Morpho-phylogenetic evidence reveals new species in Rhytismataceae (Rhytismatales, Leotiomycetes, Ascomycota) from Guizhou Province, China

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Abstract
Karst formations represent a unique eco-environment. Research in the microfungi inhabiting this area is limited. During an ongoing survey of ascomycetous microfungi from karst terrains in Guizhou Province, China, we discovered four new species, which are introduced here as Hypoderma paralinderae, Terriera karsti, T. meitanensis and T. sigmoideospora placed in Rhytismataceae, based on phylogenetic analyses and morphological characters. Molecular analyses, based on concatenated LSU-ITS-mtSSU sequence data, were used to infer phylogenetic affinities. Detail descriptions and comprehensive illustrations of these new taxa are provided and relationships with the allied species are discussed, based on comparative morphology and molecular data.

Keywords
four new taxa, Hypoderma, karst formations, taxonomy, Terriera
Introduction

Rhytismataceae (Rhytismatales) was established by Chevallier (1826), typified by Rhytisma with R. acerinum (Pers.) Fr. as the type species and belongs in Rhytismatales, Leotiomycetes, Ascomycota (Wijayawardene et al. 2020). Members of this family produce variously shaped apothecia that may be sessile, circular, navicular or hysteriform and that typically open by a longitudinal split or radial fissures. Asci are cylindrical, saccate to clavate. Ascospores are one-celled or multi-septate and vary from bacilliform to fusiform or filiform, with or without a sheath (Darker 1967; Ekanayaka et al. 2019). Species of Rhytismataceae occur on a wide range of hosts with a worldwide distribution (Cannon and Minter 1986; Johnston 1986; Hou and Piepenbring 2009; Hernández et al. 2014; Li et al. 2014; Tanney and Seifert 2017; Cai et al. 2020).

Darker (1967) proposed the generic delimitation for Rhytismataceae, based on ascoma and ascospore shapes, although this has been challenged in later studies (Cannon and Minter 1986; Johnston 1990, 2001; Hou et al. 2005). However, Darker (1967) and Cannon and Minter (1986) were followed due to lack of an alternative scheme. Molecular studies (Gernandt et al. 2001; Johnston and Park 2007; Lantz et al. 2011; Tian et al. 2013; Zhang et al. 2015) had revealed the phylogenetic relationships amongst members of Rhytismatales, but the available sequence data for this group remains limited and a phylogenetic classification of some members is unresolved. There are around 50 genera with 1000 species presently accepted in Rhytismataceae (Lumbsch and Huhndorf 2007; Wijayawardene et al. 2018; Index Fungorum 2020); however, a systematic genus-level taxonomic revision is needed to provide a clear, natural generic delimitation within this family and the relationship between Rhytismataceae and allied families within Rhytismatales needs to be resolved (Johnston et al. 2019).

Karst formations are generally characterised by sinking streams, caves, enclosed depressions, fluted rock outcrops and large springs (Ford and Williams 2007). Guizhou, as the eastern portion of the Yunnan-Guizhou Plateau, has the largest proportion of rocky desertification and karst landforms in China (Huang and Cai 2006). The flora in this area, comprising of 264 families with 1667 genera and 7505 vascular plants species, were inventoried from Guizhou Province (Liu et al. 2018). Therefore, it would be interesting to study the fungi in this area because of its unique ecological environment and rich plant resources. A series of studies have already been carried out and yielded several new species (Zhang et al. 2016, 2017a, b, 2018, 2019). The objectives of this study are to introduce four novel species of Rhytismataceae, based on phylogenetic and morphological evidence and elucidate their affinities with related species.

Materials and methods

Collection, examination, isolation and specimen deposition

Specimens were collected from Guizhou Province from 2016 to 2017 and examined in the laboratory with a Motic SMZ 168 stereomicroscope. Vertical sections of fruiting
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bodies were made by hand and mounted in water for microscopy. Macro-morphological characters were captured using a stereomicroscope (Nikon SMZ800N) with a Canon EOS 70D digital camera. Micro-morphological characters were observed by differential interference contrast (DIC) using a Nikon ECLIPSE 80i compound microscope and captured by a Canon EOS 600D digital camera. Measurements were processed in a Tarosoft (R) Image Frame Work version 0.9.7 programme and photographic plates were edited in Adobe Photoshop CS6 (Adobe Systems Inc., USA).

The single spore isolation technique described in Chomnunti et al. (2014) was followed to obtain the pure cultures of these specimens. Single germinated ascospore was picked up and transferred to potato dextrose agar (PDA; 39 g/l distilled water, Difco potato dextrose) for recording growth rates and culture characteristics.

The holotypes are deposited at the Herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand or Guizhou Academy of Agricultural Sciences (GZAAS), Guizhou, China. Ex-type living culture is deposited at Guizhou Culture Collection (GZCC), Guiyang, China. Index Fungorum and Facesoffungi numbers are provided according to Jayasiri et al. (2015) and Index Fungorum (2020). New species were established, based on the recommendations from Jeewon and Hyde (2016).

DNA extraction, PCR and phylogenetic analyses

Following the manufacturer’s instructions, the total genomic DNA was extracted from cultures using a Biospin Fungus Genomic DNA Extraction Kit (BioFlux, Hangzhou, P. R. China) or extracted from the fruiting bodies using an E.Z.N.A. Forensic DNA kit (Omega Bio-Tek, Doraville, Georgia, USA).

Polymerase chain reactions (PCR) were performed in 25 μl reaction volumes, which contained 9.5 μl distilled-deionised-water, 12.5 μl of 2 × Power Taq PCR Master Mix (TIANGEN Co., China), 1 μl of DNA template and 1 μl of each forward and reverse primers. Three different loci were used in this study. The internal transcribed spacer (ITS) and 28S large subunit of the nuclear ribosomal DNA (LSU) regions were amplified by using the primers ITS4/ITS5 and LR0R/LR5, respectively (White et al. 1990; Gardes and Bruns 1993). The primers mrSSU1 and mrSSU3R were used for amplification of the mitochondrial small subunit (mtSSU) partial regions (Zoller et al. 1999). The PCR thermal cycle programme was performed according to White et al. (1990), Gardes and Bruns (1993) and Zoller et al. (1999). Amplicon size and concentration were assessed by gel electrophoresis with 1.2% agarose stained with ethidium bromide. PCR products were purified and sequenced at Sangon Biotechnology Co. Ltd (Shanghai, P. R. China).

For phylogenetic reconstruction, newly-generated sequences were initially subjected to BLAST search (BLASTn) in NCBI (https://www.ncbi.nlm.nih.gov) and additional related sequences were selected and downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/), based on BLASTn results and recent publications (Tian et al. 2013; Wang et al. 2013; Zhang et al. 2015; Johnston et al. 2019; Cai et al. 2020). The sequences used in this study for phylogenetic analysis are listed in Table 1. All of these sequences were aligned and manually improved with BioEdit v. 7.2 (Hall 1999).
| Taxa                        | Specimen/Strain No. | GenBank accession numbers |
|-----------------------------|---------------------|---------------------------|
| Bifusella camelliae         | HOU 1094           | KF797447                  |
|                            | HOU 701B           | KF797448                  |
| Coccomyces anhuaenensis     | BJTC 201610        | MK371314                  |
| Coccomyces dematioides      | AFTOL ID-147       | AY544657                  |
| Colpoma lede                | Lantz 379 (UPS)    | HM140512                  |
| Colpoma quercinum           | Lantz 368 (UPS)    | HM140513                  |
| Cryptomyces magnus          | Lantz and Minter 424 (UPS) | HM140514 |
| Discocactis nivalis         | BJTC 201405        | KJ513473                  |
| Duplicaria phylloides       | Lantz 389 (UPS)    | HM140516                  |
| Hypoderma berberidis        | HOU 892            | JX232420                  |
|                            | HOU 942            | JX232421                  |
| Hypoderma campanulatum      | ICMP 17383         | HM140517                  |
| Hypoderma carinatum         | ICMP 18322         | HM140518                  |
| Hypoderma cordyloides       | ICMP 17344         | JF683421                  |
| Hypoderma hederae           | Lantz and Minter 421 (UPS) | HM140522 |
| Hypoderma liliensi          | ICMP 18323         | HM140523                  |
| Hypoderma obtectum          | ICMP 17365         | HM140524                  |
| Hypoderma paralinderae      | GZAA 19-1739       | MN638878                  |
| Hypoderma rubi              | Hanson 2006-451 (UPS) | HM140519 |
|                            | ICMP 17339         | JF683419                  |
|                            | ICMP 18325         | JF683418                  |
| Hypoderma sticheri          | Lantz 405 (UPS)    | HM140530                  |
| Hypohoton anhuaenensis      | BJTC 201311        | KF797443                  |
| Hypohoton scirpinum         | Lantz 394 (UPS)    | HM140531                  |
| Lirula macrospora           | Hou et al. 13 (BJTC) | HQ021959 |
|                            | BJTC 2012          | HQ021949                  |
| Laphodermium arundinaceum   | Lantz 323 (UPS)    | HM140535                  |
| Laphodermium culmigenum     | ICMP 18328         | HM140538                  |
| Marthamycies emarginata     | ICMP 22854         | MK599203                  |
| Meloderma dactyloides       | ICMP 17343         | HM140561                  |
| Nematoconcomycetes oberwinkleri | BJTC 201205       | KC312686                  |
| Nematoconcomycetes rhododendrii | HOU 469A         | KC312687                  |
| Rhytisma huangshanense      | HOU 564            | FJ491929                  |
| Rhytisma salsicum           | Lantz 370 (UPS)    | HM140566                  |
| Sporomega degenerans        | Lantz 367 (UPS)    | HM140567                  |
| Terriena camellisi           | AAUF 66555         | KP878552                  |
| Terriena cladophila         | Lantz & Minter 423 (UPS) | HM140568 |
| Terriena elliptica          | BJTC 201419        | KP878550                  |
| Terriena guizhouensis       | BJTC 2020149       | MT549890                  |
|                            | BJTC 2020147       | MT549891                  |
|                            | BJTC 2020148       | MT549889                  |
|                            | BJTC 2020149       | MT549872                  |
|                            | BJTC 2020150       | MT549871                  |
| Terriena houjiazhuangensis  | BJTC 2020145       | MT549889                  |
|                            | BJTC 2020146       | MT549886                  |
|                            | BJTC 2020192       | MT549869                  |
| Terriena ilicis             | BJTC 2020141       | MT549885                  |
|                            | BJTC 2020193       | MT549873                  |
|                            | BJTC 2020142       | MT549881                  |
| Terriena karstii            | MFLU 18-2288       | MN638871                  |
| Terriena metanensis         | MFLU 18-2299       | MN638874                  |
| Terriena meitanensis        | MFLU 18-2301       | MN638880                  |
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| Taxa                        | Specimen/Strain No. | LSU GenBank accession numbers | ITS GenBank accession numbers | mtSSU GenBank accession numbers |
|-----------------------------|---------------------|--------------------------------|-------------------------------|---------------------------------|
| *Terriera minor*            | ICMP 13973          | HM140570                       | –                             | HM143842                        |
| *Terriera pandanicola*      | MFLU 16-1931        | MH260320                       | MH275086                      | MW334971                        |
| **Terriera sigmoidespor**   | MFLU 18-2297        | MN63882                        | MN638877                      | MN638872                        |
| *Terriera thailandica*      | MFLUCC 14-0818      | KX765301                       | –                             | –                               |
| *Therrya abieticola*        | HOU 447A            | KP322580                       | KP322574                      | KP322587                        |
| *Tryblidiopsis pinastri*    | CBS 445,71          | MH871979                       | JF793678                      | AF431963                        |
| *Tryblidiopsis sichuanensis*| BJTC 201211        | KC312683                       | KC312676                      | KC312692                        |
| *Tryblidiopsis sinensis*    | BJTC 201212        | KC312681                       | KC312674                      | KC312694                        |

and then assembled as a dataset of LSU-ITS-mtSSU to infer the phylogenetic placement of newly identified taxa.

Phylogenetic analyses were performed using the algorithm of Maximum-Parsimony (MP) and Bayesian Inference (BI). MP analyses were run using PAUP v. 4.0b10 (Swofford 2002) with 1000 replications and inferred using the heuristic search option with 1000 random taxa. All characters were unordered and of equal weight and gaps were treated as missing data. Maxtrees was set as 1000, zero-length branches were collapsed and all equally parsimonious trees were saved. Clade stability was accessed using a bootstrap (BT) analysis with 1000 replicates, each with ten replicates of random stepwise addition of taxa (Hillis and Bull 1993).

BI analyses were carried out by using MrBayes v. 3.2 (Ronquist et al. 2012). The best-fit model (GTR+I+G for LSU, ITS and mtSSU) of evolution was estimated in MrModeltest 2.3 (Nylander 2008). Posterior Probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2. Six simultaneous Markov chains were run for 10,000,000 generations and trees were sampled every 100th generation. The temperature values were lowered to 0.15, burn-in was set to 0.25 and the run was automatically stopped as soon as the average standard deviation of split frequencies reached below 0.01.

The phylogram was visualised in TreeView (Page 1996) and edited in Adobe Illustrator CS v. 5 (Adobe Systems Inc., USA). The finalised alignment and tree were deposited in TreeBASE, submission ID: 27401 (http://www.treebase.org).

Results

Phylogenetic analyses

The dataset for phylogenetic analysis comprised 64 strains, with *Marthamyces emarginatus* (Cooke & Massee) Minter selected as the outgroup taxon. This dataset consists of 2078 characters (including the gaps), of which 1205 are constant, 236 are variable parsimony-uninformative, while 637 characters are parsimony-informative. The most parsimonious tree showed with length of 2843 steps (CI = 0.480, RI = 0.759, RC = 0.364 and HI = 0.520). The best tree revealed by the MP analysis was selected to represent relationships amongst taxa (Fig. 1). The tree generated from Bayesian in-
Figure 1. Phylogram of Rhytismataceae is presented as the best tree revealed by MP analysis, based on the concatenated LSU-ITS-mtSSU sequence dataset. MP bootstrap support values (MPBP ≥ 50%) and Bayesian inference posterior probabilities (BYPP ≥ 0.95) are shown near the nodes. The tree is rooted to *Marthamyces emarginatus* (ICMP 22854), the scale bar showing 10 changes. Type strains are indicated in bold and new sequences, generated in this study, are given in red.
ference analyses had similar topology. The phylogram (Fig. 1) shows that Hypoderma is non-monophyletic (Clade A, B, C and D), with H. paralinderae clusters with three existing species viz. H. cordylines P.R. Johnst., H. hederae (T. Nees ex Mart.) De Not. and H. rubi (Pers.) DC. In contrast, all of the Terriera species with available sequences (including the newly generated sequences) form a monophyletic clade with strong statistical support (MPBP 100% and BYPP 1.00). This corresponds to the phylogeny in Zhang et al. (2015). Terriera meitanensis and T. karsti group together with three reported species viz. T. camelllicola (Minter) Y.R. Lin & C.L. Hou, T. elliptica T.T. Zhang & C.L. Hou and T. thailandica Jayasiri & K.D. Hyde, while T. sigmoideoespora is placed within another clade that comprises T. houjiazhuangensis C.L. Hou & S.R. Cai and T. pandanicoila Tibpromma & K.D. Hyde.

**Taxonomy**

*Hypoderma* De Not., G. bot. ital. 2(2): 13 (1847)

De Candolle (1805) introduced *Hypoderma* to accommodate taxa resembling *Hyste-
rium* Pers., but with apothecia that are immersed in host-plant tissue and the hymenia
are exposed via a longitudinal split in the substratum. Subsequently, the nomenclature
of *Hypoderma* was challenged by various authors (Chevallier 1822, 1826; Fries 1823;
Wallroth 1833). De Notaris (1847) recognised the distinction between *Hypoderma*
and *Lophodermium* Chevall. and separated them, based on the ascospore shapes. So far, there are 214 epithets included in Index Fungorum (2020), but around half of
these species are synonymized under other genera, such as *Lophodermium*, *Meloderma*
Darker and *Terriera*.

*Hypoderma paralinderae* J.F. Zhang & Z.Y. Liu, sp. nov.

Index Fungorum number: IF556909
Facesoffungi Number No: FoF06797
Figure 2

**Etymology.** Referring to the morphological similarity with *Hypoderma linderae*.

**Holotype.** GZAAS 19-1769.

**Description.** Apothecia developing on dead stems, scattered, dark brown to black,
shiny, long elliptical to slightly fusiform, straight or somewhat curved, ends rounded
or obtuse, rising above the surface of the substrate, opening by a single longitudinal
split. Lips moderately developed, pale brown (Fig. 2a, b). In median vertical section
(Fig. 2c), apothecia subcuticular, 200–280 μm deep. Covering stroma (Fig. 2e) up to
38–45 μm thick near the opening, becoming to 12–18 μm thick towards the edges,
extending to the basal stroma, consisting of an outer layer of host cuticle and several layers of dark brown, thick-walled cells of *textura angularis*. **Lip cells** (Fig. 2d) clavate to cylindrical, 11–23 × 2–3 μm, thin-walled, hyaline to pale brown, 0–1-septate. **Basal stroma** (Fig. 2f) 10–16 μm thick, consisting of several layers of brown, thick-walled cells, arranged in *textura angularis*, becoming colourless, thin-walled cells of *textura*
prismatica towards the subhymenium. Subhymenium 19–27 μm thick, composed of several layers of hyaline, thin-walled cells of textura angularis. Paraphyses 1.5–2 μm, filiform, aseptate, unbranched, often curved, but not swollen at the apex, anastomosing at the base. Asci (81.5–)110–120(–129) × 10–14 μm (\(\bar{x} = 108 \times 12 \mu m, n = 25\)), 8-spored, unitunicate, cylindrical-clavate, round to subtruncate at the apex, with a 38–49 μm long stalk, thin-walled, J-, apical ring, without circumapical thickening. Ascospores 26–32.5 × 2.5–4.5 μm (\(\bar{x} = 30.5 \times 3.5 \mu m, n = 35\), measured without the gelatinous sheath), multi-seriate and mostly arranged in the upper half of ascus, fusiform to slightly cylindrical, straight or lightly curved, apex rounded and tapering slightly to an acute base, aseptate, hyaline, guttulate, surrounded by a 0.5–1.5 μm thick gelatinous sheath (extending to 2.5 μm at the poles). Asexual morph: Not observed.

Material examined. CHINA, Guizhou Province, Leishan County, dead stems of unidentified herbaceous plants, 2 November 2017, J.F. Zhang, LS-21 (GZAAS 19-1769, holotype).

Notes. Our phylogenetic analysis shows that Hypoderma paralinderae is placed in Hypoderma D clade (Fig. 1) and clustered with H. cordylines, H. hederae and H. rubi. Both H. paralinderae and H. cordylines have similar sized asci (110–122.5 × 5.5–7 μm vs. 90–140 × 11–16 μm); however, they can be distinguished by the different shape and size of ascospores (fusiform to slightly cylindrical, 26–32.5 × 2.5–4.5 μm in H. paralinderae vs. elliptic, 14–21 × 4.5–6 μm in H. cordylines) (Johnston 1990). Hypoderma paralinderae shares similar-sized asci with H. hederae; however, it is differentiated from the latter by larger ascospores (26–32.5 × 2.5–4.5 μm vs. 18–22 × 3.5–4 μm) (Powell 1974). Moreover, H. hederae was described with oblong-cylindrical ascospores that are bluntly round on both ends; however, the ascospores in H. paralinderae are fusiform to cylindrical, but rounded at the apex and tapering slightly to an acute base (Powell 1974), while H. paralinderae differs from H. rubi by having obviously larger asci (110–122.5 × 5.5–7 μm vs. 60–100 × 10–12.5 μm) and ascospores (26–32.5 × 2.5–4.5 μm vs. 14–18 × 3.5–4.5 μm) (Hou et al. 2007). Besides, the recommendations of delineation taxa from Jeewon and Hyde (2016) are followed and comparisons of the ITS gene region between H. paralinderae and H. cordylines (ICMP 17344), as well as H. paralinderae and H. rubi (ICMP 17339) are processed. The results showed that there are 9/468 bp (1.9%) and 9/467 (1.9%) bp differences (including gaps) between them, respectively. According to the above evidence, H. paralinderae is introduced herein as new to science.

Terriera B. Erikss., Symb. bot. upsal. 19(no. 4): 58 (1970)

Terriera was segregated from Lophodermium by Eriksson (1970) with T. cladophila as its type species. Johnston (2001) elucidated some distinctive morphological features (described as oblong to sublinear ascomata with single longitudinal opening slit, narrow-cylindrical asci and 1-septate ascospores that taper slightly at both ends and often becoming gently sigmoid on release and lacking a gelatinous sheath) for this genus and justified its monophyletic classification. There are 38 species accepted in Terriera (In-
dex Fungorum 2020) and around half of these species were discovered recently from China (Chen et al. 2011, 2013; Yang et al. 2011; Zheng et al. 2011; Gao et al. 2012; Song et al. 2012; Zhou et al. 2012; Li et al. 2015a, b; Lu et al. 2015; Wu et al. 2015; Cai et al. 2020). Here, we introduce three novel species. These three species share morphological characters typical of *Terriera* and cluster together with existing *Terriera* species in LSU-ITS-mtSSU phylogenetic analyses. In addition, a synopsis for *Terriera* species is also provided and listed in Table 2.

**Terriera karsti** J.F. Zhang & J.K. Liu, sp. nov.
Index Fungorum number: IF556901
Facesoffungi Number No: FoF06799
Figure 3

**Holotype.** MFLU 18-2288.

**Etymology.** Refers to the karst landscape where the holotype was collected.

**Description.** Apothecia developing on dead branch, elliptical or oblong-elliptical in outline, ends slightly acute to obtuse. Apothecia surface black, matt or slightly glossy, moderately raising the substratum surface, opening by a single longitudinal split that extends to the ends of the apothecium (Fig. 3a, b). *Lips* absent. In median vertical section (Fig. 3d), apothecia deeply embedded in host tissue, with host cells becoming filled with fungal tissue as the apothecium develops. *Covering stroma* (Fig. 3c) 30–45 μm thick, composed of blackish-brown to black, thick-walled cells of *textura angularis* towards the exterior and several layers of pale to nearly hyaline, thin-walled cells towards the interior. Along the edge of the apothecial opening, there is a flattened, 12–20 μm thick extension adjacent to the covering stroma that is composed of strongly melanised tissue with no obvious cellular structure. *Basal stroma* 8–18 μm thick, dark brown or blackish-brown, composed of angular to globose, thick-walled cells, 2.5–4 μm diam. A triangular space between the covering stroma and basal stroma consists of thin-walled, nearly hyaline to grey-brown cells arranged in *textura prismatica*. *Paraphyses* 1–2 μm, filiform, hyaline, septate, gradually swollen or branching once at the apex, embedded in gelatinous sheaths. *Asci* (103–)110–122.5 × 5.5–7 μm (x = 113 × 6 μm, n = 20), 8-spored, unitunicate, cylindrical, long stalk, thin-walled, apex truncate to somewhat round, J-, without circumapical thickening. *Ascospores* 55–66 × 1.5–2.0 μm (x = 61 × 1.8 μm, n = 25), fascicle, but not coiled, filiform, gradually tapering toward the ends, hyaline, aseptate, smooth-walled, straight or slightly curved, lacking gelatinous sheath. *Asexual morph*: Not observed.

**Culture characteristics.** Colonies on PDA reaching 51 mm after 14 days at 25 °C, irregular in shape, cottony with moderately dense, fluffy aerial mycelium. At first, white, becoming slightly greish in the centre, reverse side bronze in the centre and pale towards the edge.

**Material examined.** CHINA, Guizhou Province, Guiyang, Yunyan District, dead branch of unidentified ligneous plants, 6 May 2016, J.F. Zhang, SH-06 (MFLU 18-2288, *holotype*); *ibid.* (GZAAS 19-1720, *isotype*); ex-type living culture, GZCC 19-0047.
Figure 3. *Terriera karsti* a, b apothecia observed under the dissecting microscope c detail of covering stroma in vertical section d vertical section through an apothecium e, f asci in various states of maturity g apices of paraphyses h, i ascospores. Note: c–i mounted in water. Scale bar: 1 mm (a), 500 μm (b), 20 μm (c, e, f), 100 μm (d), 10 μm (g, i).

Notes. In the present study (Fig. 1), *Terriera karsti* is phylogenetically close to *T. camelliicola* and *T. thailandica* with moderate support (MPBP 63% and BYPP 1.00). *Terriera karsti* is not significantly distinguished from *T. camelliicola*, based only on morphological characters as they share similar-sized asci (110–122.5 × 5.5–7 μm vs. 85–120 × 5.5–6.5 μm) and ascospores (55–66 × 1.5–2 μm vs. 50–70 × 1 μm) (Johnston 2001). However, the ascospores of *T. camelliicola* are covered by a 0.5 μm wide gelatinous sheath, while this is not observed in *T. karsti* (Sharma 1982). In order
### Table 2. Synopsis of *Terriera* species. The new species described in this study are indicated in bold.

| Species          | Host                     | Appearance of apothecia                                                                 | Asci                                                                 | Ascospores                                                                 | Origin          | References     |
|------------------|--------------------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------|---------------------------------------------------------------------------|-----------------|----------------|
| *T. aequabilis*   | On dead leaves of *Photinia villosa* | Elliptical to sub-circular, straight or slightly curved to one side, ends rounded and opening by a single longitudinal slit | 75–105 × 4.5–5.5 μm                                                   | 55–78 × 0.8–1 μm, filiform, asperate, ends rounded, covered by a 0.3–0.5 μm wide gelatinous sheath | Jiangxi, China  | Li et al. 2015b |
| *T. angulatis*    | On leaves of *Eucalyptus tannifera* | Triangular to quadrangular, rarely elliptical and opening by 3–4 radial splits or a longitudinal split | 105–130 × 5.5–6.5 μm                                                   | 70–90 × 1–1.2 μm, filiform, asperate, slightly tapering towards the round base, covered by a 0.8–1 μm wide gelatinous sheath | Hubei, China    | Zhou et al. 2013 |
| *T. arundinacea*  | On decomposed leaves of *Rhamnus* sp. | Oblong to sublinear and opening by a single longitudinal slit                          | 130–160 × 8–9 μm                                                       | 90–100 × 2–2.5 μm, slightly tapering towards the base, lacking gelatinous sheath | Java, Indonesia | Johnston 2001  |
| *T. auricula*     | On dead leaves of *Asperula* sp. | Elliptical to oblong, ends rounded, opening by a single longitudinal split             | 75–105 × 8–10.5 μm                                                    | 45–70 × 2–2.5 μm, slightly tapering towards both ends and slightly constricted near the centre, asperate or 1-septate, gently curved, lacking gelatinous sheath | Northland, New Zealand | Johnston 2001  |
| *T. breve*        | On dead leaves of *Carex, Unicinia* and *Galatia* sp. | Oblong-elliptical, ends rounded, often sublinear, with a single longitudinal opening slit | 110–135(–160) × 6–7 μm                                                 | (55–)60–75 × 1.5–2 μm, slightly tapering towards both ends, asperate or 1-septate, gently curved or sigmoid, lacking gelatinous sheath | Campbell I, New Zealand | Johnston 2001  |
| *T. camelliae*    | On fallen leaves of *Camellia* sp. | Subcircular to irregular bleached spots, elliptical or occasionally 3-lobed and opening by a longitudinal split | 85–120 × 5.5–6.5 μm                                                   | 52–80 × 1–1.2 μm, filiform, asperate, covered by a 0.5 μm wide gelatinous sheath | Fuzhou, China   | Chen et al. 2011 |
| *T. camellicola*  | On twigs of *Camellia* sp. | Elliptical, occasionally fusing to form elongated elliptical, opening by a single longitudinal split | 80–110 × 5–7 μm                                                        | 50–70 × 1 μm, filiform, asperate, covered by a 0.5 μm wide gelatinous sheath | Assam, India    | Minter and Sharma 1982 |
| *T. cladophila*   | On dead twigs of *Vaccinium myrtillus* | Elliptical, rounded at the ends, with a longitudinal opening slit                      | 75–100 × 5.5–8 μm                                                      | 60–70 × 1 μm, filiform, asperate, lacking gelatinous sheath                | Norway          | Terrier 1942; Eriksson 1970 |
| *T. clathris*     | On dead leaves of unidentified monocotyledon | Cylindrical to linear, with longitudinal opening slit                                   | 110–120 × 6.5–7.0 μm                                                  | 60–80 × 1–1.5 μm, slightly tapering towards both ends, lacking gelatinous sheath | Rio Grande Do Sul, Brazil | Johnston 2001  |
| *T. eucalypti*    | On leaves of *Litsea cuprocyparissia* | Elliptical, sometimes branching into lobed or polygonal shapes, opening by a longitudinal split or by more than 3 lobes | 90–130 × 6.0–7.0 μm                                                   | 60–110 × 1.5–1.8 μm, filiform, asperate, covered by a 1.0–1.5 μm wide gelatinous sheath | Hainan, China   | Zheng et al. 2012 |
| *T. euchlora*     | On dead leaves or stems of *Dacarca* sp. | Oblong to oblong-elliptical, ends rounded, opening by a single longitudinal split       | 130–140 (–160) × 6–7 μm                                               | 100 × 2 μm, 1-septate, lacking gelatinous sheath                          | California, USA | Johnston 2001  |
| *T. elliptica*    | On living twigs of *Rhododendron* sp. | Elliptical, ends rounded to subacute, opening by a single longitudinal split          | 135–175 × 7–9 μm                                                      | 60–85 × 1.5–2 μm, filiform, slightly tapering towards both ends, asperate, covered by a 1–1.5 μm wide gelatinous sheath | Yunnan, China   | Zhang et al. 2015 |
| *T. fici*         | On dead leaves of *Ficus varoniana* | Rounded or subrounded, with conspicuous edge and opening by a single longitudinal split | 90–115 × 4–5.5 μm                                                      | 65–80 × 0.8–1 μm, filiform, asperate, rounded to oblate at the apex, slightly tapering towards the rounded or subacute base, covered by a 0.5 μm wide gelatinous sheath | Hainan, China   | Wu et al. 2016   |
| *T. fuegiana*     | On dead leaves of *Rostkovia grandiflora* | Oblong elliptical to broad-elliptical, ends rounded, opening by a single longitudinal slit | 75–95 × 7–10 μm                                                        | 60–65 × 1.5–2.5 μm, slightly tapering towards both ends, 1-septate, lacking gelatinous sheath | Tierra del Fuego, Argentina | Johnston 2001  |
| Species          | Host                                      | Appearance of apothecia                                      | Asci                                   | Ascospores                              | Origin         | References         |
|------------------|-------------------------------------------|-------------------------------------------------------------|----------------------------------------|-----------------------------------------|----------------|-------------------|
| T. fourcroyae    | On dead leaves of Furcraea sp.            | Oblong-elliptical, ends rounded, with a single longitudinal opening slit | 95–110 × 5–6.5 μm                     | 60–70 × 1.5–2.5 μm, slightly tapering towards both ends, gently coiled or sigmoid, 1-septate, lacking gelatinous sheath | Sri Lanka     | Johnston 2001     |
| T. guizhouensis  | On dead leaves of Eriobotrya japonica     | Elliptical, occasionally curved, opening by a longitudinal split | 88–107 × 4–6 μm                       | 50–80 × 1–1.2 μm, filiform, slightly tapering towards both ends, aseptate, pluriguttulate, covered by a thin gelatinous sheath | Guizhou, China | Cai et al. 2020   |
| T. houjiashanensis | On dead leaves of Ilex cornuta            | Elliptical, often curved, occasionally confluent, opening by a longitudinal split | 103–128 × 4–6 μm                      | 73–82 × 0.6–0.9 μm, filiform, slightly tapering towards both ends, aseptate, pluriguttulate, covered by an inconspicuous gelatinous sheath | Anhui, China  | Cai et al. 2020   |
| T. huangshanensis | On leaves of Eurya muricata var. huiana | Elliptical, fusiform or subelliptical, straight or curved (lunate), sometimes 3-lobed or triangular, ends rounded to subacute, opening by a single longitudinal slit | 100–120 × 5–7 μm                      | 58–90 × 1.5–2 μm, filiform, slightly tapering towards the base, aseptate, covered by a 1–1.5 μm thick gelatinous sheath | Anhui, China  | Yang et al. 2011  |
| T. ilicis         | On dead leaves of Ilex pernyi             | Elliptical, occasionally curved, triangular or confluent, opening by a longitudinal split | 117–139 × 4–7 μm                      | 52–84 × ca. 1 μm, filiform, slightly tapering towards both ends, aseptate, pluriguttulate, covered by a thin gelatinous sheath | Hubei, China  | Cai et al. 2020   |
| T. illícola       | On dead leaves of Lithocarpus cleistocarpus | Subcircular to broad-elliptical, opening by a longitudinal split | 90–135 × 4.0–5.0 μm                   | 65–95 × 1 μm, filiform, aseptate, covered by an inconspicuous gelatinous sheath | Anhui, China  | Zheng et al. 2011 |
| T. intropérdalí | On fallen leaves of Phytinia prunifolia   | Widely elliptical, sometimes elliptical or subcircular, occasionally triangular, straight or curved to one side slightly, ends round to obtuse, opening by a single longitudinal slit or by three radial splits | 90–135 × 5.5–7.5 μm                   | 70–105 × 1–1.5 μm, with upper end rounded to obtuse, slightly tapering towards the rounded base, covered by a 0.5 μm wide gelatinous sheath | Hunan, China  | Lu et al. 2015    |
| T. javanica       | On dead leaves of Elestaria sp.           | Oblong-elliptical to sublinear, ends acute, opening by a single longitudinal slit | 85–95 × 5.5–7 μm                      | 50–60 × 1.5 μm, but the detailed morphological characters were not seen | Java, Indonesia | Johnston 2001     |
| T. karsti         | On dead branch of unidentified host       | Elliptical or oblong-elliptical, ends slightly acute to obtuse, with a single longitudinal opening split | (103–)110–122.5 × 5.5–7 μm            | 55–66 × 1.5–2.0 μm, filiform, gradually tapering towards both ends, aseptate, lacking gelatinous sheath | Guizhou, China | In this study     |
| T. latiascus      | On dead leaves of Euterpe and Heliconia spp | Oblong-elliptical, with a single longitudinal opening slit | 80–95 × 7–8.5 μm                      | 40–50 × 2–2.5 μm, with 1(–3)-septate, slightly tapering to both ends | Amazonas, Brazil | Johnston 2001     |
| T. longiuina      | On dead leaves of Bambusaceae spp.        | Oblong to sublinear, ends rounded, opening by a single longitudinal slit | 175–210 × 6–6.5 μm                    | Approximately 120–130 μm long, but the detailed morphological characters were not seen | Potato-Siparuni region VII, Guyana | Johnston 2001     |
| T. maringifera    | On dead leaves of Aucuba japonica and Mangifera indica | Ellipsoidal, with a longitudinal opening split | 80–90 × 5–6 μm                         | 70–80 × 1 μm, filiform, lacking gelatinous sheath | Java, Indonesia | Koorders 1907; Li et al. 2014 |
| T. meitanensis    | On dead culms of unidentified host        | Elliptical to oblong-elliptical, ends slightly acute to obtuse, opening by a single longitudinal split | (98.5–)113–125.5(–131.5) × 6–7.5 μm   | 47–54.5 × 1.5–2.5 μm, filiform, gradually tapering towards both ends, aseptate, lacking gelatinous sheath | Guizhou, China | In this study     |
| Species     | Host                                      | Appearance of apothecia                                                                 | Asci                                   | Ascospores                              | Origin                  | References            |
|------------|-------------------------------------------|----------------------------------------------------------------------------------------|----------------------------------------|-----------------------------------------|-------------------------|-----------------------|
| *T. nematoidea* | On dead leaves of *Gahnia* sp.           | Elliptical to sublinear, with a single longitudinal opening slit                        | 70–80 × 5–6.5 μm                      | 30–35 × 1 μm, slightly tapering towards both ends, gently curved or sigmoid, 1-septate, lacking gelatinous sheath | Northland, New Zealand | Johnston 2001         |
| *T. nitens*   | On leaves of *Cyclobalanopsis myrsinifolia* | Suborbicular or broadly elliptical, straight or slightly curved, opening by a single longitudinal split | 95–150 × 1–1.2 μm                     | 68–115 × 0.8–1.2 μm, filiform, asceptate, round at the apex, slightly tapering towards the acute base, covered by a thin gelatinous sheath | Anhui, China           | Chen et al. 2013       |
| *T. pandani*  | On dead leaves of *Pandanus* sp.          | Oblong to oblong-elliptical, ends rounded, opening by a single longitudinal slit        | 100–120 × 5–6 μm                      | 50–70 × 1–1.5 μm, lacking gelatinous sheath | San Juan, Puerto Rico   | Johnston 2001         |
| *T. pandanicola* | On dead leaves of *Pandanus* sp.        | Elliptical, with rounded to subacute ends, opening by a longitudinal split              | 50–66 × 4–5 μm                        | 55–78 × 1–2 μm, filiform, slightly tapering towards both ends, asceptate, lacking gelatinous sheath | Prachuap Khiri Khan, Thailand | Tibpromma et al. 2018 |
| *T. petrakii*  | On fallen leaves of *Smilax* bracteata    | Elongate-elliptical, strongly curved or triangular, often coalesced, opening by a longitudinal split | 85–110 × 4–5 μm                      | (60–)70–85 × 0.8 μm, filiform, asceptate, covered by a thin gelatinous sheath | Yunnan, China          | Song et al. 2012       |
| *T. rotundata* | On fallen leaves of *Quercus* sp.        | Elliptical, occasionally triangular, ends rounded, opening by a longitudinal split or occasionally by teeth | 90–120 × 4–5.5 μm                     | 70–90(–95) × 0.8–1 μm, filiform, asceptate, lacking gelatinous sheath | Yunnan, China          | Song et al. 2012       |
| *T. sacchari*  | On dead leaves and leaf bases of *Saccharum officinarum* | Narrow-oblong to sublinear, with a single longitudinal opening split | 90–100 × 5–7 μm                      | 50–60 × 1.5 μm, lacking gelatinous sheath | Hawaii, USA             | Johnston 2001         |
| *T. samuelii* | On dead leaves of unidentified monocotyledon | Oblong to sublinear, ends rounded, opening by a single longitudinal slit               | 125–140 × 7–8 μm                      | (65–)75–90 × 2 μm, slightly tapering towards both ends, 1-septate, lacking gelatinous sheath | Amazonas, Brazil        | Johnston 2001; 2003    |
| *T. sigmoideospora* | On dead fallen leaves of unidentified host | Elliptical, ends rounded to subacute, opening by a single longitudinal split           | (93.5–)102–121 × 5–6 μm               | 79–95 × 5–2 μm, filiform, slightly tapering towards both ends, asceptate, lacking gelatinous sheath | Guizhou, China         | In this study          |
| *T. simplex*  | On fallen leaves of *Trachelospermum jasminoides* | Elliptical to ovate, ends obtruse, rounded or slightly acute, opening by a single longitudinal split which is sometimes branched in the triangular ascomata | 72–95(–105) × 4.8–5.2 μm              | (45–)56–82 × 1–1.2 μm, filiform, slightly tapering towards the rounded base, covered by a 0.8–1 μm wide gelatinous sheath | Anhui, China           | Gao et al. 2012        |
| *T. stevensii* | On dead leaves of *Vincentia* sp.        | Oblong, ends rounded, opening by a single longitudinal slit                            | 100–125 × 5–6 μm                      | 60–80 × 1.5–2 μm, lacking gelatinous sheath | Hawaii, USA             | Johnston 2001         |
| *T. thailandica* | On dead branch of unidentified host     | Elliptical, ends rounded to subacute, opening by a longitudinal split                  | 80–105 × 3.4–6.6 μm                   | 38–60 × 1–1.5 μm, filiform, slightly tapering towards both ends, asceptate, lacking gelatinous sheath | Chiang Rai, Thailand    | Hyde et al. 2016      |
| *T. transversa* | On dead leaves of *Pandanus* sp.         | Elliptical or oblong-elliptical, ends slightly acute to obtruse, opening by a single longitudinal split | 70–86 × 5–6 μm                        | 45–68 × 1–1.2 μm, filiform, slightly tapering towards both ends, asceptate, covered by a 0.5 μm wide gelatinous sheath | Hainan, China          | Li et al. 2015a        |
to clarify their affinity, the recommendations of species delineation from Jeewon and Hyde (2016) were followed and the comparison of each gene region between these two taxa is processed and showed that there are 9/840 bp (1%) and 10/694 bp (14.4%) differences in LSU and mtSSU regions, respectively, while *T. karsti* can be easily differentiated from *T. thailandica* by its larger asci (110–122.5 × 5.5–7 μm vs. 80–105 × 3.4–6.6 μm) and ascospores (55–66 × 1.5–2 μm vs. 38–60 × 1–1.5 μm) (Hyde et al. 2016). A comparison of the LSU gene region between these two taxa has also been processed and the result showed that there are 3/838 bp (base pair) differences. Based on phylogenetic analyses, coupled with morphological distinction, *Terriera karsti* is introduced herein as a new species.

*Terriera meitanensis* J.F. Zhang & Z.Y. Liu, sp. nov.
Index Fungorum number: IF556900
Facesoffungi Number No: FoF06798

Figure 4

Holotype. MFLU 18-2299.

**Etymology.** Referring to the locality of the holotype, Meitan County, Guizhou Province, China.

**Description.** Apothecia developing on dead stems (Fig. 4a), semi-immersed to superficial, elliptical or oblong-elliptical, ends slightly acute to obtuse, surface black, matt, raising the substratum surface, opening by a single longitudinal split that extends nearly the entire length (Fig. 4b, c). In median vertical section (Fig. 4d), apothecia deeply embedded in host tissue, with host cells becoming filled with fungal tissue as the apothecium develops. Covering stroma (Fig. 4e) 33–42 μm thick, composed of blackish-brown, thick-walled cells that are fused with host tissue in the outermost layers, becoming pale pigmented or nearly colourless towards the hymenium, thin-walled cells, arranged in *textura angularis* or *textura globulosa*. Along the upper edge of the apothecial opening, there is a flattened, 19–34 μm thick extension adjacent to the covering stroma that is composed of strongly melanised tissue with no obvious cellular structure. Basal stroma (Fig. 4g) 8–18 μm thick, dark-brown or blackish-brown, composed of angular to globose, thick-walled cells, 2.5–4 μm diam. Where the covering stroma meets the basal stroma, there is a triangular-shaped, 35–60 μm thick, tissue composed of thin-walled, hyaline to pale brown cells forming a *textura prismatica* (Fig. 4f). Subhymenium 12–16 μm thick, consisting of hyaline *textura angularis* to *textura intricata*. Paraphyses 1–2 μm, filiform, hyaline, septate, gradually swollen or branching once at the apex, embedded in gelatinous matrix, anastomosing at the base. Asci (98.5–)113–125.5(–131.5) × 6–7.5 μm (̄x = 117 × 6.5 μm, n = 20), 8-spored, unitunicate, cylindrical, somewhat long-stalked, thin-walled, apex generally truncate, J-, without circumapical thickening. Ascospores 47–54.5 × 1.5–2.5 μm (̄x = 50.5 × 2 μm, n = 35), fascicle, filiform, gradually tapering towards the ends, hyaline, aseptate, smooth-walled, straight or slightly curved, lacking a gelatinous sheath. Asexual morph: Not observed.
Figure 4. *Terriera meitanensis* a habit of apothecia on substrate b, c apothecia observed under the dissecting microscope in face view d vertical section through an apothecium e covering stroma f triangular space in section between the covering stroma and basal stroma g basal stroma h paraphyses with anastomoses amongst asci in various states of maturity i, j immature asci k, l ascospores. Note: d–l mounted in water. Scale bar: 1 cm (a), 1 mm (b), 500 μm (c), 100 μm (d), 10 μm (e, g, k, l), 30 μm (f), 20 μm (h–j).

Material examined. CHINA, Guizhou Province, Zunyi, Meitan County, dead stems of unidentified host, 28 August 2017, J.F. Zhang, MT-1 (MFLU 18-2299, holotype); ibid. (GZAAS 19-1731, isotype).
**Notes.** In our phylogenetic analysis (Fig. 1), Terriera meitanensis is placed in a robust clade with *T. camelliicola*, *T. elliptica*, *T. karsti* and *T. thailandica* by strong statistical support (MPBP 100% and BYPP 1.00). *Terriera meitanensis* has larger asci than *T. camelliicola* and *T. thailandica*, while the ascospores of *T. meitanensis* are smaller (Johnston 2001; Hyde et al. 2016). Both *T. meitanensis* and *T. karsti* share similar-sized asci, but *T. karsti* has larger ascospores (47–54.5 × 1.5–2.5 μm vs. 55–66 × 1.5–2.0 μm). *Terriera meitanensis* differs from *T. elliptica* by its obviously smaller asci (113–122.5 × 6–7.5 μm vs. 135–175 × 7–9 μm) and ascospores (47–54.5 × 1.5–2.5 μm vs. 60–85 × 1.5–2 μm) (Zhang et al. 2015). Moreover, the ascospores of *T. camelliicola* and *T. elliptica* are enveloped by a gelatinous sheath, respectively, while this is not observed in *T. meitanensis*. In addition, the comparison of the ITS gene region is processed between *T. meitanensis* and its closest species *T. elliptica*, based on the recommendations from Jeewon and Hyde (2016) and the results showed that there are 15/489 bp (3%) differences. Therefore, we introduce *T. meitanensis* herein as a new species, based on morphological and molecular evidence.

**Terriera sigmoideospora** J.F. Zhang & K.D. Hyde, sp. nov.

Index Fungorum number: IF556902

Facesoffungi Number No: FoF06800

Figure 5

**Holotype.** MFLU 18-2297.

**Etymology.** Refers to its sigmoidal ascospores.

**Description.** Apothecia developing on fallen leaves, scattered, dark brown to black, matt, elliptical, sometimes 3-lobed or triangular, straight or slightly curved, ends rounded to subacute, strongly raising the surface of the substrate at maturity, opening by a single longitudinal split that extends almost the whole length of the apothecium (Fig. 5a, b). Immature apothecia appearing as a single dark brown protrusion, circular to slightly elongated. In median vertical section (Fig. 5d), apothecia 185–220 μm deep. **Covering stroma** (Fig. 5c) 20–25 μm thick near the centre of the apothecium, consisting of an outer layer of host cuticle, remains of epidermal and hypodermal cells filled with thick-walled, angular fungal cells and an inner layer of textura angularis to textura globulosa with 4–7 μm diam., dark brown, thick-walled cells, slightly thinner towards the edges, extending to the basal stroma, but conspicuously thicker towards the apothecial opening, with a 15–27 μm thick extension comprising highly melanised tissue with no obvious cellular structure. **Excipulum** moderately developed, closely adhering to the covering stroma and the extension, arising from the marginal paraphyses, becoming thinner towards the base. **Basal stroma** concave, 12–15 μm thick, composed of dark brown, thick-walled, angular cells. A triangular space between the covering stroma and basal stroma is composed of thin-walled, colourless cells that are vertically arranged in rows. **Subhymenium** 6–9 μm thick, flat, consisting of hyaline cells of textura intricata. **Paraphyses** filiform, hyaline, septate, gradually or suddenly swollen to
2.5 μm near the apex, covered by a thin gelatinous sheath, forming a 4–8 μm thick epithecium. *Asci* (93.5–)102–121 × 5–6 μm (x̄ = 108.5 × 5.5 μm, n = 20), 8-spored, unitunicate, cylindrical, apex tapering to round, thin-walled, J-, without circumapical thickening. *Ascospores* 79–95 × 1.5–2 μm (x̄ = 89.5 × 1.9 μm, n = 30), fascicle, filiform, sigmoid, tapering slightly towards the ends, hyaline, aseptate, guttulate, gelatinous sheath not observed. *Asexual morph*: Not observed.

**Material examined.** CHINA, Guizhou Province, Guiyang, dead leaves of unidentified host, 5 October 2016, J.F. Zhang, GZ-28 (MFLU 18-2297, holotype); *ibid.* (GZAAS 19-1729, isotype).

**Notes.** In the present phylogenetic analysis (Fig. 1), *Terriera sigmoideospora* is placed within *Terriera* and is related to *T. houjiazhuangensis* C.L. Hou & S.R. Hou.
by strong statistical support (MPBP 99% and BYPP 1.00). *Terriera sigmoideospora* shares similar-sized asci with *T. houjiazhuangensis* (102–121 × 5–6 μm vs. 103–128 × 4–6 μm), but has larger ascospores (79–95 × 1.5–2 μm vs. 73–82 × 0.6–0.9 μm) (Cai et al. 2020). Besides, the ascospores of *T. houjiazhuangensis* are enveloped by an inconspicuous gelatinous sheath, while this is not observed in *T. sigmoideospora*. In addition, the comparison of the ITS gene region between these two taxa has been processed and showed that there are 19/815 (2.3%) bp differences. *Terriera pandanicola* is sister to the above two taxa; however, it is significantly distinguished from *T. sigmoideospora* as its obviously smaller asci (50–66 × 4–5 μm vs. 102–121 × 5–6 μm) and ascospores (55–78 × 1–2 μm vs. 79–95 × 1.5–2 μm) (Tibpromma et al. 2018).

**Discussion**

The diversity of microfungi in many parts of the world is understudied. This is evident from the numerous new species being described from Asia and South America (Hyde et al. 2018, 2019a, 2020). With this in mind, we are studying the fungi of the Karst regions in China and Thailand, where we are also finding numerous new species (Zhang et al. 2016, 2017a, b, 2018, 2019). Our study is contributing to the knowledge of fungal diversity in the region, where species may also have biotechnological potential (Hyde et al. 2019b). Additionally, as Rhytismataceae is a relatively poorly studied group, we report on one new species from *Hypoderma* and three new *Terriera* species, thereby illustrating the diversity and potential for new discoveries of these fungi in Asia.

*Hypoderma*, a large genus in Rhytismataceae, is a complicated group. There are only a few species in this genus with sequence data, but these have shown the group to be polyphyletic (Lantieri et al. 2011; Wang et al. 2013). This is also true of the phylogenies in this study (Fig. 1). *Hypoderma* is morphologically similar to *Lophodermium* and they mainly differ on the basis of ascospore shape as the former have elliptical to cylindrical-fusiform ascospores, while the latter has filiform ascospores (Powell 1974). However, there are no molecular studies that provide a natural classification for these two genera, even though more than 35 species have been synonymized under *Lophodermium* (Index Fungorum 2020). Fresh collections and molecular sequences are required to move toward a revision of these genera.

*Terriera* is one of the few genera in Rhytismataceae that can be considered a monophyletic group, based on distinctive morphology and phylogenetic characterisation (Zhang et al. 2015). Our molecular analyses corroborate this. However, there are only nine taxa with available sequences in GenBank and most of *Terriera* species were established, based only on morphological features (Yang et al. 2011; Gao et al. 2012; Song et al. 2012; Zhou et al. 2012; Chen et al. 2013; Li et al. 2015b; Lu et al. 2015; Zhang et al. 2015; Cai et al. 2020). In the latest study (Cai et al. 2020), *T. pandanicola* was distant from *Terriera* in ITS analysis, but included in this group on the basis of concatenated LSU-mtSSU sequence data. Cai et al. (2020) indicated that this taxon should
be revised in a future study. Based on their suggestion, we checked the sequence data of *T. pandanicola* and found that the ITS sequence of this species is misidentified as it is not a related *Terriera* or even a Rhytismataceae species in BLASTn results. However, the newly generated available sequences (ITS and mtSSU) of *T. pandanicola* have been uploaded in GenBank and included in our phylogenetic analysis and the results indicated that it is a unique species in *Terriera* in the present study (Fig. 1).

**Acknowledgements**

Kevin D. Hyde thanks the Thailand Research grants entitled “The future of specialist fungi in a changing climate: baseline data for generalist and specialist fungi associated with ants, *Rhododendron* species and *Dracaena* species” (Grant No. DBG6080013) and “Impact of climate change on fungal diversity and biogeography in the Greater Mekong Subregion” (Grant No. RDG6130001). Jason M. Karakehian is thanked for revising the manuscript. Dr. Shaun Pennycook (Manaaki Whenua Landcare Research, New Zealand) is gratefully thanked for advising on the fungal names. Dr. Saowaluck Tibpromma is thanked for updating the new sequences of *T. pandanicola*. Jin-Feng Zhang would like to thank Dr. Peter R. Johnston for providing literature and suggestions.

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Supplementary material 1

Dataset for molecular analyses
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Data type: phylogenetic
Explanation note: The dataset of combined of LSU_ITS_mtSSU to build the phylogenetic tree.
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