Abstract

Background

During taxonomic and phylogenetic studies of fungi on pteridophytes in Thailand, *Monilochaetes pteridophytophila* sp. nov. was collected from the frond stalks of a tree fern (*Alsophila costularis*, Cyatheaceae). The new species is introduced, based on evidence from morphology and phylogenetic analyses of a concatenated dataset of LSU, ITS, SSU and RPB2 sequences.

New information

*Monilochaetes pteridophytophila* differs from extant species of *Monilochaetes* in having darker conidiophores with fewer septae (1–4-septate). *Monilochaetes pteridophytophila*
forms a distinct clade, basal from other species of Monilochaetes in Australiascaceae. A detailed description and illustrations of the new species are provided. We also provided a synopsis of accepted species of Monilochaetes.

**Keywords**

one new taxon, Hyphomycetes, Pteridophytes, Sordariomycetes, taxonomy

**Introduction**

Studies on the diversity of fungi on pteridophytes have revealed many new taxa during the last decade (Mehltreter 2010, Braun et al. 2013, Kirschner and Liu 2014, Guatimosim et al. 2016, Kirschner et al. 2019). An estimated 670 species of fern occur in Thailand (Lindsay and Middleton 2009), making it a suitable area for studying the fungi associated with ferns. However, the study of fungi on ferns is in its infancy (Razikin et al. 2014, Kirschner et al. 2019). Cyatheaceae, a family of scaly tree ferns in Cyatheales, is widely distributed in tropical and subtropical areas (Lehnert 2011, Korall and Pryer 2014). Species of Cyatheaceae diverged ca. 150 (146–168) million years ago during the Late Jurassic period (Korall and Pryer 2014). Many taxa in this family are threatened species, including *Cyathea brunoniana*, *C. gigantea* and *C. henryi* (Balkrishna et al. 2020, Coritico and Amoroso 2020).

*Monilochaetes* Halst. ex Harter was introduced by Harter (1916) to accommodate a pathogenic fungus, *M. infuscans* Harter, that caused scurf disease of the sweet potato. *Monilochaetes infuscans* was first reported by Halsted (1890), but the species is considered invalid due to the lack of morphological description and illustrations. Réblová et al. (2011a) established the family Australiascaceae Réblová & W. Gams to accommodate *Australiasca* Sivan. & Alcorn (as a sexual morph) and *Monilochaetes* (as an asexual morph). Sivanesan and Alcorn (2002) introduced *Australiasca* with *A. queenslandica* Sivan. & Alcorn as the type species, which was linked to *Dischloridium camelliae* Alcorn & Sivan as an asexual morph. Réblová et al. (2011a) treated *Dischloridium* B. Sutton as the generic synonym of *Monilochaetes*, based on phylogenetic analysis of ITS and LSU sequences. Following the “One Fungus One Name” (1F1N) principle, *Australiasca* was synonymised under *Monilochaetes*, the latter being older (Réblová et al. 2016, Hyde et al. 2020a). Hyde et al. (2020a) and Wijayawardene et al. (2020) accepted Australiascaceae in Glomerellales with a single genus *Monilochaetes*. Index Fungorum (2021) lists nine species in *Monilochaetes*. These are *M. basicurvata* (Matsush.) Réblová & Seifert, *M. camelliae* (Alcorn & Sivan.) Réblová, W. Gams & Seifert, *M. dimorphospora* Réblová & W. Gams, *M. guadalcanalensis* (Matsush.) I.H. Rong & W. Gams, *M. infuscans*, *M. laeënsis* (Matsush.) Réblová, W. Gams & Seifert, *M. melastomae* Crous, *M. nothapodytis* S.X. Zhou, J.C. Kang & K.D. Hyde and *M. regenerans* (Bhat & W.B. Kendr.) Réblová & Seifert. Of those, seven species have molecular data in NCBI GenBank (Sivanesan and Alcorn 2002, Réblová et al. 2011a, Réblová et al. 2011b, Zhou et al. 2017, Crous et al. 2018).
The sexual morph of *Monilochaetes* is characterised by superficial, dark brown, obpyriform perithecia with or without setae, with periphysate ostioles; hyaline, branching, septate paraphyses; 8-spored, unitunicate, cylindrical-clavate, short-pedicellate asci; and hyaline, ellipsoidal to ovoid, 0–3-septate ascospores (Sivanesan and Alcorn 2002, Réblová et al. 2011a). The asexual morph of *Monilochaetes* is characterised by solitary, erect, sometimes curved or geniculate, septate, pale brown to dark brown conidiophores; phialidic, terminal, hyaline to pale brown, ampulliform to cylindrical conidiogenous cells with a shallow collarette; and hyaline, aseptate or rarely septate, oval conidia (Harter 1916, Bhat and Kendrick 1993, Réblová et al. 2011a, Réblová et al. 2011b, Zhou et al. 2017, Crous et al. 2018).

In this study, a new species of *Monilochaetes*, *M. pteridophytophila*, is described, illustrated and compared with closely-related taxa. Morphological study and multilocus phylogenetic analyses confirm the identity of the new species and confirm its placement in *Monilochaetes*.

**Materials and methods**

**Sample collection, isolation and conservation**

Frond stalks of *Alsophila costularis* (tree fern) were collected in a disturbed forest near the roadside in Tak Province, Thailand. Specimens were packed into a plastic bag for transportation to the laboratory and the associated metadata were noted (date, locality and host). Fungal colonies on the host surface were observed and examined using a stereomicroscope (Leica EZ4, Leica Microsystems AG, Singapore). Micro-morphological characters were documented with a Nikon DS-Ri2 digital camera fitted to a Nikon ECLIPSE Ni compound microscope (Nikon, Japan). Measurements of morphological structures (conidiophores, conidiogenous cells and conidia) were made with the Tarosoft (R) Image Frame Work. Figures were processed and combined with Adobe Illustrator CS6 (Adobe Systems, USA).

Single spore isolation was carried out to obtain a pure culture, following the method described by Dai et al. (2017). Germinated conidia were aseptically transferred to potato dextrose agar (PDA) plates and incubated at 25°C. Cultures were grown for 2 weeks and culture characteristics, such as size, shape, colour and texture, were recorded. The holotype specimen and ex-type living culture are deposited in the Herbarium of Mae Fah Luang University (MFLU) and Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand, respectively. An isotype specimen is deposited at the Herbarium of Guizhou Academy of Agricultural Sciences (GZAAS), Guiyang, China.

**DNA extraction, PCR amplification and sequencing**

Fresh fungal mycelium grown on PDA at 25°C for 2 weeks was used to extract DNA. Genomic DNA was extracted by using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux, China), following the manufacturer’s instructions. We amplified the internal transcribed spacer (ITS) region, the small and large subunits of the ribosomal RNA gene
(SSU, LSU) and the second largest subunit of RNA polymerase II (RPB2). Primer pairs and PCR thermal cycle conditions are listed in Table 1. The quality of PCR products was checked on 1% agarose gel electrophoresis stained with ethidium bromide. Successful PCR products were sent to Sangon Biotech (Shanghai, China) for purification and sequencing. Forward and reverse sequence reads were assembled using SeqMan v. 7.0.0 (DNASTAR, Madison, WI). Consensus sequences were submitted to NCBI GenBank (Table 2).

**DNA sequence alignments and phylogenetic analysis**

Closely-related taxa were selected for phylogenetic analyses, based on BLASTn searches in NCBI GenBank ([https://blast.ncbi.nlm.nih.gov/Blast.cgi](https://blast.ncbi.nlm.nih.gov/Blast.cgi)), as well as recent publications (Réblová et al. 2011a, Hongsanan et al. 2017, Zhou et al. 2017, Crous et al. 2018, Dissanayake et al. 2020, Table 2). Sequences of each locus were aligned using the online multiple alignment programme MAFFT version 7 ([https://mafft.cbrc.jp/alignment/server/](https://mafft.cbrc.jp/alignment/server/), Katoh et al. 2019) and then manually adjusted in BioEdit 7.1.3.0 (Hall 1999). Phylogenetic relationships were inferred, based on a combined LSU–ITS–SSU–RPB2 dataset. Sequences of each locus were combined to form a concatenated super matrix using SequenceMatrix 1.7.8 and analysed with Maximum Likelihood (ML) and Bayesian Inference (BI) criteria.

| Locus                      | Primers (forward/reverse) | PCR amplification condition | Reference(s) |
|----------------------------|---------------------------|-----------------------------|---------------|
| Large subunit ribosomal RNA (LSU) | LR0R/LR5                 | 1. 95°C – 3 min             | Vilgalys and Hester (1990), Hopple (1994), Lu et al. (2017) |
|                            |                           | 2. 94°C – 30 sec            |               |
|                            |                           | 3. 51°C – 50 sec            |               |
|                            |                           | 4. 72°C – 1 min             |               |
|                            |                           | 5. Repeat 2–4 for 30 cycles|               |
|                            |                           | 6. 72°C – 7 min             |               |
|                            |                           | 7. 4°C on hold              |               |
| Internal transcribed spacer region of ribosomal DNA (ITS) | ITS1/ITS4                | 1. 95°C – 3 min             | White et al. (1990), Lu et al. (2017) |
|                            |                           | 2. 95°C – 30 sec            |               |
|                            |                           | 3. 51°C – 1 min             |               |
|                            |                           | 4. 72°C – 45 sec            |               |
|                            |                           | 5. Repeat 2–4 for 34 cycles|               |
|                            |                           | 6. 72°C – 10 min            |               |
|                            |                           | 7. 4°C on hold              |               |
| Small subunit ribosomal RNA (SSU) | NS1/NS4                  | 1. 94°C – 3 min             | White et al. (1990) |
| Locus | Primers (forward/reverse) | PCR amplification condition | Reference(s) |
|-------|---------------------------|----------------------------|--------------|
|       |                           | 2. 94°C – 45 sec            |              |
|       |                           | 3. 56°C – 50 sec            |              |
|       |                           | 4. 72°C – 1 min             |              |
|       |                           | 5. Repeat 2–4 for 40 cycles |              |
|       |                           | 6. 72°C – 10 min            |              |
|       |                           | 7. 4°C on hold              |              |
| RNA polymerase II second largest subunit (RPB2) | fRPB2-5f/ fRPB2-7cR | 1. 95°C – 5 min | Liu et al. (1999) |
|       |                           | 2. 95°C – 1 min             |              |
|       |                           | 3. 55°C – 2 min             |              |
|       |                           | 4. 72°C – 90 sec            |              |
|       |                           | 5. Repeat 2 – 4 for 40 cycles |              |
|       |                           | 6. 72°C – 10 min            |              |
|       |                           | 7. 4°C on hold              |              |

Table 2.
Taxa used to infer the phylogenetic tree and their GenBank accession numbers.

Notes: "-" as meaning no data available in GenBank. The newly-generated sequences are underlined. The ex-type strains are in bold.

| Taxa                     | Strain/ Voucher No. | GenBank Accession no. |
|--------------------------|---------------------|------------------------|
|                          |                     | ITS | LSU | SSU | RPB2 |
| Acrostalagmus luteoalbus | strain V205         | KJ443271 | KJ443141 | KJ443096 | KJ443184 |
| Acrostalagmus luteoalbus | strain V206         | KJ443272 | KJ443142 | KJ443097 | KJ443185 |
| Collariella bostrychodes | CBS 586.83          | KX976642 | KX976739 | -     | KX976838 |
| Colletotrichum acutatum  | BBA 67875           | AJ301926 | AJ301926 | AJ301926 | -     |
| Colletotrichum circinans | CBS 221.81          | NR_111457 | NG_069094 | NG_062845 | -     |
| Colletotrichum gloeosporioides | CBS 79672      | -     | AY705727 | -     | -     |
| Colletotrichum gloeosporioides | MCA 2498       | DQ286198 | DQ286199 | -     | -     |
| Colletotrichum sansevieriae | MFLU 19–2898  | MT177931 | MT177958 | MT177985 | MT432208 |
| Colletotrichum truncatum  | BBA 70523          | AJ301937 | AJ301937 | AJ301937 | -     |
| Corynascus fumimontanus  | CBS 137294         | MK919291 | LK932706 | -     | MK919347 |
| Cylindrotrichum clavatum | CBS 125296         | GU180627 | GU180643 | GU180622 | -     |
| Cylindrotrichum clavatum | DLUCC 0575         | MH120193 | MH120184 | -     | MH120179 |
| Cylindrotrichum gori           | DLUCC 0614         | MH120195 | MH120189 | -     | MH120183 |
| Cylindrotrichum oligospermum  | CBS 570.76         | MH861002 | MH872775 | -     | -     |
| Taxa                              | Strain/ Voucher No. | GenBank Accession no. |   |   |
|-----------------------------------|---------------------|----------------------|---|---|
| Cylindrotrichum oligospermum     | CBS 561.77          | GU291801             | - | - |
| Cylindrotrichum setosum          | DAOM 229246         | -                    | GU180652 | GU180617 | - |
| Gibellulopsis nigrescens         | CBS 120949          | NR 149327            | NG 067330 | - | LR026149 |
| Gibellulopsis nigrescens         | DAOM 226890         | GU180631             | GU180648 | GU180613 | GU180664 |
| Kylindria chinensis              | MFLUCC 16–0965      | MH120190             | MH120186 | - | MH120181 |
| Kylindria peruamazonensis        | CBS 838.91          | GU180628             | GU180638 | GU180609 | GU180656 |
| Kylindria peruamazonensis        | CBS 421.95          | GU291800             | HM237325 | - | - |
| Lectera nordwiniana              | CBS 144921          | NR 161150            | NG 066300 | - | MK047549 |
| Lectera nordwiniana              | JW231013            | MK047462             | MK047512 | - | MK047550 |
| Lectera sambuci                  | CPC 36475           | NR 170055            | MT223905 | - | - |
| Leptosillia pistaciae            | CBS 128196          | NR 160064            | MH798901 | - | MH791334 |
| Malaysia sc phai                 | CBS 141321          | KX228280             | KX228331 | - | - |
| Malaysia sc phai                 | MFLUCC 16–0256      | MH275069             | MH260302 | MH260342 | - |
| Monilochaetes camelliae          | BRIP 24607          | HM237327             | HM237324 | - | - |
| Monilochaetes camelliae          | BRIP 2433c          | HM237326             | HM237323 | - | - |
| Monilochaetes dimorphospora      | MUCL 40959          | NR 137765            | HQ609480 | NG 062390 | - |
| Monilochaetes guadalcanalensis   | CBS 346.76          | GU180625             | GU180640 | - | - |
| Monilochaetes infuscans          | CBS 379.77          | -                    | GU180645 | GU180619 | GU180658 |
| Monilochaetes infuscans          | CBS 870.96          | -                    | GU180644 | GU180621 | - |
| Monilochaetes infuscans          | CBS 869.96          | GU180626             | GU180639 | GU180620 | GU180657 |
| Monilochaetes laeensis           | MR 2875             | GU180624             | GU180642 | - | - |
| Monilochaetes laeensis           | DAOM 226788         | GU180623             | GU180641 | GU180610 | - |
| Monilochaetes melastomae         | CBS 145059          | NR 161124            | NG 068601 | - | - |
| Monilochaetes nothapodytis       | TRY2 34             | MF153475             | MF153476 | - | - |
| Monilochaetes pteridophytophila  | MFLUCC 21 – 0022    | MW826218             | MW826219 | MW826220 | MW829186 |

Maximum Likelihood (ML) analysis was performed using IQ-TREE (Nguyen et al. 2015, Chernomor et al. 2016) under partitioned models. The optimal nucleotide substitution model for each locus was selected under the corrected Akaike Information Criterion (AICc) using jModelTest2 (Darriba et al. 2012) on XSEDE via the CIPRES Science Gateway 3.3 (https://www.phylo.org/portal2/home.action, Miller et al. 2010). The TIM3+I+G model (-lnL = 3601.7319) was selected for LSU, GTR+I+G (-lnL = 4351.9427) for ITS, TIM1+G (-lnL = 2071.9778) for SSU and TIM2+I+G (-lnL = 7734.2580) for RPB2. A non-parametric bootstrap (BS) analysis was implemented with 1000 replicates (Hoang et al. 2018).

The aligned fasta file was converted to nexus file format for BI analyses using AliView. BI analyses were performed in CIPRES (Miller et al. 2010) with MrBayes on XSEDE 3.2.7a (Ronquist et al. 2012). The best-fit evolutionary model for BI analysis was determined.
using MrModeltest v.2 (Nylander 2004). For the LSU, ITS and RPB2 datasets, GTR+I+G was selected, whereas GTR+G was selected for SSU. Bayesian posterior probabilities (PP) (Rannala and Yang 1996) were evaluated, based on Markov Chain Monte Carlo (MCMC) sampling. Four simultaneous Markov chains were run for 10,000,000 generations and trees were sampled every 1,000th generation (yielding 10,000 total trees). The first 2,500 trees, which represented the burn-in phase of the analysis, were discarded. The remaining 7,500 trees were used to calculate PP in the majority rule consensus tree.

Phylogenetic trees were visualised using FigTree v. 1.4.0 (Rambaut and Drummond 2008) and edited using Microsoft Office PowerPoint 2010 and Adobe Illustrator CS6 (Adobe Systems, USA). The final alignments and trees were deposited in TreeBASE (http://www.treebase.org/), accession number: 27987).

Taxon treatment

**Monilochaetes pteridophytophila** J.Y. Zhang, K.D. Hyde & Y.Z. Lu, sp. nov.

- IndexFungorum [IF558296](http://www.indexfungorum.org)
- Species-ID [Facesoffungi number: FoF 09708](https://www.facesoffungi.org)

**Materials**

**Holotype:**
- **scientificName:** *Monilochaetes pteridophytophila*; **phylum:** Ascomycota; **class:** Sordariomycetes; **order:** Glomerellales; **family:** Australiascaceae; **locationRemarks:** THAILAND, Tak Province, Umphang District, Mo Kro Subdistrict, 16°12'11"N, 98°52'5"E, 21 August 2019; **habitat:** Terrestrial; **fieldNotes:** on dead frond stalks of *Alsophila costularis* Baker (Cyatheaceae) in a disturbed forest nearby the roadside; **recordedBy:** Jing Yi Zhang; **collectionID:** MFLU 21–0023; **collectionCode:** Y26

**Isotype:**
- **collectionID:** GZAAS 21-0015

**Description**

Saprobic on dead frond stalks of *Alsophila costularis*. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous (Fig. 1), colonies on natural substrate superficial, effuse, gregarious, white. Conidiophores (268–)360–565 μm high (x̄ = 465 μm, n = 15), 9–14.5 μm wide (x̄ = 12 μm, n = 15) near the base, macronematous, unbranched, solitary, erect, straight or slightly flexuous, monophiladic, subcylindrical, thick-walled, 1–4-septate, dark brown to black, darker near the base, becoming paler brown towards the apex. Conidiogenous cells 25–54 × 7–11.5 μm (x̄ = 38 × 9.5 μm, n = 20), enteroblastic, monophiladic, terminal, swollen, with a shallow collarette, subcylindrical with apical taper to truncate apex, pale brown, rough. Conidia 20–24 × 10–12 μm (x̄ = 22 × 11.7 μm, n = 30), oblong to obvoid or ellipsoidal, occasionally with a median or submedian constriction, thick-walled, hyaline, aseptate, rough-walled.
Culture characteristics: Conidia germinating on PDA within 12 hours at 25°C, with hyaline germ tube germinating from the base of conidia. Colonies growing on PDA at 25°C, circular, flat surface, planar, thin, dark brown, reaching 2 cm diam. in 7 days, edge entire, emission at margin, dark brown to pale brown in reverse from the centre to margin of the colony.

Material: ex-type living culture, MFLUCC 21–0022.

Etymology
Referring to the host, which is a pteridophyte.

Notes
Monilochaetes pteridophytophila formed a distinct phylogenetic clade, which clustered with other species of Monilochaetes (Fig. 2). Following BLASTn searches, the closest matches of *M. pteridophytophila* are *M. melastomae* (LSU, NG_068601, 98.21% shared identity; ITS, NR_161124, 84.5%), *M. laeensis* (SSU, GU180610, 99.4%) and *M. infuscans* (RPB2, GU180658, 80.64%). *Monilochaetes pteridophytophila* is most similar to *M. regenerans* in the shape of conidiophores, conidiogenous cells and conidia (Bhat and Kendrick 1993). However, *M. pteridophytophila* has darker and longer conidiophores [(268–)360–565 μm vs. 300 μm high], shorter conidiogenous cells (25–54 μm vs. 70–100 μm) and smaller conidia (20–24 × 10–12 μm vs. 25–38 × 12–16 μm). Therefore, we introduce *M. pteridophytophila* as a new species, based on both phylogenetic and morphological evidence.
Analysis

Analysis I: Phylogenetic reconstruction of a combined LSU, ITS, SSU and RPB2 sequence dataset

The aligned, concatenated sequence matrix comprised sequence data for 39 taxa from seven families of the following loci: LSU (853 bp), ITS (489 bp), SSU (1,014 bp) and RPB2 (1,061 bp). Included sequences represented taxa of Glomerellales and three outgroup taxa, Collariella bostrychodes (CBS 586.83), Corynascus fumimontanus (CBS 137294) and Leptosillia pistaciae (CBS 128196). The sequence matrix comprised 3,417 characters (including gaps), of which 2,317 characters were constant, 185 variable characters were parsimony-uninformative and 915 characters were parsimony-informative. The matrix had 1,188 distinct alignment patterns, with 40.80% undetermined characters or gaps. The ML and BI analyses of the concatenated LSU–ITS–SSU–RPB2 dataset resulted in similar tree topologies (Fig. 2).

The phylogenetic tree shows that all strains of Monilochaetes clustered within Australiascaceae. The new species M. pteridophytophila forms a distinct clade, basal to other species of Monilochaetes with BS = 98% MLBS and PP = 1.00 (Fig. 2).
Discussion

*Monilochaetes* is a widespread genus, with species occurring as endophytes, pathogens or saprobes on various plants in terrestrial environments (Rashmi et al. 2019, Table 3). All currently-described species of *Monilochaetes* have hyphomycetous asexual morphs. Only *M. camelliae*, *M. dimorphospora* and *M. nothapodytis* have dimorphic hyphomycetous asexual forms (Réblová et al. 2011a, Réblová et al. 2011b, Zhou et al. 2017). *Monilochaetes camelliae* and *M. laeensis* are represented also by sexual morphs (Sivanesan and Alcorn 2002, Réblová et al. 2011a).

| Species                  | Hosts                              | Distribution                                      | Macroconidiophores/ Microconidiophores (µm) | Macroconidia/ Microconidia (µm) | Reference(s)                        |
|--------------------------|------------------------------------|--------------------------------------------------|---------------------------------------------|---------------------------------|-------------------------------------|
| *Monilochaetes basicurvata* | Palm petiole                        | Peru                                             | 200–300(−600) × 5−7 / -                     | 9–25 × 3.5–6(−7) / -            | Matsushima (1995)                   |
| *M. camelliae*          | Branch of *Camellia sinensis*      | Australia                                        | 200−720 × 9−10(−10.5) / 40−60 × 2−2.5        | 20.5–24(−26.5) × (10−)11−12 / 4–5.5 × 3–3.5 | Sivanesan and Alcorn (2002), Réblová et al. (2011a) |
| *M. dimorphospora*      | Decayed wood                        | Cuba                                            | 230–450 × 6.5–7 / 40 × 3                     | 21–25(−27) × 6.5–7 / 4.5–6(−6.5) × 2.5–3 | Réblová et al. (2011b)              |
| *M. guadalcanalensis*   | Decaying leaf of *Musa* sp.         | Solomon Islands                                  | 150–220(−400) × 4–7 / -                      | 18–21 × 6–9 / -                 | Rong and Gams (2000)                |
| *M. infuscans*          | Ipomoea batatas (sweet potato)     | Asia, Australia, Europe, New Zealand, South Africa, Pacific Islands, USA | 60–400 / -                          | 15–20 × 4–6 / -                 | Harter (1916), Lawrence et al. (1981), Rong and Gams (2000) |
| *M. laeensis*           | Leaf litter, dead stipes and spathes of a tree fern, rotting frond stems of *Victoria regia*, dead stipes of *Dicksonia antarctica* and dead palm spathes | Australia, British Isles, Cuba, Ethiopia, India, Malaysia, Papua New Guinea, Sabah and Sri Lanka. | 40–160(−280) × 7–8 / -            | (15.5–)18–22.5(−23.5) × 7.5–9(−10) / - | Bhat and Sutton (1985), Kirk (1986), Rong and Gams (2000), Réblová et al. (2011a) |
| *M. melastomae*         | Leaf spots of *Melastoma* sp.       | Malaysia                                         | 90 – 250 × 6 −10 / -                        | (17–)18–19(−20) × (7.5–)8 / -  | Crous et al. (2018)                 |
### Table 1: Characteristics of Bacillus species

| Species                  | Hosts                                      | Distribution | Macroconidiophores/ Microconidiophores (μm) | Macroconidia/ Microconidia (μm) | Reference(s) |
|--------------------------|--------------------------------------------|--------------|---------------------------------------------|---------------------------------|--------------|
| *M. nothapodytis*        | Healthy leaf of *Nothapodytes pittosporoides* | China        | 300–640 × 7.5–13 / 18–35 × 4–5.5            | 16.5–24 × 9.5–15.5 / 3–4.9 × 2.9–4 | Zhou et al. (2017) |
| *M. pteridophytophila*   | Dead frond stalks of *Alsophila costularis* | Thailand     | (268–)360–565 × 9–14.5 / -                 | 20–24 × 10–12 / -               | This study   |
| *M. regenerans*          | Dead twigs of *Ficus sp.*                  | India        | 300 × 8–10 / -                             | 25–38 × 12–16 / -              | Bhat and Kendrick (1993) |

*Monilochaetes pteridophytophila* is the second species found on a fern; *M. laeensis* occurs on tree ferns in Australia and the UK (Kirk 1986, Réblová et al. 2011a). *Monilochaetes pteridophytophila* forms a distinct clade with *M. laeensis*, basal to other *Monilochaetes* species. However, *M. pteridophytophila* differs from *M. laeensis* in having darker and longer conidiophores [(268–)360–565 μm vs. 40–160(–280) μm]. Hyde et al. (2018) and Hyde et al. (2020b) showed high fungal diversity in Thailand and suggested that studies on new hosts and new areas would lead to discovery of further new fungal species. Further studies of fungi on pteridophytes are likely expected to reveal more novel species.

Glomerellales was proposed by Réblová et al. (2011a) to accommodate three families, based on morphology and multilocus phylogenetic data: Australiascaceae, Glomerellaceae and Reticulascaceae. Later, Maharachchikumbura et al. (2016) accepted Plectosphaerellaceae in Glomerellales, based on the analysis of a combined LSU–SSU–TEF1–RPB2 dataset. Malaysiascaceae was added to Glomerellales by Tibpromma et al. (2018), based on a combined ribosomal DNA dataset (SSU, ITS, LSU). Our phylogenetic study confirms Glomerellales as a robust clade (ML = 100, PP = 1.00) comprising five lineages: Australiascaceae (ML = 98, PP = 1.00), Glomerellaceae (ML = 95, PP = 1.00), Malaysiascaceae (ML = 100, PP = 1.00), Plectosphaerellaceae (ML = 100, PP = 1.00) and Reticulascaceae (ML = 99, PP = 1.00). The phylogenetic relationships of families in Glomerellales are in agreement with Tibpromma et al. (2018) and Hyde et al. (2020a).

The tree topologies resulting from the phylogenetic reconstruction of a combined LSU–ITS dataset (analysis II, Suppl. material 1) and the concatenated LSU–ITS–SSU–RPB2 dataset (analysis I, Fig. 2) were overall similar and not significantly different. A comparison of phylogenetic analysis I and II with the analysis by Hyde et al. (2020a) showed negligible variation in tree topologies in Glomerellales, even with the inclusion of SSU and RPB2 data. The phylogeny in the current study suggests that LSU and ITS sequences can resolve interspecific relationships within *Monilochaetes*, as well as interfamilial relationships within Glomerellales.
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References

- Balkrishna A, Arya V, Kushwaha AK (2020) Population structure, regeneration status and conservation measures of threatened Cyathea spp. Journal of Tropical Forest Science 32 (4). https://doi.org/10.26525/jtfs2020.32.4.414
- Bhat DJ, Sutton BC (1985) Some phialidic Hyphomycetes from Ethiopia. Transactions of the British Mycological Society 84 (4).
- Bhat DJ, Kendrick B (1993) Twenty-five new conidial fungi from the Western Ghats and the Andaman Islands (India). Mycotaxon 49 (1).
- Braun U, Nakashima C, Crous PW (2013) Cercosporoid fungi (Mycosphaerellaceae) 1. Species on other fungi. Pteridophyta and Gymnospermae. IMA Fungus 4 (2). https://doi.org/10.5598/imafungus.2013.04.02.12
- Chernomor O, Von Haeseler A, Minh BQ (2016) Terrace aware data structure for phylogenomic inference from supermatrices. Systematic Biology 65 (6). https://doi.org/10.1093/sysbio/syw037
- Coritico F, Amoroso V (2020) Threatened lycophytes and ferns in four protected areas of Mindanao, Philippines. Nature Conservation Research 5 (4). https://doi.org/10.24189/ncr.2020.061
- Crous PW, Luangsa-Ard JJ, Wingfield MJ, Carnegie AJ, Hernández-Restrepo M, Lombard L, Roux J, Barreto RW, Baseia IG, Cano-Lira JF, Martín MP, Morozova OV, Sttchigel AM, Summerell BA, Brandrud TE, Dimia B, Garcia D, Giraldo A, Guarro J, Giummão LFP, Khamisumton P, Noordeloos ME, Nuankaew S, Pinruan U, Rodríguez-Andrade E, Souza-Motta CM, Thangavel R, van Iperen AL, Abreu VP, Accioly T, Alves JL, Andrade JP, Bahram M, Baral HO, Barbier E, Barnes CW, Bendiksen E, Bernard E, Bezerra JDP, Bezerra JL, Bizio E, Blair JE, Bulyonkova TM, Cabral TS, Caiafa MV, Cantillo T, Colmán AA, Conceição LB, Cruz S, Cunha AOB, Darveaux BA, da Silva AL, da Silva GA, da Silva GM, da Silva RMF, de Oliveira RJV, Oliveira RL, De Souza JT, Dueñas M, Evans HC, Epifáni F, Felipe MTC, Fernández-López J, Ferreira BW, Figueiredo redo CN, Filippova NV, Flores JA, Gené J, Ghorbani G, Gibertoni TB, Glushakova AM, Healy R, Huhndorf SM, Iturrieta-González I, Javan-Nikkham M, Juciano RF, Jurjevič Ž, Kachalkin AV, Keoachapeng K, Krisai-Greilhuber I, Li YC, Lima AA, Machado AR, Madrid H, Magalhães OMC, Marbach PAS, Melanda GCS, Miller AN, Mongkolsamrit S, Nascimento RP, Oliveira TGL, Ordoñez ME, Orzes R, Palma MA, Pearce CJ, Pereira OL, Perrone G, Peterson SW, Pham THG, Piontelli E, Pordel A, Quijada L, Raja HA,
Rosas de Paz E, Ryvarden L, Salitaa A, Salcedo SS, Sandoval-Denis M, Santos TAB, Seifert KA, Silva BDB, Smith ME, Soares AM, Sommai S, Sousa JO, Suetrong S, Susca A, Tedersoo L, Telleria MT, Thanakitpipattana D, Valenzuela-Lopez N, Visagie CM, Zapata M, Groenewald JZ (2018) Fungal Planet description sheets: 785–867. Persoonia 41 https://doi.org/10.3767/persoonia.2018.41.12
• Dai DQ, Phookamsak R, Wijayawardene NN, Li WJ, Bhat DJ, Xu JC, Taylor JE, Hyde KD, Chukeatirote E (2017) Bambusicolous fungi. Fungal Diversity 82 https://doi.org/10.1186/s13052-016-0286-z
• Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9 (8). https://doi.org/10.1038/nmeth.2109
• Dissanayake AJ, Bhunjun C, Maharachchikumbura SSN, Liu JK (2020) Applied aspects of methods to infer phylogenetic relationships amongst fungi. Mycosphere 11 (1). https://doi.org/10.5943/mycosphere/11/1/18
• Guatimosim E, Schwartsburd PB, Barreto RW, Crous PW (2016) Novel fungi from an ancient niche: cercosporoid and related sexual morphs on ferns. Persoonia 37 https://doi.org/10.3767/003158516X690934
• Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. 41. Nucleic Acids Symposium Series. [London]: Information Retrieval Ltd., c1979-c2000.
• Halsted BD (1890) Some fungus diseases of the sweet potato. New Jersey Agricultural Experiment Station Bulletin (76).
• Harter LL (1916) Sweet-potato scurf. Journal of Agricultural Research 5.
• Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35 (2): 518-522. https://doi.org/10.1093/molbev/msx281
• Hongsanan S, Maharachchikumbura SSN, Hyde KD, Samarakoon MC, Jeewon R, Zhao Q, Al-Sadi AM, Bahkali AH (2017) An updated phylogeny of Sordariomycetes based on phylogenetic and molecular clock evidence. Fungal Diversity 84 (1). https://doi.org/10.1007/s13225-017-0384-2
• Hopple JS (1994) Phylogenetic investigations in the genus Coprinus based on morphological and molecular characters. PhD thesis, Duke University, Durham, NC
• Hyde KD, Norphanphoun C, Chen J, Dissanayake AJ, Doilom M, Hongsanan S, Jayawardena RS, Jeewon R, Perera RH, Thongbai B, Wanasinghe DN, Wisitrassameewong K, Tibepromma S, Stadler M (2018) Thailand’s amazing diversity: up to 96% of fungi in northern Thailand may be novel. Fungal Diversity 93 (1). https://doi.org/10.1007/s13225-018-0415-7
• Hyde KD, Norphanphoun C, Maharachchikumbura SSN, Bhat DJ, Jones EBG, Bundhun D, Chen YJ, Bao DF, Boonmee S, Calabon MS (2020a) Refined families of Sordariomycetes. Mycosphere 11 (1). https://doi.org/10.5943/mycosphere/11/1/7
• Hyde KD, Jeewon R, Chen YJ, Bhunjun CS, Calabon MS, Jiang HB, Lin CG, Norphanphoun C, Sysouphanthong P, Pem D, Tibepromma S, Zhang Q, Doilom MK, Jayawardena RS, Liu JK, Maharachchikumbura SSN, Phukhamsakda C, Phookamsak R, Al-Sadi AM, Thongklang N, Wang Y, Gaforov Y, Jones EBG, Lumyong S (2020b) The numbers of fungi: is the descriptive curve flattening? Fungal Diversity 103 (1). https://doi.org/10.1007/s13225-020-00458-2
• Index Fungorum (2021) http://www.indexfungorum.org [accessed 16 July 2021]
Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20 (4). https://doi.org/10.1093/bib/bbx108

Kirk PM (1986) New or interesting microfungi: XV. Miscellaneous hyphomycetes from the British Isles. Transactions of the British Mycological Society 86 (3). https://doi.org/10.1016/S0007-1536(86)80185-1

Kirschner R, Liu LC (2014) Mycosphaerellaceous fungi and new species of Venustosynnema and Zasmidium on ferns and fern allies in Taiwan. Phytotaxa 176 (1). https://doi.org/10.11646/phytotaxa.176.1.29

Kirschner R, Lee PH, Huang Y (2019) Diversity of fungi on Taiwanese fern plants: review and new discoveries. Taiwania 64 (2). https://doi.org/10.1016/j.tai.2019.64.163

Korall P, Pryer KM (2014) Global biogeography of scaly tree ferns (Cyatheaceae): evidence for Gondwanan vicariance and limited transoceanic dispersal. Journal of Biogeography 41 (2). https://doi.org/10.1111/jbi.12222

Lawrence GW, Moyer JW, Van Dyke CG (1981) Histopathology of sweet potato roots infected with Monilochaetes infuscans. Phytopathology 71 (3). https://doi.org/10.1094/Phyto-71-312

Lehnert M (2011) The Cyatheaceae (Polypodiopsida) of Peru. Brittonia 63 (1).

Lindsay S, Middleton DJ (2009) Development of a multi-access key to the ferns of Thailand. Thai Forest Bulletin (Botany) (37).

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16 (12).

Lu YZ, Boonmee S, Dai DQ, Liu JK, Hyde KD, Bhat DJ, Ariyawansa H, Kang JC (2017) Four new species of Tubeufia (Tubeufiaceae, Tubeufiales) from Thailand. Mycological Progress 16 (4). https://doi.org/10.1007/s11557-017-1280-6

Maharachchikumbura SSN, Hyde KD, Jones EBG, McKenzie EHC, Bhat JD, Dayarathne MC, Huang SK, Norphanphoun C, Senanayake IC, Perera RH, Shang QJ, Xiao Y, D'souza MJ, Hongsanan S, Jayawardena RS, Daranagama DA, Konta S, Goonasekara ID, Zhuang WY, Jeewon R, Phillips AJL, Abdel-Wahab MA, Al-Sadi AM, Bahkali AH, Boonmee S, Boonyuen N, Cheewangkoon R, Dissanayake AJ, Kang J, Li QR, Liu JK, Liu XZ, Liu ZY, Luangsa-ard JJ, Pang KL, Phookamsak R, Promputtha I, Suetrong S, Stadler M, Wen TC, Wijayawardene NN (2016) Families of Sordariomycetes. Fungal Diversity 79 (1). https://doi.org/10.1007/s13225-016-0369-6

Matsushima T (1995) Matsushima mycological memoirs no. 8. Matsushima Fungus Collection, Kobe, Japan.

Mehltreter K (2010) Interactions of ferns with fungi and animals. Fern Ecology https://doi.org/10.1017/CBO9780511844898.008

Miller MA, Pfeiffer W, Schwartz T (2010) "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway. Computing Environments Workshop (GCE). New Orleans https://doi.org/10.1109/GCE.2010.5676129

Nguyen L, 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 (1). https://doi.org/10.1093/molbev/msu300

Nylander JAA (2004) MrModeltest Version 2. Program distributed by the author. (Uppsala University, Uppsala, Sweden).
• Rambaut A, Drummond A (2008) FigTree: Tree figure drawing tool, version 1.2. 2.
Institute of Evolutionary Biology, University of Edinburgh.

• Rannala B, Yang ZH (1996) Probability distribution of molecular evolutionary trees: a
new method of phylogenetic inference. Journal of Molecular Evolution 43 (3).
https://doi.org/10.1007/BF02338839

• Rashmi M, Kushveer JS, Sarma VV (2019) A worldwide list of endophytic fungi with
notes on ecology and diversity. Mycosphere 10 (1). https://doi.org/10.5943/mycosphere/10/1/19

• Razikin MZM, Nagao H, Zakaria R (2014) First report of pteriodocolous discomycetes,
Lachnum lanariceps and L. oncospermatum, on decayed tree fern in Bukit Bendera
(Penang Hill), Pulau Pinang, Malaysia. SongklaNakarin Journal of Science and
Technology 36 (4).

• Réblová M, Gams W, Seifert KA (2011a) Monilochaetes and allied genera of the
Glomerellales, and a reconsideration of families in the Microascales. Studies in
Mycology 68 https://doi.org/10.3114/sim.2011.68.07

• Réblová M, Gams W, Štěpánek V (2011b) The new hyphomycete genera Brachyalara
and Infundichalara, the similar Exochalara and species of 'Phialophora sect.
Catenulatae' (Leotiomycetes). Fungal Diversity 46 (1). https://doi.org/10.1007/s13225-010-0077-6

• Réblová M, Miller AN, Rossman AY, Seifert KA, Crous PW, Hawksworth DL, Abdel-
Wahab MA, Cannon PF, Daranagama DA, De Beer ZW (2016) Recommendations for
competing sexual-asexually typified generic names in Sordariomycetes (except
Diaporthales, Hypocreales, and Magnaporthales). IMA Fungus 7 (1). https://doi.org/10.5598/imafungus.2016.07.01.08

• Rong I, Gams W (2000) The Hyphomycete genera Exochalara and Monilochaetes. Mycotaxon 76 https://doi.org/10.1007/s13225-016-0369-6

• Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L,
Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic
inference and model choice across a large model space. Systematic Biology 61 (3).
https://doi.org/10.1093/sysbio/sys029

• Sivanesan A, Alcorn JL (2002) Australiasca queenslandica gen. et sp. nov.
(Chaetosphaeriaceae: Ascomycota) and its anamorph Dischloridium camelliae sp. nov.
from Australia. Australian Systematic Botany 15 (5). https://doi.org/10.1071/SB01049

• Tibpromma S, Hyde KD, McKenzie EHC, Bhat DJ, Phillips AJL, Wanasinghe DN,
Samarakoon MC, Jayawardena RS, Dissanayake AJ, Tennakoon DS, Doilm M,
Phookamsak R, Tang AMC, Xu JC, Mortimer PE, Promputtha I, Maharachchikumbura
SSN, Khan S, Karunaratna SC (2018) Fungal diversity notes 840–928: micro-fungi
associated with Pandanaceae. Fungal Diversity 93 (1). https://doi.org/10.1007/s13225-018-0408-6

• Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically
amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology
172 (8). https://doi.org/10.1128/jb.172.8.4238-4246.1990

• White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal
ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and
applications 18 https://doi.org/10.1016/B978-0-12-372180-8.50042-1

• Wijayawardene NN, Hyde KD, Al-Ani LKT, Tedersoo L, Haelewaters D, Rajeshkumar
KC, Zhao RL, Apteet A, Leontyev V, Saxena D,RK, Tokarev YS, Dai DQ, Letcher PM,
Supplementary material

Suppl. material 1: Phylogenetic analysis of a combined LSU and ITS sequence data

Authors: Jingyi Zhang
Data type: phylogenetic tree
Brief description: Analysis II: Phylogenetic analysis of a combined LSU and ITS sequence data

The aligned sequence matrix comprises LSU (853 bp) and ITS (489 bp) sequence data for 39 taxa from GenBank. The aligned sequence matrix comprises 1,342 characters after alignment including the gaps, of which 873 characters were constant, 67 variable characters were parsimony-uninformative and 402 characters were parsimony informative. The matrix had 518 distinct alignment patterns, with 10.95% undetermined characters or gaps. The RAxML and BI analyses, based on combined LSU and ITS sequence data, provided similar tree topologies and the result of ML analysis is shown in FIGURE S1.

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