**Mendogia diffusa** sp. nov. and an updated key to the species of *Mendogia* (Myriangiaceae, Dothideomycetes)

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Academic editor: Danny Haelewaters

Received: 21 Apr 2021 | Accepted: 05 Aug 2021 | Published: 07 Sep 2021

Citation: Thiyagaraja V, Lücking R, Ertz D, Samarakoon MC, Wanasinghe DN, Karunarathna SC, Cheewangkoon R, Hyde KD (2021) *Mendogia diffusa* sp. nov. and an updated key to the species of *Mendogia* (Myriangiaceae, Dothideomycetes). Biodiversity Data Journal 9: e67705. [https://doi.org/10.3897/BDJ.9.e67705](https://doi.org/10.3897/BDJ.9.e67705)

**Abstract**

**Background**

*Mendogia* belongs to Dothideomycetes and its members are epiphytic on living bamboo culms or palms and distributed in tropical regions. Currently, the genus comprises seven species. Another collection resembling *Mendogia* was collected from the leaves of *Fagales* sp. in Thailand. Morphological characteristics and multilocus phylogenetic analyses, using ITS, LSU and SSU sequences, showed that the fungus is new to science, described herein...
as *Mendogia diffusa*. *Mendogia diffusa* is characterised by apothecial ascostromata, a carbonised epithecium, dark brown setae on the ascostromatal surface, hyaline paraphysoids, ovoid to clavate asci and oblong to elliptical, muriform ascospores. The fungus has a dark pigmented surface and is occasionally facultatively associated with patches of green algae, but not actually lichenised. Instead, the fungus penetrates the upper leaf surface, forming dark pigmented isodiametric cells below the epidermis.

**New information**

Re-examination of specimens of *M. chiangraiensis*, *M. macrostroma* and *M. yunnanensis* revealed the absence of algal associations. The status of *Mendogia philippinensis* (= *M. calami*) and *M. bambusina* (= *Uleopeltis bambusina*) was established, based on morphological comparisons and previous studies. Comprehensive morphological descriptions with phylogenetic analyses support *M. diffusa* as a novel species in *Myriangiaceae*. An updated key to the known species of the genus is also provided.

**Keywords**

one new species, morphology, multilocus phylogeny, saprotroph, taxonomy

**Introduction**

Dothideomycetes is the largest class in Ascomycota, comprising 19,000 species, including saprotrophs, pathogens, endophytes, epiphytes, fungicolous, lichenised and lichenicolous taxa (Hyde et al. 2013, Hongsanan et al. 2020). *Myriangiales* was introduced by Starbäck (1899), based on species producing crustose ascostromata and muriform ascospores in the Dothideomycetes (Hyde et al. 2013). These species occur as pathogens, saprobes or epiphytes on bark, leaves and branches of plants (Dissanayake et al. 2014, Jayawardena et al. 2014), while some are rock-inhabiting (Ruibal et al. 2009). Kirk et al. (2008) included *Cookellaceae*, *Elsinoaceae* and *Myriangiaceae* in *Myriangiales*. Based on molecular phylogenetic studies, Lumbsch and Huhndorf (2010) accepted only *Elsinoaceae* and *Myriangiaceae* within *Myriangiales*, whereas *Cookellaceae* was treated as Dothideomycetes *incertae sedis*. This classification was accepted in subsequent studies (Hyde et al. 2013, Dissanayake et al. 2014, Jayawardena et al. 2014, Wijayawardene et al. 2014 Dai et al. 2017, Wijayawardene et al. 2017, Wijayawardene et al. 2018, Hongsanan et al. 2020, Jiang et al. 2020, Wijayawardene et al. 2020). *Myriangiaceae* is a poorly known family (Dissanayake et al. 2014) and comprises 11 genera. These are *Anhellia*, *Ascostratum*, *Butleria*, *Dictyocyclus*, *Eurytheca*, *Hemimyriangium*, *Mendogia*, *Micularia*, *Myriangium*, *Uleomyces* and *Zukaliopsis* (Hongsanan et al. 2020, Wijayawardene et al. 2020). Members in *Myriangiaceae* occur mainly in tropical and sub-tropical areas (Boedijn 1961, Barr 1979).
**Mendogia** was introduced by Raciborski (1900), based on the single species *M. bambusina* collected on bamboo in Indonesia. This genus was, for some time, placed in *Schizothyriaceae* (von Arx and Müller 1975). However, Dai et al. (2017) provided the first molecular data for *M. macrostroma* and transferred this genus to *Myriangiaceae*, based on morphological and phylogenetic analyses. Seven species are currently recognised within this genus (Jiang et al. 2020). They are characterised by small to large, black, flattened, solitary to scattered, superficial ascostromata with a centrally raised area, subglobose to clavate, bitunicate, (6–)8(–10)-spored asci with a distinct ocular chamber and elliptical, muriform, hyaline ascospores (Jiang et al. 2020). The species of **Mendogia**, thus far known, are exclusively epiphytic on living bamboo culms or palms and are found in Brazil, China, Indonesia, Philippines and Thailand (Raciborski 1900, Hennings 1904, von Arx and Müller 1975, Dai et al. 2017). **Mendogia** is distinguished from other genera of this family by its larger ascostromata, thick peridium, carbonaceous outer cells, pseudoparenchymatous inner cells and muriform ascospores (Phookamsak et al. 2016).

This study introduces a new species of **Mendogia** that appeared unusual due to its growth on leaves and its occasional, facultative association with patches of green algae. We conducted a detailed investigation to resolve the identity of our newly-collected material, including morphological and chemical assessments. The phylogenetic position of the taxon was investigated, based on Maximum Likelihood and Bayesian analyses of combined ITS, LSU and SSU sequences. We further re-examined herbarium collections of **Mendogia chiangraiensis**, *M. macrostroma* and *M. yunnanensis* to test potential associations with algae. Additionally, morphological comparisons between closely-related taxa have led to reclassify several species in **Mendogia** (*M. philippinensis* (= *M. calami*) and *M. bambusina* (= *Uleopeltis bambusina*)). We, therefore, provided an updated key to the genus.

**Materials and methods**

**Morphological analysis**

The fungal material was collected in Phayao, Thailand. Herbarium specimens of **Mendogia chiangraiensis**, *M. macrostroma* and *M. yunnanensis* were loaned from Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand. Fungal structures on the substrate were observed with a stereomicroscope and micro-morphological features were examined and photographed using a Nikon Eclipse E600 fluorescence microscope with a Canon 750D digital camera. Hand sections of the ascomata were mounted in water, 5% potassium hydroxide (KOH), 5% Lugol's solution and Trypan blue. All microscopic measurements were measured in water and images were made with Tarosoft Image Frame Work (0.9.0.7) and processed with Adobe Photoshop CS6 Extended 10.0 software (Adobe Systems, San Jose, CA, USA). The newly-proposed synonymies were established, based on revision of available data from previous studies. The holotype specimen of *M. diffusa* was deposited in the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand.
DNA extraction, PCR amplification and sequencing

The E.Z.N.A. Forensic DAT (D3591 – 01, Omega Bio–Tek, Guangzhou, China) kit was used to extract DNA, following the manufacturer’s instructions. DNA samples that were intended for use as a template for PCR were stored at 4°C for use in regular work; long-term storage was at -20°C. The small and large subunits (SSU, LSU) of the nuclear ribosomal RNA gene, as well as the internal transcribed spacer (ITS) region were amplified with primer pairs NS1/NS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990, Hopple 1994) and ITS5/ITS4 (White et al. 1990), respectively. PCR amplification was performed using a final volume of 25 µl, comprised of 2.0 µl of DNA template, 1 µl of each forward and reverse primer, 12.5 µl of Taq PCR Super Mix and 8.5 µl of sterilised water. Cycling conditions were as follows: initial denaturation at 94°C for 3 min; followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 50 s, elongation at 72°C for 1 min; and a final extension at 72°C for 10 min. PCR products were examined on 1% agarose electrophoresis gels and stained with ethidium bromide. Purification and DNA sequencing were performed at Shanghai Sangon Biological Engineering Technology and Services Co. (Shanghai, P.R. China). Forward and reverse sequence reads were assembled and manually edited in Bioedit. Generated sequences were submitted to NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Alignments and phylogenetic trees were submitted to TreeBASE with Submission ID: 28050.

Phylogenetic analyses and species recognition

The newly-generated sequences were BLAST-searched against the NCBI GenBank standard nr/nt database (https://blast.ncbi.nlm.nih.gov/BLAST.cgi). Sequences of closely-related taxa for Myriangiales were downloaded from GenBank. We failed to generate sequences for the translation elongation factor 1-alpha (TEF1) using the primer pair EF1-983F/EF1-2218R with the PCR conditions recommended in Jiang et al. (2020). As a result, our phylogenetic analyses were carried out using ITS, LSU and SU sequences (Table 1). Columnosphaeria fagi (CBS 171.93), Dothidea insculpta (CBS 189.58), D. sambuci (DAOM 231303), Dothiora cannabinae (CBS 737.71) and Sydowia polyspora (CBS 116.290) were used as outgroup taxa (Jiang et al. 2020).

Phylogenetic analyses of both individual and combined aligned data were performed under Maximum Likelihood (ML) and Bayesian Inference (BI) criteria. Multiple alignments were automatically performed for each locus with MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html, Katoh et al. 2017). Terminal ends of sequences and ambiguous regions were trimmed manually using BioEdit v.7.0.5.2 (Hall 2001) and excluded from the analysis. The phylogenetic web tool “ALTER” (Glez-Peña et al. 2010) was used to convert sequence alignment from FASTA to NEXUS format for Bayesian analysis. The estimated model of ML and Bayesian analyses were performed independently for each locus using MrModeltest v. 2.2 (Nylander 2008). ML analysis was performed in IQ-TREE web server under different partitions (Nguyen et al. 2015) for SSU, LSU, ITS1, 5.8S and ITS2 gene regions, with default parameters. MrBayes v. 3.1.2 was used to perform Bayesian analysis (Huelsenbeck and Ronquist 2001). Markov Chain Monte Carlo sampling (MCMC) was run
for 5,000,000 generations and the trees were sampled every 100th generation. The first 10% of trees that represented the burn-in phase were discarded and only the remaining 90% of trees were used for calculating posterior probabilities (PP) for the majority rule consensus tree. The resulting trees were visualised in FigTree v.1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/), then edited in Microsoft PowerPoint 2013 and converted to a jpeg file using Adobe Photoshop CS6 (Adobe Systems, USA).

| Species               | Strain     | ITS       | LSU       | SSU       |
|-----------------------|------------|-----------|-----------|-----------|
| Anhellia nectandrae   | VIC 31767  | NR_111700 | NG_042604 | -         |
| Cylindrospora fagi    | CBS 171.93 | KT693737  | AY016359  | AY016342  |
| Dothidea insculpta    | CBS 189.58 | AF027764  | NG_027643 | DQ247810  |
| Dothidea sambuci      | DAOM 231303| NR_111220 | NG_027611 | NG_012432 |
| Dothiora cannabinae   | CBS 737.71 | NR_144904 | DQ470984  | NG_062696 |
| Elsinoe brasiensis    | CPC 18528  | NR_148130 | JN940394  | NG_064989 |
| Elsinoe caleae        | CBS 221.50 | NR_148131 | NG_064001 | -         |
| Elsinoe centrolobii   | CBS 222.50 | NR_148132 | KX886969  | NG_062717 |
| Elsinoe citricola     | CPC 18535  | NR_148133 | KX886970  | JN940559  |
| Elsinoe embeliae      | CBS 472.62 | NR_148136 | KX886974  | -         |
| Elsinoe erythrinae    | CPC 18542  | KX887214  | KX886977  | JN940550  |
| Elsinoe eucalypticola | CBS 124765 | NR_132834 | KX886978  | -         |
| Elsinoe eucalyptorum  | CBS 120084 | NR_155080 | KX886979  | -         |
| Elsinoe euphorbiae    | CBS 401.63 | NR_148137 | KX886980  | -         |
| Elsinoe fagarae       | CBS 514.50 | NR_148138 | KX886981  | -         |
| Elsinoe fawcettii     | CBS 139.25 | NR_148139 | KX886982  | -         |
| Elsinoe krugii        | CPC 18531  | NR_148150 | KX886998  | NG_064987 |
| Elsinoe lagoa-santensis | CBS 518.50 | NR_148151 | KX887002  | -         |
| Elsinoe leucopogonis  | CPC 32097  | NR_159836 | NG_064551 | -         |
| Elsinoe leucospermi   | CBS 111207 | NR_148154 | KX887005  | -         |
| Elsinoe lippiae       | CBS 166.40 | NR_148155 | NG_063985 | -         |
| Species                        | Strain    | GenBank Accessions Number |
|-------------------------------|-----------|---------------------------|
| Elsinoe mangiferae            | CBS 226.50 | NR_148156, KX887012        |
| Elsinoe perseae               | CBS 406.34 | NR_148160, NG_063977      |
| Elsinoe phaseoli              | CBS 165.31 | NR_148161, KX887026, NG_062718 |
| Elsinoe quercus-licis         | CBS 232.61 | NR_148164                  |
| Elsinoe perseae               | CPC 18549  | KX887298, KX887051, JN940561 |
| Elsinoe sicula                | CBS 398.59 | NR_148170, KX887052      |
| Elsinoe solidaginis           | CBS 191.37 | NR_148171, KX887053      |
| Elsinoe tectiflaca            | CBS 124777 | NR_148172, KX887055      |
| Elsinoe terminaliae           | CBS 343.39 | NR_148173, KX887056      |
| Elsinoe terminaliae           | CPC 18538  | JN943497, JN940371, JN940560 |
| Elsinoe theae                 | CBS 228.50 | NR_148174, KX887058      |
| Elsinoe tiliae                | CBS 350.73 | KX887296, KX887059      |
| Elsinoe veneta                | CBS 164.29 | NR_148175, NG_059194, NG_062714 |
| Elsinoe verbena               | CPC 18561  | NR_148176, NG_059208, NG_064988 |
| Endosporium aviarium          | UAMH 10530 | NR_111286, NG_059195, NG_016524 |
| Endosporium aviarium          | UAMH 10531 | EU304352, EU304353      |
| Endosporium populi-tremuloidis| UAMH 10529 | EU304347, EU304348, EU304346 |
| Mendogia diffusa              | MFLU 20-0541 | MW854639, MW854637, MW854638 |
| Mendogia chiaangraensis       | MFLU 19-0005 | MK433591                  |
| Mendogia macrostroma          | MFLU 13-0642 | NR_154192, KU863104, NG_065082 |
| Mendogia yunnanensis          | MFLU 19-0006 | -                         |
| Myriangium citri              | MAaK       | KU720544, KU720541       |
| Myriangium citri              | MAsS1      | KU720543, KU720539       |
| Myriangium citri              | MAsS2      | KU720542, KU720540       |
| Myriangium duriae             | CBS 260.36 | MH855793, NG_027579, AY016347 |
| Myriangium hispanicum         | CBS 300.34 | MH855532, MH867034      |
| Myriangium hariame             | CBS 247.33 | MH855426, KX887067      |
| Myriangium sp.                | HK         | KR909171                  |
| Sydowia polyspora             | CBS 116.29 | MH 855019, DQ678058, DQ678005 |
**Taxon treatments**

*Mendogia diffusa* Thiyagaraja, Ertz, Lücking, Samarak. and K.D. Hyde, sp. nov.

- IndexFungorum [IF 558292](#)
- Facesoffungi number [FoF 09466](#)

**Material**

**Holotype:**
- kingdom: Fungi; phylum: Ascomycota; class: Dothideomycetes; order: Myriangiales; family: Myriangiaceae; genus: *Mendogia*; specificEpithet: *diffusa*; scientificNameAuthorship: Thiyagaraja, Ertz, Lücking, Samarak. and K.D. Hyde; continent: Asia; country: Thailand; stateProvince: Phayao; locality: Phu Sang; recordedBy: Milan C. Samarakoon; identificationID: MFLU 20-0541; identifiedBy: Vinodhini Thiyagaraja; dateIdentified: 4 Dec 2018; modified: 4 December 2018; institutionID: MFLU; institutionCode: Mae Fah Luang University; occurrenceID: MFLU 20-0541

**Description**

*Saprotrophic* on dead leaves. *Thallus* absent (Fig. 1 and Fig. 2). **Sexual morph:** *Ascomata* scattered in dense, pseudostromatic, irregularly stellate groups over an effuse, thallus-like, dark structure, with thin covering layer, superficial, solitary or gregarious, easily removed from the host surface, carbonaceous, ovoid to sub-globose, black, abundant, with numerous external dark brown setae on the epithecium, which are branched at the end, individual loci (120-)225–410 µm wide, 250–180 µm high. *Epithecium* 16–33 µm thick, distinct, dark brown. *Hymenium* 40–95 µm high, hyaline.
Hypothecium 35–75 µm thick, distinct, thicker in the centre, brownish, infrequently with free-living unicellular algae below the hypothecium. Excipulum inconspicuous. Paraphysoids 1.1–3.3 µm thick, abundant, anastomosing, branched, not or slightly enlarged at the apex. Asci 45–70 × 25–35 µm (x̄ = 57.5 × 30 µm, n = 20), 8-spored, bitunicate, fissitunicate, ovoid to clavate, tholus thickened, tip blunted, with poorly developed stipe, ascus wall apically thickened with well-developed ocular chamber, concave. Ascospores 15–25 × 6–10 µm (x̄ = 20 × 8 µm, n = 20), irregularly arranged, hyaline, oblong to elliptical, both ends bluntly tapered, muriform, with 5–6 transverse septa, 3–6 longitudinal septa, slightly constricted at each septum, smooth-walled, without gelatinous sheath, occasionally asymmetrical. Hymenium I–, KI–, Asci I–, KI–.

Asexual morph: Undetermined.

Etymology

Referring to the morphology of the fungus with ascostromata that are diffuse and spread extensively on the leaves.

Figure 2. Mendogia diffusa (MFLU 20-0541, holotype) a–e. Vertical sections of ascomata in water (upper surface); f. Vertical section of an ascoma in water (lower surface); fh, hair-like structure on leaf; g. Ascomata in trypan blue; h, i. (a1, a2) Algae; j. Paraphysoids in water; k–m. Asci in water; n. Asci in 5% KOH stained with Lugol's solution; o1–o8. Ascospores in water. Scale bars: (a–g) = 200 µm, (h–j) = 5 µm, (k–n) = 30 µm, (g–j) = 30 µm, (o1–o8) = 10 µm

Habitats and Distribution: On dead leaves of Fagales sp. Thus far, only known from Thailand, Phayao Province, Phu Sang District.

Notes

Mendogia diffusa is the first reported species in the genus from dead dicotyledonous leaves. Other species were mostly reported from bamboo culms, with the exception of M. manaosensis that is reported from palm leaves (Vitória 2012, Dai et al. 2017) and
M. philippinensis (= M. calami) that is found on living leaves of Calamus palms (Jiang et al. 2020). In those species, ascostromata do not penetrate the leaf surface and they also differ from M. diffusa in the sharply delimited ascostromata; and M. philippinensis further differs in the smaller ascospores. The new taxon shares morphological characteristics with Mendogia bambusina: carbonaceous peridium, paraphysoid-like filaments, similar asci and ascospores. However, M. diffusa differs in the absence of ascostromata, presence of setae (Dai et al. 2017), the type of habitat (Fagales leaves vs. bamboo or palms culms) and its distribution (Thailand vs. Indonesia) (Hyde et al. 2013, Dai et al. 2017).

**Mendogia philippinensis** (Syd. & P. Syd.) Arx & E. Müll., Stud. Mycol. 9: 29 (1975).

**Nomenclature**
Basionym: Pleiostomella philippinensis Syd. & P. Syd., Annls mycol. 15(3/4): 221 (1917); Type: The Philippines, Biliran, 1914, RC McGregor 18371 (S-F61491).

Syn. nov.: Mendogia calami H.B. Jiang, Phookamsak and K.D. Hyde, in Jiang, Phookamsak, Xu, Karunaratna, Mortimer and Hyde, Mycol. Progr. 19: 47 (2020); Type: The Philippines, Mt. Makiling, S. A. Reyes 3367a, (S-F48343).

**Notes**
Mendogia calami was recently introduced from leaves of Calamus sp. in the Philippines (Jiang et al. 2020). However, there are no discernible differences between M. philippinensis and M. calami, neither in phenotype nor in substrate ecology. Jiang et al. (2020) did not discuss M. philippinensis when establishing M. calami and the difference implied in the table and key (ascostroma size, number of longitudinal septa) are either due to age (ascostroma size) or they are non-existent (the ascospores of M. calami have mostly one, rarely two longitudinal septa in the photographs and the protologue of M. philippinensis also indicates mostly one longitudinal septum) (Sydow and Sydow 1917). This synonymy needs further testing with molecular data as previous studies on palms have shown that the taxa on different palm species differ (Konta et al. 2016, Konta et al. 2017) as they may have derived from endophytes.

**Mendogia bambusina** Racib., Parasit. Alg. Pilze Java’s (Jakarta) 3: 31 (1900)

**Nomenclature**
Syn. nov.: Uleopeltis bambusina Syd. & P. Syd., Annls mycol. 12(6): 565 (1914)

*Ital. 1 (Fasc. 3): 159 (1862). Type: The Philippines, Luzon, Bulacan Prov., Angat, 1913, M Ramos, Bur. Sci. 21852 (GZU, S-F5988).*
Notes

_Uleopeltis_ was introduced to accommodate _U. manaosensis_ and later the second species _U. bambusina_ added to this genus (Hennings 1904, Dai et al. 2017). _Uleopeltis manaosensis_ was synonymised under _Mendogia_, while _U. bambusina_ remained in _Uleopeltis_ which was collected from bamboo culms in the Philippines (Hennings 1904, von Arx and Müller 1975, Dai et al. 2017). The species lacks molecular data and shares similar morphological characteristics with the type species of _Mendogia_ (von Arx and Müller 1975). Dai et al. (2017) gave spores of the type material of _Mendogia bambusina_ as 13.5–25 × 5–8 μm, but mature ascospores in the photographs are 15–21 × 7–9 μm. Raciborski (1900) gave the ascospores as 17–19 × 8 μm for _M. bambusina_. This supports the assessment of von Arx and Müller (1975) that _M. bambusina_ and _Uleopeltis bambusina_ are conspecific. The synonymisation is formalised here. The report of _M. bambusina_ from Brazil on palm leaves (Vitória 2012) has been documented with morphological and anatomical photographs and agrees well with the material from the Paleotropics. The African _Pleiostomella halleriae_ (Doidge 1921) will also key out close to _M. bambusina_ and may represent another synonym. It is the only other species described in _Pleiostomella_, a synonym of _Mendogia_, but has apparently never been dispositioned. Unfortunately, no type was indicated and a total of six collections on two host species (leaves of _Halleria elliptica_ and _H. lucida_) were listed. The ascus and ascospore dimensions (50–70 × 20–33 μm; 22–24 × 9–10 μm) partly fit _M. bambusina_, but Doidge described two types of asci, one ovate and ca. 50 × 30 μm and the other clavate and ca. 65–70 × 20–25 μm. The latter fits _M. bambusina_, whereas the former does not conform to any of the species recognised here. Revision of all paratypes is necessary to assess the taxonomic status of this material (Sydow and Sydow 1917).

Identification keys

| Key to the species of _Mendogia_ |
|----------------------------------|
| 1. Ascomata scattered in dense, pseudostromatic, irregularly stellate groups over an effuse, thallus-like, dark structure, with thin covering layer, interascal hyphae forming distinct paraphysoids, asci 45–70 × 25–35 μm, ascospores 15–25 × 6–10 μm, on dead dicotyledonean leaves, Thailand | _Mendogia diffusa_ |
| 2. Ascomata one to many immersed in sharply delimited, rounded ascostromata, without associated thallus-like structure, interascal hyphae, asci and ascospores variable, on living bamboo culms or palm leaves |  |
| 2 | Ascospores narrowly oblong, transversely septate, 30–55 × 3.5–4.5 μm, interascal hyphae forming sparsely branched paraphysoids, asci cylindrical-clavate, 85–120 × 10–12 μm, Brazil | Mendogia manaosensis (≡ Uleopeltis manaosensis) |
| --- | --- | --- |
| - | Ascospores broadly oblong to somewhat tapering, muriform, interascal hyphae variable, asci broadly oblong to obclavate | 3 |
| 3 | Ascostromata with distinct chambers appearing peritheciiform in cross section, but forming dense, concentric structures, with the asci in a single layer formed at the bottom of the chambers (type II), interascal hyphae forming more or less distinct paraphysoids, asci 45–55 × 16–20 μm, ascospores 14–18 × 5–6.5 μm, on living palm leaves, Philippines | Mendogia philippinensis (≡ Pleiostomella philippinensis) (≡ Mendogia calami) |
| - | Ascostromata indistinctly chambered (arthothelioid) or asci in concentric structures mostly towards the periphery, with the asci irregularly dispersed in irregular layers (type I), on bamboo culms (rarely on palm leaves) interascal hyphae forming indistinct paraphysoids or textura angulate | 4 |
| 4 | Interascal hyphae forming indistinct paraphysoids, asci developing in concentric structures mostly towards the periphery, 17–25 μm broad, ascospores 15–28 × 7–11 μm, without gelatinous caps, on bamboo culms or palm leaves, USA, Brazil, Indonesia, Philippines | Mendogia bambusina (≡ Uleopeltis bambusina) |
| - | Interascal hyphae forming a textura angulata, asci and ascospores variable | 5 |
| 5 | Ascostromata 5–20 mm diam., asci 70–85 × 28–35 μm, ascospores 20–27 × 9–11 μm, without gelatinous sheath or caps, on bamboo culms, Thailand | Mendogia macrostroma |
| - | Ascostromata 1–5 mm diam., asci and ascospores variable in size, but ascospores with thin gelatinous sheath and distinct gelatinous caps | 6 |
| 6 | Ascii 55–75 × 25–30 μm, ascospores 19–23 × 8–11 μm, on bamboo culms, China | Mendogia yunnanensis |
| - | Ascii 75–165 × 30–40 μm, ascospores 25–35 × 12–16 μm, on bamboo culms, Thailand | Mendogia chiangraiensis |
Analysis

Phylogenetic analyses

The genera of Myriangiaceae were well recovered, as studied in Jiang et al. (2020). The final alignment comprised 50 strains including the new strain and 2469 nucleotide positions. The topologies of the single gene markers tree and the tree topology obtained from the combined five-locus (SSU, LSU, ITS1, 5.8S, ITS2) dataset were congruent. Our phylogenetic analyses supported the placement of Mendogia diffusa within Mendogia. The average standard deviation of split frequencies at the end of total MCMC generations was calculated as 0.0024 in the Bayesian analysis.

Discussion

Mendogia has previously been recorded from monocotyledons, but, in the present case, was collected on a dicotyledon, indicating many more species are likely to be discovered. Other species currently recognised in Mendogia (see key above) differ from the new species in the sharply delimited ascostroma (Dai et al. 2017, Jiang et al. 2020), which renders the diffusely delimited ascomata (Fig. 1) as the most diagnostic feature of M. diffusa. In terms of ascospore size, M. bambusina, M. macrostroma and M. yunnanensis are closely related to M. diffusa. Apart from the sharply delimited ascostromata and the usually bambusicolous habit of all three species, M. bambusina has narrower asci and M. macrostroma differs in the much larger ascostromata (Raciborski 1900, Dai et al. 2017, Jiang et al. 2020). The internal anatomy of the ascomata of M. diffusa is also distinctive, with easily discernible paraphysoids (Fig. 2). Mendogia manaosensis and M. philippinensis (= M. calami) also form paraphysoid-like interascal hyphae, whereas in M. bambusina, these are less distinctive and, in M. chiangraiensis, M. macrostroma and M. yunnanensis, the interascal hyphae form a textura angularis (Raciborski 1900, Hennings 1904, Sydow and Sydow 1917, von Arx and Müller 1975, Dai et al. 2017, Jiang et al. 2020). This variation in morphology and internal anatomy of such closely-related species is remarkable, especially given that, in our phylogenetic analysis, M. diffusa and M. chiangraiensis formed a sister clade to M. macrostroma and M. yunnanensis (Fig. 3), although without support. The new taxon shows more than 2% nucleotide differences in the ITS region compared to other Mendogia species. This, along with the discussed morphological differences, supports recognition as a new species (Jeewon and Hyde 2016). Unfortunately, DNA sequences are lacking for three of the seven recognised species in the genus: M. bambusina, M. manaosensis and M. philippinensis (= M. calami). Mendogia diffusa should not be confused with the superficially similar Diplotheca tunae in the same family (Dissanayake et al. 2014). The latter also forms ascomata scattered in dense groups instead of sharply delimited ascostromata, but differs in the broad, globose asci and the much thicker covering layer of the ascomata.

Mendogia diffusa was found on dead leaves and the fungal structures penetrate the upper epidermis of the leaf surface, turning the epidermal cells into a dark pigmented layer (Fig. 2). Such dark pigmented cells are absent where the ascomata are not observed.
Some ascostromata observed were found to loosely associate with algal colonies (Fig. 1). The algae are probably trentepohlioid, 3–5 µm thick, rounded to slightly elongate and greenish. However, since these are absent from most of the ascostromata and no closer anatomical associations or penetration structures were detected, we assume that this association is opportunistic, the algae is taking advantage of the microrelief formed by the ascostromata to colonise the otherwise smooth leaf surface. While the ascostromata were detected on dead leaves, it is unclear whether the fungus is also present on living leaves and how common is the observed opportunistic association with algae. It is possible that *M. diffusa* indirectly benefits from the presence of the algae as an additional carbon source, through leaching or by decomposing dead algal cells. Similar cases of loose associations have been reported from saxicolous biocoenoses where rock-inhabiting fungi are often growing together with algae or cyanobacteria (Muggia et al. 2013). Muggia et al. (2016) found alpine rock lichens to be associated with members of *Myriangiales*.

**Acknowledgements**

We thank the Thailand Research Fund (“The future of specialist fungi in a changing climate: baseline data for generalist and specialist fungi associated with ants, *Rhododendron* species and *Dracaena* species DBG6080013” and “Impact of climate change on fungal diversity and biogeography in the Greater Mekong Sub-region RDG6130001”) and “The 2019 high-end foreign expert introduction plan to Kunming Institute of Botany (granted by the Ministry of Science and Technology of the People’s...
Republic of China (Grant Number G20190139006)) for funding this research. Kevin D. Hyde thanks Chiang Mai University for the award of Visiting Professor. S.C. Karunarathna would like to thank the CAS President’s International Fellowship Initiative (PIFI) young staff under grant number: 2020FYC0002 and the National Science Foundation of China (NSFC) under the project code 31851110759, the CAS President’s International Fellowship Initiative (PIFI) under the following grant: 2018PC0006 and the National Science Foundation of China (NSFC, project code 31851110759). We also thank Udeni Jayalal, Nalin Wijayawardene, Diana Sandamali and Danushka Sandaruwan for their support during this research. We thank Shaun Pennycook, for helping in the nomenclature. Dhanushka Wanasinghe would like to thank CAS President’s International Fellowship Initiative (PIFI) for funding his postdoctoral research (number 2021PC0008), the National Science Foundation of China and the Chinese Academy of Sciences for financial support under the following grants: 41761144055, 41771063 and Y4ZK111B01.

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