μm, flask-shaped or ellipsoidal, ostiolate, superficial. *Peridium* 30–45 ($\bar{x}$ = 33, n = 20) μm, comprised of hyaline cell of *texture angularis*. *Paraphyses* absent. *Asci* 200–400 × 6–16 ($\bar{x}$ = 300 × 10, n = 20) μm, cylindrical, with a thickened apex. *Ascospores* 10–15 × 2–3.2 ($\bar{x}$ = 13 × 2.5, n = 20) μm, hyaline, ellipsoidal to slightly fusiform, aseptate. Asexual morphs: *Stromata* up to 0.5–1.5 mm in diam., sessile, yellow, flatly pulvinate, scattered. *Conidiomata* immersed. *Conidiomatal wall* composed of hyaline cells of *textura epidermoidea*. *Paraphyses* absent. *Conidiophores* cylindrical, terminally or internally bearing whorl of phialides. *Phialides* 11–19 × 1–2.5 ($\bar{x}$ = 14 × 1.7, n = 30) μm, hyaline, cylindrical, straight, aseptate, holoblastic. *Conidia* 10–15 × 1.4–2.6 ($\bar{x}$ = 12 ×2, n = 30) μm, hyaline, fusiform, aseptate, smooth-walled, first conidium becoming conidiogenous by apically wall-building to form an unbranched chain.

Culture characteristics – Colonies on PDA reaching 2.2 cm after 30 days at 30 °C, white to pale yellow, surface minutely velvety, wrinkled, reverse yellow, conidial mass yellow, fluid.

Material examined – Thailand, NaKhon Si Thammarat Province, Lan Saka District, Kam Lone (99°46'37"E, 8°27'6"N, elevation 180 m), on scale insects attached to decayed leaves of a dicotyledonous plant, 27 April 2019, De-Ping Wei, HC03 (MFLU 22-0262), living culture MFLUCC 22-0184. Chiang Mai Province, Mushroom researcher centre, on scale insects attached to living leave of a dicotyledonous plant, 13 August 2020, De-ping Wei, MRC0851B (MFLU 22-0261), living culture MFLUCC 22-0191.

Notes – *Hypocrella raciborskii* was revisited by Liu et al. (2006) in which the authors provided the updated morphological description and linked its asexual morphs to *Aschersonia placenta*. *Hypocrella raciborskii* later was transferred to *Moelleriella* by Chaverri et al. (2008). The most distinctive character of *M. raciborskii* is the formation of tubercles on pulvinate stroma, inside which a single perithecium is embedded. The multigene phylogenetic analysis reveals a robust clade of our specimens (MFLU 22-0262 and MFLU 22-0261) and *M. raciborskii* (P. C 533). The sexual specimen (MFLU 22-0262) fits well with the concept of *M. raciborskii* in producing the tuberculate stromata and filiform ascospores, which break into many cylindrical fusiform secondary ascospores inside the asci. The asexual specimen (MFLU 22-0261) is diagnosed as the asexual

morph of *M. raciborskii* based on phylogenetic evidence, its pulvinate stroma and the aschersonia-like conidiophore.

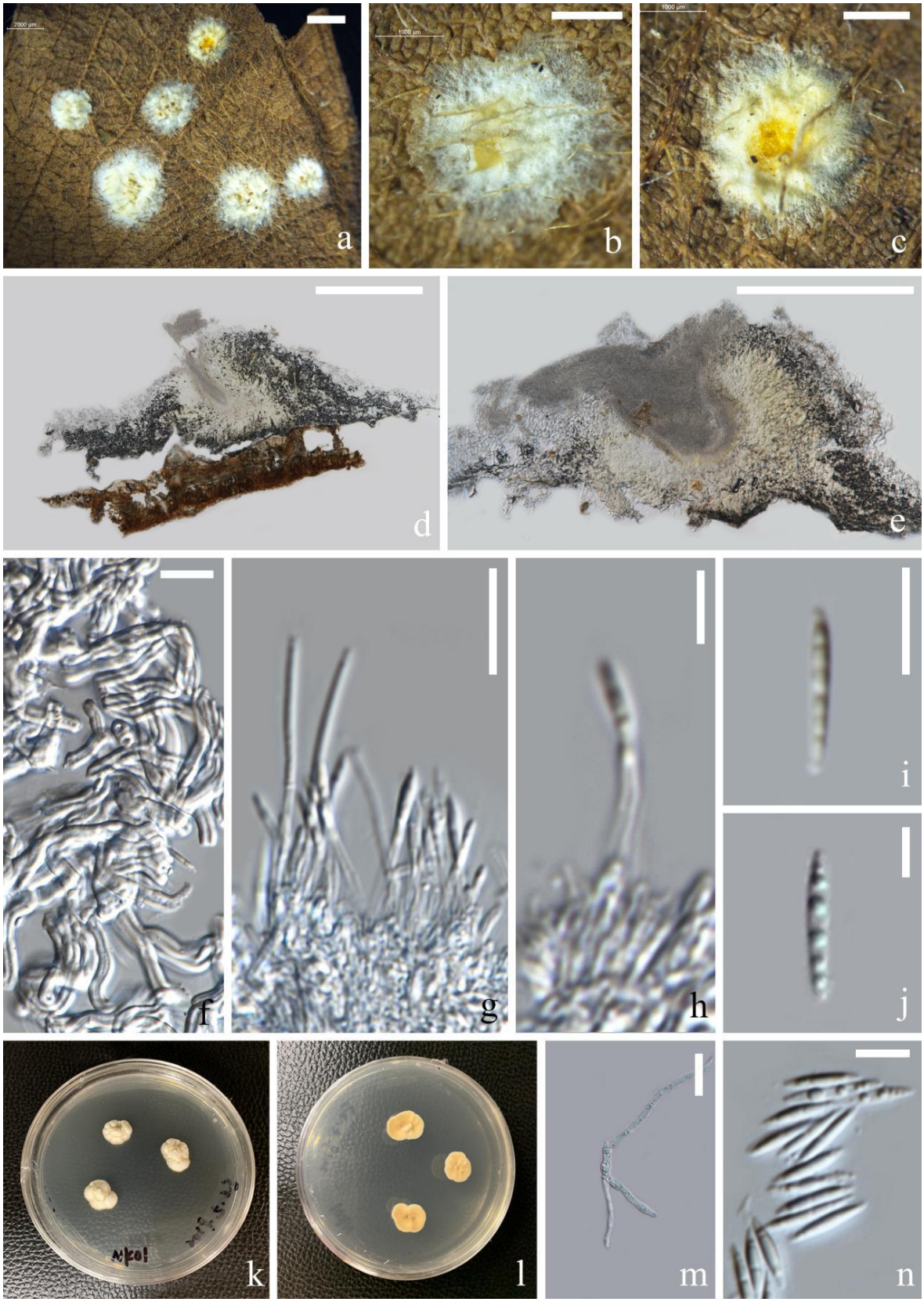

Figure 8 – *Moelleriella chiangmaiensis* (MFLU 22-0263). a–c Stromata on dead leaves. d, e Section through stroma showing conidiomata. i, j, n Conidia. k, l Upper and reverse view of

culture on PDA after 68 days incubation. m Germinating condium. Scale bar: a = 2000 µm, b, c = 1000 µm, d, e = 500 µm, f, g, m = 15 µm, h–j, n = 5 µm.

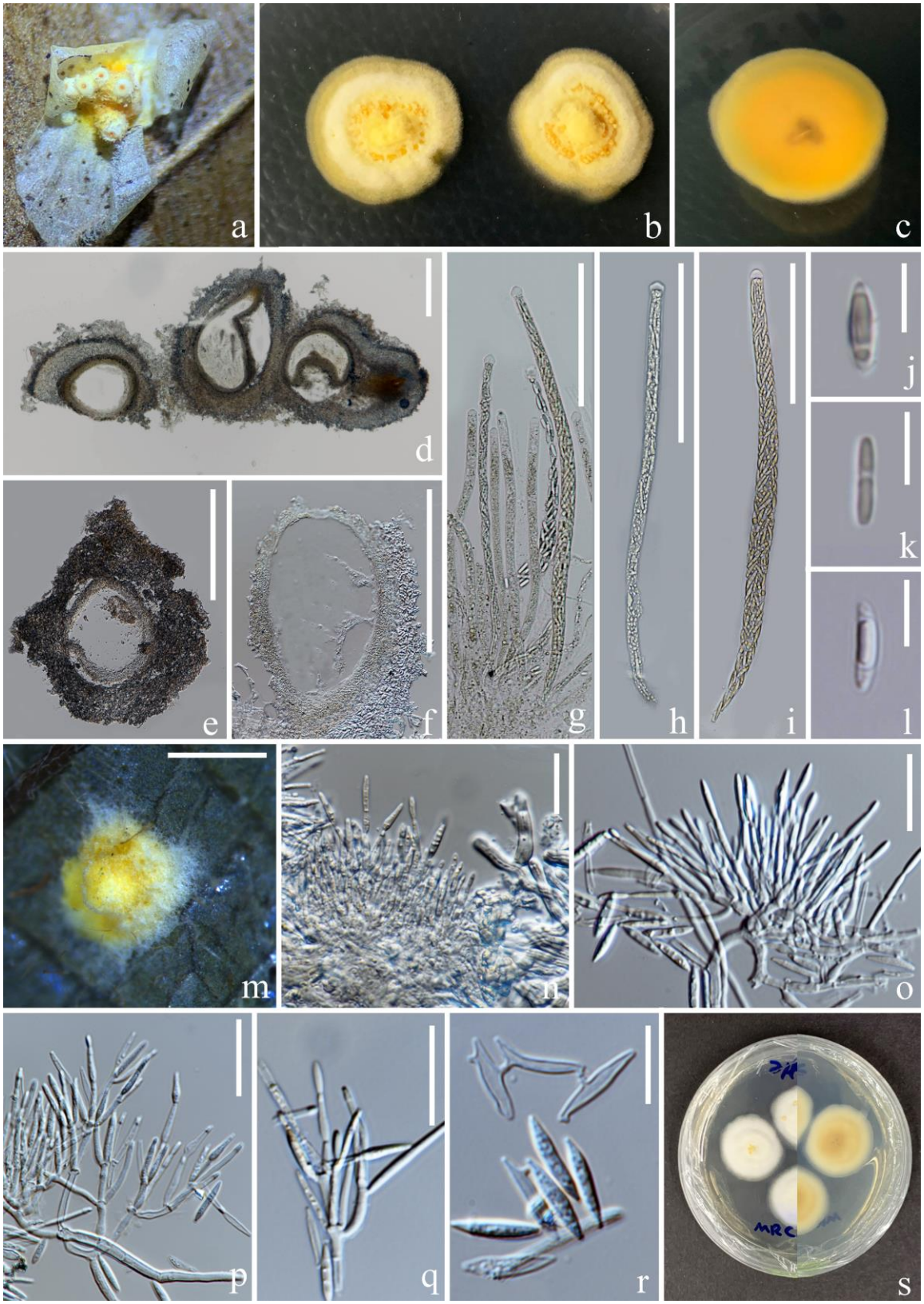

Figure 9 – *Moelleriella raciborskii* (MFLU 22-0262, a–l), (MFLU 22-0263, m–s). a Stroma on substrate. b, c, s Upper and lower view of culture. d–f Perithecia. g–i Asci. j–l Ascospores. m Conidioma. n–q Conidiophores and phialides. r Conidia. Scale bar: m = 1000 µm, d–f = 300 µm, g–i = 100 µm, n–q = 20 µm, j–l, r = 10 µm.

Orbiocrella D. Johnson, G.H. Sung, Hywel-Jones & Spatafora, Mycol. Res. 113(3): 286 (2009)
Orbiocrella petchii (Hywel-Jones) D. Johnson, G.H. Sung, Hywel-Jones & Spatafora, Mycol. Res. 113(3): 287 (2009) Fig. 10

Index Fungorum number: IF 512032; Facesoffungi number: FoF 13950

Parasitic on scale insects. Sexual morph: *Stromata* 3.5–5.5 ($\bar{x}$ = 4.3, n = 10) mm, entirely covering the insect hosts, ochraceous, sessile, scattered, rink-like. Stromal hyphae are coarse and filled with abundant yellow guttules. *Perithecia* 330–425 × 75–120 ($\bar{x}$ = 384 × 85, n = 5) μm, flask-shaped, superficial, ostiolate, not confluent. *Peridium* 25–130 ($\bar{x}$ = 139, n = 10) μm, composed of thick-walled cells of *textura angularis*. *Asci* 160–300 × 5–7 ($\bar{x}$ = 236 × 6, n = 10) μm, cylindrical, with thickened apical cap (6–7 × 3–3.5, $\bar{x}$ = 6.5 × 3.4, n = 10 μm). *Ascospores* 200–250 × 1–2 ($\bar{x}$ = 222 × 1.6, n = 10) μm, hyaline, filiform, aseptate, not disarticulate into secondary ascospores. Asexual morph: Undetermined.

Figure 10 – *Orbiocrella petchii* (MFLU 22-0260). a, c Stromata on the surface of bamboo leaf. b Vertical section through stroma. d Perithecium. e Peridium. f, g Asci. h, i Ascospores. Scale bars: b, c = 1000 μm, d = 200 μm, e–i = 50 μm.

Material examined – Thailand, Chiang Rai Province, Wiang Chai District, Ban Don Mun, on scale insects attached to bamboo leaves, 10 April 2018, De-Ping Wei, BD18041001 (MFLU 22-0260).

Notes – *Orbiocrella petchii* initially was recognized as a member of *Torrubiella* based on the sessile stroma (Hywel-Jones 1997). Phylogenetic analysis rejected the monophyly of torrubielloid fungi thus *Orbiocrella* was established to accommodate the single species *O. petchii*, which is specific to bamboo scale insects (Johnson et al. 2009). The detailed asexual and sexual morphs of *O. petchii* have been provided by Hywel-Jones (1997). Our collection morphologically resembles *O. petchii* in ochraceous, ring-like, reduced stromata, long-cylindrical asci and hyaline, filiform, non-disarticulating ascospores. In the multigene phylogenetic analyses, our collection groups with *O. petchii* (NHJ 6209) with significant support. A comparison of ITS nucleotide sequences showed that our collection (MFLU 22-0260) differed from the reference stain *O. petchii* (NHJ 6209) in 2/365 bp (0.5%). Based on the morphological observation and molecular analysis, we determine our collection as *O. petchii*.

Cordycipitaceae Kreisel ex G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Stud. Mycol. 57: 48 (2007)

Notes – We describe 16 species representing six genera of Cordycipitaceae, including one *Akanthomyces* species, four *Beauveria* species, one *Blackwellomyces* species, one *Gibellula* species, eight *Cordycpe*s species and one *Samsoniella* species. Species in *Akanthomyces* and *Gibellula* were found on spider hosts and produce isaria-like and gibellula-like conidiophores, respectively. The asexual morphs of *Beauveria* are found on various order of Insecta and often form white powdery colonies on surface of hosts. They produce distinctive beauveria-like conidiophores. *Blackwellomyces*, *Cordycpe*s and *Samsoniella* often parasitize Hemiptera and Lepidoptera, producing isaria-like conidiophores and fleshy, stalked stromata, subterminal fertile parts, semi-immersed to superficial perithecia and filiform, multiseptate ascospores which disarticulate into cylindrical secondary ascospores.

Akanthomyces Lebert, Z. Wiss. Zool. 9: 449 (1858)

Akanthomyces kanyawimiae Mongkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard, Mycologia 110(1): 237 (2018) Fig. 11

Index Fungorum number: IF 823788; Facesoffungi number: FoF 13951

Parasitic on spiders. Sexual morph: Undetermined. Asexual morph: Colony entirely covering the host, white, powdery. *Mycelium* 1.2–3.8 ($\bar{x}$ = 2.1, n = 60) μm in wide, septate, hyaline, smooth-walled. *Conidiophores* micronematous, septate, branched, bearing clusters of metulae or phialides. *Metulae* 4.5–8 × 2–3 ($\bar{x}$ = 6.2 × 2.6, n = 20) μm cylindrical, hyaline, smooth-walled. *Phialides* 6–11 × 2–4 ($\bar{x}$ = 8 × 3, n = 60) μm, monophialidic, ampulliform, typically swollen basal portion tapering into a long neck. *Conidia* 1.5–3.5 × 1.2–2.5 ($\bar{x}$ = 2.7 × 1.9, n = 100) μm, globose to fusiform, unicellular, smooth-walled, hyaline, adhering in chains.

Culture characteristics – *Colonies* grow on PDA reaching 2 cm diameter after 5 days at 30 °C, white, velvety, nearly circular, umbonate; pale yellow from below.

Material examined – Thailand, Chiang Mai Province, Chiang Dao District, on spider lying on leaf litter, 9 February 2018, De-Ping Wei, CMCD01 (MFLU 22-0273), living culture MFLUCC 18-1389.

Notes – *Akanthomyces kanyawimiae* was introduced by Mongkolsamrit et al. (2018) from a spider host in Thailand. Our collection was also found on a spider host lying on leaf litter in Thailand. It was identified as *A. kanyawimiae* based on its isaria-like asexual morph and the close phylogenetic affinity to the type strain (TBRC 7244).

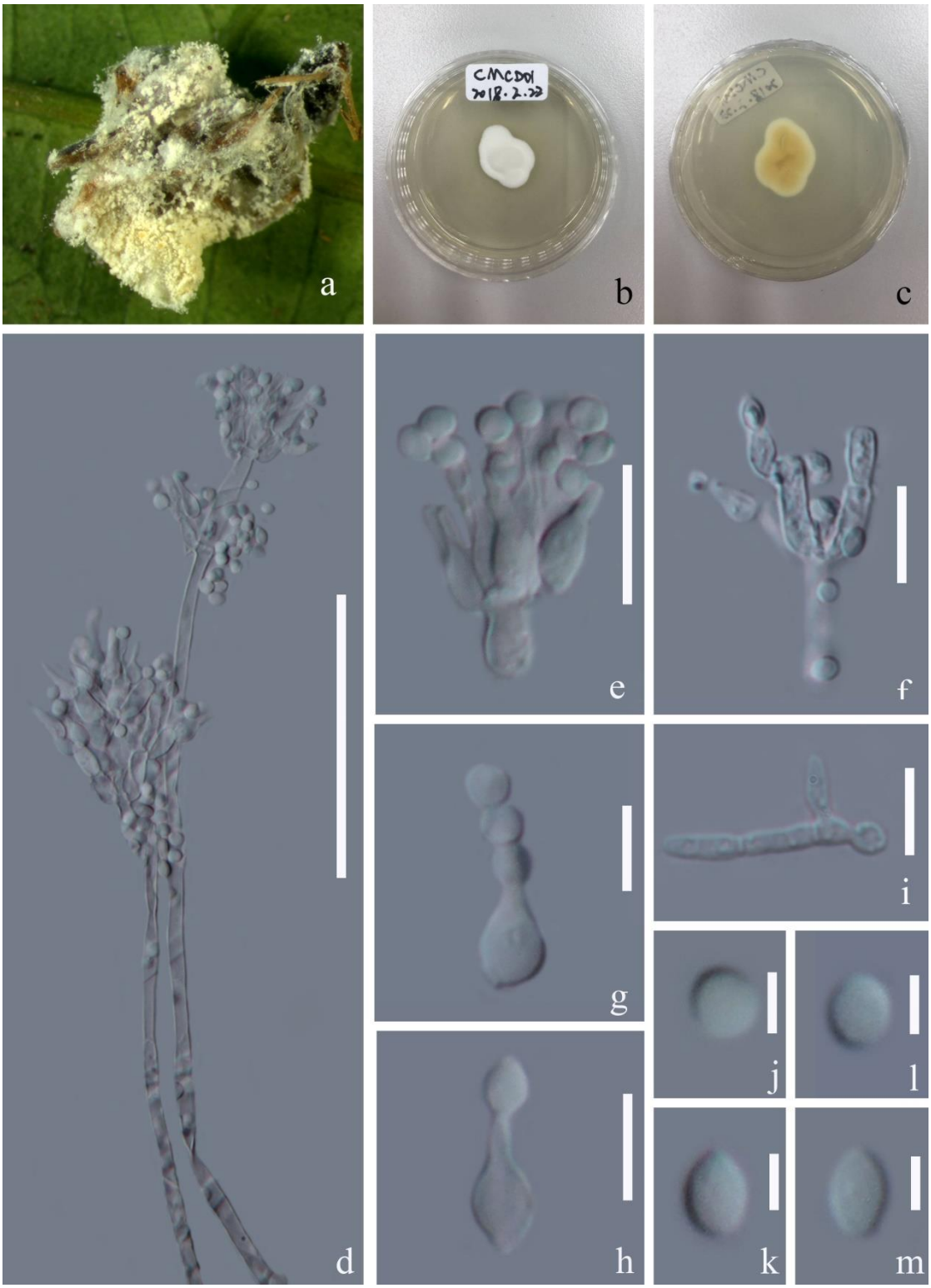

Figure 11 – *Akanthomyces kanyawimiae* (MFLU 22-0273). a host. b, c Upper and reverse view of cultures on PDA after 5 days incubation. d–f Conidiophore g, h Conidia produced from the apex of phialides. i–m Conidia. (i is germinating conidium). Scale bars: d = 50 µm, e, f, i = 10 µm, g, h = 5 µm, j–m = 2 µm.

Beauveria Vuill., Bull. Soc. bot. Fr. 59: 40 (1912)
Beauveria bassiana (Bals.-Criv.) Vuill., Bull. Soc. bot. Fr. 59: 40 (1912) Fig. 12a–i
Index Fungorum number: IF 199430; Facesoffungi number: FoF 13952

Parasitic or saprobic on dead insects. Sexual morph: Undetermined. Asexual morph: *Colony* entirely covering the host, white, woolly. *Mycelium* 1–2.3 ($\bar{x}$ = 1.8, n = 40) µm, hyaline, septate, branched, smooth-walled to slightly verruculose. *Conidiogenous cells* 2.5–3.7 × 1.9–3 ($\bar{x}$ = 3.3 × 2.4, n = 20) µm, occurring in tight clusters, base subglobose, apex with an indeterminate, geniculate, denticulate rachis. *Conidia* 2–2.6 ($\bar{x}$ = 2.2, n = 30) µm in diam., forming from the denticulate rachis, globose, hyaline, aseptate.

Culture characteristics – Colonies on PDA reaching 4.5 cm after 15 days at 25 °C, white, powdery, irregularly-shaped, reverse creamy white.

Material examined – China, Yunnan Province, Honghe County, Amushan natural reserve, on a decay insect lying on leaf litter, 15 June 2018, De-Ping Wei, TSQ17 (HKAS 102446), living culture KUNCC 21-10504.

Notes – *Beauveria bassiana* is a facultative parasite of a wide range of insects. This species is also saprobic on various decaying substrates or lives as an endophyte. The updated morphological illustration of *B. bassiana* was provided by Rehner et al. (2011) based on the neotype (ARSEF 1564). Our collection (HKAS 102446) was found on the remnants of an unidentified insect and its morphological features fit well with the description of *B. bassiana.*

Beauveria majiangensis W.H. Chen, M. Liu, Z.X. Huang, G.M. Yang, Y.F. Han, J.D. Liang & Z.Q. Liang, Phytotaxa 333(2): 246 (2018) Fig. 13

Index Fungorum number: IF 823150; Facesoffungi number: FoF 13953

Parasitic on coleopteran larvae. Sexual morph: *Stroma* 6 cm in high, bright yellow, fleshy, single, stipitate. *Fertile part* 3.5 cm in length, 7 mm in wide, developing from upper part of stroma, broadest at middle part, slightly tapering toward the apex. *Perithecia* 530–570 × 250–330 ($\bar{x}$ = 550 × 300, n = 5) µm, ovoid, superficial, ostiolate. *Peridium* 20–60 ($\bar{x}$ = 35, n = 30) µm, composed of hyaline, thick-walled cells of *textura angularis*. *Asci* 200–420 × 3.5–5.5 ($\bar{x}$ = 334 × 4.5, n = 20) µm, filiform, 8-spored, with thickened apex. *Ascospores* filiform, as long as asci, transversely multiseptate, breaking into secondary ascospore when mature. *Secondary ascospores* 5–7.5 × 0.7–1.6 ($\bar{x}$ = 6.2 × 1.2, n = 20) µm, cylindrical, hyaline, aseptate, smooth-walled, with truncated ends. Asexual morph: *Colony* rapidly growing on PDA media, reaching 3 cm after 5 days, white, velutinous, margin entire, sporulation in *vitro*. *Mycelium* 1.4–2.2 ($\bar{x}$ = 1.7, n = 35) µm in wide, hyaline, septate, branched, smooth-walled. *Conidiophores* 15–60 × 1.5–2.5 ($\bar{x}$ = 29 × 1.8, n = 10) µm, arising from procumbent mycelia, micronematous, solitary, cylindrical, aseptate, flexuous. *Conidiogenous cells* 2.6–4.6 × 2–2.6 ($\bar{x}$ = 3.4 × 2.3, n = 25) µm, producing from apex region of conidiophores, solitary, base globose to broadly ellipsoidal, apex with an indeterminate, geniculate, denticulate rachis. *Conidia* 3–3.5 × 1.5–2.2 ($\bar{x}$ = 3.8 × 1.8, n = 35) µm, forming from denticulate rachis, ellipsoidal, hyaline, aseptate.

Culture characteristics – Colonies on PDA reaching 3 cm after 7 days at 25 °C, white, velvety, circular, margin entire, mycelia sparse, reverse similar with upper view in colour.

Material examined – Thailand, Chiang Mai Province, mushroom research center, on larva of Coleoptera buried in soil, 13 August 2020, De-Ping Wei, MRC0825 (MFLU 22-0272), living culture MFLUCC 22-0190.

Notes – Our collection (MFLU 22-0272) groups with the type strain of *Beauveria majiangensis* with high support (Fig. 1). *Beauveria majiangensis* was introduced by Chen et al. (2018) based on an asexual morph, which was found on a grub in China. Our collection was discovered in its sexual morph in the natural substrate, a coleopteran larva that was buried in soil. The culture-based asexual morph was observed and it morphologically resembles *B. majiangensis* in the beauveria-like conidiophores and ellipsoidal conidia. We tentatively assign our collection to be a new geographic record and first record of the sexual morph of *B. majiangensis.* The most noteworthy is that neither sexual nor asexual characters are highly informative for species delimitation in *Beauveria*. The Bloc gene is a useful marker frequently used for identifying *Beauveria* species. Thus, the accurate identification of *Beauveria* should be based on additional taxon sampling with use of Bloc sequences.

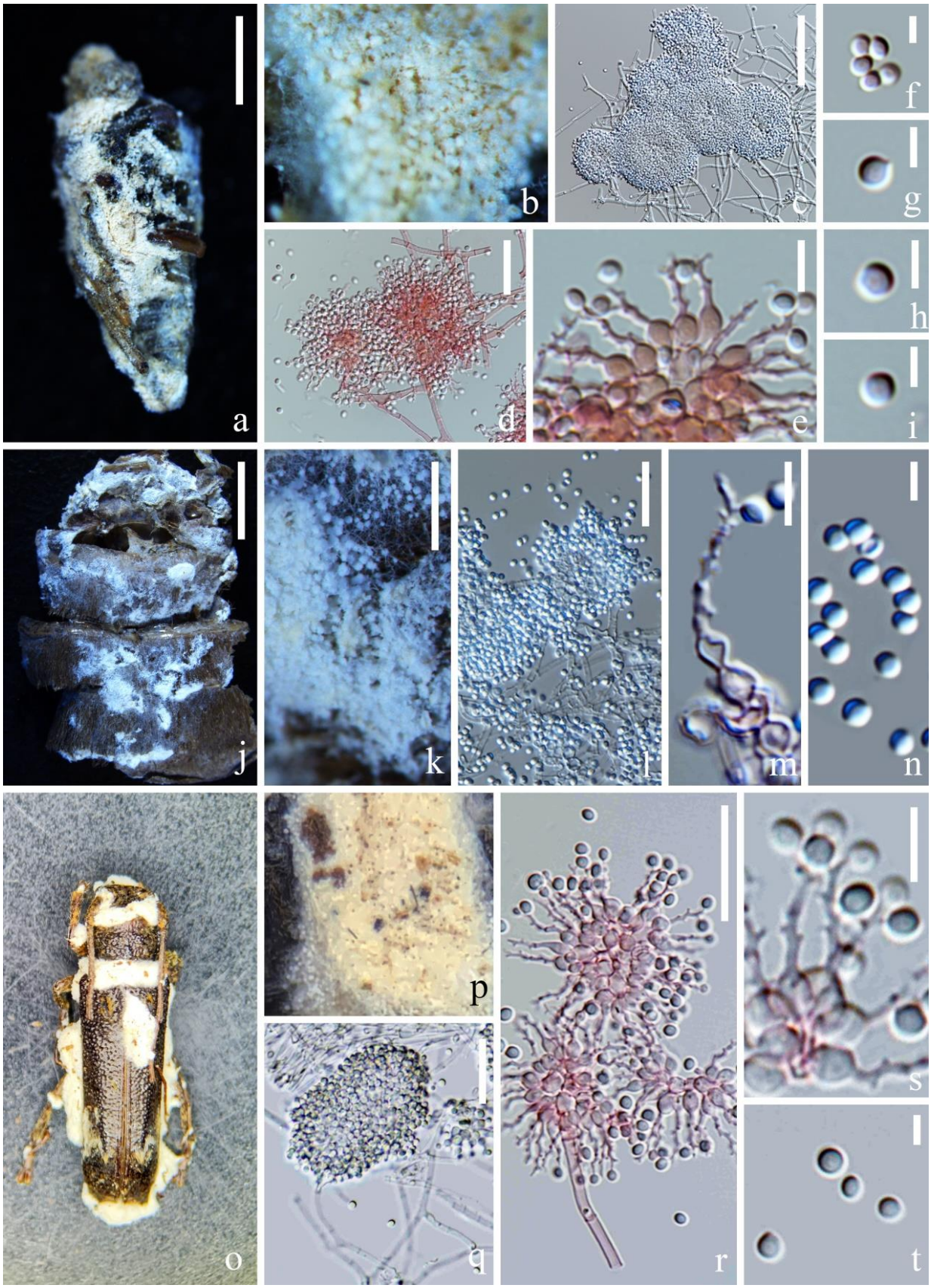

Figure 12 – *Beauveria bassiana* (HKAS 102446, a–i), *Beauveria medogensis* (HKAS 102452, j–n), *Beauveria pseudobassiana* (HKAS 102456, o–t). a, j, o Colonies on hosts. b, k, p Close-up of colonies. c, l, q Conidial mass. d, e, m, r, s Conidiophores and phialides. f–i, n, t Conidia. Scale bar: j = 5000 μm, a = 2000 μm, k = 500 μm, c = 50 μm, d, l, q, r = 20 μm, e, m, s = 5 μm, f–i, n, t = 3 μm (d, e, m, r, s stained with Congo red solution).

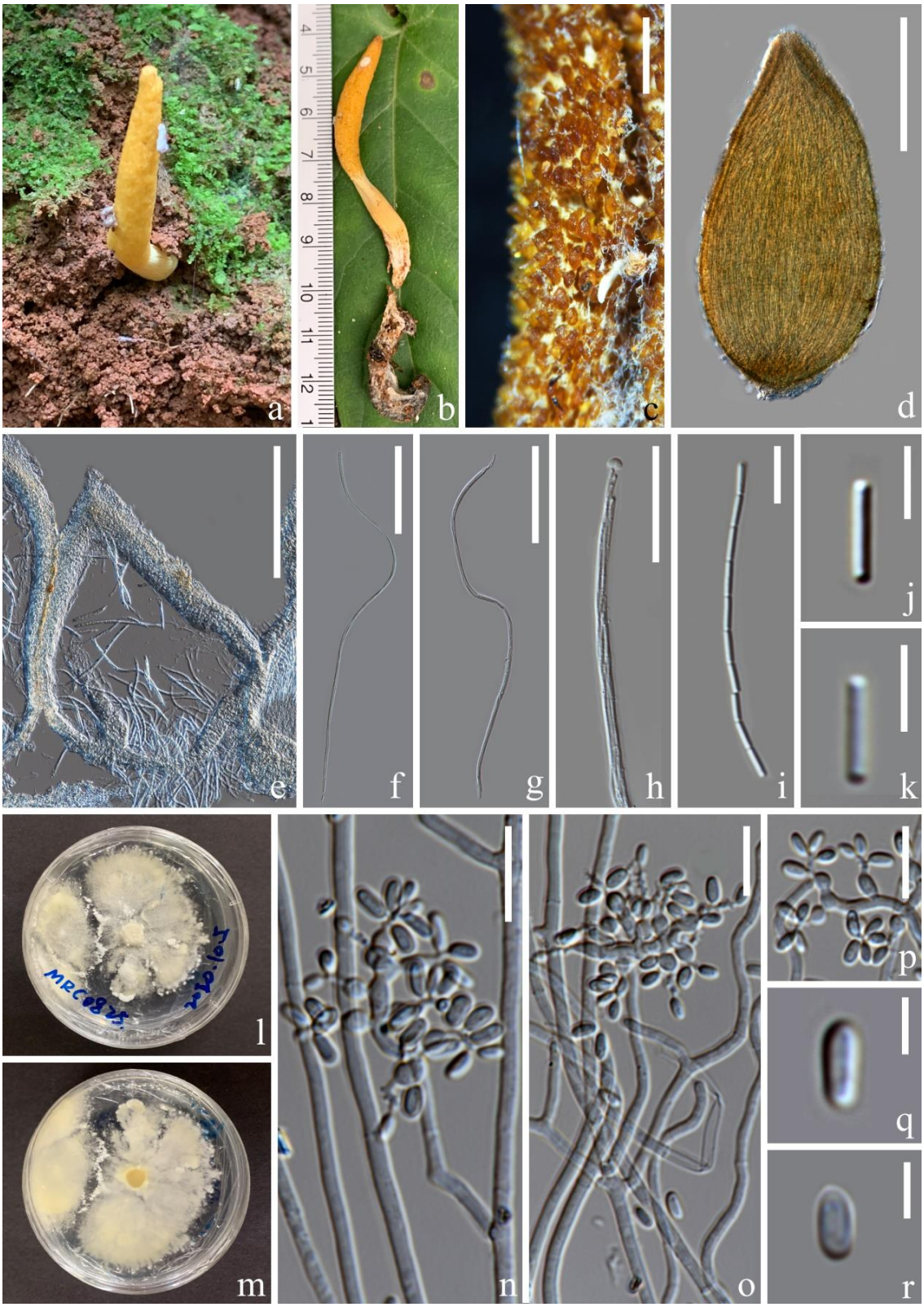

Figure 13 – *Beauveria majiangensis* (MFLU 22-0272). a Stroma emerging from soil. b Stroma on host. c Close-up of fertile part showing perithecia. d Perithecium. e Vertical section through perithecia. f, g Asci. h Part of ascus. i Part of ascospore. j, k Secondary ascospores. l, m Upper and lower view of culture. n–p Conidiophores bearing phialides and conidia. q, r Conidia. Scale bar: c = 1000 µm, d, e = 200 µm, f, g = 100 µm, h = 30 µm, i, n–p = 10 µm, j, k = 5 µm, q, r = 3 µm.

Beauveria medogensis Imoulan & Y.J. Yao, Invert. Path. 139: 79 (2016) Fig. 12j–n
Index Fungorum number: IF 570256; Facesoffungi number: FoF 13954

Saprobic on insect cuticle. *Colony* woolly white. *Mycelium* 1.1–2.2 ($\bar{x}$ = 1.6, n = 30) µm in wide, hyaline, branched, smooth-walled. *Conidiophores* up to 10 µm long, 2.4 µm wide, micronematous, cylindrical, aseptate, arising from anastomosing mycelia. *Conidiogenous cells* 2–3 ($\bar{x}$ = 2.3, n = 20) µm in diam., occurring in dense cluster at the apex of conidiophores, giving an appearance of globose head, base globose, apex with an indeterminate, geniculate, denticulate rachis. *Conidia* 1.5–2.5 ($\bar{x}$ = 2, n = 30) µm in diam., hyaline, globose, aseptate, smooth-walled.

Culture characteristics – Colonies on PDA reaching 3.5 cm after 7 days at 25 °C, white, fluffy, circular, margin entire, mycelia loose, reverse similar with upper view in colour.

Material examined – China, Yunnan Province, Kunming City, Xishan District, Western hill park, on cuticle of decaying insect on leaf litter, 27 July 2018, De-Ping Wei, XS2710 (HKAS 102452), living culture KUNCC 21-10506.

Notes – *Beauveria medogensis* was isolated from soil in Tibet, China by Imoulan et al. (2016). Our strain (HKAS 102452) phylogenetically clusters with *B. medogensis* with strong support (Fig. 1). Morphologically, it shares similar characteristics with *B. medogensis* in having beauveria-like conidiophores and globose hyaline conidia.

Beauveria pseudobassiana S.A. Rehner & Humber, in Rehner, Minnis, Sung, Luangsa-ard, Devotto & Humber, Mycologia 103(5): 1068 (2011) Fig. 12o–t

Index Fungorum number: IF 519125; Facesoffungi number: FoF 13955

Saprobic on adults of Coleoptera. *Colony* woolly, white, superficial on host body. *Mycelium* 1.4–2.4 ($\bar{x}$ = 1.8, n= 20) µm in wide, hyaline, branched, smooth-walled. *Conidiophores* micronematous, cylindrical, septate, thin-walled, arising from anastomosing mycelia. *Conidiogenous cells* 2.5–4 ($\bar{x}$ = 3.2, n= 30) µm in diam., aggregated in dense cluster at the apex of conidiophores, forming a globose head, base globose, apex with an indeterminate, geniculate, denticulate rachis. *Conidia* 1.5–2.5 ($\bar{x}$ = 2.1, n= 30) µm in diam., hyaline, globose, aseptate, smooth-walled.

Culture characteristics – Colonies on PDA reaching 4.3 cm after 15 days at 20 °C, white, fluffy, circular, margin entire, mycelia loose, reverse creamy yellow.

Material examined – China, Yunnan Province, Kunming City, Panlong District, forest near to Songhuaba reservoir, on a coleopteran adult lying on leaf litter, 11 December 2021, De-Ping Wei, SHB1221 (HKAS 102456), living culture KUNCC 21-10509.

Notes – The DNA-based analysis reveals that our isolate (HKAS 102456) group with the type strain of *Beauveria pseudobassiana* (ARSEF 3405) with maximum support (Fig. 1). *Beauveria pseudobassiana* was introduced from a larva of *Lymantria dispar* (Lepidoptera) in the USA by Rehner et al. (2011). This species produces conidiogenous cells with globose base, which apically connects with geniculate, denticulate rachis and broadly ellipsoidal conidia. Our isolate is similar to *B. pseudobassiana* in all aspects except for the associated host being a coleopteran adult.

Blackwellomyces Spatafora & Luangsa-ard, IMA Fungus 8(2): 345 (2017)

Blackwellomyces roseostromatus Mongkols., Noisrip., Khonsanit & Luangsa-ard, Mycol. Progr. 19(9): 968 (2020) Fig. 14

Index Fungorum number: IF 835362; Facesoffungi number: FoF 13956

Parasitic on larva of insect cocoon. Sexual morphs: *Stroma* up to 10 mm long, yellowish orange, clavate, solitary, simple, fleshy, apically enlarged, stipitate. *Stipe* up to 5.6 mm long, 0.8 mm wide, cylindrical, orange. *Rhizoids* flexuous, multiple, arising from body of a lepidopteran larva. *Fertile part* up to 4.4 mm long, 1.3 mm wide, yellowish orange, clavate. *Perithecia* 260–370 × 140–200 ($\bar{x}$ = 314× 164, n = 5) µm, semi-immersed, ovoid, spare, with periphyses, prominent ostioles. *Peridium* 10–20 ($\bar{x}$ = 16, n = 30) µm in wide, two-layered, with the outermost layers comprised of yellow cells *textura angularis*, the inner layer comprised of hyaline cells of *textura prismatica*, the area near to ostioles encompassed by palisade cells. *Asci* 160–260 × 3–5 ($\bar{x}$ = 204× 4.3, n = 20) µm, cylindrical, eight-spored, with thickened apex (measuring 2.4–3.5 × 1.5–2.9 ($\bar{x}$ =

3× 2.2, n = 30) µm). *Ascospores* 140–200 × 0.8–1.5 ($\bar{x}$ = 164× 1.1, n = 5) µm, hyaline, filiform, aseptate. Asexual morphs: Conidia arranged as evlachovaea-like.

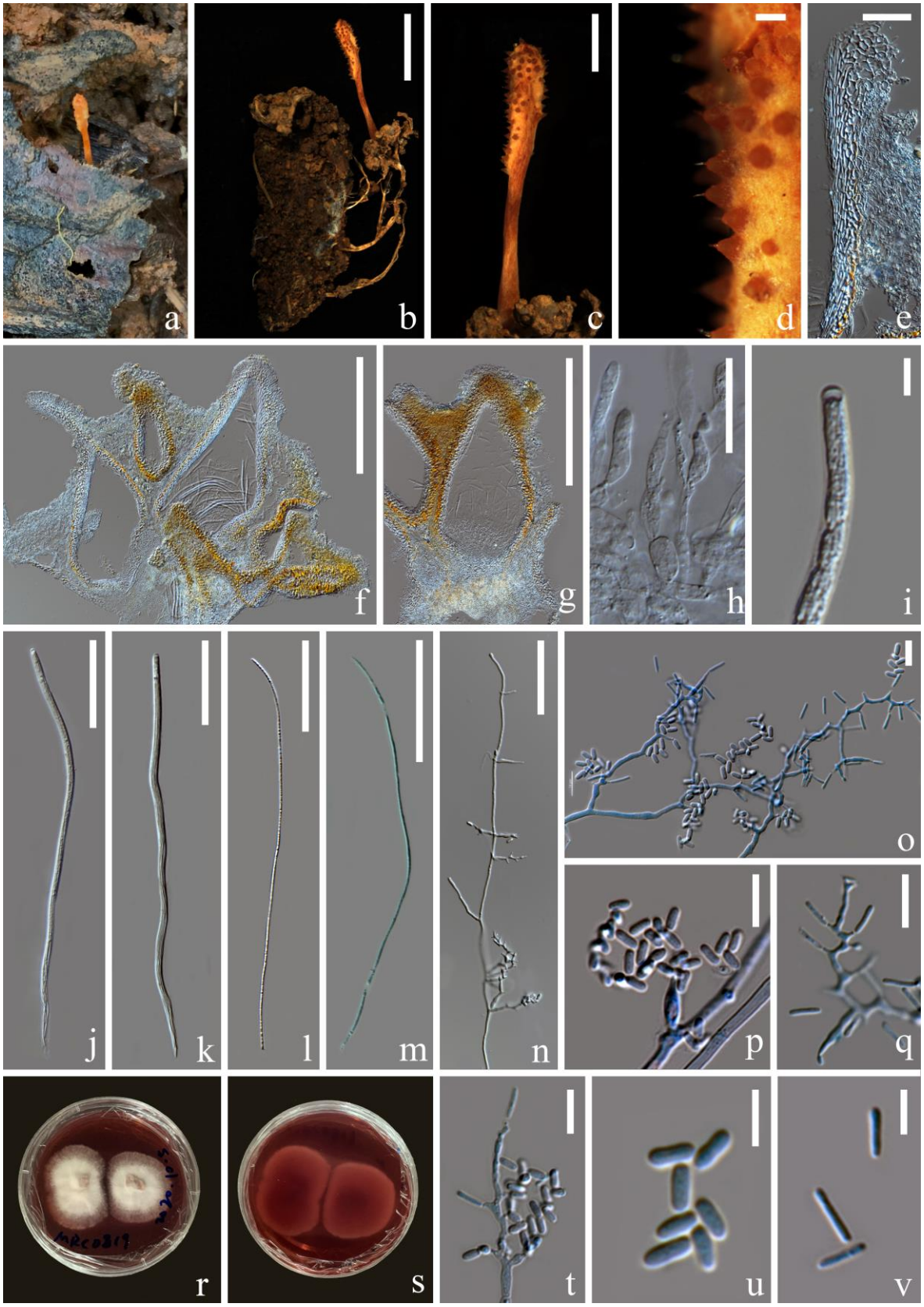

Figure 14 – *Blackwellomyces roseostromatus* (MFLU 22-0274) a Substrate. b, c Stroma growing from host. d Perithecia on stroma. e Peridium. f, g Vertical section through stroma showing perithecia. i Ascus cap. h, j, k Immature and mature asci. l, m Ascospores. n, o, t Conidiophores.

p, q Conidiogenous cells. r, s Upper and reverse of culture. u, v Conidia. Scale bar: b = 5 mm, c = 2 mm, d, f, g = 200 μm, e, h = 30 μm, i, u, v = 5 μm, j–n = 50 μm, o–t = 10 μm. (m, o, p, q, t –v mounted in cotton blue solution).

Culture characteristics – Culture was obtained by cultivating a small piece of tissue isolated from fertile part of the natural specimen. Colony on PDA medium moderately growing, reaching 3.5 cm within 49 days, white, circular, velvety, producing reddish pigment diffusing in agar medium. *Mycelium* 1.5–2.9 ($\bar{x}$ = 2.2, n = 20) μm hyaline, smooth-walled, branched, occasionally septate, guttulate. *Phialides* 5.5–13 × 1.2–3.5 ($\bar{x}$ = 10 × 2.5, n = 20) μm, arising from procumbent hyphae, hyaline, cylindrical to flask-shaped to enlongated-ovoid, phialidic, smooth-walled, aseptate, solitary or in whorls of two. Sometimes, phialides reduce into small denticles (measuring 1–4 × 0.6–1.6 ($\bar{x}$ = 2.1 × 1.1, n = 20) μm), which percurrently proliferate from the apex of cylindrical phialides or sympodially develop from flexuous hyphae. *Conidia* hyaline, aseptate, smooth-walled, ellipsoidal or cylindrical. *Ellipsoidal conidia* 4.2–5.6 × 1.5–2.2 ($\bar{x}$ = 4.8 × 1.8, n = 40) μm, usually forming from cylindrical or flask-shaped or elongated-ovoid phialides as well as from small denticles, often aggregated in wheat ear-like. *Cylindrical conidia* 5.3–9.1 × 0.7–1.3 ($\bar{x}$ = 6.6 × 1, n = 30) μm, typically produced from tip of small denticles, solitary.

Material examined – Thailand, Chiang Mai Province, Mushroom research center, on an insect cocoon bruied in soil, 12 August 2020, De-ping Wei, MRC0819 (MFLU 22-0274), living culture MFLUCC 22-0189.

Notes – *Blackwellomyces roseostromatus* was introduced by Mongkolsamrit et al. (2020b) from a lepidopteran larva in Chiang Mai Province, Thailand. In this study, a new collection of *B. roseostromatus* was discovered on the pupa of an unidentified insect from the same area as the holotype (BBH 47570). Although our isolate (MFLU 22-0274) phylogenetically groups with extant strains of *B. roseostromatus* with significant support, it morphologically differs from the holotype in having straight, orange stipe, unbranched fertile part, spare perithecia and aseptate ascospores, as opposed to cream, slightly flexuous stipe and branched fertile part, crowded perithecia and septate ascospores. Our isolate resembles the ex-type of *B. roseostromatus* in producing reddish pigment diffusing in PDA media, but differs in producing synanamorphs, which have not been noted in Mongkolsamrit et al. (2020b).

Cordyceps Fr., Observ. mycol. (Havniae) 2: 316 (cancellans) (1818)

Cordyceps cateniobliqua (Z.Q. Liang) Kepler, B. Shrestha & Spatafora, IMA Fungus 8(2): 346 (2017) Fig. 15

Index Fungorum number: IF 820977; Facesoffungi number: FoF 13957

Parasitic on insect larvae. Sexual morph: Undetermined. Asexual morph: *Synnemata* multiple, cylindrical, branched, erected, pink at base, paler toward the apex. *Conidiophores* developing from the upper part of synnemata, septate, smooth-walled, unbranched, bearing cluster of metulae or phialides. *Metulae* 4.5–8.5 × 1.5–2.7 ($\bar{x}$ = 6.4 × 2.2, n = 20) μm, cylindrical to clavate, hyaline, aseptate, carrying a group of phialides. *Phialides* 3.5–7 × 1.7–3.4 ($\bar{x}$ = 5 × 2.3, n = 30) μm, flask-shaped, hyaline. *Conidia* 2.5–4 × 1–2 ($\bar{x}$ = 3.2 × 1.5, n = 35) μm, hyaline, ellipsoidal to hammerhead, unicellular, smooth-walled, irregularly arranged in chain.

Culture characteristics – Colonies on PDA reaching 2.5 cm after 15 at 25 °C, pink, velvety, circular, margin entire, reverse orange-yellow.

Material examined – China, Yunnan Province, Kunming City, Panlong District, Kunming botanical garden, on an insect larva packed in decay leaf, 9 July 2021, Lei Lei, Lei01 (HKAS 102458), living culture KUNCC21-10510.

Notes – *Paecilomyces cateniobliquus* was introduced by Liang (1981) from *Adoxophyes privatana* (Lepidoptera) in Guizhou Province, China. Kepler et al. (2017) transferred this species to *Cordyceps* based on multigene phylogenetic analysis. Liang (1981) defined this species as having white to rosey erected branched synnemata, cylindrical metulae, phialides with swollen base, ellipsoidal to ovoid conidia arranged in chains and pink colonies. The morphology of our isolates is largely consistent with those described by Liang (1981), except for the conidial shape. Our isolate

produces dominantly hammerhead conidia, while these are scarcely observed in the original illustration. The multigene phylogenetic analysis confirms that our isolate is conspecific with *Cordyceps cateniobliqua* (Fig. 1).

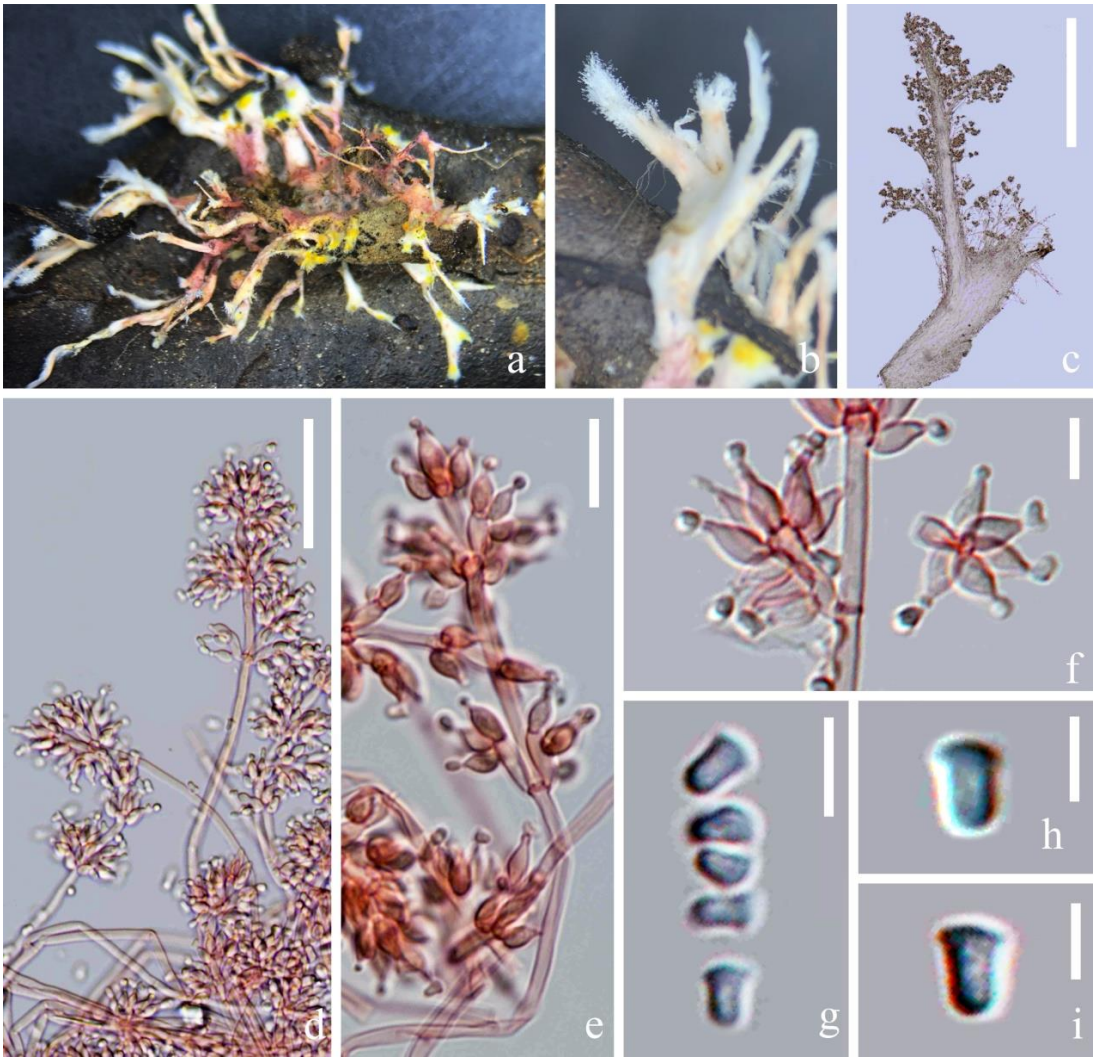

Figure 15 – *Cordyceps cateniobliqua* (HKAS 102458). a, b Synnemata growing from host. c Synnema bearing conidiophores. d, e Conidiophores. f Phialides bearing conidia. g–i Conidia. Scale bar: c = 500 μm, d = 30 μm, e = 10 μm, f, g = 5 μm, h, i = 3 μm (c–f stained with Congo red solution).

Cordyceps cateniannulata (Z.Q. Liang) Kepler, B. Shrestha & Spatafora, IMA Fungus 8(2): 346 (2017) Fig. 16

Index Fungorum number: IF 820976; Facesoffungi number: FoF 13958

Parasitic on insect cocoon. Sexual morph: Undetermined. Asexual morph: *Synnemata* sessile, white, gregarious, powdery. *Conidiophores* differentiating from hyphae of synnemata, septate, smooth-walled, unbranched, bearing cluster of metulae or phialides. *Metulae* 3–5.5 ($\bar{x}$ = 4.3, n = 20) μm in diam., subglobose, hyaline, aseptate, carrying a group of phialides. *Phialides* 3.5–7 × 2–3.5 ($\bar{x}$ = 5 × 2.8, n = 25) μm, with globose basal portion, tapering abruptly into narrow neck, hyaline. *Conidia* 3.5–5 × 1.3–2.1 ($\bar{x}$ = 4.1 × 1.7, n = 25) μm, hyaline, allantoid, unicellular, smooth-walled.

Culture characteristics – Colonies on PDA reaching 4.5 cm after 30 days at 25 °C, white, velvety, circular, margin entire, reverse pale white.

Material examined – China, Yunnan Province, Kunming City, Xishan District, Western hill park, on an insect cocoon laying on leaf litter, 27 July 2018, De-Ping Wei, XS2701 (HKAS 102451), living culture KUNCC21-10505.

Notes – The isolate (HKAS 102451) was obtained from an infected insect cocoon on leaf litter in Yunnan Province, China. The host was covered with abundant white powdery conidia. In the phylogenetic tree, it clusters with *Cordyceps cateniannulata* (TBRC 7258) with maximum support (Fig. 1). *Cordyceps cateniannulata* was originally described as *Paecilomyces cateniannulatus* by Liang (1981) and it was moved to the former genus by Kepler et al. (2017) based on multigene phylogenetic analysis. This species has been reported to infect *Pieris* sp. *Euproctis pseudoconspersa*, *Nilaparvata lugens* (Lepidoptera), *Cacoecia ingentana* (Coleoptera) in Guizhou Province, China (Liang 1981). Our isolate bears similarity to *C. cateniannulata* in the nearly reduced synnemata, phialides with subglobose base and narrow neck, allantoid conidia as well as white, fluffy colonies.

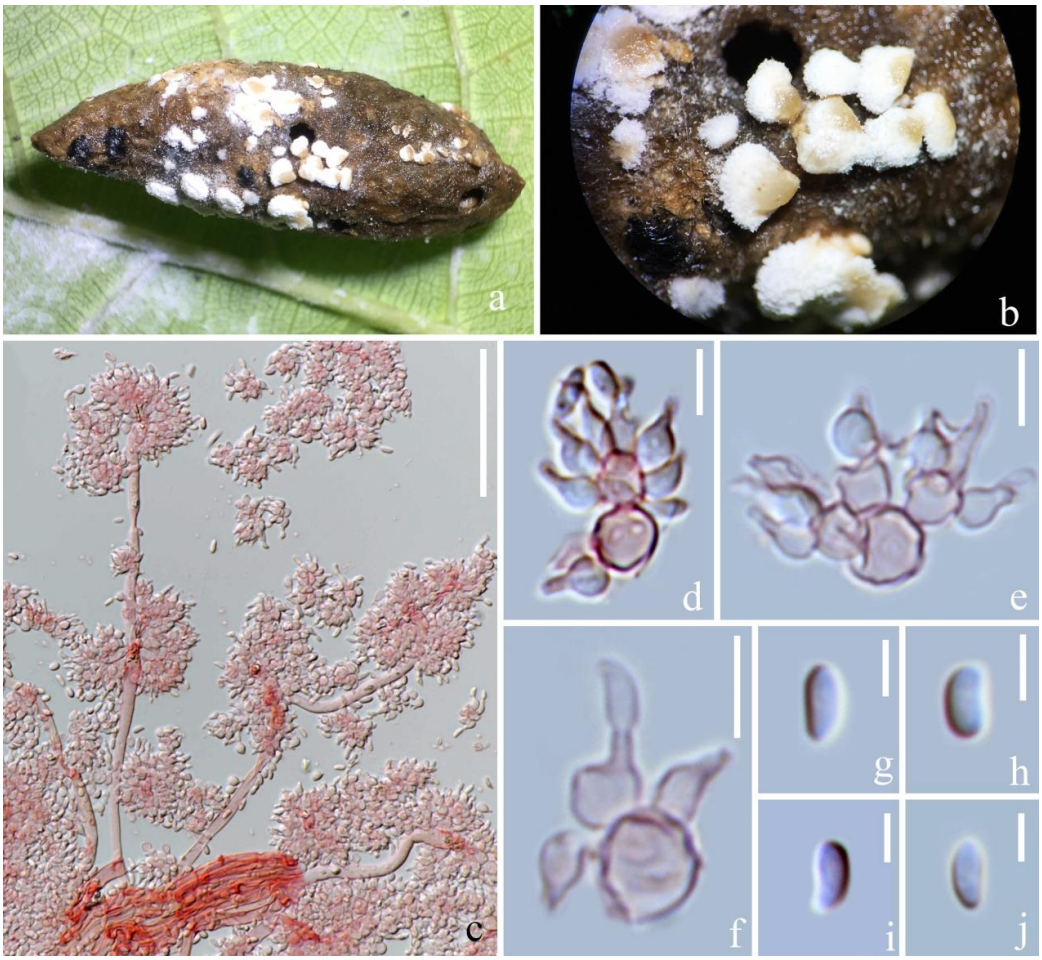

Figure 16 – *Cordyceps cateniannulata* (HKAS 102451). a Synnemata on insect cocoon. b Enlargement of synnemata. c Conidiophores. d–f Metulae bearing phialides and conidia. g–j Conidia. Scale bar: c = 50 μm, d–f = 5 μm, g–j = 3 μm (c–f stained with Congo red solution).

Cordyceps cicadae (Miq.) Massee, Ann. Bot., Lond. 9: 38 (1895) (Fig.17, a–d)

Index Fungorum number: IF 311793; Facesoffungi number: FoF 13961

Parasitic on nymph of cicada. Sexual morph: Undetermined. Asexual morph: *Synnema* single, cylindrical, flesh color, rhizote, unbranched, apically white, powdery due to sporulation. *Conidiophores* 3–8 ($\bar{x}$ = 5.9, n = 20) μm in wide, macronematous, apically swollen, forming cellular hyphae where they give rise to metulae. *Metulae* 4.5–8 ($\bar{x}$ = 5.6, n = 20) μm, occurring in dense clusters on apical regions of conidiophores, subglobose to globose, thin-walled, bearing

groups of phialides. *Phialides* 4–7 × 2.5–4 ($\bar{x}$ = 5.4 × 3.3, n = 30) μm, ampulliform, smooth-walled, phialidic. *Conidia* 5.5–9 × 2–3.5 ($\bar{x}$ = 7.3 × 2.7, n = 50) μm, hyaline, ellipsoidal, aseptate, guttulate, aggregated in chains.

Culture characteristics – Colonies on PDA reaching 5.3 cm after 30 days at 25 °C, white, powdery, circular, margin entire, reverse white.

Material examined – China, Guizhou Province, Qianxinan Buyei and Miao Autonomous Prefecture, Ceheng County, Gaofeng Villige, on cicada nymph buried in soil, 6 August 2018, De-Ping Wei, GFC 607 (HKAS 102460), living culture KUNCC21-10512.

Notes – Our collection nested in a well-supported monophyletic clade consisting of *C. lepidopterorum* and *C. jakajanicola*. *Cordyceps lepidopterorum* was introduced from a lepidopteran larva in Thailand and this species is characterized by forming white colonies on host, isaria-like conidiophores, phialides with globose base, narrow neck and hyaline ellipsoidal conidia (Mongkolsamrit et al. 2018). *Cordyceps jakajanicola* was described from its sexual and asexual morphs found on a cicada nymph in Thailand (Crous et al. 2019). *C. jakajanicola* produces white, powdery conidiophores on the terminal part of the stroma, has isaria-like conidiophores, phialides with a globose basal portion, which tapers into a thin neck and ellipsoidal to cylindrical conidia (Crous et al. 2019). *Cordyceps jakajanicola* was separated from *C. lepidopterorum* based on the smaller phialides and conidia and the associated hosts. However, in the phylogenetic tree constructed in this study and Crous et al. (2019), these two species have a close affiliation. Notably, the asexual morph of *C. jakajanicola* is similar with *Cordyceps cicadae* in the stromal form and the morphology of conidiophores, phialides and conidia. However, the molecular data of *C. cicadae* was not included in the phylogenetic analysis of Crous et al. (2019), which hindered inference of its phylogenetic relationship with *C. jakajanicola.* In this study, we have collected a sample whose morphology resembles *C. cicadae* in all aspects (Kobayasi 1949, Samson 1974, Luangsa-Ard et al. 2005, Zha et al. 2018). Thus, we determined our collection as *C. cicadae* rather than *C. lepidopterorum* and *C. jakajanicola* given that the establishment of the latter two species is not based on sufficient evidence from phylogenetic analysis.

Cordyceps fumosorosea (Wize) Kepler, B. Shrestha & Spatafora, IMA Fungus 8(2): 347 (2017)
Fig. 17m–p

Index Fungorum number: IF 820980; Facesoffungi number: FoF 13959

Parasitic on insect cocoon. Sexual morph: Undetermined. Asexual morph: *Synnemata* paired, unbranched, clavate, white, stipitate. *Conidiophores* septate, apically branched, terminating in cluster of metulae. *Matulae* 4.5–7 × 2.4–4.6 ($\bar{x}$ = 5.7 × 3.4, n = 20) μm, oblong, smooth-walled, producing a group of phialides on the apex. *Phialides* 4.5–8.5 × 2–3 ($\bar{x}$ = 6.2 × 2.5, n = 35) μm, hyaline, with globose basal portion, tapering abruptly into narrow neck, phialidic. *Conidia* 2–3 ($\bar{x}$ = 2.4, n = 40) μm, broadly fusiform to globose, hyaline, aseptate, aggregated in short chain.

Culture characteristics – Colonies on PDA reaching 5 cm after 20 days at 20 °C, pale yellow-green at centre, white at periphery, velvety, circular, mycelia loose, margin entire, reverse creamy yellow.

Material examined – China, Yunnan Province, Kunming City, Panlong District, forest near to Songhuaba reservoir, oninsect cocoon buried in soil, 27 November 2021, De-Ping Wei, SHB2704 (HKAS 102455), living culture KUNCC21-10508.

Notes – *Isaria fumosorosea* firstly was found on the larva of the beetle *Cleoni punctiventris* in Ukraine (Wize 1904). It was transferred to *Spicaria* by Vasilyevskii (1929). Both *Isaria fumosorosea* and *Spicaria fumosorosea* were merged under *Paecilomyces fumosoroseus* by Brown and Smith (1957) who provided the line drawings of conidial structures and conidia from culture materials. Samson (1974) observed several herbarium specimens and living cultures of *P. fumosoroseus* obtained from herbarium (K, IMI and CBS) and provided its description and drawings of microscopic characteristics. Kepler et al. (2017) rejected the genus name *Isaria* in favour of *Cordyceps*, and thus *I. fumosorosea*, *S. fumosorosea* and *P. fumosoroseus* were treated as synonyms of *Cordyceps fumosorosea*. Our isolates share similarities with those described by

Samson (1974) in having synnematous conidiophores, flask-shaped phialides with a globose basal portion and cylindrical to fusiform conidia. The megablast search using ITS gene as query revealed that our isolates highly hit many strains of *Cordyceps fumosorosea* available in GenBank. The phylogenetic tree shows our new isolates grouped with *C. fumosorosea* with maximum support. Following comprehensive morphological observations and phylogenetic inference, we determined our isolate as *C. fumosorosea*.

Figure 17 – *Cordyceps cicada* (HKAS 102460, a–d), *Cordyceps qingchengensis* (HKAS 102444, e–h), *Cordyceps tenuipes* (HKAS 102445, i–l), *Cordyceps fumosorosea* (HKAS 102455, m–p). a, e, i, m Stromata growing from hosts. b, f, j, n Conidiophores. c, g, k, o Phialides. d, h, l, p Conidia. Scale bar: b, f, j, n = 20 μm, c, d, g, h, k, l, o, p = 5 μm (b–c, f–h, j–l, n, o stained with Congo red solution).

Cordyceps inthanonensis Mongkols., Tasan., Thanakitp. & Luangsa-ard, Mycol. Progr. 19(9): 973 (2020) Fig. 18a–j

Index Fungorum number: IF 835365; Facesoffungi number: FoF 13960

Parasitic on insect cocoon. Sexual morph: *Stromata* 3.5 cm in high, multiple, cylindrical, orange-yellow, fleshy, stipitate, branched. *Fertile part* 1 cm high, 3 mm wide, subterminal, slightly attenuate toward the apex. *Perithecia* 680–790 × 370–550 ($\bar{x}$ = 374 × 479, n = 10) μm, ovoid, superficial, ostiolate. *Peridium* 15–35 ($\bar{x}$ = 24, n = 30) μm, composed of thick-walled, yellow cells of *textura angularis*. *Asci* 320–530 × 3.2–5.1 ($\bar{x}$ = 24 × 435, n = 25) μm, filiform, eight-spored, with a thickened apical cap which pierced by a channel. *Ascospores* filiform, multiseptate, hyaline, as long as the asci, fragmenting into secondary spores at maturity. *Secondary ascospores* 2.6–4.6 × 0.8–1.4 ($\bar{x}$ = 3.5 × 1.1, n = 55) μm, cylindrical, hyaline, aseptate, with truncated ends. Asexual morph: Undetermined.

Material examined – China, Yunnan Province, Honghe County, Amushan natural reserve, on insect cocoon lying on leaf litter, 19 July 2017, Samantha C. Karunarathna, Y27 (HKAS 102448).

Notes – Based on phylogeny, the collection (HKAS 102448) clusters with *Cordyceps inthanonensis* with great support (Fig. 1). Our collection morphologically resembles *C. inthanonensis* in producing orange, fleshy, stipitate stroma, subterminal fertile part, superficial perithecia, filiform, multiseptate, disarticulating ascospores and cylindrical secondary ascospores (Mongkolsamrit et al. 2020b).

Cordyceps neopruinosa Mongkols., Noisrip., Khons. & Luangsa-ard, Mycol. Progr. 19(9): 976 (2020) Fig. 18k–o

Index Fungorum number: IF 835366; Facesoffungi number: FoF 13962

Parasitic on insect. Sexual morph: Stromata 3 cm high, cylindrical, orange, multiple, branched, fleshy, stipitate. *Fertile part* 4 mm in length, 0.7 mm in wide, occurring in the middle and upper part of stroma. *Perithecia* 230–300 × 84–143 ($\bar{x}$ = 273 × 115, n = 10) μm, ovoid, superficial. *Peridium* 15–35 ($\bar{x}$ = 24, n = 20) μm, composed of thin-walled, orange cells of *textura angularis*. *Asci* 100–160 × 2–3.5 ($\bar{x}$ = 129 × 3, n = 20) μm, filiform, with thickened apical cap. *Ascospores* immature.

Culture characteristics – Colonies on PDA reaching 3.5 cm after 45 days at 20 °C, orange-yellow, circular, margin entire, reverse yellow, with erected cylindrical, yellow synemata in circular arrangement near to the margin of colony.

Material examined – China, Yunnan Province, Kunming City, Xishan District, Western hill Park, probably on pupa of insect lying on leaf litter, 20 November 2021, De-Ping Wei, XS1101 (HKAS 102453), living culture KUNCC 21-10507.

Notes – Our specimen (HKAS 102453) was found on leaf litter in Yunnan Province, China. Regrettably, its host was not found with the fruiting bodies in the field and this specimen was not matured yet. We have obtained the pure culture by cultivating the tissue. The new collection groups with *Cordyceps neopruinos*a with significant support (Fig. 1). *Cordyceps neopruinosa* was introduced from a pupa of Limacodidae (Lepidoptera) in Thailand. The colonies growing on PDA are yellow, floccose, giving rise to awl-shaped synnemata, verticillate conidiophores and mariannaea-like conidial arrangement (Mongkolsamrit et al. 2020b). Our specimen has similar culture characteristics with those of *C. neopruinosa*.

Cordyceps qingchengensis L.S. Zha & T.C. Wen, Phytotaxa 416(1): 18 (2019) Fig. 17e–h

Index Fungorum number: IF 556460; Facesoffungi number: FoF 03405

Parasitic on insect cocoon. Sexual morph: See Zha et al. (2019). Asexual morph: *Synnemata* numerous, cylindrical, fleshy, unbranched, erected, apically white, powdery due to sporulation. *Conidiophores* 3–5.5 ($\bar{x}$ = 4.2, n = 25) μm, developing from outer layer of synnemata, macronematous, septate, apically swollen and branched, forming cellular hypha where give rise to metulae. *Metulae* 3–7 ($\bar{x}$ = 4.5, n = 20) μm, occurring in dense clusters on apical regions of conidiophores, subglobose to globose, thin-walled, bearing group of phialides. *Phialides* 3.5–6 × 2–

3.5 ($\bar{x}$ = 4.5 × 2.7, n = 30) µm, ampulliform, smooth-walled, phialidic. *Conidia* 2.8–4 × 1.3–2 ($\bar{x}$ = 3.4 × 1.7, n = 40) µm, hyaline, ellipsoidal, aseptate, aggregated in chains.

Culture characteristics – Colonies on PDA reaching 4.5 cm after 15 days at 25 °C, white, circular, fluffy, margin entire, mycelia loose reverse white.

Material examined – China, Yunnan Province, Honghe County, Amushan natural reserve, on insect cocoon packed by dead leaf on ground, 15 June 2018, De-Ping Wei, TSQ05 (HKAS 102444), living culture KUNCC 21-10502.

Notes – *Cordyceps qingchengensis* was known from its sexual morph, which was found on a silk moth (Lepidoptera) in Sichuan Province, China (Zha et al. 2019). However, our collection was found in its asexual morph, which occurred on insect cocoon buried in soil. Thus, it is impossible to compare their morphologies. The nucleotide comparison between our collection (HKAS 102444) and type strain of *C. qingchengensis* shows 2/568 bp, 3/822 bp, 1/988 bp and 2/896 bp differences in ITS, LSU, SSU and *tef*1sequences, respectively. Based on the similarity of nucleotide sequences, we determined our isolate as the asexual morph of *C. qingchengensis* and amended the species description.

Figure 18 – *Cordyceps inthanonensis* (HKAS 102448, a–j). *Cordyceps neopruinosa* (HKAS 102453, k–o). a, k Stromata. b Stromata growing from host. c, l Close-up of stroma. d Perithecium. e, m Vertical section of perithecia. f, n Peridium. g , o Asci. h Ascospore. j Secondary ascospores.

Scale bar: c = 3000 μm, d, e = 300 μm, m = 100 μm, g, h, o = 50 μm, f, n = 30 μm, i, j= 5 μm (o stained with Congo red solution).

Cordyceps tenuipes (Peck) Kepler, B. Shrestha & Spatafora, IMA Fungus 8(2): 347 (2017) Fig. 17i–l

Index Fungorum number: IF 820986; Facesoffungi number: FoF 13963

Parasitic on insect cocoon. Sexual morph: Undetermined. Asexual morph: *Mycelial felt* white, enveloping the insect from which several erect synnemata arise. *Synnemata* white, stipitate, cylindrical, branched, apically bearing powdery conidia. *Conidiophores* 3–5.5 ($\bar{x}$ = 4.7, n = 10) μm in wide, developing from outer layer of synnemata, macronematous, septate, apically swollen and branched, forming cellular hypha where they give rise to metulae. *Metulae* 3.5–6.5 ($\bar{x}$ = 4.7, n = 30) μm, born on apical regions of conidiophores, subglobose to globose, thin-walled, bearing group of phialides. *Phialides* 3.5–6 × 2.5–3.5 ($\bar{x}$ = 4.7 × 3, n = 30) μm, ampulliform, smooth-walled, phialidic. *Conidia* 3–5 × 1.3–2.3 ($\bar{x}$ = 3.9 × 1.7, n = 20) μm, hyaline, ellipsoidal to reniform, smooth-walled, aseptate, adhering in chain.

Culture characteristics – Colonies on PDA reaching 3.5 cm after 20 days at 25 °C, white, circular, fluffy, margin entire, mycelia loose, reverse white.

Material examined – China, Yunnan Province, Honghe County, Amushan natural reserve, on insect cocoonburied in soil, 15 June 2018, De-Ping Wei, TSQ12 (HKAS 102445), living culture KUNCC 21-10503.

Notes – Samson (1974) listed many synonyms under *Paecilomyces tenuipes* and provided line drawing of the conidiophores, phialides and conidia for this species. Kepler et al. (2017) transferred *P. tenuipes* to *Cordyceps* on account of the molecular evidence. Our collection (HKAS 102445) phylogenetically groups with *C. tenuipes* with great support (Fig. 1). Morphologically, it well fit with *C. tenuipes* in having white, conspicuous synnmeta, cellular condiophores, phialies with a globose basal portion and a narrow neck and the hyaline, cylindrical, straight to slightly curved conidia (Samson 1974).

Gibellula Cavara, Atti Ist. bot. R. Univ. Pavia, 2 Sér. 3: 347 (1894)

Gibellula gamsii Kuephadungphan, Tasan. & Luangsa-ard, Mycol. Progr. 18(1-2): 138 (2018) [2019] Fig. 19

Index Fungorum number: IF 825141; Facesoffungi number: FoF 13964

Parasitic on spider. Sexual morph: Undetermined. Asexual morph: Host was completely covered by mycelium, forming a yellow cushion. *Vegetative mycelium* 2–4.5 ($\bar{x}$ = 3.1, n = 40) μm, hyaline, septate, smooth-walled. *Synnema* up to 5.5 cm in high, 0.72 mm at the broadest part, 0.22 mm in the narrowest part, yellowish white to pale yellow, solitary, with clavate, brush-like fertile area, apically narrowing to a short stipe and terminating in a subglobose, yellow sterile tip, stipitate. *Conidiophores* 30–80 × 5.5–7.5 ($\bar{x}$ = 59 × 6.1, n = 20) μm, developing from surface hyphae of synnema, scattered, verrucose, hyaline, septate, narrowing to a slender apex and terminating in a swollen vesicle. *Conidial head* 30–50 ($\bar{x}$ = 42, n = 10) μm in diam., globose. *Vesicles* 7.5–9 × 5–5.5 ($\bar{x}$ = 8.1 × 5.2, n = 5) μm, ellipsoidal to globose, bearing a group of metulae. *Metulae* 3–8 ($\bar{x}$ = 5.4, n = 5) μm in diam., subglobose to ovoidal, giving rise to a cluster of phialides. *Phialides* 7.5–11 × 2–3 ($\bar{x}$ = 9 × 2.5, n = 25) μm, oblong-elliptical, with thickened apex, monophialidic. *Conidia* 3.5–5.5 × 1.8–2.6 ($\bar{x}$ = 4.6 × 2.2, n = 50) μm, ellipsoidal-fusiform, smooth-walled, hyaline, aseptate. granulomanus-like synanamorph not observed.

Material examined – Thailand, Chiang Mai Province, Mushroom researcher centre, on spider attached to lower side of living dicotyledonous leaf, 19 February 2018, De-Ping Wei, MRC18021929 (MFLU 22-0275).

Notes – Phylogenetically our collection clusters with the type strain of *Gibellula gamsii* (BCC 27968). This species has been reported to specifically infect the spiders in Thailand. Its distinctive characters are the brush-like, green-yellow, erect, single synnema, typical gibellula-like

conidiophores, cylindrical-clavate phialides and ellipsoidal to fusiform conidia (Kuephadungphan et al. 2019). Our collection (MFLU 22-0275) morphologically fits with the concept of *G. gamsii*.

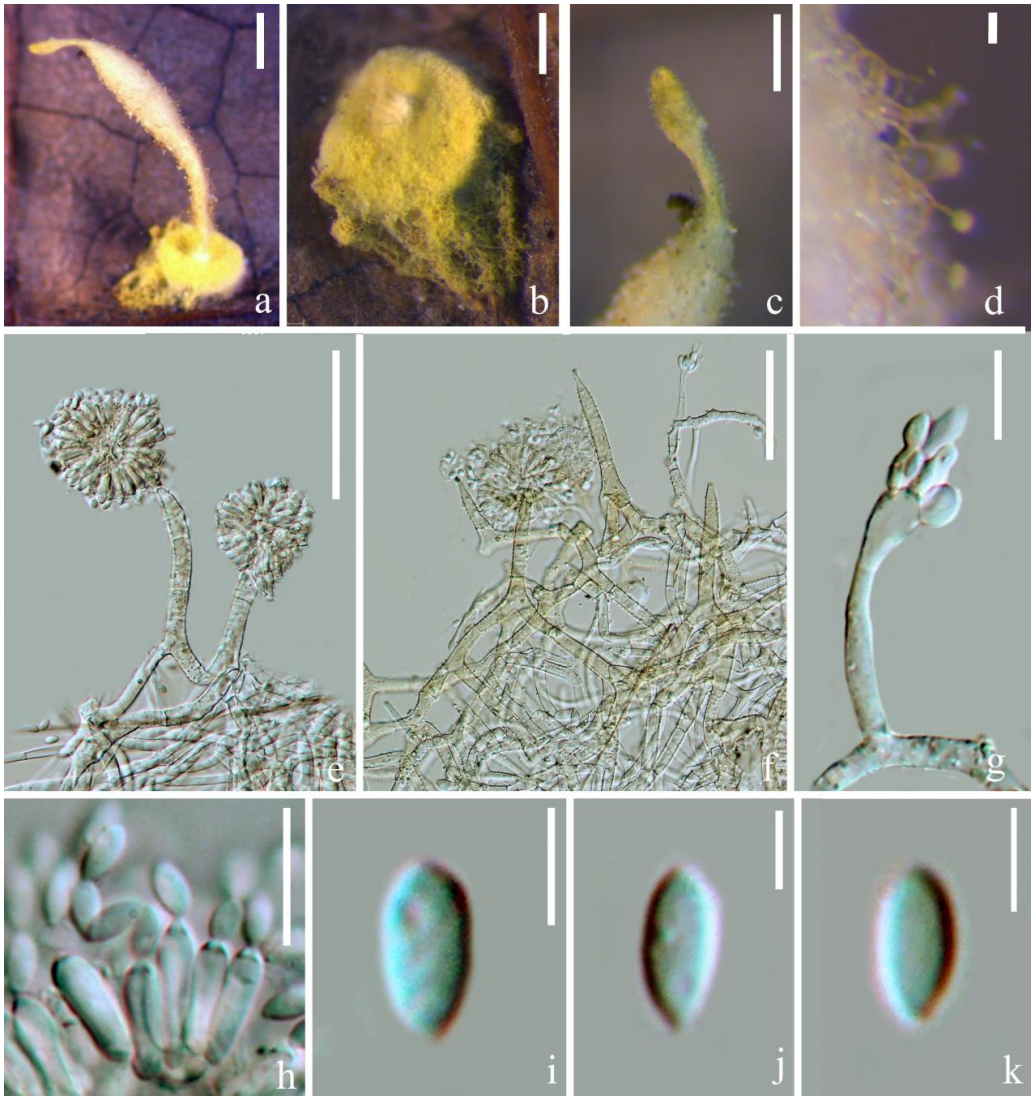

Figure 19 – *Gibellula gamsii* (MFLU 22-0275). a Synnema. b Mycelial mat. c Sterile tip. d Conidiophores arising from synnema. e–g Conidiophores. h Phialides. i–k Conidia. Scale bars: a = 1000 µm, b, c = 500 µm, d–f = 50 µm, g, h = 10 µm, i–k = 3 µm.

Samsoniella Mongkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard, Mycologia 110(1): 248 (2018)

Samsoniella hepiali (Q.T. Chen & R.Q. Dai ex R.Q. Dai, X.M. Li, A.J. Shao, Shu F. Lin, J.L. Lan, Wei H. Chen & C.Y. Shen) H. Yu, R.Q. Dai, Y.B. Wang, Y. Wang & Zhu L. Yang, Fungal Diversity 103: 31 (2020) Fig. 20

Index Fungorum number: IF 833114; Facesoffungi number: FoF 13965

Parasitic on pupa of Limacodidae. Sexual morph: Undetermined. Asexual morph: *Synnemata* 2 cm in high, 1.6 mm in wide, multiple, cylindrical, white, branched. *Conidiophores* 12–50 × 1.4–2.7 ($\bar{x}$ = 31 × 2, n = 15) µm, micronematous, divergent, septate, smooth-walled, branched, apically carrying whole of matulae or phialides. *Matulae* 3–5.5 × 2.1–3.5 ($\bar{x}$ = 4.2 × 2.7, n = 30) µm, cylindrical, smooth-walled. *Phialides* 4.5–7 × 1.5–3 ($\bar{x}$ = 5.6 × 2.3, n = 35) µm, flask-shaped, monophialidic. *Conidia* 2–3.5 × 1.3–2 ($\bar{x}$ = 2.9 × 1.6, n = 30) µm, hyaline, broadly fusiform, aseptate, aggregated in chain.

Culture characteristics – Colonies on PDA reaching 5.2 after 30 days at 25 °C, white, circular, velvety, margin entire, reverse white.

Material examined – China, Yunnan Province, Honghe County, Amushan natural reserve, on pupa of Limacodidae lying on leaf litter, 23 October 2018, De-Ping Wei, AMS09 (HKAS 102443), living culture KUNCC 21-10501.

Notes – *Samsoniella hepiali* was introduced as a new combination with the basionym, *Paecilomyces hepialid* (Wang et al. 2020). This species was reported to infect the larvae of *Hepialus* parasitized by *Ophiocordyceps sinensis* in Diqing Tibetan Autonomous Prefecture, China and the pupa or larva of Lepidoptera in Laocai Province, Vietnam. In this study, we collected a specimen which occurs on pupa of Limacodidae in Honghe County, Yunnan Province, China. Phylogenetically, it has close affinity with *S. hepiali* with high support (Fig. 1). Our collection morphologically resembles *S. hepiali* in the erect, branched synnemata with white conidia in the apex, the isaria-like conidiophores and flask-shaped phialides and fusiform or oval conidia (Wang et al. 2020). The morphological observation and the molecular analysis support our collection as *S. hepiali*.

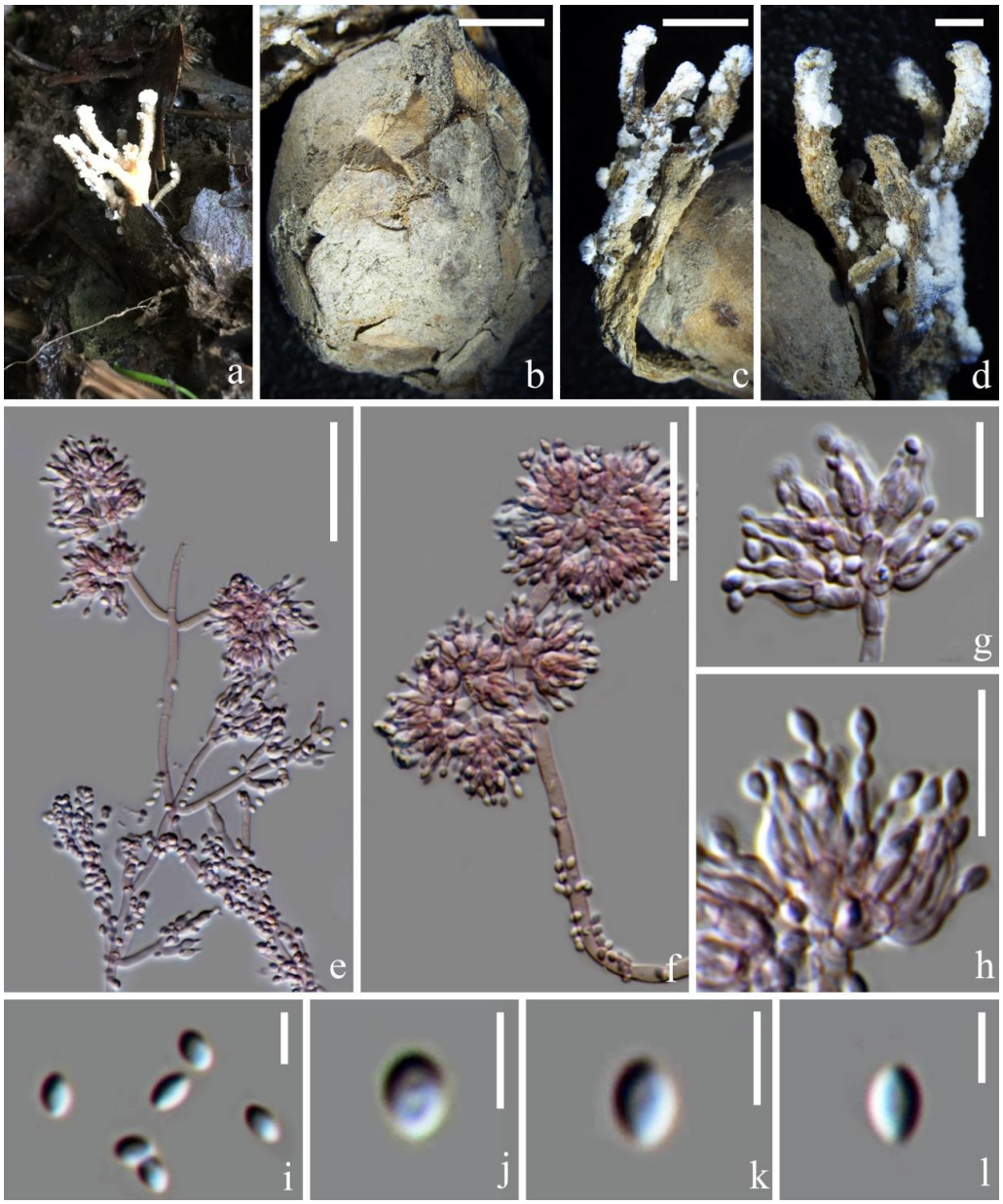

Figure 20 – *Samsoniella hepiali* (HKAS 102443). a Synnemata. b Host. c, d Synnemata growing from host. e, f Conidiophores. g, h Phialides bearing conidia. i–l Conidia. Scale bars: b, c = 5000μm, d = 2000 μm, e, f = 30 μm, g, h = 10 μm, i–l = 3 μm (e–h stained with Congo red solution).

Ophiocordycipitaceae G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Stud. Mycol. 57: 35 (2007)

Notes – In this study, we described eight species of *Ophiocordyceps* from insect hosts in Blattodea, Coleoptera, Diptera, Lepidoptera and Hymenoptera. These entomopathogenic species produces stalked, wiry, pliant to fibrous stromata, intercalary to terminal fertile part, superficial to immersed perithecia, elongated fusiform to filiform ascospores which break into secondary ascospores or not and the hirsutella-like and hymenostilbe-like conidiophores.

Ophiocordyceps Petch, Trans. Br. mycol. Soc. 16(1): 73 (1931)

Ophiocordyceps blattae (Petch) Petch, Trans. Br. mycol. Soc. 16(1): 74 (1931) Fig. 21

Index Fungorum number: IF 431869; Facesoffungi number: FoF 13966

Parasitic on cockroach nymphs (Blattaria). Sexual morph: *Stromata* 1.5–3 ($\bar{x}$ = 2, n = 5) μm, up to 460 μm in wide, multiple, cylindrical, white, unbranched, fragile. *Perithecia* 120–300 × 100–200 ($\bar{x}$ = 206 × 151, n = 15) μm, occurring on middle and upper portions of stroma, pale yellow, ovoid, partly immersed. *Peridium* 10–22 ($\bar{x}$ = 15, n = 20) μm, composed of yellow cells of *textura angularis*. *Asci* 70–110 × 10–20 ($\bar{x}$ = 91 × 13, n = 20) μm, fusiform, with round caps. *Ascospores* 45–60 × 2.5–4 ($\bar{x}$ = 52 × 3.2, n = 20) μm, hyaline, fusiform, 7-sepate, with the secondary cells near to the both ends swollen into subglobose, whole when mature. Asexual morph: *Hymenium* covering the stromata, white, co-occurring with perithecia. *Conidiophores* 6.5–11 × 4.5–8 ($\bar{x}$ = 8.5 × 5.5, n = 15) μm, laterally developing from superficial cells of stromata, subglose, smooth-walled. *Phialides* 10–25 × 6–9 ($\bar{x}$ = 18 × 7.3, n = 20) μm, polyphialidic, hyaline, oblong to cylindrical, with numerous, cylindrical conidiogenous loci on the apex. *Conidia* 9–12 × 4.5–6.5 ($\bar{x}$ = 11 × 5.3, n = 20) μm, hyaline, broadly fusiform, aseptate, slightly guttulate.

Material examined – Thailand, Chiang Mai Province, Chiang Dao District, on cockroach nymph lying on leaf litter, 12 August 2020, De-Ping Wei, CD0802 (MFLU 22-0268).

Notes – *Ophiocordyceps blattae* initially was introduced as a *Cordyceps* by Petch (1921) wherein the author illustrated this fungus. Petch (1931) established *Ophiocordyceps* and selected *Cordyceps blattae* as the type species. This species shows preference to cockroach nymphs and fastens its host to the underside of living leaves in the forest. It has been reported from Sri Lanka and Thailand (Araújo et al. 2021). In a survey of entomopathogenic fungi in Chiang Mai Province, Thailand, we found a cockroach parasite attached to a decaying leaf on the forest floor. Our specimen shares similarity with *O. blattae* in the stalked stromata, partly immersed perithecia, cylindrical asci with inconspicuous apical cap and elongated-fusiform whole ascospores. The phylogenetic analysis also confirms its close affinity with *O. blattae* (Fig. 1).

Ophiocordyceps camponoti-leonardi Kobmoo, Mongkols., Tasan., Thanakitp. & Luangsa-ard, Mol. Ecol. 21(12): 3029 (2012)

Fig. 22

Index Fungorum number: IF 564215; Facesoffungi number: FoF 13967

Parasitic on ants. Sexual morph: *Stroma* arising from the dorsal pronotum of host, solitary, simple, cylindrical. *Fertile cushion* hemisphaerical, brown, surface rough due to projecting perithecial necks. *Perithecia* 230–260 × 80–130 ($\bar{x}$ = 250 × 104, n = 10) μm, immersed, flask-shaped. *Peridium* 15–40 ($\bar{x}$ = 24, n = 20) μm, comprised of hyaline, thick-walled cells of *textura angularis*. *Asci* 100–150 × 6–9 ($\bar{x}$ = 118 × 7.4, n = 20) μm, eight-spored, hyaline, slightly fusiform with hemispherical apical cap measuring 4.5–7 × 2–4.5 ($\bar{x}$ = 5.3 × 3.6, n = 20) μm. *Ascospores* 70–95 × 2–3 ($\bar{x}$ = 86 × 2.6, n = 20) μm, hyaline, needle-like, 4–5-septate. Asexual morph: *Hisutella*-A type produced laterally on upper part of stromata. *Phialide* 4.5–9 × 2.5–4.5 ($\bar{x}$ = 6.2 × 3.7, n = 25) μm, base cylindrical, arbitrarily tapering into a narrow neck 10–20 × 0.8–1.4 ($\bar{x}$ = 15.6 × 1.1, n = 25) μm. *Conidia* 3.5–5 × 1.5–2.5 ($\bar{x}$ = 4.2 × 2, n = 25) μm hyaline, one-celled, oval. Hisutella-C type produced from brown sporodochia on the joint of legs and antennal joints. *Phialide* 10–40 × 3–5 ($\bar{x}$ = 22 × 4.1, n = 35) μm, base cylindrical, gradually attenuate into neck 14–32 × 1.3–2.1 ($\bar{x}$ =

2.1 × 1.7, n = 35) µm. *Conidia* 10–15 × 1.6–3 ($\bar{x}$ = 12 × 2.2, n = 15) µm, hyaline, cylindrical to slightly fusiform, aseptate smooth-walled.

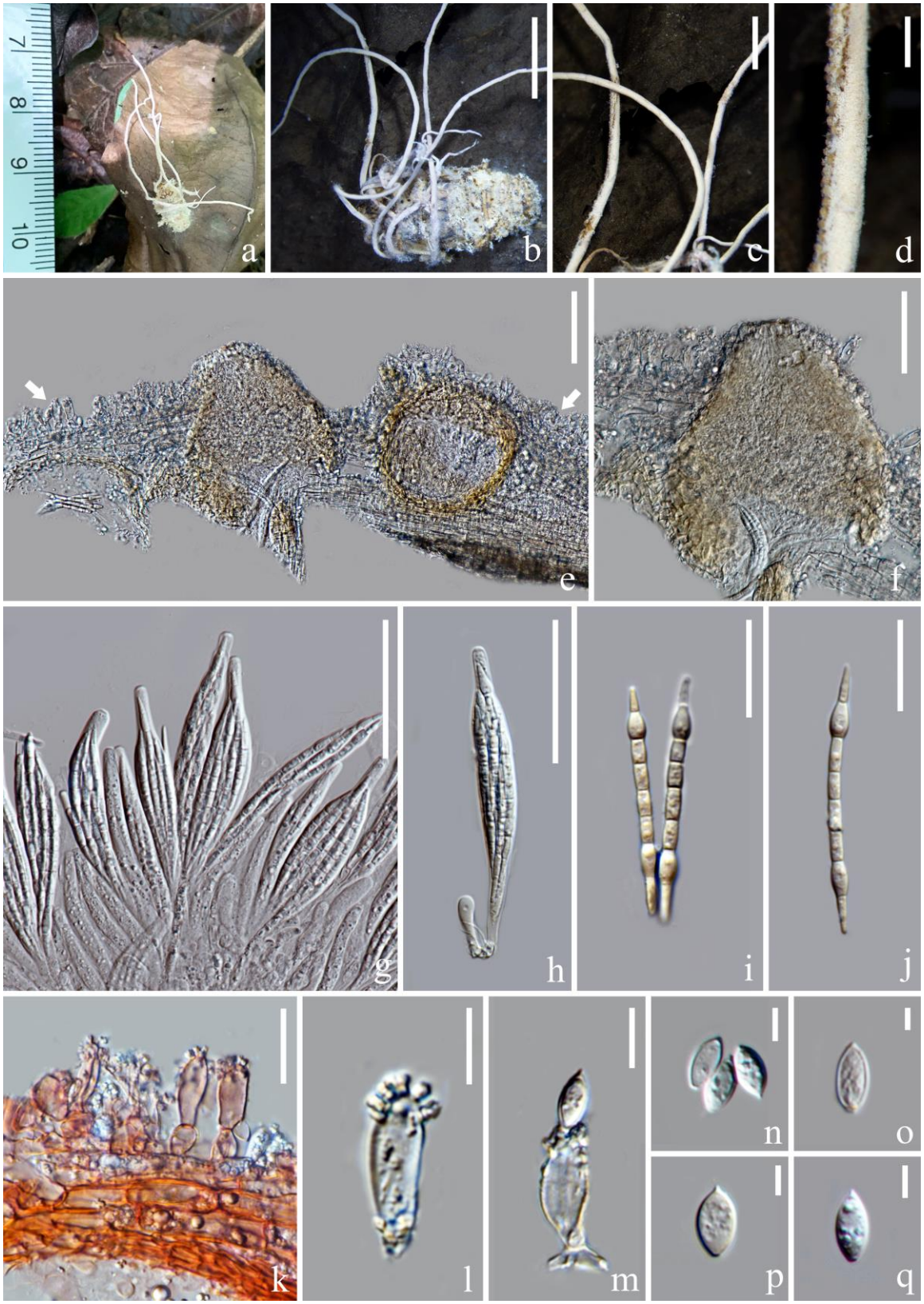

Figure 21 – *Ophiocordyceps blattae* (MFLU 22-0268). a, b Stroma growing from cockroach nymphs (Blattaria). c, d Stromata showing perithecia and conidiophores (indicated with white arrows). f Perithecium. g, h Asci. i, j Ascospores. k Conidiophores. l, m Phialides bearing

conidium. n–q Conidia. Scale bar: b = 5 mm, c = 2 mm, d = 500 µm, e–h = 50 µm, i–k = 20 µm, l, m = 10 µm, n–q = 5 µm. (k was mounted in Congo red solution).

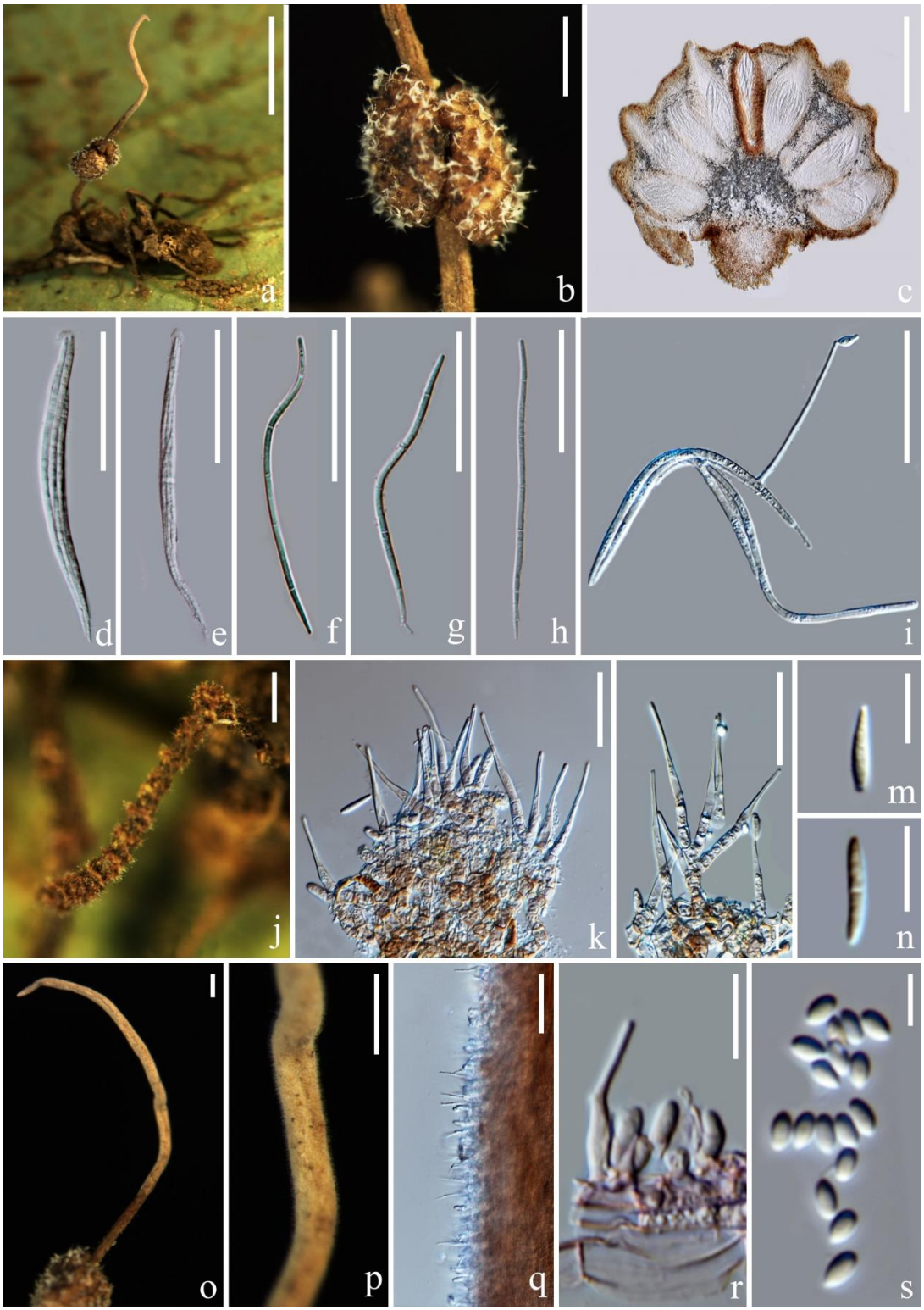

Figure 22 – *Ophiocordyceps camponoti-leonaridi* (MFLU 22-0269). A Stroma arising from neck of ant. b Ascomatal cushions. c Vertical section through ascoma. d, e Asci. f–h Ascospores. i Ascospore with capilliconidiophore bearing one capilliconidium at its apices. j *Hirsutella* hymenium on antenna. k, l, r Phialides. m, n, s Conidia. o, p Upper part of stroma. q *Hirsutella* hymenium on upper part of stroma. Scale bars: a = 5000 µm, b = 1000 µm, j, o, p = 500 µm, c =

200 µm, d–i = 50 µm, k, l, q = 30 µm, m, n, r = 10 µm, s = 5 µm (r stained with Congo red solution).

Material examined – Thailand, Chiang Mai Province, Mushroom researcher centre, on ant (*Camponotus leonardi*) attached to living dicotyledonous leaf, 13 August 2020, De-Ping Wei, MRC0805B-1 (MFLU 22-0269).

Notes – Kobmoo et al. (2012) recognized three cryptic species in *Ophiocordyceps unilateralis* sensu lato based on phylogenetic analysis of individual *tef*1 and *Beta-Tubulin* genes. The result shows that species parasitic on *Camponotus leonardi* form a distinct clade well-separated from those on *Camponotus saundersi* and *Polyrhachis furcate*. Thereby *Ophiocordyceps camponoti-leonardi* was proposed to accommodate species in the clade of *Camponotus leonardi*. In this study, we collected a zombie ant fungus parasitic on *Camponotus leonardi.* It produces typical morphologies of *Ophiocordyceps unilateralis* in having stalked stroma arising from the ant neck, brown fertile part laterally attached to the stroma, immersed perithecia, fusiform asci, long fusiform whole ascospores as well as the hisutella-like conidiophores. Thereby, we determined our collection as *O. camponoti-leonardi* and provided the clear microphotolate that has not been given by Kobmoo et al. (2012). The accurate identification of this specimen should be confirmed with phylogenetic analysis of *tef*1 and *beta-Tubulin* genes.

Ophiocordyceps crinalis (Ellis ex Lloyd) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Stud. Mycol. 57: 41 (2007) Fig. 23

Index Fungorum number: IF 504243; Facesoffungi number: FoF 13968

Parasitic on Lepidopteran larva. Sexual morph: *Stromata* up to 7 mm in high, 1 mm wide, brown, cylindrical, with acute ends, numerous, unbranched, erected. *Perithecia* 300–350 × 250–310 ($\bar{x}$ = 334 × 279, n = 5), brown, ovoid, superficial. *Peridium* 15–30 µm ($\bar{x}$ = 24, n =20), composed of thick-walled, brown cells of *textura angularis*. *Asci* 120–190 × 4.5–7 ($\bar{x}$ = 153 × 5.8, n = 20) µm, slightly fusiform, with thickened caps measuring 3.7–5 × 2–5.5 ($\bar{x}$ = 4.3 × 2.8, n = 20) µm. *Ascospores* 90–130 × 1.5–2.7 ($\bar{x}$ = 111 × 2.0, n = 20) µm, hyaline, cylindrical-fusiform, multiseptate, whole when mature. Asexual morph: Undetermined.

Material examined – China, Yunnan Province, Honghe County, Amushan natural reserve, on lepidopteran larva lying on leaf litter, 19 July 2017, Samantha C. Karunarathna, Y12 (HKAS 102447).

Notes – *Cordyceps crinalis* was initially regarded as *C. sphingum* by Ellis and Everhart (1892) until Lloyd (1920) concluded it as a valid species. Mains (1940, 1958) described this species as having caespitose, brownish-gray, filiform stromata, chestnut-brown, ovoid, superficial perithecia, fusoid asci and filiform, multiseptate ascospores. Sung et al. (2007b) transferred this species to *Ophiocordyceps* based on multigene phylogenetic analysis. The recently updated macro- and micro-morphological feature of *O. crinalis* was provided by Wang et al. (2014) based on a Chinese specimen, wherein its morphological characters are same as those described by Mains (1940, 1958). Our collection resembles *O. crinalis* in the stromatal form, perithecial arrangement, asci and ascospores morphologies as well as the association with lepidopteran larva. The phylogenetic analysis also reveals its close affinity to *O. crinalis.*

Ophiocordyceps globiceps Y.P. Xiao, T.C. Wen & K.D. Hyde, MycoKeys 47: 60 (2019) Fig. 24k–t

Index Fungorum number: IF 555323; Facesoffungi number: FoF 04864

Parasitic on fly. Sexual morph: *Stromata* 9.5–11 mm, laterally emerging from abdomen of host, yellow, multiple, branched, stipitate. *Fertile head* 2–3 mm in diam., globose, lateral surface furrowed. *Stipe* 7–10 mm, cylindrical, straight. *Perithecia* 480–860 × 110–330 µm ($\bar{x}$ = 631 × 183, n = 15), ovoid, immersed, ostiolate. *Asci* up to 215 µm long, 4 µm wide, filiform. *Ascospores* filiform, multiseptate, as long as asci, disarticulating into secondary ascospores when mature.

Secondary ascospores 6.5–9 × 1.2– 1.7 µm ($\bar{x}$ = 7.5 × 1.4, n = 55), hyaline, slightly fusiform, aseptate, smooth-walled.

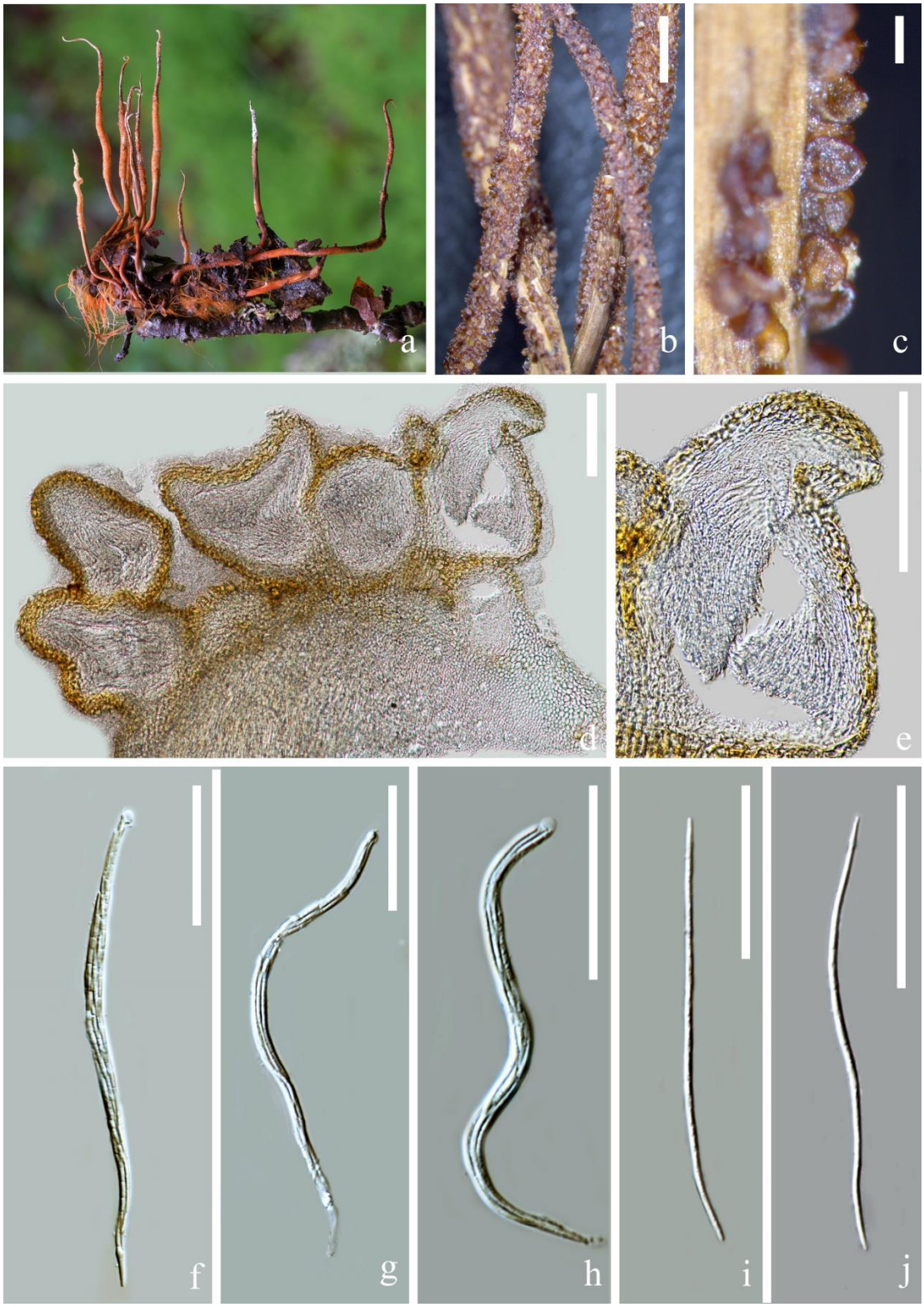

Figure 23 – *Ophiocordyceps crinalis* (HKAS 102447). a Stromata arising from host. b, c Perithecia on stromata. d, e Vertical section of perithecia. f–h Asci. i, j Ascospores. Scale bar: b = 2000 µm, c = 200 µm, d, e = 100 µm, f–j = 50 µm.

Material examined – Thailand, Chiang Mai Province, Chiang Dao District, on fly attached to a dead twig on leaf litter, 12 August 2020, De-Ping Wei, CD0803 (MFLU 22-0267).

Figure 24 – *Ophiocordyceps pseudolloydii* (MFLU 22-0266, a–j), *Ophiocordyceps globiceps* (MFLU 22-0267, k–t). a, k Stromata emerging from hosts. b, c, l Fertile parts. d, m Section through fertile parts. n, o Perithecia. p Ascus. q Part of ascus. h–j, r–t Secondary ascospores. Scale bars: a, b, l = 1000 µm, c, d, m = 500 µm, n, o = 300 µm, g, p = 50 µm, e = 30 µm, f, q, r = 10 µm, h–j, s, t = 3 µm.

Notes – In the multigene phylogenetic tree, our collection (MFLU 22-0267) is sister to the type of *O. globiceps* with maximum support (Fig. 1). It shares similar morphologies with *O. globiceps* in producing yellow, stipitate stroma with terminal globose fertile head, immersed

perithecia, narrow cylindrical asci and filiform multiseptate ascospores (Xiao et al. 2019). However, our collection has branched stromata and infects flies with green dorsal color, whereas the type specimen of *O. globiceps* has unbranched stromata and infects flies with reddish brown dorsal color. Additionally, our collectionhas longer stroma (9.5–11 mm *vs.* 4–8 mm) and larger secondary ascospores (6.5–9 × 1.2– 1.7 μm *vs.* 4–5.4 × 1.2–1.9 μm) compared with that of *O. globiceps*. Nucleotide comparison between our collection and *O. globiceps* shows that there are 12 bp differences and two gaps within the 439 bp ITS region. Considering the insufficient differences upon the morphology, nucleotide sequence and the phylogenetic distance, we determine our collection as *O. globiceps*. This finding indicates that this species is not host-specific, but can infect other aligned fly species.

Ophiocordyceps nutans (Pat.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Stud. Mycol. 57: 45 (2007) Fig. 25

Index Fungorum number: IF 504313; Facesoffungi number: FoF 13969

Parasitic on adult stinkbug. Sexual morph: *Stroma* 50 mm high, 2 mm wide, single, erected, simple, yellow, fibrous. *Fertile head* 18 mm long, 3 mm wide, bright yellow, fusiform, encompassed with a layer of palisade cells. *Perithecia* 310–540 × 130–350 ($\bar{x}$ = 434 × 200, n = 15) μm, obliquely immersed, flask-shaped. *Peridium* 25–50 ($\bar{x}$ = 34, n = 30) μm wide, comprising hyaline, thick-walled cells of *textura angularis*. *Asci* 100–190 × 2.5–4.5 ($\bar{x}$ = 140 × 3.5, n = 15) μm, unitunicate, hyaline, narrow cylindrical, eight-spored, with a thickened cap. *Apical cap* 3–5.5 × 2–5 ($\bar{x}$ = 4.4 × 3.5, n = 25) μm thick, hemispherical, pierced by a canal. *Ascospores* narrow filiform, as long as the asci in long, multiseptate, breaking into numerous secondary ascospores at maturity. *Secondary ascospores* 3–5 × 0.4–1.3 μm ($\bar{x}$ = 4 × 0.8, n = 100), cylindrical, with truncated ends, hyaline, smooth, aseptate, straight.

Material examined – China, Guizhou Province, Guiyang City, Huaxi District, Tongxin Village, on stinkbug lying on ground, 23 June 2018, De-Ping Wei, TXC03 (HKAS 102461).

Notes – Four cryptic species including *Ophiocordyceps asiana*, *O. tessaratomidarum*, *O. nutans*, *O. neonutans* have been recognized in *Ophiocordyceps nutans* complex (Khao-ngam et al. 2021). These species specifically infect stink bugs (Hemiptera) and are widely distributed (Friedrich et al. 2018, Khao-ngam et al. 2021). Species of this complex are characterized by producing dark brown to black stipe, pale yellow to orange yellow fertile head, obliquely immersed perithecia, narrow cylindrical asci and filiform multiseptate ascospores fragmenting into numerous cylindrical secondary ascospores at maturity (Friedrich et al. 2018, Khao-ngam et al. 2021). It is difficult to distinguish species in this complex solely based on macro-morphology due to their morphological variation. Our collection groups sister to *O. nutans* with maximum support. Herein we tentatively design it as *O. nutans* based on the phylogenetic analysis. The individual ITS-based phylogenetic analysis with sufficient taxa sampling is needed to confirm the natural taxonomic placement of our specimen.

Ophiocordyceps pseudolloydii (H.C. Evans & Samson) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Stud. Mycol. 57: 46 (2007) Fig. 24a–j

Index Fungorum number: IF 504324; Facesoffungi number: FoF 02828

Parasitic on ant. Sexual morph: *Stroma* arising from the thorax of the ant, light yellow, single, stipitate. *Fertile head* 0.5–0.6 mm in high, 0.7–0.9 mm diam., hemispherical or oblate globoid, upper surface flat and rough, lateral surface ridged and furrowed. *Stipe* 0.5–1 mm long, 80–100 μm wide, clavate, straight. *Perithecia* 310–570 × 78–173 ($\bar{x}$= 456 × 130, n = 20) μm, flask-shaped, immersed, ostiolate. *Peridium* 45–140 ($\bar{x}$= 80.6, n = 35) μm in wide, composed of hyaline cells of *textura angularis*. *Asci* 110–300 × 3–5.5 ($\bar{x}$= 216 × 4.5, n = 20) μm, filiform, eight-spored, with a thickened cap measuring 2–4 ($\bar{x}$= 2.8, n = 60) μm thick. *Ascospores* filiform, multiseptate, as long as asci, disarticulating into secondary ascospores when mature. *Secondary ascospores* 3–5 × 1.2–2.2 ($\bar{x}$= 4.7 × 1.7, n = 100) μm, hyaline, cylindrical, aseptate, smooth-walled, with truncated ends.

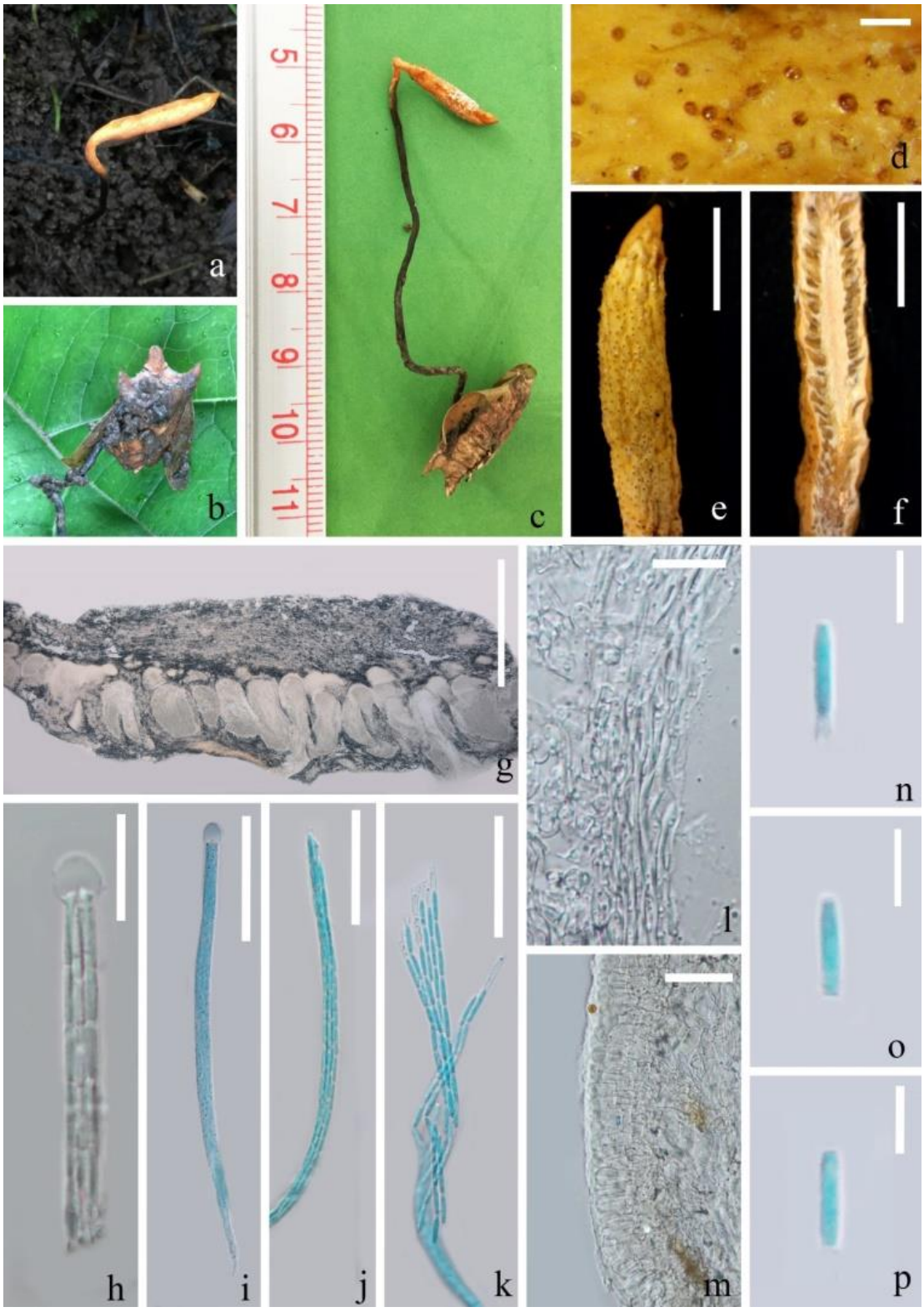

Figure 25 – *Ophiocordyceps nutans* (HKAS 102461). a Substrate. b Host. c Stroma emerging from host. d Apex of perithecia. e Fertile part. f, g Vertical section of fertile part. h Ascus cap. i–k Part of asci. l Peridium. m palisade cell. n–q Secondary ascospores. Scale bars: e–g = 1000 µm, d = 200 µm, m = 50 µm, h–l = 30 µm, n–p = 5 µm (i–k, n–p mounted in cotton blue solution).

Material examined – Thailand, Chiang Mai Province, Mushroom researcher centre, on ant attached to lower side of living dicotyledonous leaf, 19 February 2018, De-Ping Wei, MRC18021902 (MFLU 22-0266).

Notes – Xiao et al. (2017) have updated the description of *Ophiocordyceps pseudolloydii* based on specimens from Chiang Rai Province, Thailand. Our collection was obtained from Chiang Mai Province, Thailand. It bears similarity with *O. pseudolloydii* in having yellow stroma, clavate stipe, terminal, hemispherical fertile head with upper surface being somewhat flat, narrow cylindrical asci and filiform multiseptate ascospores which easily break into cylindrical secondary ascospores when mature (Xiao et al. 2017). The muti-loci phylogenetic analysis also revealed it as a sister clade to *O. pseudolloydii*.

Ophiocordyceps sporangifera Y.P. Xiao, T.C. Wen & K.D. Hyde, MycoKeys 47: 63 (2019)

Fig. 26

Index Fungorum number: IF 555324; Facesoffungi number: FoF 04865

Parasitic on larva of Elateridae (Coleoptera). Sexual morph: Undetermined. Asexual morph: *Epigeal synnema* up to 4.2 cm in high, 940 μm in wide, gray, cylindrical, straight, single, rhizote, producing numerous small branches, terminating in a subglobose, gray fertile head. *Fertile head* 4.9 mm in diam., gray, globose, composed of numerous sporangia, terminating in tip of epigeal synnema. *Sporangia* 80–130 ($\bar{x}$ = 109, n = 10) μm in diam., brown, globose, containing numerous spores. *Spores in sporangia* 10–15 ($\bar{x}$ = 12, n = 25) μm, hyaline, subglobose to irregularly shaped, thick-walled, aseptate. *Hypogeal synnemata* 800–1200 ($\bar{x}$ = 999, n = 5) μm in long, up to 35μm in wide, forming from surface of host body, cylindrical, dark brown, unbranched. *Conidiophores* hirsutella-like. *Phialides* 20–50 × 2–5 ($\bar{x}$ = 33 × 3.2, n = 20) μm, laterally forming from synnemata, hyaline to pale brown, 0-1-sepate, solitary, monophialidic. *Conidia* 6–8.5 × 2–3.5 ($\bar{x}$ = 7.3 × 2.7, n = 25) μm, hyaline, ellipsoidal to reniform, aseptate, bound in mucilaginous spheres. *Mucilaginous spheres* 9–25 ($\bar{x}$ = 12.7, n = 25) μm in diam., forming on tip of phialides, globose, various in size, hyaline when immature, becoming yellow-brown when mature.

Culture characteristics – Colonies on PDA reaching 3.5 cm after 30 days at 25 °C, brown, circular, velvety, margin entire, reverse white.

Material examined – Thailand, Chiang Mai Province, Mushroom researcher centre, on larva of Elateridae buried in soil, 13 August 2020, De-Ping Wei, MRC0812-1 (MFLU 22-0270), living culture MFLUCC 22-0188.

Notes – *Ophiocordyceps sporangifera* was introduced by Xiao et al. (2019) from larvae of Elateridae (Coleoptera) in Mushroom research center, Chiang Mai Province, Thailand. Our collection was obtained from the same area of the type specimen of *O. sporangifera* and its morphologies well fits with those described by Xiao et al. (2019) in the fibrous, brownish gray erect stromata which carry many small secondary branches and terminate in gray globose sporangia. The conidiophores were formed from the surface of host bodies under the ground, while it was observed from the small secondary branches and synnemata on culture in Xiao et al. (2019)'s study. The conidia are hyaline reinform and restricted in mucilaginous spheres that are hyaline in the young stage and become brown in the later stage. This conidial characteristic was found on this and Xiao et al. (2019)'s illustration. The multigene phylogeny also reveals our collection form a well-supported clade with the type specimen of *O. sporangifera* (MFLU 18-0658).

Ophiocordyceps thanathonensis Y.P. Xiao, T.C. Wen & K.D. Hyde, Phytotaxa 328(2): 120 (2017)

Fig. 27a–d, i–k

Index Fungorum number: IF 552731; Facesoffungi number: FoF 02827

Parasitic on ants. Sexual morph: *Stroma* 3.4 cm in long, 0.6 mm in wide, arising from thorax of ant, yellow, single, stipitate. *Fertile head* 3.7 mm in long, 2.1 mm in wide, ellipsoidal. *Stipe* cylindrical, flexuous. *Perithecia* 500–900 × 150–500 ($\bar{x}$ = 648 × 225, n = 10) μm, flask-shaped, obliquely immersed, ostiolate. *Peridium* 25–35 ($\bar{x}$ = 30, n = 10) μm composed of hyaline cells of *textura angularis*. *Asci* 270–530 × 5–8 ($\bar{x}$ = 430 × 6.4, n = 20) μm, filiform, eight-spored, with a thickened cap. *Ascospores* filiform, multiseptate, as long as asci, disarticulating into secondary ascospores when mature. *Secondary ascospores* 6–8.5 × 1.4–2.5 ($\bar{x}$ = 7.1 × 1.8, n = 20) μm, hyaline, cylindrical, aseptate, smooth-walled, with truncated ends.

Material examined – Thailand, Chiang Mai Province, Mushroom research centre, on dead ant lying on leaf litter, 13 August 2020, De-Ping Wei, MRC0842 (HKAS 102442).

Notes – The specimen (HKAS 102442) has a sister phylogenetic affinity to *Ophiocordyceps thanathonensis* with maximum statistical support. This species was introduced by Xiao et al. (2017) from an adult ant in Chiang Rai Province, Thailand. It has a single yellow stroma emerging from the neck of the ant, cylindrical stipe, terminal fusiform fertile head, narrow cylindrical asci and filiform ascospores which break into numerous barrel-shaped aseptate secondary ascospores (Xiao et al. 2017). Our specimen bears high similarity with *O. thanathonensis* in macro and micro-morphologies as well as the associated host. The combination of morphology and phylogeny support our specimen as *O. thanathonensis.*

Fig. 26 – *Ophiocordyceps sporangifera* (MFLU 22-0270). a Epigeal synnema. b Synnema growing from host. c Fertile head bearing sporangia. d Braches of synnema. e Sporangia. f Spores in sporangia. g Germinating spores. h Hypogeal synnemata directly arising from surface of host body. i, j Close-up of synnemta. k Part of synnema. l, m Phialides bearing conidial mass. n Conidia enclosed by mucilaginous substance. o–q Conidia. Scale bars: h = 5000 µm, c, d = 2000 µm, i = 500 µm, e, j = 200 µm, f, g, k–n = 20 µm, o–q = 5 µm.

Ophiocordyceps vespulae F.Y. Long, Y.P. Xiao & T.C. Wen, Phytotaxa 478 (1): 35 (2021) Fig. 27e–h, l–o

Index Fungorum number: IF 556626; Facesoffungi number: FoF 13970

Parasitic on wasp. Sexual morph: *Stroma* 105 mm in long, growing from thorax of wasp, light yellow, single, stipitate. *Stipe* 1 mm wide, cylindrical, flexuous. *Fertile head* 12.5 mm long, 1.6 mm wide, cylindrical and gradually attenuated toward the apex. *Perithecia* 550–960 × 200–240 ($\bar{x}$ = 699 × 228, n = 10) μm, flask-shaped, obliquely immersed, ostiolate. *Peridium* 25–40 ($\bar{x}$ = 32, n = 20) μm, composed of hyaline cells of *textura angularis*. *Asci* 400–650 × 4.5–9 ($\bar{x}$ = 517 × 6.3, n = 15) μm, filiform, eight-spored, with a thickened cap. *Ascospores* filiform, multiseptate, as long as asci, disarticulating into secondary ascospores when mature. *Secondary ascospores* 8.5–11 × 1.9–2.6 ($\bar{x}$ = 10 × 2.2, n = 20) μm, hyaline, fusiform, aseptate, smooth-walled.

Material examined – China, Yunnan Province, Honghe County, Amushan natural reserve, on wasp lying on leaf litter, 25 July 2017, Samantha C. Karunarathna, Y47 (HKAS 102449).

Notes – Based on a megablast search against GenBank nucleotide database, the closest hits using ITS sequence is *Ophiocordyceps vespulae* (GenBank: MN044857, Identities = 486/488 (99%), Gaps = 1/488 (0%)). *Ophiocordyceps vespulae* was introduced by Long et al. (2021) from a wasp host in Jilin Province, China. Our specimen is similar with *O. vespulae* in having yellow stroma, terminal cylindrical fertile part which tapers toward the apex, narrow cylindrical asci, filiform multiseptate ascospores and fusiform secondary ascospores (Long et al. 2021). However, they were found on different wasp species. Phylogenetic analysis shows that our collection (Y47) clusters with the type specimen of *O. vespulae*. Thus, we considered our collection as a new host record of *O. vespulae* from Yunnan Province, China.

Purpureocillium takamizusanense (Kobayasi) S. Ban, Azuma & Hiroki Sato, Int. J. Syst. Evol. Microbiol. 65: 2463 (2015) Fig. 28

Index Fungorum number: IF 811842; Facesoffungi number: FoF 13971

Parasitic on stinkbug. Sexual morph: Undetermined. Asexual morph: *Synnemata* 550–940 × 50–120 ($\bar{x}$ = 730 × 87, n = 5) μm, gray, gregarious, branched, stipitate, apically powdery due to sporulation. *Conidiophores* penicillate, composed of septate, smooth-walled hyphae, terminating in group of phialides. *Phialides* 7–9.5 × 1.5–2.3 ($\bar{x}$ = 7.8 × 2, n = 20) μm, hyaline, flask-shaped, smooth-walled, with a narrow neck, monophialidic. *Conidia* 2–3.5 × 1–2 ($\bar{x}$ = 2.7 × 1.4, n = 25) μm, hyaline, broadly fusiform, aseptate.

Culture characteristics – Colonies on PDA reaching 5 cm after 30 days at 25 °C, gray-purple, circular, powdery, margin entire, reverse white gray.

Material examined – Thailand, Chiang Mai Province, Chiang Dao District, on stinkbug lying on ground, 12 August 2020, De-Ping Wei, CD0805 (MFLU 22-0271), living culture MFLUCC 22-0186.

Notes – *Purpureocillium takamizusanense* was originally found on a cicada in Japan and was described as an *Isaria* species by Kobayasi (1941). Ban et al. (2015) transferred this species to *Purpureocillium* and linked its teleomorph to *Cordyceps ryogamimontana* based on phylogenetic analyses. *Purpureocillium takamizusanense* growing on stinkbug has been documented on the website "Atlas of Invertebrate-Pathogenic fungi of Thailand". In this study, we discovered *P. takamizusanense* from a stinkbug in northern Thailand and it phylogenetically clusters with *P. takamizusanense* (BCC49261) with strong support (Fig. 1). Morphologically, our isolate resembles *P. takamizusanense* in producing numerous, lilac-colored synnemata, bottle-shaped phialides and catenulate, fusiform to ellipsoidal conidia. Hence, we determined our isolate as a new collection of this species.

Polycephalomycetaceae Y.P. Xiao, Y.B Wang, T.C. Wen, H. Yu & K.D. Hyde (in press)

Notes – We described two fungicolous fungi representing *Pleurocordyceps* and *Perennicordyceps* in Polycephalomycetaceae. The asexual morph of *Pleurocordyceps* produces the typical polycephalomyces-like conidiophores. The sexual morph of *Perennicordyceps* has stalked,

fibrous stromata, intercalary fertile part, superficial perithecia and filiform, multiseptate ascospores, which break into cubic secondary ascospores.

Fig. 27 – *Ophiocordyceps thanathonensis* (HKAS 102442, a–d, i–k), *Ophiocordyceps vespulae* (HKAS 102449, e–h, l–o). a, e Stroma raising from hosts. b Ant. c, g Fertile parts. d, h Perithecia. f Vesp. j, l Asci. k, n, o Secondary ascospores. Scale bars: b, c, g = 2000 µm, d, h = 300 µm, i, l =

100 µm, j, m = 30 µm, k, n, o = 10 µm (i–k stained with Congo red solution, l stained with cotton blue solution).

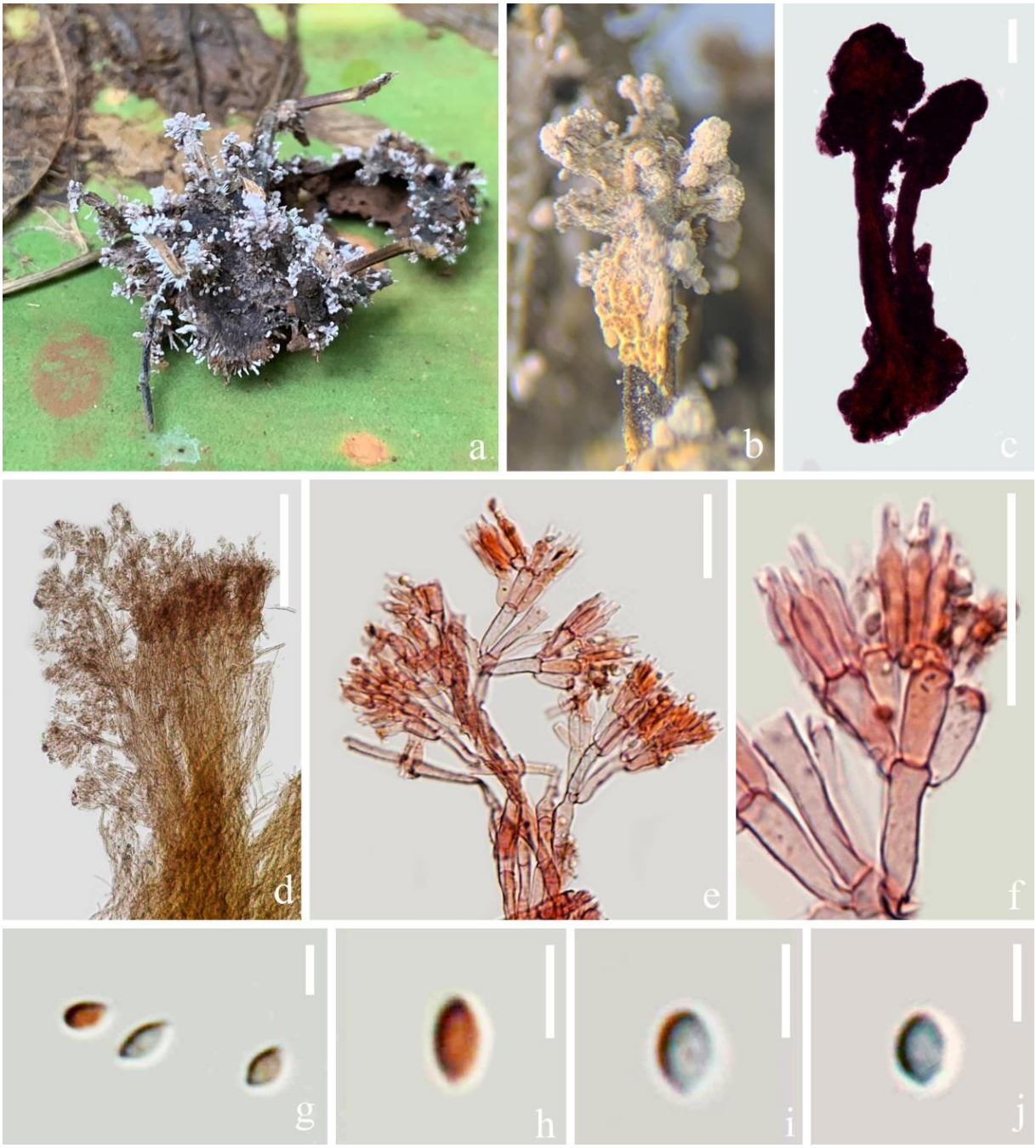

Fig. 28 – *Purpureocillium takamizusanense* (MFLU 22-0271). a Synnemata growing on hosts. b, c Enlargement of synnemata. d Synnema bearing conidiophores. e, f Conidiophores and phialides. g–j Conidia. Scale bars: c, d = 100µm, e, f = 20 µm, g–j = 3 µm (e–j stained with Congo red solution).

Pleurocordyceps Y.J. Yao, Y.H. Wang, S. Ban, W.J. Wang, Yi Li, Ke Wang & P.M. Kirk, Journal of Systematics and Evolution 59(5): 1074 (2021)

Pleurocordyceps ophiocordycipiticola D.P. Wei & K.D. Hyde, sp. nov. Fig. 29a–g

Index Fungorum number: 900189; Facesoffungi number: FoF 13972

Etymology – The specific epithet refers to the genus name of the host, *Ophiocordyceps*.

Parasitic on *Ophiocordyceps* sp. Sexual morph: Undetermined. Asexual morph: *Synnemata* 1.3–1.5 ($\bar{x}$ = 1.4, n = 5) mm in high, arising from stroma of *Ophiocordyceps* sp., comprised of a globose base measuring 490–680 ($\bar{x}$ =595, n = 5) μm in diam., and a white, cylindrical part measuring 70–150 ($\bar{x}$ = 112, n = 5) μm in wide. *Conidiophores* dimorphic. *α-Phialides* 7.5–17 × 1–2 ($\bar{x}$ = 12 × 1.5, n = 30) μm, forming from base of synnemata, compact, awl-shaped, hyaline, smooth-walled, monophialidic, producing *α*-conidia. *β-Phialides* 9.5–16 ($\bar{x}$ = 13, n = 30) μm in long, hirsutella-like, forming from upper part of synnemata, base cylindrical (1.6–3.1 ($\bar{x}$ = 2.5, n = 30) μm in wide)), narrow into a neck (0.7–1.5 ($\bar{x}$ = 1, n = 30) μm in wide)), monophialidic, producing β-conidia. *α-conidia* 1.8–2.5 ($\bar{x}$ = 2, n = 40) μm in diam., hyaline, globose, aseptate, aggregated at base of synnemata, forming yellow, subglobose conidial mass. *β-conidia* 2.5–4.8 × 1–1.8 ($\bar{x}$ = 3.6 × 1.5, n = 40) μm, hyaline, fusiform, aseptate, aggregated in short chain.

Culture characteristics – Colonies on PDA reaching 4 cm after 30 days at 25 °C, white, circular, flat, mycelia dense, margin entire, reverse yellow, producing yellow conidia mass.

Material examined – Thailand, Chiang Mai Province, Mushroom researcher centre, on stroma of *Ophiocordyceps cylindrospora*, 13 August 2020, De-Ping Wei, MRC0801 (MFLU 22-0265, holotype), ex-type MFLUCC 22-0187.

Notes – A clade of maximum support consisting of *Pleurocordyceps ophiocordycipiticola*, *P. marginaliradian* and *P. aurantiacus* was observed in our multigene phylogenetic tree (Fig. 1). Both the reference species mentioned above were originally assigned to *Polycephalomyces* by Xiao et al. (2018) and later were transferred to *Pleurocordyceps* by Wang et al. (2021) based on phylogenetic analysis. The asexual and sexual morphs of *P. marginaliradian* simultaneously occured on Cossidae larvae (Lepidoptera) in Thailand. *Pleurocordyceps ophiocordycipiticola* can be distinguished from *P. marginaliradian* in forming white velvety synnemata with its base being enclosed by thick yellow conidial mass, while the latter species has yellow, cottony synnemata without conidial mass in the basal part (Xiao et al. 2018). *Pleurocordyceps aurantiacus* is distinct from *P. ophiocordycipiticola* by its flat-shaped, orange synnemata, oval to globose α-conidia (Xiao et al. 2018), whereas the new species has erected, cylindrical, white synnemata and globose α-conidia. We determined our collection as a new species on the basis of morphological differences and phylogenetic analysis.

Perennicordyceps elaphomyceticola W.Y. Chuang, H.A. Ariyaw., J.I. Yang & Stadler, Mycol. Progr. 19(1): 102 (2020) Fig. 29h–q

Index Fungorum number: 830670; Facesoffungi number: FoF 10735

Parasitic on *Elaphomyces* sp. Sexual morph: *Stroma* up to 80 mm long, yellow-brown, single, branched, wiry, stipitate. *Stipe* up to 38 mm, 3.6 mm wide, cylindrical, terminally branched. *Branches* up to 41 mm long, 1.5 mm wide, cylindrical, paler toward the apex. *Perithecia* 380–550 × 260–310 ($\bar{x}$ = 473 × 285, n = 15) μm, producing from the branches, superficial, ovoid, brown, ostiolate. *Asci* 230–525 × 4–6.1 ($\bar{x}$ = 280 × 5.4, n = 25) μm, narrow cylindrical, with a thickened apex. *Apical cap* 2.5–5 × 1.5–3.5 ($\bar{x}$ = 3.9 × 2.4, n = 25) μm, hemispherical. *Ascospores* hyaline, filiform, transversely multiseptate, breaking into numerous secondary ascospores. *Secondary ascospores* 1.7–3.2 × 1.1–1.9 ($\bar{x}$ = 2.3 × 1.5, n = 70) μm, hyaline, cubic, with truncate ends, smooth-walled. Asexual morph: Undetermined.

Material examined – China, Yunnan Province, Honghe County, Amushan natural reserve, on *Elaphomyces* sp. buried in soil, 19 July 2017, Samantha C. Karunarathna, Y57 (HKAS 102450).

Notes – *Perennicordyceps elaphomyceticola* was initially placed in *Polycephalomyces* by Yang et al. (2020) and subsequently was transferred to the former genus by Xiao et al. (2023) based on phylogenetic analysis. The sexual morph of *P. elaphomyceticola* was discovered as a parasite on *Elaphomyces* sp. in Taiwan and Dadugang Village, Jinghong City, Yunnan Province, China (Yang et al. 2020, Xiao et al. 2023). Its asexual morph was found as a hyperparasite on *Ophiocordyceps* sp. in Yunnan Province. Our specimen was collected from Honghe County, Yunnan Province, China. It has similar morphology to *P. elaphomyceticola* in that it parasitizes *Elaphomyces* buried in soil and having dark brown, branched stromata, intercalary fertile part, superficial, ovoid

perithecia, narrow cylindrical asci with thick apical cap and filiform, multiseptate ascospores, which disarticulate into numerous cubic secondary ascospores at maturity (Yang et al. 2020, Xiao et al. 2023). The multigene phylogenetic analysis shows it clusters with the type specimen of *P. elaphomyceticola*, confirming its taxonomic assignment.

Fig. 29 – *Pleurocordyceps ophiocordycipiticola* (MFLU 22-0265, a–g). Infected *Ophiocordyceps* sp. b Synnemata growing on fertile part of the host. c Synnemata enclosed by conidial mass. d a-phialides. e β-phialides. f *α*-conidia. g β-conidia. *Perennicordyceps elaphomyceticola* (HKAS

102450, h–q). h Stroma emerging from *Elaphomyces* sp. i Perithecia on stromata. j, k Vertical section of perithecia. l Peridium. m, n Asci. o Asci cap. p Part of ascospore. q Secondary spore. Scale bars: a = 5000μm, b = 1000 μm, c = 500 μm, j, k = 200 μm, m, n = 50 μm, d, e = 20 μm, f, g, o–q = 5 μm. (d, e stained with Congo red solution)

Discussion

Problematic genera/species in entomopathogenic families

In this study, the phylogeny of Hypocreales with a focus on entomopathogenic families was inferred using increased taxon-sampling and six gene regions (LSU, SSU, 5.8S, *tef*1, *rpb*1 and *rpb*2). The analyses revealed some problematic species in the following genera.

(1) *Metarhizium* Sorokīn, Veg. Parasitenk. Mensch Tieren 2: 268 (1879)

Metarhizium is a speciose genus in Clavicipitaceae with 101 species being listed in Index Fungorum (2022). This genus was resolved as a monophyletic group by Mongkolsamrit et al. (2020a) wherein the authors classified its asexual morphs into four categories: metarhizium-like, nomuraea-like, paecilomyces-like and unknown. Our specimen (HKAS 102454) produces nomuraea-like asexual morphs from natural substrates. In the phylogeny, it groups with *Metarhizium rileyi* (CBS 806.71), forming a clade sister to *Nigelia* (Fig. 1). *Nigelia* was introduced by Luangsa-ard et al. (2017) to accommodate *Nigelia aurantiaca* and *N. martiale*. In the phylogenetic tree of Luangsa-ard et al. (2017) and Mongkolsamrit et al. (2020a), *Nigelia* grouped separately and distantly from the core clade of *Metarhizium* within which the strain *M. rileyi* (CBS 806.71) was nested. The topology in Luangsa-ard et al. (2017) significantly differs from the one in our study. The asexual structure of *Nigelia* differs from that of *Metarhizium rileyi* in producing phialides consisting of a cylindrical to globose basal portion tapering into a long neck or may proliferate and form 2–3 lateral necks. However, *M. rileyi* has ellipsoidal phialides born on subglobose to ellipsoidal metulae, but no necks. Based on the difference of the asexual morph, *M. rileyi* is not congeneric with *Nigelia*. *Metarhizium rileyi* was recognized by Samson (1974) as *Nomuraea* due to producing nomuraea-like conidiophores, while this species was transferred to *Metarhizium* by Mongkolsamrit et al. (2020a) based on phylogenetic analyses. Collective consideration of the phylogenetic result in this study and the treatment of Samson (1974), we suggest resurrecting the genus name *Nomuraea* to accommodate *N. rileyi*.

(2) *Ophiocordyceps* Petch, Trans. Br. mycol. Soc. 16(1): 73 (1931)

Ophiocordyceps is the type genus of Ophiocordycipitaceae, containing highly diverse species, which infect various arthropod hosts (Sung et al. 2007b). The asexual morphs associated with *Ophiocordyceps* are *Hirsutella*, *Hymenostilbe*, *Paraisaria*, and *Synglіocladium* (Sung et al. 2007b). These asexual generic names were subsequently synonymized under *Ophiocordyceps* by Quandt et al. (2014) based on multigene phylogenies. Mongkolsamrit et al. (2019) resurrected *Paraisaria* to accommodate eight species that clustered as a monophyletic clade within *Ophiocordycpes*. *Paraisaria* is defined by fleshy, stipitate stromata, terminal fertile head, flask-shaped to ovoid, immersed perithecia, cylindrical asci with thickened apex, filiform ascospore fragmenting into cylindrical secondary ascospores at maturity and paraisaria-like asexual morphs (Mongkolsamrit et al. 2019). Recognition of *Paraisaria* has resulted in a polyphyletic *Ophiocordyceps* (Mongkolsamrit et al. 2019). In this study, *Paraisaria* was also monophyletic and sister to a clade formed by *O. nigrella*, *O. ravenelii*, *O. barnesii*, *O. clavata*, and *O. krachonicola* (Fig. 1). The morphological characteristics of these *Ophiocordyceps* species do not conform to the concept of *Paraisaria*, thus it is unreasonable to assign them to this genus. In the case of accepting *Paraisaria* as a valid genus, corresponding new genera need to be established to accommodate the *Ophiocordyceps* species that branch off *Paraisaria*. Alternatively, *Paraisaria* could be synonymized under *Ophiocordyceps.*

(3) *Lecanicillium* W. Gams & Zare, Nova Hedwigia 72(3-4): 332 (2001)

Lecanicillium was typified with *L. lecanii* by Zare and Gams (2001). Kepler et al. (2017) transferred *L. lecanii* to *Akanthomyces* based on phylogenetic analysis, while the authors rejected *Lecanicillium* to protect *Akanthomyces*. Even though the polyphyly of *Lecanicillium* was revealed in Kepler's study, subsequent authors still introduced new species into this genus, such as *L. cauligalbarum* (Zhou et al. 2018), *L. gracile* (Ponizovskaya et al. 2020), *L. huhutii* (Zhou et al. 2022), *L. magnisporum* (Zhang et al. 2021) and *L. praecognitum* (Ferrer et al. 2020). This current study also revealed that *Lecanicillium* species cluster into multiple distinct clades within *Cordycipitaceae* (Fig. 1) and this result is in agreement with the one in Zhou et al. (2022). The taxonomic positions of some *Lecanicillium* species are uncertain yet thus it is necessary to integrate them into the existing genera or to propose new genera to accommodate them.

Significant aspects of host affiliation, macro- and micro characters

Insecta is the most speciose class in Arthropoda, with 1,013,825 extant species representing 29 orders (Stork 2018). Hypocrealean entomopathogenic fungi have a broad range of hosts in Insecta, including Araneae, Blattodea, Coleoptera, Dermaptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Mantodea, Neuroptera, Odonata, Orthoptera, Phasmatodea and Thysanoptera (Shrestha et al. 2016). Lists of fungal pathogens on Coleoptera, Lepidoptera (Shrestha et al. 2016), Hymenoptera, Hemiptera (Shrestha et al. 2017), Orthoptera (Zha et al. 2020) and Araneae (Shrestha et al. 2019) have been compiled. These entomopathogenic species attack different growth stages of insects, such as larvae, pupae, nymphs and adults. They are obligate parasites that have adapted to become either specialists infecting specific insect species or generalists capable of infecting many host species (Goettel et al. 2005). The associated host can be taxonomically informative for the former. For instance, entomopathogenic species in the *Ophiocordyceps unilateralis* core clade are specific to *Camponotus* ants, thus species in this clade could be diagnosed based on the ant host (Wei et al. 2020). In practice, many entomopathogenic species are generalists. *Beauveria* and *Metarhizium* are two such taxa that have been found parasitizing a wide range of hosts hence it is not suitable to identify these fungi according to the associated hosts.

The fungal stroma is made of compacted vegetative hyphae, which has structural or connective roles (Bandyopadhyay et al. 1990). The specific function of stromal connective tissue is to secure perithecia (Rogers 1979). Two major stromal types including sessile and stalked are recognized based on the presence or absence of stipe, a stem or stalk-like feature that supports the fertile part (Miller 1949, Ekanayaka et al. 2017). The benefit of having a stipe is generally considered to be in mediating spore dispersal. An elevated stroma will release its spores more easily into wind currents or onto passing animals (Webster & Weber 2007). Nevertheless, many entomopathogenic taxa do not have stipes, including *Aschersonia*, *Conoideocrella*, *Moelleriella*, *Orbiocrella* and *Samuelsia* (Chaverri et al. 2008, Johnson et al. 2009, Mongkolsamrit et al. 2016). These genera are lumped in Clavicipitaceae and preferentially infect epiphyllous and sap-sucking insects, such as scale insects and white flies (Chaverri et al. 2008). These same genera usually form sessile, pulvinate to tuberculate stromata (see Figs 5, 8, 9, 10). The subicular stroma is also sessile and composed of loosely intricate hyphae, appearing as a flat mycelial cushion on which perithecia sit. The subiculum is the typical stromal form in *Akanthomyces*, *Epichloë* and *Gibellula*. *Akanthomyces* usually infect moths and spiders on the underside of living plant leaves. *Epichloë* species commonly form a layer of subiculum on leaves of Poaceae (see Fig. 6). *Gibellula* species are known to infect spider hosts and in most cases the cadavers are attached to plant leaves (see Fig. 19). Considering the above one could speculate that reduced stipe, to some extent, is beneficial for the attachment of the sessile stroma on leaves as it increases the contact area. The texture of stroma varies from fleshy, brittle to fibrous in hypocrealean entomopathogenic taxa. This is another important character used for species circumscriptions at the family level. Most species in Cordycipitaceae possess brightly coloured, fleshy stromata, while those in Ophiocordycipitaceae have darkly pigmented, tough to pliant stromata (Sung et al. 2007b).

The fertile part refers to a specific region of the stroma from which gregarious perithecia emerge (Sung et al. 2007b, Luangsa-Ard et al. 2008). Four forms of fertile parts have been described according to their position on the stroma: lateral, intercalary, subterminal and terminal (Shimizu 1997, Liang 2007, in Chinese). The latter resembles a swollen head and is found on the tip of the stroma (see Fig. 24, 25, 27). In subterminal fertile parts, the perithecia-producing region starts below the apex of the stroma (see Fig. 18). In the lateral type, the perithecia are restricted in cushion-like ascomata and can be attached to any part of the stroma (see Fig. 22). In the intercalary type the perithecia are found along the length of the stroma (see 15, 23, 29). The type of fertile part is a vital character in identifying entomopathogenic taxa to species level. Most members of Ophiocordycipitaceae and Polycephalomycetaceae have intercalary, lateral and terminal fertile parts, while those in Clavicipitaceae and Cordycipitaceae typically possess subterminal fertile parts.

Perithecia of entomopathogenic taxa in Hypocreales are often obpyriform to ovoid with an ostiole through which the asci and ascospores are released (Sung et al. 2007b, Chaverri et al. 2008, Khonsanit et al. 2019). Perithecia can be divided into two major categories based on their arrangement on mature stromata: superficial and immersed. The immersed perithecia can be further divided into vertically and obliquely immersed forms (Liang 2007, in Chinese). Pseudo-immersed perithecia are superficial structures that falsely appear as immersed either because they cling tightly together or are embedded inside the immature stroma surrounded by loosely interlaced hyphae. When the stroma matures, the interlaced hyphae disappear and the perithecia are exposed (Liang 2007, in Chinese). Perithecia are regarded as semi-immersed when approximately half protrude from the stromal surface (Liang 2007, in Chinese). The arrangement of perithecia together with ascospore morphology were considered as critical diagnostic characters in subgenus level classification even before the molecular era. For example, species of *Cordyceps* subg. *cordyceps* were recognized by presence of superficial/immersed perithecia and disarticulating ascospores. *Cordyceps* subg. *neocordyceps* is characterized by immersed perithecia and ascospores fragmenting into secondary ascospores upon maturity. Nonetheless, perithecial arrangement is not a phylogenetically informative character (Sung et al. 2007) and this is also shown in our analysis.

Ascospores are generally defined by their color, shape, septation, disarticulation and ornamentation. Most hypocrealean entomopathogens produce hyaline smooth-walled ascospores, thus the ascospore colour and ornamentation, to some extent, have limited taxonomic value (Araújo et al. 2018, Wang et al. 2020). Traditional classification of *Cordyceps sensu lato* has emphasized the importance of ascospore disarticulation. For example, Kobayasi (1941, 1982) proposed *Cordyceps* subg. *cordyceps* to accommodate species with disarticulating ascospores, while species in *Cordyceps* subg. *ophiocordyceps* are characterized by whole ascospores. The ascospore shape and septation is also useful in distinguishing different species and/or complexes. For example, species in the *Ophiocordyceps unilateralis* complex usually produce long-fusiform, few-celled, intact ascospores, while those in *O. irangiensis* complex have filiform, multiseptate ascospores that break into numerous secondary ascospores. In our analysis, the ascospores with similar shape and septation are distributed randomly in many genera in the four entomopahtogenic families, thus this character is not informative at family or genus level.

Secondary ascospores can be observed in members of all hypocrealean entomopathogenic families (Khonsanit et al. 2019, 2021, Kuephadungphan et al. 2020, Yu et al. 2021). Usually, filiform multiseptate ascospores are more likely to break into secondary ascospores at maturity, as opposed to long fusiform, few-celled ascospores, which remain intact when mature (Araújo et al. 2018, Luangsa-Ard et al. 2018). The main characters of these secondary ascospores are smooth-walled, hyaline and aseptate, but with a variety of shapes ranging from cylindrical, fusiform, barrel-shaped to cubic. The shape and size of secondary ascospore is a vital character used for species delimitation in the *O. irangiensis* complex (Khonsanit et al. 2019).

The life cycle of entomopathogenic taxa in Hypocreales often has two states, asexual (anamorph) and sexual (teleomorph) (Mora et al. 2017). The sexual state of some species may never be or is rarely produced. For example, most species in *Beauveria*, *Metarhizium* and *Simplicillium* were introduced based solely on their asexual morphs. In this case, anamorph

morphology can be useful for providing taxonomic information. The conidiophore serves as the supporting structure of conidiogenous cells and conidia. A highly diverse array of conidiophore structures has been recognized and described.

Ancestral character reconstruction

Members of Hypocreales are saprobes, endophytes, and pathogens of plants, fungi, and arthropods, while some taxa are hyperparasites of other fungi. In this study, Hypocreales diverged in the Jurassic, ca. 200 Mya. The ancestral nutritional mode of this order was unequivocally supported as being plant-based. Interkingdom host shifting to fungi and animals occurred in Clavicipitaceae, Ophiocordycipitaceae, Polycephalomycetaceae and Cordycipitaceae. The ancestral ecologies of the former three families were animal-based. In our analysis, the ancestral ecology of Cordycipitaceae was denoted as fungal-based. In previous investigations this particular result was unclear (Sung et al. 2008). The difference could be due to additional taxa having been discovered leading to broader and denser taxon sampling. Discovery and description of more taxa will further clarify and/or confirm the ancestral ecologies of all these families.

Subicular and pulvinate stromata are commonly found on parasites of scale insects and white flies and fungal symbionts that are associated with stems, leaves or culms of Poaceae. Stalked stromata with various fertile parts usually occur on parasitic fungi that infect coleopteran and lepidopteran larvae or pupae, and hemipteran nymphs that live in soil, decaying woody logs or leaf litter. In these environments, the stroma must protrude from the covering substrates to effectively release its spores. Nonetheless, not all species with stalked stroma are embedded into soil. For example, species in the *Ophiocordyceps unilateralis* complex comprise a distinctive group whose members manipulate their hosts to bite living leaves or grab the plant stem with their legs. Their conspicuous feature is the tiny fruiting body. This characteristic could benefit fungi for attaching themselves to the substrate for longer time with light weights. Hence, stromal types are generally shaped by the ecological niches of their hosts.

The perithecial arrangement and stromal texture and type are directly correlated. Fungi with subicular stromata usually also have superficial perithecia. The texture of pulvinate to tuberculate stromata is often pliant with immersed perithecia as in *Aschersonia*, *Samuelsia* and *Moelleriella* (Figs 5, 8, 9, 10). Stalked stromata with wiry texture and lateral fertile pads have been reported in the *O. unilateralis* complex whose species have immersed perithecia (see Fig. 22). Species with stalked stroma, pliant texture and terminal fertile heads form immersed perithecia, such as species of the *O. nutans* complex (see Fig. 25). Alternatively, species with stalked stroma, fibrous texture and intercalary fertile parts have superficial perithecia, such as species of *O. crinalis* (see Fig. 23) and *Perennicordyceps elaphomyceticola* (see Fig. 29).

Spore shape commonly relates to ascus apex morphology and the two determine the mechanics of discharge (Read & Beckett 1996). The asci of hypocrealean entomopathogens can be largely classified into filiform and fusiform forms. Their ascospores are usually slender, transversely multiseptate, arranged in a fasciculate manner in the ascus, with the exception in *Regiocrella* which produces a few-celled, short-fusiform (Chaverri et al. 2005). Filiform and fusiform asci have similarly-shaped ascospores as exemplified by *Orbiocrella petchii* (see Fig. 10) and *Ophiocordyceps blattae* (see Fig. 21). Based on the variety of ascospore morphology in these entomopathogenic families it seems that this character is not significant for classification at the family level. Some filiform ascospores will break into numerous tiny secondary ascospores at maturity. Cylindrical secondary ascospores are the most common in these entomopathogenic families. Generally, conidiophore morphology is genus-level specific, which is also shown in our analysis. Hence this is a useful character in generic delimitation.

Conclusion

A total of 36 species were described based on the morphological and phylogenetic evidence. Of these 35 species are known, while *Pleurocordyceps ophiocordycipiticola* is new. These species are taxonomically distributed in 16 genera (including *Akanthomyces*, *Aschersonia*, *Beauveria*,

Blackwellomyces, *Cordyceps*, *Epichloë*, *Gibellula*, *Metarhizium*, *Moelleriella*, *Nomuraea*, *Ophiocordyceps*, *Orbiocrella*, *Perennicordyceps*, *Pleurocordyceps*, *Purpureocillium*, *Samsoniella*) and four families (including Clavicipitaceae, Cordycipitaceae, Ophiocordycipitaceae and Polycephalomycetaceae). The ancestral ecology of Cordycipitaceae is inferred as fungal-based, while the rest of the entomopathogenic families were animal-based. Conidiophore morphologies of most genera in entomopathogenic families are unique and thus this character could be useful in genus level taxonomy. However, morphologies of stromata, perithecia, ascospores and secondary ascospores have limitation in classification of entomopathogenic species at the family and genus level, but are taxonomically informative in species identification.

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References

Altinok HH, Altinok MA, Koca AS. 2019 – Modes of action of entomopathogenic fungi. Current Trends in Natural Sciences 8, 117–124.

Anisimova M, Gil M, Dufayard JF, Dessimoz C et al. 2011 – Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. Systematic Biology 60, 685–699.

Araújo JPM, Evans HC, Kepler R, Hughes DP. 2018 – Zombie-ant fungi across continents: 15 new species and new combinations within *Ophiocordyceps*. I. Myrmecophilous hirsutelloid species. Studies in Mycology 90, 119–160.

Araújo JPM, Hughes DP. 2016 – Diversity of Entomopathogenic Fungi. which groups conquered the insect body? Advances in Genetics 94, 1–39.

Araújo JPM, Moriguchi MG, Uchiyama S, Kinjo N et al. 2021 – *Ophiocordyceps salganeicola*, a parasite of social cockroaches in Japan and insights into the evolution of other closely-related Blattodea-associated lineages. IMA Fungus 12, 1–17.

Bandyopadhyay R, Mughogho LK, Manohar SK, Satyanarayana MV. 1990 – Stroma development, honeydew formation, and conidial production in *Claviceps sorghi*. Phytopathology 80, 812–818.

Beimforde C, Feldberg K, Nylinder S, Rikkinen J et al. 2014 – Estimating the Phanerozoic history of the Ascomycota lineages: combining fossil and molecular data. Molecular Phylogenetics and Evolution 78, 386–398.

Bisson KR, Liu M. 2021 – Fungi Canadenses No. 350: EPICHLOË GLYCERIAE. Canadian Journal of Plant Pathology 43, 855–858.

Brown AHS, Smith G. 1957 – The genus *Paecilomyces* Bainier and its perfect stage *Byssochlamys* Westling. Transactions of the British Mycological Society 40, 17-IN3.
Charleston MA, Robertson DL. 2002 – Preferential host switching by primate lentiviruses can account for phylogenetic similarity with the primate phylogeny. Systematic biology 51, 528–535.
Chaverri P, Bischoff J, Evans H, Hodge K. 2005 – *Regiocrella*, a new entomopathogenic genus with a pycnidial anamorph and its phylogenetic placement in the Clavicipitaceae. Mycologia 97, 1225–1237.
Chaverri P, Liu M, Hodge KT. 2008 – A monograph of the entomopathogenic genera *Hypocrella*, *Moelleriella*, and *Samuelsia* gen. nov. (Ascomycota, Hypocreales, Clavicipitaceae), and their aschersonia-like anamorphs in the Neotropics. Studies in mycology 60, 1–66.
Chen WH, Liu M, Huang ZX, Yang GM et al. 2018 – *Beauveria majiangensis*, a new entomopathogenic fungus from Guizhou, China. Phytotaxa 333, 243–250.
Clewley JP 1995–Macintosh sequence analysis software. Molecular biotechnology 3, 221–224.
Crous PW, Wingfield MJ, Lombard L, Roets F et al. 2019 – Fungal Planet description sheets: 951–1041. Persoonia Molecular Phylogeny and Evolution of Fungi 43, 223–425.
Cruickshank RD, Ko K. 2003 – Geology of an amber locality in the Hukawng Valley, northern Myanmar. Journal of asian earth Sciences 21, 441–455.
Driver F, Milner RJ, Trueman JWH. 2000 – A taxonomic revision of *Metarhizium* based on a phylogenetic analysis of rDNA sequence data. Mycological research 104, 134–150.
Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. 2006 – Relaxed phylogenetics and dating with confidence. PLoS Biology 4, e88.
Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012 – Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29, 1969–1973.
Ekanayaka AH, Ariyawansa HA, Hyde KD, Jones EB et al. 2017 – Discomycetes: the apothecial representatives of the phylum Ascomycota. Fungal Diversity 87, 237–298.
Ellis JB, Everhart BM. 1892 – The North American Pyrenomycetes. Newfield.
Engler A, Prantl K. 1896 – Die Natürlichen Pflanzenfamilien, Teil 4, Abt. 3a; Wilhelm Engelmann: Leipzig, Gemany, pp. 302–303.
Friedrich RCS, Shrestha B, Salvador-Montoya CA, Tome LM et al. 2018 – *Ophiocordyceps neonutans* sp. nov., a new neotropical species from *O. nutans* complex (Ophiocordycipitaceae, Ascomycota). Phytotaxa 344, 215–227.
Goettel MS, Eilenberg J, Glare T. 2005 – Entomopathogenic Fungi and their role in regulation of insect populations. In: Ginert LI, Iatrou K, Gill S (Eds), Comprehensive Molecular Insect Science, Elsevier, Amsterdam, Netherlands 387–431.
Gueidan C, Ruibal C, De Hoog GS, Schneider H. 2011 – Rock-inhabiting fungi originated during periods of dry climate in the late Devonian and middle Triassic. Fungal Biology 115, 987–996.
Gurulingappa P, McGee PA, Sword G. 2011 – Endophytic *Lecanicillium lecanii* and *Beauveria bassiana* reduce the survival and fecundity of *Aphis gossypii* following contact with conidia and secondary metabolites. Crop Protection 30, 349–353.
Hongsanan S, Sánchez-Ramírez S, Crous PW, Ariyawansa HA et al. 2016 – The evolution of fungal epiphytes. Mycosphere 7, 1690–1712.
Huelsenbeck JP, Ronquist F. 2001 – Mabayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Hyde KD, Norphanphoun C, Maharachchikumbura SSN, Bhat DJ et al. 2020 – Refined families of Sordariomycetes. Mycosphere 11, 305–1059.
Hyde KD, Xu J, Rapior S, Jeewon R et al. 2019 – The amazing potential of fungi: 50 ways we can exploit fungi industrially. Fungal Diversity 97, 1–136.
Hywel-Jones NL. 1997 – *Torrubiella petchii*, a new species of scale insect pathogen from Thailand. Mycological Research 101, 143–145.

Hywel-Jones NL, Evans HC. 1993 – Taxonomy and ecology of *Hypocrella discoidea* and its anamorph, *Aschersonia samoensis*. Mycological Research 97, 871–876.
Imoulan A, Wu J, Lu WL, Li Y et al. 2016 – *Beauveria medogensis* sp. nov., a new fungus of the entomopathogenic genus from China. Journal of invertebrate pathology 139, 74–81.
Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat J et al. 2015 – The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. Fungal Diversity 74, 3–18.
Johnson D, Sung GH, Hywel-Jones NL, Luangsa-Ard JJ et al. 2009 – Systematics and evolution of the genus *Torrubiella* (Hypocreales, Ascomycota). Mycological Research 113, 279–289.
Kabaluk JT, Ericsson JD. 2007 – *Metarhizium anisopliae* seed treatment increases yield of field corn when applied for wireworm control. Agronomy Journal 99, 1377–1381.
Kaya HK, Vega FE. 2012 – Scope and basic principles of insect pathology. Insect pathology, pp.1-12.
Kepler RM, Humber RA, Bischoff JF, Rehner SA. 2014 – Clarification of generic and species boundaries for *Metarhizium* and related fungi through multigene phylogenetics. Mycologia 106, 811–829.
Kepler RM, Luangsa-ard JJ, Hywel-jones NL, Quandt CA et al. 2017 – A phylogenetically-based nomenclature for Cordycipitaceae (Hypocreales). IMA Fungus 8, 335–353.
Kepler RM, Sung G, Harada Y, Tanaka K et al. 2012 – Host jumping onto close relatives and across kingdoms by *Tyrannicordyceps* (Clavicipitaceae) gen. nov. and *Ustilaginoidea* (Clavicipitaceae). American Journal of Botany 99, 552–561.
Kerry BR. 2000 – Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-parasitic nematodes. Annual review of phytopathology 38, 423.
Khao-ngam S, Mongkolsamrit S, Rungjindamai N, Noisripoom W et al. 2021 – *Ophiocordyceps asiana* and *Ophiocordyceps tessaratomidarum* (Ophiocordycipitaceae, Hypocreales), two new species on stink bugs from Thailand. Mycological Progress 20, 341–353.
Khonsanit A, Luangsa-ard JJ, Thanakitpipattana D, Kobmoo N et al. 2019 – Cryptic species within *Ophiocordyceps myrmecophila* complex on formicine ants from Thailand. Mycological Progress 18, 147–161.
Khonsanit A, Noisripoom W, Mongkolsamrit S, Phosrithong N et al. 2021 – Five New Species of *Moelleriella* With Aschersonia-like Anamorphs Infecting Scale Insects (Coccidae) in Thailand. Research Square 1–29.
Kobayasi Y. 1949 – Several species of the genus *Cordyceps* and their conidial forms. Journal of Japanese Botany 24, 176–180.
Kobmoo N, Mongkolsamrit S, Tasanathai K, Thanakitpipattana D et al. 2012 – Molecular phylogenies reveal host-specific divergence of *Ophiocordyceps unilateralis* sensu lato following its host ants. Molecular Ecology 21, 3022–3031.
Komaki A, Aghajani MA, Hassan ZU. 2017 – First report of *Epichloë sylvatica* on *Brachypodium pinnatum* grass from Iran. International Journal of Agriculture Sciences 9, 4209–4210.
Koroch A, Juliani H, Bischoff J, Lewis E et al. 2004 – Examination of plant biotrophy in the scale insect parasitizing fungus *Dussiella tuberiformis*. Symbiosis 37, 267–280.
Kuephadungphan W, Macabeo APG, Luangsa-Ard JJ, Tasanathai K et al. 2019 – Studies on the biologically active secondary metabolites of the new spider parasitic fungus *Gibellula gamsii*. Mycological Progress 18, 135–146.
Kuephadungphan W, Tasanathai K, Petcharad B, Khonsanit A et al. 2020 - Phylogeny-and morphology-based recognition of new species in the spider-parasitic genus *Gibellula* (Hypocreales, Cordycipitaceae) from Thailand. MycoKeys. 72, 17.
Leuchtmann A, Bacon CW, Schardl CL, White JJF et al. 2014 – Nomenclatural realignment of *Neotyphodium* species with genus *Epichloë*. Mycologia 106, 202–215.
Liang ZQ. 1981 – Two new species of *Paecilomyces* from insect. Acta Microbiological Sinica 21, 31–34.
Liang ZQ. 2007 – *Cordyceps*. Flora Fungorum Sinicorum, vol 32. Science Press, Beijing, pp 1–190.

Litwin A, Nowak M, Różalska S. 2020 – Entomopathogenic fungi: unconventional applications. Reviews in Environmental Science and Bio/Technology 19, 23–42.
Liu M, Chaverri P, Hodge KT. 2006 – A taxonomic revision of the insect biocontrol fungus *Aschersonia aleyrodis*, its allies with white stromata and their *Hypocrella* sexual states. Mycological Research 110, 537–554.
Lloyd CG 1920 – Mycological Notes 62. Mycological Writings 6, 904–944.
Long FY, Qin LW, Xiao YP, Hyde KD et al. 2021 – Multigene phylogeny and morphology reveal a new species, *Ophiocordyceps vespulae*, from Jilin Province, China. Phytotaxa 478, 33–48.
Luangsa-ard JJ, Hywel-Jones NL, Manoch L, Samson RA. 2005 – On the relationships of *Paecilomyces* sect. *Isarioidea* species. Mycological Research 109, 581–589.
Luangsa-ard JJ, Mongkolsamrit S, Thanakitpipattana D, Khonsanit A et al. 2017 – Clavicipitaceous entomopathogens: new species in *Metarhizium* and a new genus *Nigelia*. Mycological Progress 16, 369–391.
Luangsa-ard JJ, Tasanathai K, Mongkolsamrit S, Hywel-Jones NL. 2008 – Atlas of invertebratepathogenic fungi of Thailand. National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency.
Luangsa-Ard J, Tasanathai K, Thanakitpipattana D, Khonsanit A et al. 2018 – Novel and interesting *Ophiocordyceps* spp. (Ophiocordycipitaceae, Hypocreales) with superficial perithecia from Thailand. Studies in mycology 89, 125–142.
Lucking R, Huhndorf S, Pfister DH, Plata ER et al. 2009 – Fungi evolved right on track. Mycologia 101, 810–822.
Mains EB. 1958 – North American entomogenous species of *Cordyceps*. Mycologia 50:169–222.
Mains EB. 1940 – Species of *Cordyceps*. Mycologia 32, 310–320.
Miller JH. 1949 – A revision of the classification of the Ascomycetes with special emphasis on the Pyrenomycetes. Mycologia 41, 99–127.
Minh BQ, Nguyen MAT, von Haeseler A. 2013 – Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution 30, 1188–1195.
Mongkolsamrit S, Thanakitpipattana D, Khonsanit A, Promharn R et al. 2016 – *Conoideocrella krungchingensis* sp. nov., an entomopathogenic fungus from Thailand. mycoscience 57, 264–270.
Mongkolsamrit S, Khonsanit A, Thanakitpipattana D, Tasanathai K et al. 2020a – Revisiting *Metarhizium* and the description of new species from Thailand. Studies in Mycology 95, 171–251.
Mongkolsamrit S, Noisripoom W, Tasanathai K, Khonsanit A et al. 2020b – Molecular phylogeny and morphology reveal cryptic species in *Blackwellomyces* and *Cordyceps* (Cordycipitaceae) from Thailand. Mycological Progress 19, 957–983.
Mongkolsamrit S, Noisripoom W, Arnamnart N, Lamlertthon S et al. 2019 – Resurrection of *Paraisaria* in the Ophiocordycipitaceae with three new species from Thailand. Mycological Progress 18, 1213–1230.
Mongkolsamrit S, Noisripoom W, Thanakitpipattana D, Wutikhun T et al. 2018 – Disentangling cryptic species with isaria-like morphs in Cordycipitaceae. Mycologia 110, 230–257.
Mora MAE, Castilho AMC, Fraga ME. 2017 – Classification and infection mechanism of entomopathogenic fungi. Arquivos do Instituto Biológico (Sao Paulo) 84.
Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. 2015 – IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32, 268–274.
Nicoletti R, Becchimanzi A. 2020 – Endophytism of lecanicillium and akanthomyces. Agriculture 10, 205.
Nikoh N, Fukatsu T. 2000 – Interkingdom host jumping underground: phylogenetic analysis of entomoparasitic fungi of the genus *Cordyceps*. Molecular Biology and Evolution 17, 629–638.

Ownley BH, Gwinn KD, Vega FE. 2010 – Endophytic fungal entomopathogens with activity against plant pathogens: Ecology and Evolution. BioControl 55, 113–128.
Pérez-Ortega S, Garrido-Benavent I, Grube M, Olmo R et al. 2016 – Hidden diversity of marine borderline lichens and a new order of fungi: Collemopsidiales (Dothideomyceta). Fungal Diversity 80, 285–300.
Petch T. 1921 – Studies in entomogenous fungi. 1. The nectriae parasitic on scale insects. Transactions of the British Mycological Society 7, 89–167.
Petch T. 1931 – Notes on entomogenous fungi. Transactions of the British mycological Society 16, 55–75.
Ponizovskaya VB, Grum-Grzhimaylo AA, Georgieva ML, Kokaeva LY et al. 2020 – *Lecanicillium gracile* (Cordycipitaceae), a new species isolated from mineral building materials. Phytotaxa 443, 265–278.
Prieto M, Wedin M. 2013 – Dating the diversification of the major lineages of Ascomycota (Fungi). PLoS One 8, e65576.
Quandt CA, Kepler RM, Gams W, Araújo JPM et al. 2014 – Phylogenetic-based nomenclatural proposals for Ophiocordycipitaceae (Hypocreales) with new combinations in *Tolypocladium*. IMA Fungus 5, 121–134.
Rambaut A. 2012 – FigTree v1. 4.0. A graphical viewer of phylogenetic trees. See http//tree bio ed ac uk/software figtreetree.
Read ND, Beckett A. 1996 – Ascus and ascospore morphogenesis. Mycological Research 100, 1281–1314.
Rehner SA, Minnis AM, Sung GH, Luangsa-ard JJ et al. 2011 – Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. Mycologia 103, 1055–1073.
Rogers JD 1979 – The Xylariaceae: systematic, biological and evolutionary aspects. Mycologia 71, 1–42.
Samarakoon MC, Hyde K, Promputtha I, Hongsanan S et al. 2016 – Evolution of Xylariomycetidae (Ascomycota: Sordariomycetes). Mycosphere 7, 1746–1761.
Samson RA. 1974 – Paecilomyces and some allied Hyphomycetes. Studies in Mycology 6, 1–119
Senanayake IC, Rathnayaka AR, Marasinghe DS, Calabon MS et al. 2020 – Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. Mycosphere 11, 2678–2754.
Shimizu D. 1997 – Illustrated vegetable wasps and plant worms in colour. Ie-No-Hikari Association, Tokyo
Shrestha B, Kubátová A, Tanaka E, Oh J et al. 2019 – Spider-pathogenic fungi within Hypocreales (Ascomycota): their current nomenclature, diversity, and distribution. Mycological Progress 18, 983–1003.
Shrestha B, Tanaka E, Hyun MW, Han JG et al. 2017 – Mycosphere Essay 19, *Cordyceps* species parasitizing hymenopteran and hemipteran insects. Mycosphere 8, 1424–1442.
Shrestha B, Tanaka E, Hyun MW, Han JG et al. 2016 – Coleopteran and Lepidopteran hosts of the entomopathogenic genus *Cordyceps sensu lato*. Journal of Mycology 2016.
Song H, Nan Z 2015 – Origin, divergence, and phylogeny of asexual *Epichloë endophyte* in *Elymus* species from western China. PLoS One 10, e0127096.
Spatafora JW, Sung G, Sung J, Hywel-Jones NL et al. 2007 – Phylogenetic evidence for an animal pathogen origin of ergot and the grass endophytes. Molecular Ecology 16, 1701–1711.
Stork NE. 2018 – How many species of insects and other terrestrial arthropods are there on Earth. Annual Review of Entomology 63, 31–45.
Sung GH, Poinar Jr GO, Spatafora JW. 2008 – The oldest fossil evidence of animal parasitism by fungi supports a Cretaceous diversification of fungal–arthropod symbioses. Molecular Phylogenetics and Evolution 49, 495–502.
Sung GH, Sung JM, Hywel-Jones NL, Spatafora JW. 2007a – A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): Identification of localized incongruence using a combinational bootstrap approach. Molecular Phylogenetics and Evolution 44, 1204–1223.

Sung GH, Hywel-Jones NL, Sung JM, Luangsa-ard JJ et al. 2007b – Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Studies in Mycology 57, 5–59.
Thines M. 2019 – An evolutionary framework for host shifts–jumping ships for survival. New Phytologist 224, 605–617.
Vasilyevskii NI. 1929 – Rozovaya muskardina i ee vozbuditeli-*Spicaria* aphodii Vuill. i *S. fumoso-rosea* (Wize). Summary: Die Rose Muscardine und ihre Erreger *Spicaria aphodii* Vuill. und *Spicaria fumoso-rosea* (Wize). Bolezni Rastenij 18, 113–148.
Vega FE, Goettel MS, Blackwell M, Chandler D et al. 2009 – Fungal entomopathogens: new insights on their ecology. Fungal Ecology 2, 149–159.
Wang L, Li HH, Chen YQ, Zhang WM et al. 2014 – *Polycephalomyces lianzhouensis* sp. nov., a new species, co-occurs with *Ophiocordyceps crinalis*. Mycological Progress 13, 1089–1096.
Wang YB, Wang Y, Fan Q, Duan DE et al. 2020 – Multigene phylogeny of the family Cordycipitaceae (Hypocreales): new taxa and the new systematic position of the Chinese cordycipitoid fungus *Paecilomyces hepiali*. Fungal Diversity 103, 1–46.
Wang Y, Ban S, Wang W, Li Y et al. 2021 – *Pleurocordyceps* gen. nov. for a clade of fungi previously included in *Polycephalomyces* based on molecular phylogeny and morphology. Journal of Systematics and Evolution 59, 1065–1080.
Webster J, Weber R. 2007 – Introduction to Fungi Cambridge University Press, Cambrige, U.K.
Wei DP, Wanasinghe DN, Hyde KD, Mortimer PE et al. 2020 – *Ophiocordyceps tianshanensis* sp. nov. on ants from Tianshan mountains, PR China. Phytotaxa 464, 277–292.
Wei DP, Gentekaki E, Hyde KD, Xiao YP et al. 2021a – https://invertebratefungi. org: an expert-curated web-based platform for the identification and classification of invertebrate-associated fungi and fungus-like organisms. Database, 2022.
Wei DP, Wanasinghe DN, Xu JC, To-Anun C et al. 2021b – Three novel entomopathogenic fungi from China and Thailand. Frontiers in Microbiology 11, 608991.
White Jr JF, Bultman TL 1987 – Endophyte-host associations in forage grasses. VIII. Heterothallism in *Epichloë typhina*. American Journal of Botany 74, 1716–1721.
Wijayawardene NN, Hyde KD, Al-Ani LKT, Tedersoo L, Haelewaters D et al. 2020 –Outline of Fungi and fungus-like taxa. Mycosphere 11, 1060–1456.
Wize MC. 1904 – Die durch Pilze hervorgerufenen Krankheiten des Rübenrusselka fers (*Cleonus punctiventris* Germ.) mit besonderer Berucksichtigung neuer Arten. In Bulletin International de l'Académie des Sciences de Cracovie, Classe des Sciences Mathématique et Naturelles; Polska Akademia Umiejętności: Cracovie, Poland, Bulletin Number 713727.
Xiao YP, Hongsanan S, Hyde KD, Brooks S et al. 2019 – Two new entomopathogenic species of *Ophiocordyceps* in Thailand. MycoKeys 53–74.
Xiao YP, Wang YB, Hyde KD et al 2023 – Polycephalomycetaceae, a new family of clavicipitoid fungi segregates from Ophiocordycipitaceae. Fungal Diversity (under review)
Xiao YP, Wen TC, Hongsanan S, Sun JZ et al. 2017 – Introducing *Ophiocordyceps thanathonensis*, a new species of entomogenous fungi on ants, and a reference specimen for *O. pseudolloydii*. Phytotaxa 328, 115–126.
Xiao YP, Wen TC, Hongsanan S, Jeewon R et al. 2018 – Multigene phylogenetics of *Polycephalomyces* (Ophiocordycipitaceae, Hypocreales), with two new species from Thailand. Scientific Reports 8, 1–12.
Yan K, Yanling J, Kunran Z, Hui W et al. 2011 – A new *Epichloë* species with interspecific hybrid origins from *Poa pratensis* ssp. pratensis in Liyang, China. Mycologia 103, 1341–1350.
Yang J, Stadler M, Chuang WY, Wu S et al. 2020 – In vitro inferred interactions of selected entomopathogenic fungi from Taiwan and eggs of *Meloidogyne graminicola*. Mycological Progress 19, 97–109.
Yu FM, Chethana KWT, Wei DP, Liu JW et al. 2021 – Comprehensive Review of *Tolypocladium* and Description of a Novel Lineage from Southwest China. Pathogens 10, 1389.
Zare R, Gams W. 2001 – The genera *Lecanicillium* and *Simplicillium* gen. nov. Nov Hedwigia 73, 1–50.

Zha LS, Ye L, Huang SK, Saranyaphat B et al. (2020) Species diversity and host associations of cordyceps (Hypocreales) parasitic on *Orthoptera* insects. Mycosystema 39, 707–722.

Zha LS, Wen TC, Huang SK, Boonmee S et al. 2019 – Taxonomy and biology of *Cordyceps qingchengensis* sp. nov. and its allies. Phytotaxa 416, 14–24.

Zha L, Huang SK, Xiao YP, Boonmee S et al. 2018 – An Evaluation of Common *Cordyceps* (Ascomycetes) Species Found in Chinese Markets. International Journal of Medicinal Mushrooms 20, 1149–1162.

Zhang D, Gao F, Jakovlić I, Zou H et al. 2020 – PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20, 348–355.

Zhang ZF, Zhou SY, Eurwilaichitr L, Ingsriswang S et al. 2021 – Culturable mycobiota from Karst caves in China II, with descriptions of 33 new species. Fungal Diversity 106, 29–136.

Zhou YM, Zhi JR, Mao Y, Zhi YZ et al. 2018 – *Lecanicillium cauligalbarum* sp. nov. (Cordycipitaceae, Hypocreales), a novel fungus isolated from a stemborer in the Yao Ren National Forest Mountain Park, Guizhou. Mycokeys 59–74.

Zhou YM, Zhi JR, Qu JJ, Zou X. 2022 – Estimated Divergence Times of *Lecanicillium* in the Family Cordycipitaceae Provide Insights into the Attribution of *Lecanicillium*. Frontiers in Microbiology 13, 859886.