รายงานวิจัยฉบับสมบูรณ์

โครงการ
การศึกษาโรคราดำของไม้ผลในเขตภาคเหนือของประเทศไทย

โดย
ดร. พุทธรักษ์ ชมนันติ

มิถุนายน 2559

สัญญาเลขที่TRG5780008

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัยและต้นสังกัด

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Investigator: ดร. พุทธรักษ์ ชมนันติ มหาวิทยาลัยแม่ฟ้าหลวง

E-mail Address: putarak.cho@mfu.ac.th

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Abstract (บทคัดย่อ)

Sooty moulds are plant pathogens that have ecology complex with host and insect, they are produce black mycelium which covers plants and interfere with the photosynthetic capability, causes plant stunting and yield and thereby reduce the marketability of post-harvest products. In view of high diversity, complex ecology of growing, mixing together on the host and lack of proper taxonomic records, the relationships of sooty moulds are poorly known also the sexual-asexual connections. In this study we emphasis on the taxonomy and molecular studies to resolve their life cycle complex, established xerophlic property and bioactive compound. We obtain 80 strains of sooty mould species from difference geographical regions and difference hosts; *Mangifera* sp., *Chrysophyllum cainito*, *Psidium* sp. and *Cofea* sp., from Chiang Rai, Chiang Mai, Payao and Songkla Province. Eighth genera were identified based on morphological characters including of *Phragmocapnias*, *Phaeosaccardinula*, *Capnodium, Leptoxyphium, Polychaeton*, and species in *Trichomeriaceae* and *Chaetothyriaceae* respectively. Six insect types have been found together with sooty moulds habitat included of soft scale insect, whitefilies, mealybugs, acarid, aleyrodid and aphid. Totally 30 morphological characters were prepared in photo plates with descriptions. Three sooty mould fungi; *Leptoxyphium cacuminum* (MFLUCC 14-0276), *Capnodium coffeae* (MFLUCC 14-0182), *Phragmocapnias siamensis* (MFLUCC 14-0277) were selected to present a test for verify sooty moulds are xerophilic and will be analysesed by ANOVA and linear growth rate. Thus, the sooty moulds are Xerophilic fungi and such knowledge will contribute to the future work of secondary metabolism and may have biotechnological potential in enzyme production or bioremediation. The holotypes were deposited at the herbarium of Mae Fah Luang University (MFLU). Fungi isolated by single spore technique were used for molecular work, and deposited in in Mae Fah Luang university Culture collection (MFLUCC) and BRIP culture collection. Genomic DNA was extracted from fungal mycelium using Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) according to the manufacturer's protocol for maximum yield and amplified by using PCR technique. Five primers including ITS, LSU, SSU TEF and RPB2 genes were used in this process. The RAxML phylogenetic tree was contributed and sequences data were deposited in

GenBank. Three poster presentations were presented in IMC 2014 and three SCI papers were published.

Keywords: Biodiversity, *Capnodiaceae* phylogeny, sooty moulds, Taxonomy

โรคราดำเป็นโรคพืชที่พบได้ทั่วไปในเขตร้อนและเขตกึ่งร้อน มีความหลากหลายสูงและสัมพันธ์กับพืชและแมลงหลายชนิด ลักษณะเฉพาะของเชื้อราดำคือ จะสร้างเส้นใยปกคลุมบริเวณผิวใบและผลของพืชอาศัย ลักษณะดังกล่าวส่งผลต่ออัตราการสังเคราะห์แสงของพืชลดลง ปัจจุบันการศึกษาทางด้านอนุกรมวิธานและลักษณะสัณฐานวิทยาของเชื้อราดำยังไม่มีความชัดเจน อีกทั้งไม่มีความหลากหลายในด้านวงศ์วานวิวัฒนาการ ดังนั้นวัตถุประสงค์ของการวิจัยนี้คือเพื่อศึกษาถึงอนุกรมวิธานและวงศ์วานวิวัฒนาการโดยใช้ลักษณะสัณฐานวิทยาและชีวโมเลกุลในการจัดจำแนกและการวิเคราะห์ความสามารถของเชื้อราในสภาพที่ไม่เหมาะสมต่อการเจริญหรือขาดน้ำโดยวิธี Xerophilic จากการศึกษาดังกล่าวสามารถรวบรวมเชื้อราดำได้จำนวน 80 ตัวอย่างจากพืชอาศัยที่หลากหลายได้แก่ *Mangifera* sp., *Chrysophyllum cainito*, *Psidium* sp. และ *Cofea* sp ซึ่งพบได้ต่างพื้นที่เช่นใน จังหวัดเชียงราย เชียงใหม่และ สงขลา จากผลการวิจัยพบว่าจากการศึกษาลักษณะทางด้านสันฐานวิทยาร่วมกับการวิเคราะห์ทางด้านชีวโมเลกุลเชื้อราโดยทำการวิเคราะห์หาลำดับเบสของเชื้อราดำ โดยใช้ multi genes analysis ได้แก่การวิเคราะห์ลำดับเบสตำแหน่ง LSU, SSU, TEF, ITS และ RPB2 ตามลำดับ และสร้างแผนภูมิวิวัฒนาการโดยใช้โปรแกรม RAxML เพื่อทำการจัดจำแนกเชื้อราดำดังกล่าวได้อย่างแม่นยำและถูกต้อง ซึ่งสามารถจัดจำแนกตัวอย่างเชื้อราดำจำนวน 80 ตัวอย่าง ออกเป็น 8 สกุลคือ *Phragmocapnias*, *Phaeosaccardinula*, *Capnodium, Leptoxyphium, Polychaeton* และพบว่ามีเชื้อราดำบางชนิดที่ถูกจัดอยู่ในวงศ์ *Chaetothyriaceae* และ *Trichomeriaceae* ทั้งนี้ยังพบแมลงทั้งหมด 6 ชนิด ที่มีความสัมพันธ์กับเชื้อราดำได้แก่ เพลี้ยหอย แมลงหวี่ขาว เพลี้ยแป้ง เพลี้ยอ่อน เป็นต้น จากการศึกษาด้านสัญฐานวิทยา รายงานฉบับนี้ได้จัดทำรูปภาพราดำและคำอธิบายถึงลักษณะเฉพาะของเชื้อราดำไว้อย่างละเอียดเพื่อสามารถนำไปใช้ในการจัดจำแนกเบื้องต้น นอกจากนี้ได้ทำการวิเคราะห์ความสามารถของเชื้อราในสภาพที่ไม่เหมาะสมต่อการเจริญหรือขาดน้ำโดยวิธี Xerophilic ผลการศึกษาเบื้องต้นถึงประสิทธิภาพในการผลิตเอนไซม์ พบว่าเชื้อราดำ *Leptoxyphium cacuminum* (MFLUCC 14-0276), *Capnodium coffeae* (MFLUCC 14-0182), *Phragmocapnias siamensis* (MFLUCC 14-0277) สามารถเจริญได้ในสภาวะที่ขาดแคลนน้ำและไม่เหมาะสม ดังนั้นผู้วิจัยจึงเลือกศึกษาประสิทธิภาพในการสร้างเอนไซม์ในเชื้อราดำทั้งสามชนิดในขั้นตอนต่อไป ซึ่งผลดังกล่าวยังสามารถพัฒนาและนำไปใช้ประโยชน์ทางด้านอุตสาหกรรมและการบำบัดทางชีวภาพ จากการวิจัยครั้งนี้ทำให้สามารถจัดจำแนกเชื้อราดำได้อย่างถูกต้องและมีข้อมูลพื้นฐานทางด้านสัณฐานวิทยา ซึ่งสามารถนำไปใช้ประกอบการศึกษาและวินิจฉัยเชื้อราดำได้ อีกทั้งเพิ่มข้อมูลที่ทางด้านลำดับเบสของ DNA ใน GenBank ซึ่งเป็นข้อมูลที่เป็นประโยชน์ต่อการวิจัยทางด้านโรคพืชและอนุกรมวิธานของเชื้อรา นอกจากนี้ผู้วิจัยได้เก็บรักษาสายพันธุ์เชื้อราดังกล่าวใน MFLU culture collection (MFLUCC), Biotech Culture Collection (BCC), และ BRIP culture collection เพื่อให้นักวิจัยและผู้ที่สนใจศึกษานำไปใช้ประโยชน์ต่อไป ผลงานของโครงการวิจัยนี้ได้มีการเผยแพร่ในการประชุมนานาชาติในรูปแบบของโปสเตอร์จำนวน 3 ผลงาน และในรูปแบบของเอกสารตีพิมพ์ในวารสารนานาชาติจำนวน 3 ผลงาน

คำสำคัญ: ความหลากหลายทางชีวภาพ, วงศ์วานวิวัฒนาการ, ราดำ, อนุกรมวิธาน, *Capnodiaceae*

Executive Summary

Over 24 months period we carried on the taxonomy of sooty moulds research based on morphology and molecular technique to identified sooty moulds from various host and identification of the species (Table 1). 38 sooty moulds DNA have been extracted from fresh fungal mycelium using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China) following the instructions of the manufacturer (Hangzhou, P.R. China). and phylogenetic analyses were based on partial sequences of five loci, internal transcribed spacers (ITS), small subunit rDNA (SSU), large subunit (LSU), translation elongation factor 1-alpha gene (TEF 1α) and second largest subunit of RNA polymerase II (RPB2). Fresh samples of sap feeding insects associated with sooty moulds are mostly found in the family *Capnodiaceae* which is largest family, *Chaetothryiaceae* and *Trichomeriaceae* were also found on the surface of various host in Northern Thailand and Southern China. Three sooty mould fungi; *Leptoxyphium cacuminum* (MFLUCC 14-0276), *Capnodium coffeae* (MFLUCC 14-0182), *Phragmocapnias siamensis* (MFLUCC 14-0277) were selected to present a test for verify sooty moulds are xerophilic and will be analyzed by ANOVA and linear growth rate. Thus, the sooty moulds are Xerophilic fungi and such knowledge will contribute to the future work of secondary metabolism and may have biotechnological potential in enzyme production or bioremediation. Sooty moulds are a morphologically diverse group, associated with various host and insect, and a study on evolution based on molecular data is essential to infer phylogenetic relationships between main groups of fungi and to recognize taxa towards a natural classification. The relationship among plant, insect and fungi is playing an important role in plant disease control. During this time, specimens were observed under microscope and isolated by single spore isolation. Cultures were observed the principle possibility to secret enzymes or basic chemical on agar by agar changed color. Cultured were deposited in MFLUCC and BRIP culture collection, and the herbarium in MFLU herbarium for future study on taxonomy and secondary metabolite. The sequences which have been identified were contributed to GenBank. During 24 months the results of sooty mould were published in three SCI journal and three poster presentation in international conferences “The 10th International Mycological Congress”(see index Over the previous 6 months, we have completed the characterization of two new species of sooty moulds, leading to the peer-reviewed publication “Hongsanan S, Tian Q,

Hyde KD, Chomnunti P (2015) Two new species of sooty moulds, *Capnodium coffeicola* and *Conidiocarpus plumeriae* in *Capnodiaceae*. Mycosphere. Vol. 6(6), 814-824. Doi 10.5943/mycosphere/6/6/14. This paper contributes to our continual understanding of the natural diversity of sooty moulds fungi. Moreover we contributed the network and have collaborated with Chinese Academy of Sciences, Beijing on the study of molecular clock and phylogeny of sooty moulds. We have collaborated with Dr. Narit Thaochan; entomologist at Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University to identified insect. Furthermore, discussions have taken place with Prof Craig Faulds, Director of the INRA-Aix Marseille University "Biodiversity and Biotechnology of Fungi" laboratory in Marseille, France, on future joint research on exploiting the bioactivity properties of sooty mould fungi and their metabolites.

Objectives

1. To classify various sooty moulds from fruit tree by morphology and molecular analysis.
2. To studies the relationship of asexual and sexual statesof sooty moulds growing in complex colonies on host surface
3. To establish xerophilic property of sooty moulds
4. To establish the antagonistic of sooty moulds and the fungi can then be screened for novel metabolites in a future study.
5. To establish the specific relationship of sooty moulds with insects and/or plant hosts

Methodology

1. Collecting and isolation of sooty moulds

Random collections of the sooty moulds from orchards of fruit trees in Northern Thailand will be made. Collected material will be placed in Zip-locked plastic bags and processed quickly in the laboratory. Sections of ascomata will be made free hand and mounted in lactophenol, and morphology characteristics and measurements were made in water under a microscope.

Isolation, using singles spores, will be made to obtain the pure cultures. First, spore masses are transferred with a sterilized wire loop or fine forceps and suspended in sterilized water. The spore suspension is diluted to a reasonable concentration and spread onto the surface of PDA agar, followed by incubation overnight at room temperature (25°C). Single germinated spores are picked up with a sterilized needle and transferred onto new PDA plate for morphological or molecular study (Chomnunti et al., 2011). Isolates will be grown on 2% difco potato-dextrose agar (PDA) at 28°C for 12 hr. of light/12 hr. of dark for routine maintenance. Colony colour and characteristics will be assessed after 2 weeks. Cultures are incubated at 20, 25 and 30°C under

constant fluorescent light. Three replicate cultures of each isolate will be maintained. After 7 days, conidial size and shape from >25 conidia will be measured and recorded, while colony characters be recorded and photographed (Than et al., 2008). Observation and measurements (e.g. conidial size, appressoria size and conidiogenous cells) will be made in water mounts. The holotype will be deposited at the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand. Fungi isolated in our study will be deposited at Mae Fah Luang University (MFLUCC) and BIOTEC culture collection (BCC).

2. Identification of host plants

The host plants are easy to identify in the field as they are fruit tress. Dried voucher specimens will be deposited in the Mae Fah Luang University herbarium. The insects will also be collected and identified with help from entomologists; Dr.Narit Thaochan at Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University.

3. DNA isolation, amplification and sequencing for fungi

DNA extraction : Selected fungal isolates will be grown on PDA for 21 d at 28 °C in the dark. Genomic DNA will be extracted from the fresh mycelium using Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) according to the manufacturer's protocol at molecular laboratory, Mae Fah Luang University.

PCR amplification and sequencing: The methods for PCR amplification and sequencing will follow those used in previous studies (Jeewon et al. 2002; Pang et al 2010). DNA amplification will be performed by polymerase chain reaction (PCR). Primer pairs NS1 and NS4 will be used to amplify a region spanning the small subunit rDNA. LROR and LR5 primer pairs will be used to amplify a segment of the large subunit rDNA. Primer pairs ITS5 and ITS4 will be used to amplify the internal transcribed spacers. Other primers for gene regions will be used as needed. The amplifications will be performed in a 50 μl reaction volume as follows: 1X PCR buffer, 0.2 mM d'NTP, 0.3 μM of each primer; 1.5 mM MgCl2, 0.8 units Taq Polymerase and 5–10 ng DNA (Cai et al. 2009). The amplification conditions will be as follows: initial denaturation of 5 min at 95 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 48 °C and 90 s at 72 °C, and a final extension period of 10 min at 72 °C. The PCR products will be checked on 1 % agarose electrophoresis gels stained with ethidium bromide.

PCR products will then be purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amersham product code: 27–9602–01). DNA sequencing of PCR products will be carried out by a reliable company or BIOTEC.

4. Phylogenetic analysis: The methods for phylogenetic analysis will follow those used in previous studies (Jeewon et al. 2002; Pang et al 2010). Sequences generated from different

primers will be analyzed with other sequences obtained from GenBank. A Blast search will be performed to reveal the closest matches with taxa in the various fungal groups. In addition, fungal members from different families and related orders will be included in the analyses. Sequences will be aligned using Bioedit and ClustalX v. 1.83. The alignments will be visually checked and improved manually where necessary. Phylogenetic analyses will be performed by using PAUP v. 4.0b10 for Maximum-parsimony (MP) and Neighbour-joining (NJ) analyses, MrBayes v. 3.0b4 for Bayesian analyses and MEGA 5 or RAMxL where needed. Trees will be rooted to appropriate outgroup taxa. Maximum-parsimony analyses will be performed using the heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters will be unordered and of equal weight and gaps will be treated as missing data. Maxtrees will be unlimited, branches of zero length will be collapsed and all multiple, equally parsimonious trees will be saved. Clade stability will be assessed using bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa. The model of evolution will be estimated by using MrModeltest 2.2. Posterior probabilities (PP) will be determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4. Six simultaneous Markov chains will be run for 1000000 generations and trees will be sampled every 100th generation (resulting in 10000 total trees). The first 2000 trees, representing the burn-in phase of the analyses, will be discarded and the remaining 8000 trees will be used for calculating posterior probabilities (PP) in the majority rule consensus tree. Phylogenetic trees will be viewed using Treeview. Sequences derived in this study will be deposited in GenBank, and the alignments in TreeBASE (www.treebase.org)

5. Testing whether sooty moulds are xerophilic

The linear growth rates of sooty mould strains will be assessed at 20, 25, 30 and 37°C on solid agar media containing a mixture of glucose and fructose to reduce aw to 0.94, 0.88, 0.84, 0.80, 0.76 and 0.66 (Leong et al. 2011) to establish if sooty moulds are xerophilic.

Results

Morphology of Sooty moulds

Basically sooty moulds will be occurring on various hosts and they are often present asexual and sexual morph on the same host, moreover more than in some case there are three species occur together. The relationship of plant and insect hosts and fungi are very important for this study (Fig.1). Asexual morph belong to Coelomycetes and Hyphotmycetes and sexual morph belong to Ascomycetes group especially in *Capnodiaceaeae* which is the large group of sooty moulds and *Chaetothyriaceae* or some of them belong to *Trichomeriaceae*. The morphology results of sooty moulds are presented in photoplate with descriptions.

Fig 1. Various of host plant with insects

***Leptoxyphium* sp.**

Saprobic on sugary exudates from insects growing on the surface of living leaves *Thallus*, brown to dark brown, septate, branched, irregular, superficial mycelium, constricted at the septa. *Pycnidia* 250–303 μ*m* high ($\bar{X}$ = 275 μ*m*, n=10), and 43–60 μ*m* at base ($\bar{X}$ = 52 mm, n=10), superficial, gregarious, arising from aggregated hyphae on the surface of host, bulbous base, comprising straight to flexuous hyphae, deeply pigment at the base. *Stalked pycnidia* comprising cylindrical hyphae, expanding at the end into a funnel-shape, resembling a cupula, 70–95 μ*m* high × 48–68 μ*m* wide ($\bar{X}$ = 84 × 55 μ*m*, n=10). *Conidiogenous cells* arising from the inner cell wall of the cupulate apex. *Conidia* 4.5–11 μ*m* high × 3–5.5 μ*m* wide ($\bar{X}$ = 6.5 × 3.5 μ*m*, n=10), hyaline, broadly ellipsoidal to ovoid, aseptate, guttulate.

Material examined: THAILAND, Chiang Rai Province, on living leaf of *Kaffir lime*, 14 December 2013, Qing Tian, MFLUCC 14-0189 (TS05, Fig. 2).

Fig 2. *Leptoxyphium* sp. a. Sooty moulds on the surface of host. b, c. Gregarious pycnidia on host surface. d, g-i. Stalked pycnidia with wider base. e-f. Brown stalked funnel cupulate apex, note conidiogenous boundary with hyaline hyphae surrounding the ostiole. j. Conidia. k. Germinating conidia. l. Colony on MEA from above. Scale Bars: b, c = 100 μ*m* , d = 50 μ*m*, e–i = 20 μ*m*, j–k = 10 μ*m*.

***Trichomerium* sp.**

Saprobic on sugary exudates from insects growing on the upper surface of living leaves. *Ascomata* 360–450 μ*m* diam ($\bar{X}$ = 410 μ*m*, n=10), scattered, superficial, subglobose to globose, cupulate when dry, dull black, lacking setae, with a central ostiole. *Peridium* 40–52 μ*m* wide ($\bar{X}$ =45 μ*m*, n=10), 2–layered with the spine on the surface, glabrous, comprising of brown to dark brown cells of *textura globulosa* at the outside, and flattened inwardly hyaline cells of *textura angularis*. *Pseudoparaphyses* lacking. *Asci* 130–210 μ*m* high × 37–39 μ*m* diam ($\bar{X}$ =175 × 38.5 μ*m*, n=10), 8-spored, bitunicate, pyriform to clavate or oboviod to ellipsoid, sessile or short pedicellate, early evanescent, lacking an ocular chamber. *Ascospores* 40–52 μ*m* high × 10–13 μ*m* diam ($\bar{X}$ = 48 × 11 μ*m*, n=10), overlapping 1–2- seriate, hyaline, 2–3-sepate, constricted at the septum oblong-ellipsoid, tapering at apex, wall- smoothed.

Material examined: THAILAND, Chiang Rai Province: Mae Fah Luang University, on living leaf of Mangifera sp., 31 January 2014, Chonticha Singtripop, MFLUCC 14-0176 (MFU01/1, Fig 3).

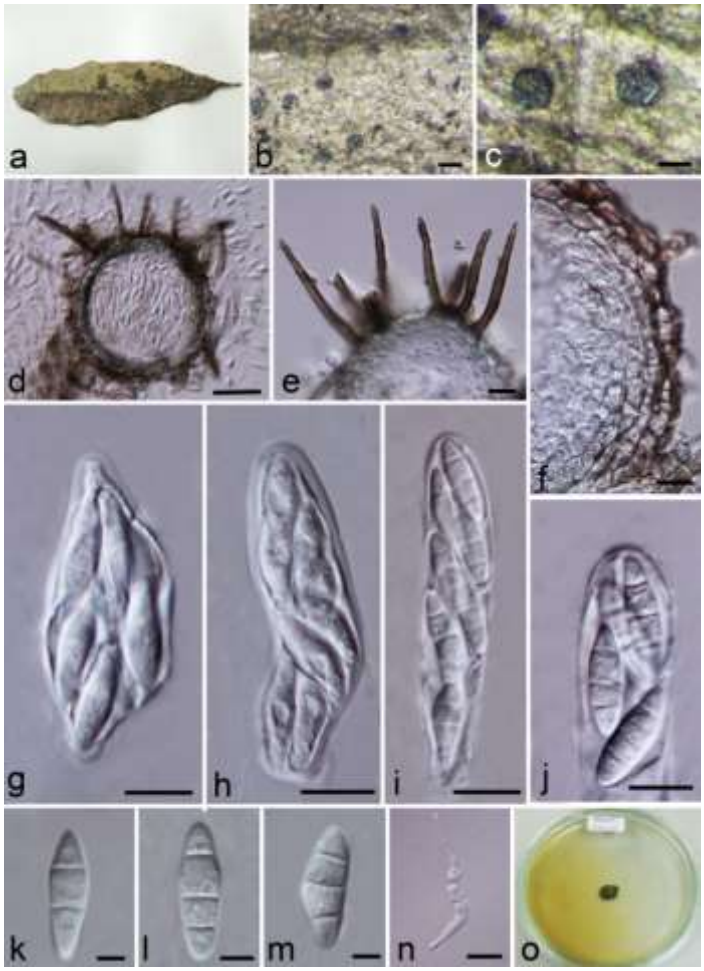

Fig 3. *Trichomerium* sp. a. Sooty moulds on the surface pf host. b, c. Ascomata on the surface of host, easily removed. d. Vertical hand section of ascoma. e, f. Vertical hand section through peridium. g-j. Asci with ascospores. k-m. Ascospores. n Germinating spore. o. Colony on PDA from above. Scale bars: b = 200 *μm*, c = 100 *μm*, d = 50 *μm*, e = 20 *μm*, f-j = 10 *μm*, k-m = 5 *μm*.

***Trichomerium* sp.**

Saprobic on upper surface of living guava leaves. *Thallus* thin, dark brown, composed of reticulately branched, dense, cylindrical to somewhat constricted, radiating, septate hyphae. *Ascomata* 115–164 *μm* high × 102–158 *μm* diam ($\bar{X}$ =136 × 133 *μm*, n=5), superficial, globose to broadly subglobose, dark brown to black, shiny, with several setae surrounded 106–176 × 5–8 *μm* ($\bar{X}$ =6 × 139 *μm*, n=10), dark brown, septate, tapering with rounded ends. *Peridium* thin, comprising cells arranged in a *textura angularis*. *Asci* 53–75 × 13–14 *μm* ($\bar{X}$ = 65 × 13 *μm*, n=10), 8-spored, bitunicate, ellipsoid to broadly fusiform, with ocular chamber. *Ascospores* 14–21 × 6–7 *μm* ($\bar{X}$ =20 × 6 *μm*, n=20), 2–4 overlapping seriate, broadly ellipsoid to obovoid, middle cell mostly

larger, straight to curved apically rounded end, surrounded by a mucilaginous sheath, hyaline and 3-septate observed in mature stage.

Material examined: THAILAND, Chiang Rai Province, Tar Sud district, on living leaf of *Psidium* sp., 3 February 2014, Chonticha Singtripop, (MRF 01, Fig. 4)

Fig 4. *Trichomerium* sp. a Disease symptom on leave. b Appearance of ascomata on host. c–d Vertical section through ascomata. e Peridium. f Setae. g Setae base. h–i Asci. j–l Ascospores surrounded by a mucilaginous sheath. Scale bars: c = 500 *μm*, d = 200 *μm*, e, f = 200 *μm*, g = 50 *μm*, h, i = 20 *μm*, j, k = 10 *μm*.

***Phragmocapnias* sp**

Habit on living leaves of host plant, covering the upper leaf surface with dark mycelium without penetrating host tissues. *Mycelial* mat subiculum-like, comprising hyphae that are mostly narrow, brown or brownish, dense, radiating outward, anastomosing at their tips with cells of the hyphal network. *Ascomata* superficial, solitary or scattered, pale brown to greenish brown, with setae, globose to subglobose, somewhat flattened when dry, covered by a subiculum or layer, composed of a few hyaline to subhyaline, septate hyphae. *Peridium* hyaline, cells brown to dark brown at the base of *textura angularis*. *Hamathecium* of cellular, hyaline, pseudoparaphyses. *Asci* eight-spored, bitunicate, clavate or pyriform, short pedicellate, mostly evanescent. *Asco- spores* overlapping 3–5-seriate, hyaline, cylindro- clavate, with 3–5 transverse septa, constricted at the septa, smooth-walled.

Material examined: THAILAND: Chiang Rai Province, on living leaf of Unidentified plant, Chayanard Phukhamsakda (STC 003).

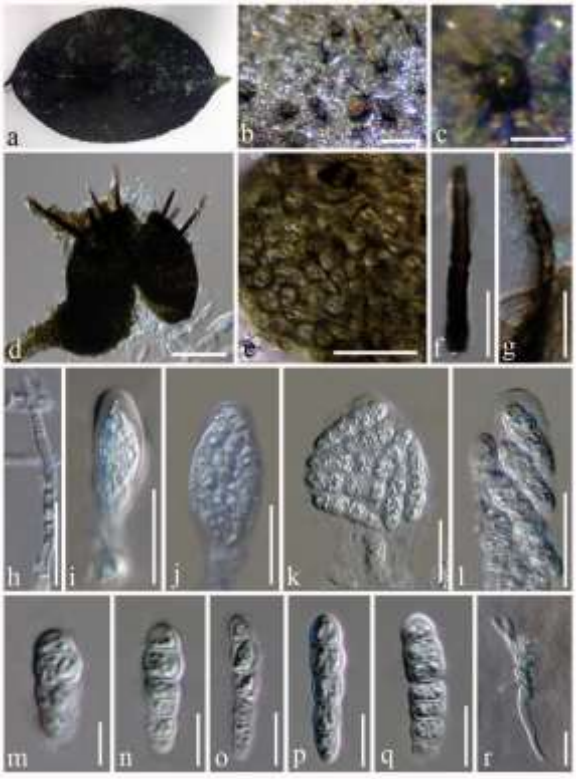

Fig 5 *Phragmocapnias* sp. a. Black mycelium covering the leaf surface. b, c, d. Appearance of ascomata on host. f, g. Stalked pycnidia. i-l. Ascospores surrounded by a mucilaginous sheath. m-q. Ascospores. r. Germinating ascospore. Scale bar: b = 200 µm, c = 100 µm, d = 50 µm, e–l = 20 µm, m-r = 10 µm

Habit forming a soot-like coating on the upper surface of leaves. *Thallus* superficial, consisting of a network of cylindrical and septate, thick, hyphae, constricted at the septum, pale brown. *Pycnidia* thick at base, arising from capnodiaceous type hyphae, black at the base. *Stalk* long, the conspicuous oval swelling which produce conidia, brown, comprising of cylindrical septate cells. *Conidiogenous cells* formed in the inner cells of the oval part. *Conidia* oblong to ellipsoid, 1-celled, hyaline, rounded ends

Material examined: THAILAND: Chiang Rai Province, on living leaf of Unidentified plant , Chayanard Phukhamsakda (STC 004).

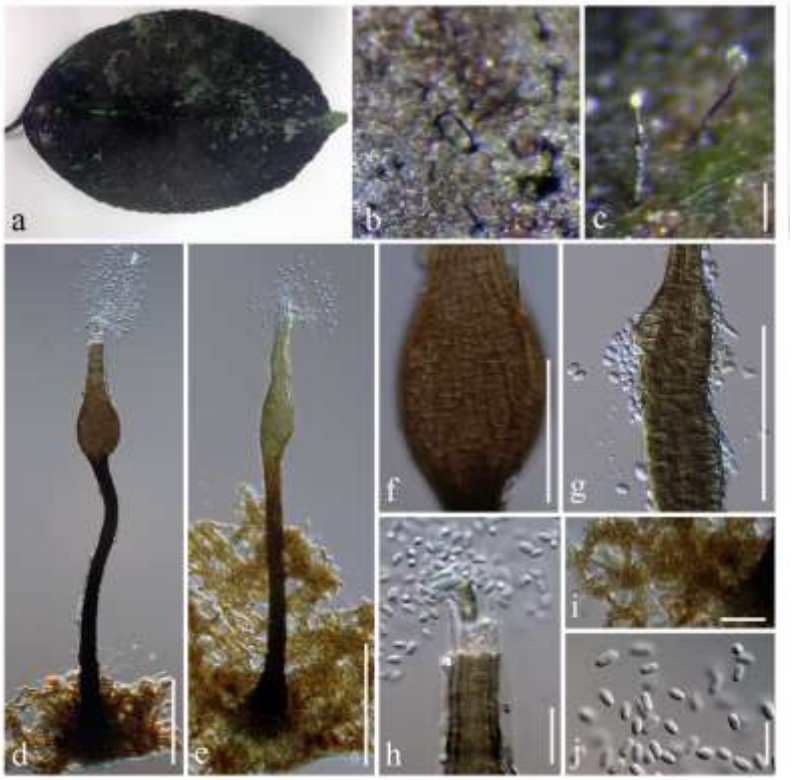

Fig 6 *Phragmocapnias* sp. a. Black mycelium covering the leaf surface. b, c. Pycnidia on the surface of host. e-f. Conical pycnidia and pycnidia wall. d-e. Stalked pycnidia with wider base. h. stalked funnel shaped at apex. f-g. stalked funnel shaped at apex. j. Conidia. Scale bar: c-e = 100 µm, f-g = 50 µm, h-j = 10 µm

***Polychaeton* sp.**

Saprobic on sugary exudates from insects growing on host. *Thallus* comprising 3.8–5.5 µm wide ($\bar{X}$ = 4.7 mm, n = 10) wide, black, dense, septate mycelium. Sexual state: *Ascomata* 36–59 µm diam, 89–132 µm high ($\bar{X}$ = 52 × 110 mm, n=10), covering the surface of thallus, gregarious, dark brown to black, shiny, subglobose to broadly ellipsoidal, with a rounded apex. Peridium composed of cells of *textura angularis*. *Asci* 28.5–33.2 × 6.8– 12.5 µm ($\bar{X}$ = 30.5. × 9.7 µm, n=10), 8-spored, bitunicate, oblong, apedicellate, apex with a long ocular chamber. *Ascospores* 13.5–17.2 × 4.2–5.8 µm ($\bar{X}$ = 16.5 ×5.2 µm, n=10), hyaline, fusiform, 1–2 transverse septate, upper cells slightly wider than the lower cells. Asexual state: *Pycnidia* 136–294 × 20–37 ($\bar{X}$ = 250 × 32 µm, n=10), long stalked, tapering to the apex, frequency with immature ascomata, dark brown to black at the base, brown to pale brown towards the tapering apex. *Conidia* 3.1–4.2 × 1.6–2.4 ($\bar{X}$ =3.7 × 2 µm, n=10), ellipsoidal, unicellular, hyaline, ends rounded.

Material examined: THAILAND, Doi wawee, Chiang Rai Province, Tar Sud district, on living leaf of *coffee* sp., 12 May 2014, Qing Tian (TQ14015).

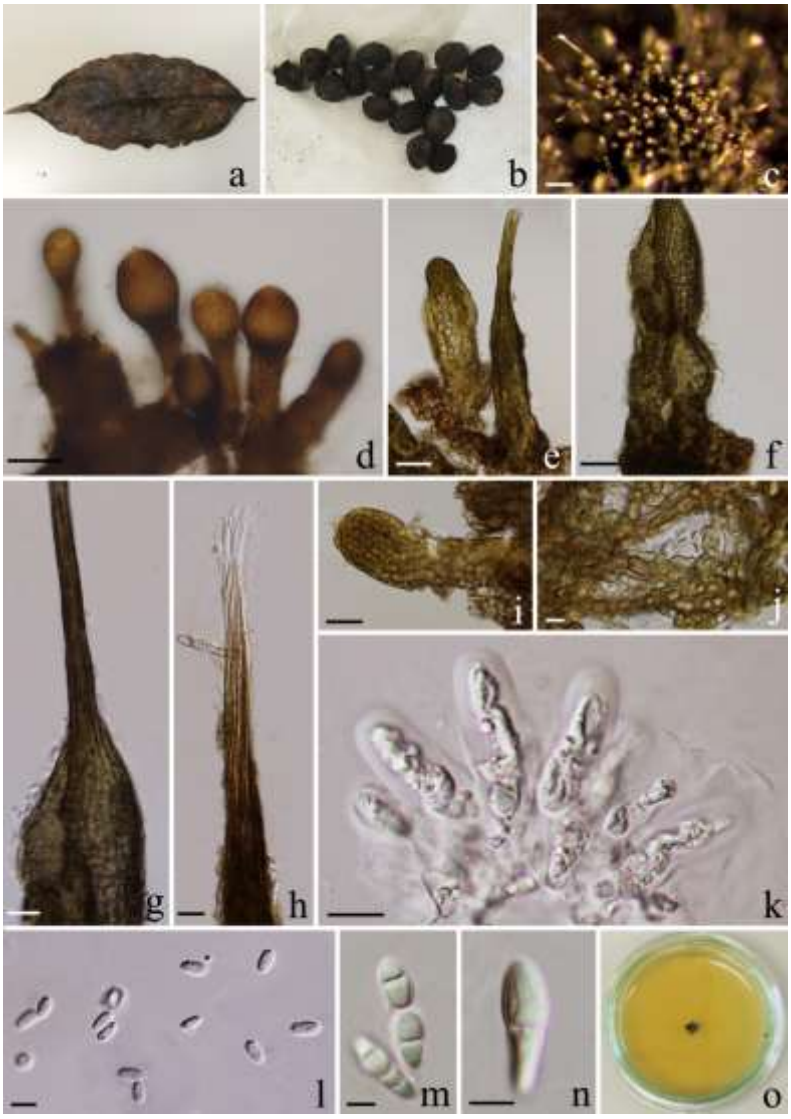

Fig. 7. *Polychaeton* sp. a, b. Sooty mould on the surface of leaves and seeds. c. Ascomata and pycnidia on surface of host. d, i. Matured ascomata. e-h, Pycnidia. j. V Mycelial network. k. Asci with ascospores. l. Conidia. m-n. Ascospores. o. Colony on MEA from above. Scale bars: c = 100 μm, d = 50 μm, e-f, i = 25 μm, g-h, k = 10μm, j, l-m = 5μm

***Polychaeton* sp.**

Saprobic sugary exudates from insects growing on coffee leaves. Thallus of dark brown mycelium growing on the surface with abundant pycnidia, produced on 3–5 μm wide ($\bar{X}$ = 4 μm, n = 10), irregularly branched, pale brown to brown, septate, cylindrical , constricted at the septum. *Pycnidia* 332–401 × 34–56 μm ($\bar{X}$ = 366 × 45 μm, n = 10), superficial, scattered or gregarious,dark brown, ovoid to flask–shaped, elongate, with slightly swollen or flattened base, ostiole surrounded by hyaline hyphae. *Conidia* 4.2–4.6 × 1.9–2.4 μm ($\bar{X}$ = 4.4 × 2.1 μm, n = 10),

produced within the swollen base, gathering in a terminal droplet, ellipsoidal, smooth, round ends, hyaline .

Material examined: THAILAND, Doi pui, Chiang Rai Province, on living leaf of *coffee* sp., 17 June 2014, Qing Tian (MFLUCC14-0610).

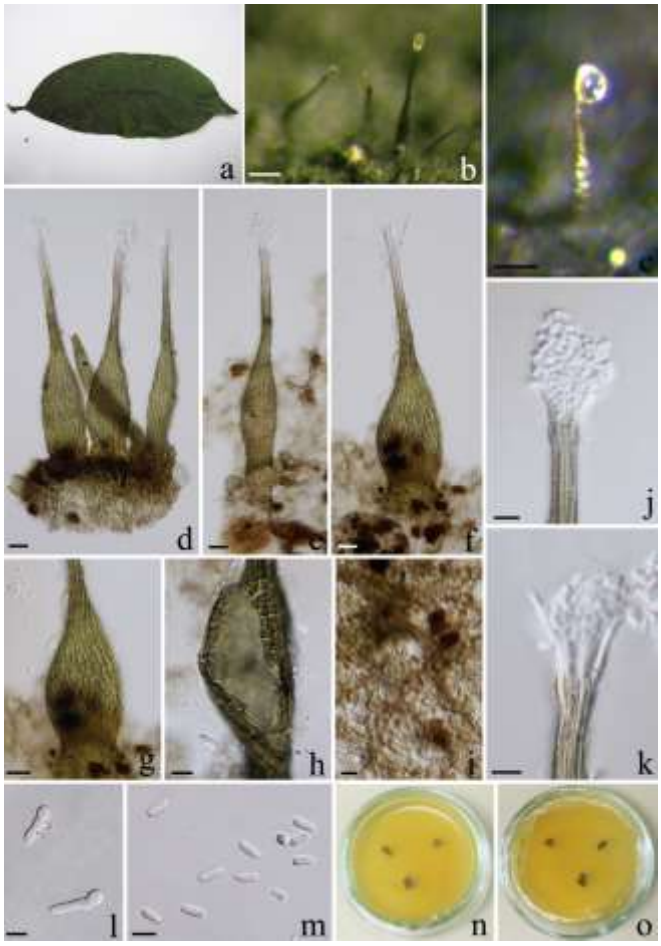

Fig. 8. *Polychaeton* sp. a. Black mycelium covering the leaf surface. b, c. Pycnidia on the surface of host. g-h. Conical pycnidia and pycnidia wall. i Mycelial network. d-f. Stalked pycnidia with wider base. j-k. stalked funnel shaped at apex. l. Germinating conidia. m. Conidia. n. Colony on MEA from above. o. Colony on MEA from blow. Scale Bars: b, c = 100 μm, d-h = 20 μm, j, k, i = 20 μm, l-m = 5 μm.

***Phragmocapnias* sp.**

Saprobic on sugary exudates from insects growing on the surface of living leaves. *Thallus* light brown, superficial, septate, broadly cylindrical to oval, branched, irregular, constricted at the septa and pale brown. *Pycnidia* 420–530 μm high ($\bar{X}$ = 495 μm, n=10), and 95–125 μm at base ($\bar{X}$ = 112μm, n=10), brown, superficial, gregarious, arising from aggregated hyphae on the surface of host, comprised of cylindrical septate cells of flexuous hyphae, deeply pigment at the base. *Stalk* comprising cylindrical hyphae, expanding at the end into oval part produces conidia, 65–96 μm

high × 45–52 μm wide ($\bar{X}$ = 87 × 47 μm, n=10). *Conidiogenous cells* arising from the inner cell wall of the oval part. Conidia 4.5–5.2 μm × 2.5–3.2 μm ($\bar{X}$ = 4.9 × 3.9 μm, n=10), ellipsoidal to ovoid, hyaline, aseptate, smooth walled, ends rounded.

Material examined: THAILAND, Doi Pui, Chiang Rai Province, on living leaf of *coffee* sp., 17 June 2014, Qing Tian (MFLUCC14-0611).

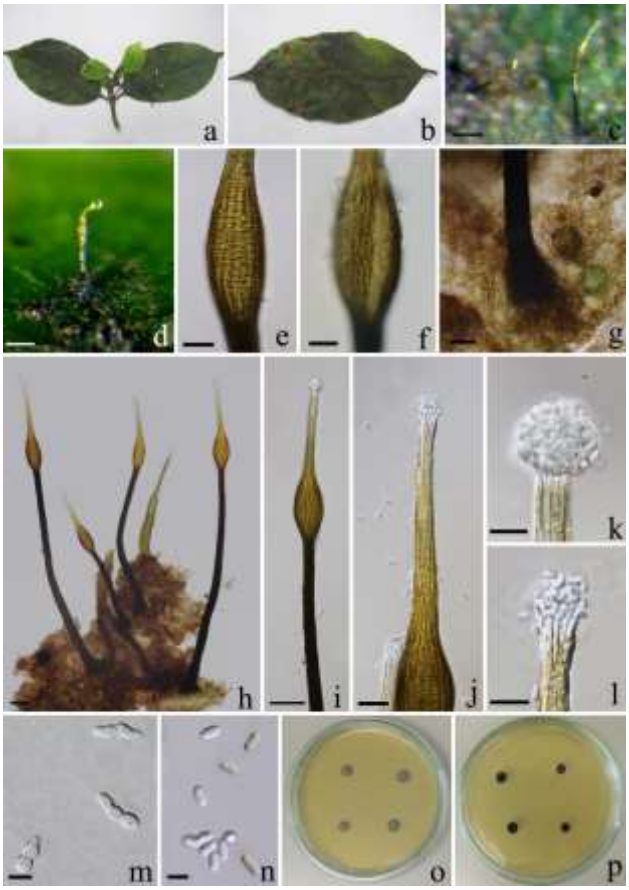

Fig. 9. *Phragmocapnias* sp. a, b. Black mycelium covering the leaf surface. c, d. Pycnidia on the surface of host. e-f. Conical pycnidia and pycnidia wall. h-j. Stalked pycnidia with wider base. j-k. stalked funnel shaped at apex. k-l. stalked funnel shaped at apex. m. Germinating conidia. n. Conidia. o. Colony on MEA from above. p. Colony on MEA from blow. Scale Bars: c, d = 100 μm, h-j = 50 μm, e-g = 20 μm, k-l = 10 μm, m-n = 5 μm

Molecular and phylogeny results of sooty moulds.

PCR amplification and DNA sequencing analysis on 5 loci; LSU, SSU, ITS, TEF1 and RPB2 regions and DNA sequences are showed in Table 1.

Table 1. Molecular results of 38 sooty mould strains

No.	MFLUCC	Host	Regions				
			LSU	SSU	ITS	TEF1	RPB2
1	15-0230	*Mangifera* sp.	x	x	x	x	/
2	15-0204	*Plumeria* sp.	x	x	x	x	/
3	15-0205	*Plumeria* sp.	x	x	x	x	/
4	15-0206	*Coffae* sp.	x	x	x	x	/
5	15-0218	*Coffae* sp.	x	x	x	x	/
6	15-0228	*Ficus* sp.	x	x	x	x	/
7	15-1084	*Syzygium* sp.	/	/	x	x	x
8	15-1085	*Citrus maxima*	/	/	x	x	x
9	15-1087	*Coffae* sp.	/	/	x	x	x
10	15-1088	*Lansium domesticum*	/	/	x	x	x
11	15-1089	*Lansium domesticum*	/	/	x	x	x
12	15-1091	*Syzygium aqueum*	/	/	x	x	x
13	15-1093	*Mangifera sp.*	/	/	x	x	x
14	15-1095	*Artocarpus heterophyllus*	/	/	x	x	x
15	15-1096	*Artocarpus heterophyllus*	/	/	x	x	x
16	15-1090	*Syzygium aqueum*	/	/	x	x	x
17	15-1094	*Artocarpus heterophyllus*	/	/	x	x	x
18	14-0175	*Mangifera* sp.	x	x	x	x	x
19	14-0176	*Mangifera* sp.	x	x	x	x	x
20	14-0282	Unknown	x	x	x	x	x
21	14-0280	*Psidium* sp.	x	x	x	x	x

22	14-0608	Unknown	x	x	x	x	x
23	14-0609	*Coffae* sp.	x	x	x	x	x
24	14-0610	Unknown	x	x	x	x	x
25	14-0611	*Coffae* sp.	x	x	x	x	x
26	14-0569	*Coffae* sp.	x	x	x	x	x
27	14-0570	*Coffae* sp.	x	x	x	x	x
28	14-0571	*Coffae* sp.	x	x	x	x	x
29	14-0572	*Coffae* sp.	x	x	x	x	x
30	14-0801	Unknow	x	x	x	x	x
31	14-0874	*Coffae* sp.	x	x	x	x	x
32	14-0875	*Coffae* sp.	x	x	x	x	x
33	14-0876	*Coffae* sp.	x	x	x	x	x
34	14-0877	*Coffae* sp.	x	x	x	x	x
35	14-0800	*Chrysophyllum cainito*	x	x	x	x	x
36	S-041	On decaying wood	x	x	x	/	/
37	S-050	On decaying wood	x	x	x	/	/
38	MTK 062	*Tectona grandis*	x	x	x	x	x

Thirty two strains are included in the combined ITS, LSU and SSU rDNA analises with *Dothidea insculpta* (CBS 189.58) as the outgroup taxon. The best scoring RAxML tree with a likelihood value is shown in Figure 7. The phylogenetic hypothesis strongly supports three monophyletic groups, the six samples described here cluster within the *Phragmocapnias* clade, three specimens cluster within *Capnodium* and five samples formed a high supported group *Leptoxyphium*.

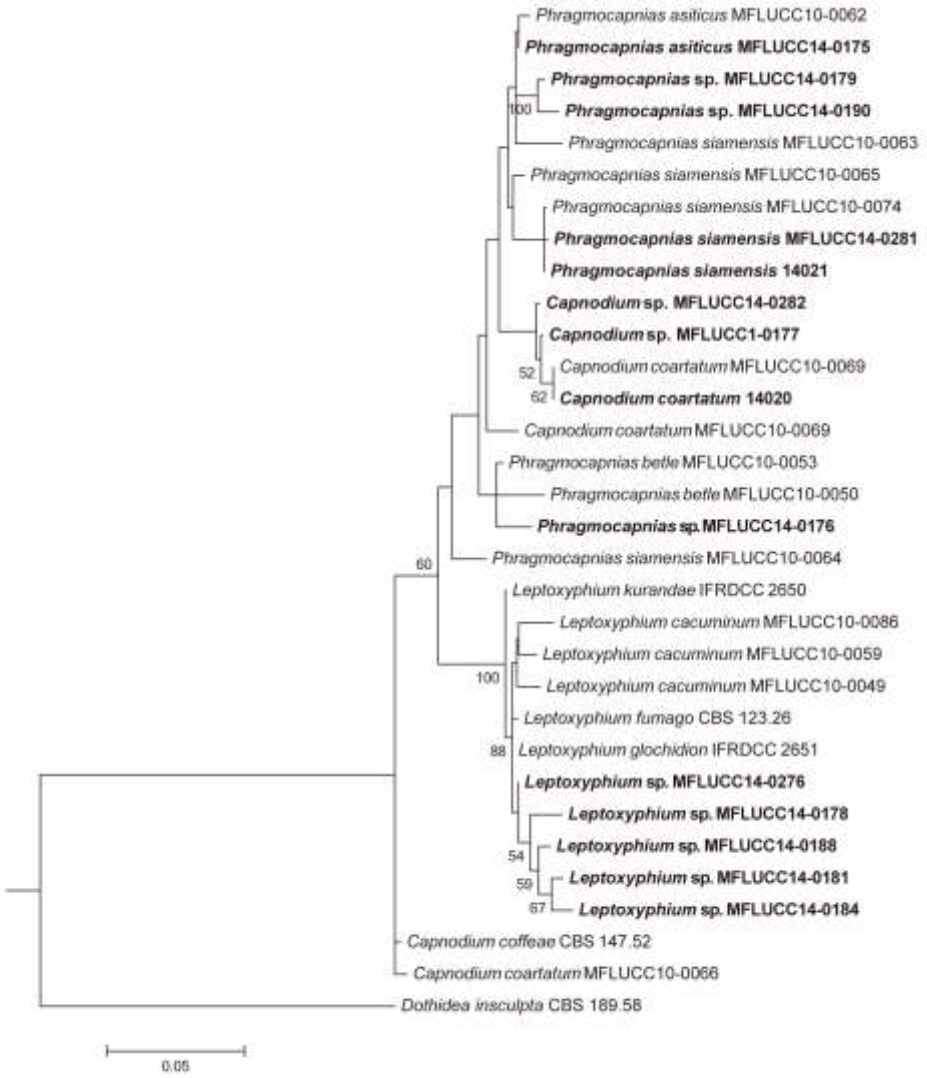

Fig. 10 Phylogram generated from Maximum likelihood (RAxML) analysis of combined LSU, SSU and ITS genes. Bootstrap support values for maximum likelihood equal or greater than 50 % are given above the nodes. Newly generated sequences are in bold. The tree is rooted with *Dothidae insculpta* CBS 189.58.

Phragmocapnias plumeriae Hongsanan & K.D. Hyde, **sp. nov.**

Saprobic on sugary exudates from Mealy bug growing on the upper surface of *Plumeria* sp. *Thallus* thin, black, composed of cylindrical hyphae. *Superficial hyphae* 5 *μm* wide, branched, septate, slightly constricted and dark at the septum, pale brown to brown. **Sexual morph** *Ascomata* 90–95 *μm* diam. ($\bar{x}$ =94 *μm*, n=10), superficial on surface of the plant, solitary, subglobose, narrowly rounded above, black and constricted at the base, ostiole present at mature, thin-walled, brown to dark brown, with 3–4 ascomatal setae at the upper part of ascomata. *Ascomatal setae* 5 *μm* diam., aseptate, brown to reddish brown, pale brown to hyaline at the apex. *Peridium* 10–13 *μm* ($\bar{x}$ =11 *μm*, n=10), comprising of *textura angularis* cells, inner layer hyaline, outer layer dark brown or reddish brown. *Hamathecium* lacking pseudoparaphyses. *Asci* 37–42 × 13–16 *μm* ($\bar{x}$ =39

× 14 μm, n=10), 8-spored, bitunicate, fissitunicate, subcylindrical to obovoid, short pedicellate or sometimes apedicellate, an ocular chamber not observed. *Ascospores* 19–25 × 4–6 μm ($\bar{x}$ =22 × 5 μm, n=10), bi to tri-seriate, cylindrical to clavate, 5-septate, slightly constricted at the septum, narrow ends, sometimes tapering toward the base, hyaline, smooth-walled. **Asexual morph** Undetermined.

***Culture characters*:** Ascospores germinating on PDA at 28°C for 12 h of dark, hyphae germinated at each cells of the ascospores, septate, constricted at the septum, hyaline to brown at the beginning, and become brown to black or greenish, with darker at the margin. Colonies slow growing reaching 2 cm diam. after 7 days on PDA, colony superficial to erumpent, verrucose surface, velvety.

Material examined: THAILAND, Chiang Rai, Tasud, Mae Fah Luang University, AD2, on leaves of *Plumeria* sp. (*Apocynaceae*), 10 January 2015, C. Singhapop PST1-2, ex-type living culture = MFLUCC 15-0205.

Notes: *Phragmocapnias plumeriae* is most typical of *P. imperspicua* (Sacc.) Cif. & Bat., but differs in having long, hyaline ascospores, with 5-septate at maturity, while short, brownish ascospores, with 4-septate at maturity in *P. imperspicua*. *Phragmocapnias plumeriae* is also similar to *P. betle* (Syd., P. Syd. & E.J. Butler) Theiss. & Syd., it however differs in having subcylindrical to obovoid asci, 5-septate ascospores, without hyaline sheath, while boradly clavate asci, 4-septate ascospores, surrounded by hyaline sheath in *P. betle*. Phylogenetic analyses based on LSU and ITS regions indicate that *P. plumeriae* is closely related to *P. betle* (Syd., P. Syd. & E.J. Butler) Theiss. & Syd. but should be illustrated as a distinct species.

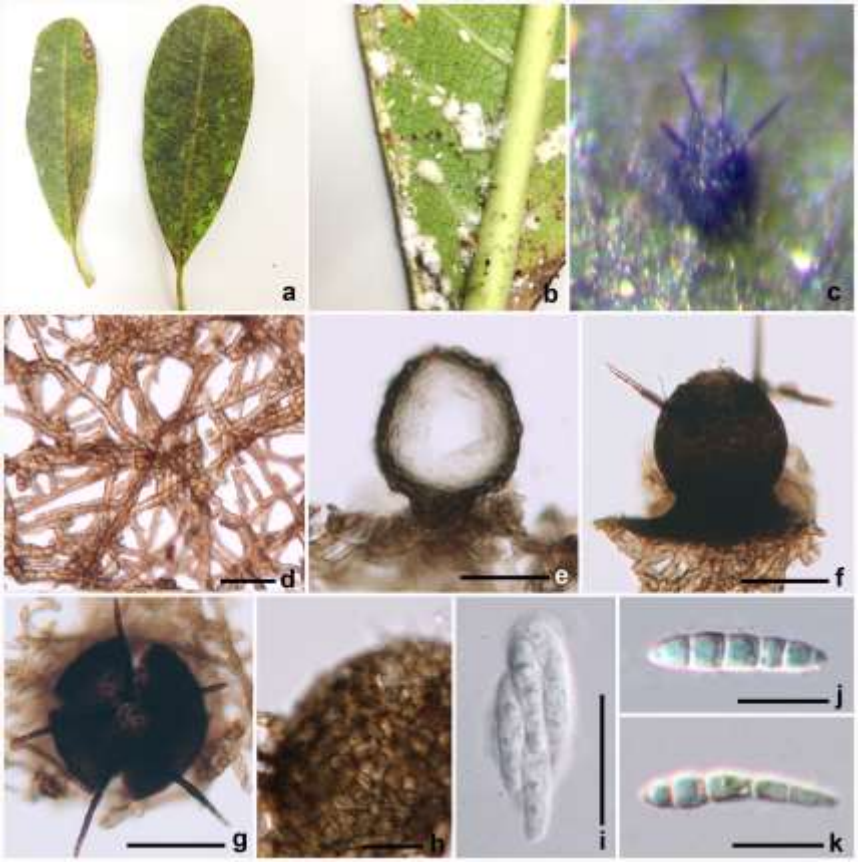

Fig 11. ***Phragmocapnias plumeriae*** (**holotype**). **a** Substrates. **b** Mealy bug on the lower surface of leaves. **c** Ascomata solitary on surface of leaves. **d** Hyphal networks. **e** Section through ascomata. **f, g** Ascomata when viewed in squash mounts. **h** Upper wall when viewed in squash mounts. **i** Asci with 8-spored. **j** Ascospores. **k** Ascospores in Melzer's reagent. Scale bars: **e, f, g**=50 *μm*, **d, h, i**=20 *μm*, **k, j**=10 *μm*.

Capnodium coffeanum Hongsanan & K.D. Hyde, **sp. nov.**

Saprobic on sugary exudates from Coccidae insects growing on the surface of leaves, branches, and stems of *Coffea* sp. *Thallus* of dark brown hyphae growing over plant surface. *Superficial hyphae* 3–5 *μm* wide ($\bar{x}$=4 *μm*, n=20), septate, branched, brown to dark brown, subcylindrical hyphal cells, constricted at the septum. **Asexual morph** *Pycnidia* 165–178 *μm* long ($\bar{x}$=170 *μm*, n=10), superficial, scattered or gregarious, blackish brown, cylindrical, with swollen at the central part, 14–16 *μm* diam. ($\bar{x}$=34 *μm*, n=10), comprising mostly cylindrical cells, stalk black, 19–24 long × 18–23 *μm* diam. ($\bar{x}$=23 × 21 *μm*, n=20), the swollen part produces conidia. *Ostiole* 14–16 *μm* diam. ($\bar{x}$=15 *μm*, n=10), surrounded by hyaline hyphae, 23–26 × 2–3 *μm* ($\bar{x}$=25 × 2.5 *μm*, n=20). *Conidiogenous* cells formed on the inner cell walls of the swollen part. *Conidia* 5–7 × 1–3 *μm* ($\bar{x}$=6 × 2 *μm*, n=20) cylindrical to oblong, smooth, round ends, hyaline.

Asexual morph Undetermined.

***Culture characters*:** Conidia germinating on PDA at 30°C for 12 h of dark, hyphae germinated from conidia, septate, constricted at the septum, hyaline to grayish at the beginning, and become black to greenish later. Colonies slow growing reaching 2 cm diam. after 5 days on PDA, colony superficial to erumpent, sometimes hyphae growing down and immersed in media, verrucose surface, velvety, branched at the margin, asexual structures was produced in PDA after 5 days incubation.

***Material examined**:* THAILAND, Chiang Rai, Tasud, Mae Fah Luang University, AD2, on leaves of *Coffea* sp. (*Rubiaceae*), 10 January 2015, S. Hongsanan PST2-1, ex-type living culture = MFLUCC 15-0207.

Notes: *Capnodium coffeanum* is most typical of *C. coartatum* Chomnunti & K.D. Hyde, but it has black, short stalk at the base, swollen at the central part of pycnidia, cylindrical to oblong conidia, while *C. coartatum* has brown pycnidia, swollen part at the base, and ellipsoidal conidia. In addition, *C. coffeanum* is also similar to species in the genus *Phragmocapnias* based on its swollen at the central part of pycnidia and black stalk lower the swollen part. However, there is very short stalk in *C. coffeanum*, and phylogenetic analyses demonstrat that *C. coffeanum* belongs in *Capnodium*, within the family *Capnodiaceae*.

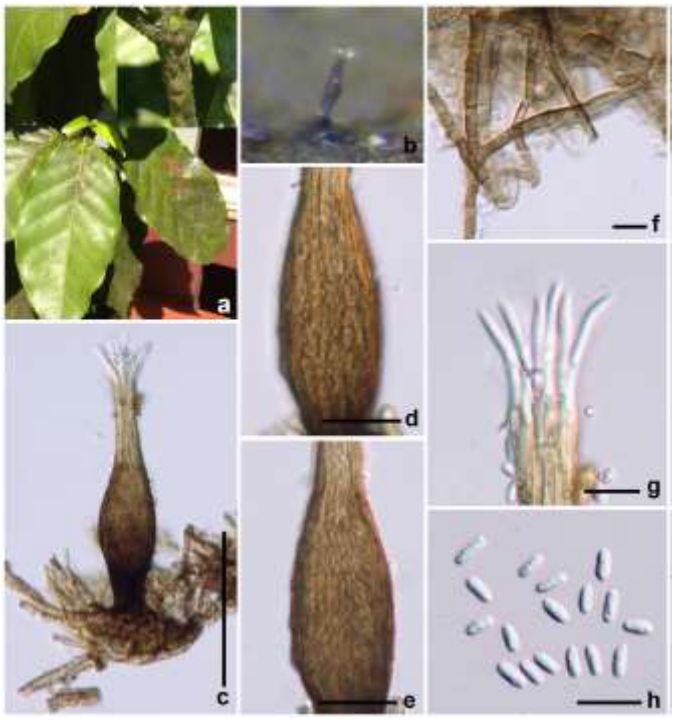

Fig 12. *Capnodium coffecola* (**holotype**). **a** Substrate. **b** Pycnidia on surface of leave. **c** Pycnidia when viewed in squash mounts. **d, e** Upper wall of swollen part when viewed in squash mounts. **f** Septate hyphae with constricted at the septum. **g** Ostiole surrounded by hyaline hyphae. **k** Unicellular conidia. Scale bars: **c**=100 *μm*, **d, e**=20 *μm*, **f**–**h j**=10 *μm*.

Ceramothyrium ficus Hongsanan, Q. Tian, A.H. Bahkali & K.D. Hyde, **sp. nov.**

GenBank: KT588601 (ITS), KT588599 (LSU).

Epiphytic on the upper surface of living leaves, appearing as small black dots, leaves remaining healthy. *Superficial hyphae* branched, septate, slightly constricted at the septa, brown to dark brown, radiating outwardly, and covering the ascomata as a subiculum, without penetrating host tissues. **Sexual morph**: *Ascomata* 475-550 high × 120-130 μm diam. ($\bar{x}$ = 530 × 124 μm, n = 5), solitary or scattered, superficial on the surface of leaves, globose to subglobose, flattened when dry, brown to dark brown, easily removed, covered by a subiculum or hyphal layer. *Peridium* 5-10 μm wide, comprising two strata, the outer stratum comprising brown to dark brown cells of *textura angularis*, the inner stratum comprising pale brown to hyaline flattened cells. *Hamathecium* comprising pseudoparaphyses embedded in mucilage. *Asci* 95-110 × 30-39 μm ($\bar{x}$ = 104 × 33 μm, n = 10), 8-spored, bitunicate, clavate, with long pedicellate, ocular chamber not seen, evanescent. *Ascospores* 36-39 × 7-8 μm ($\bar{x}$ = 38 × 7.5 μm, n = 10), 3-5-seriate, hyaline, subcylindrical, muriform, with 7-8 transverse septa and 1 longitudinal septa, sometimes with 2 longitudinal septa in the end cells, slightly constricted at the septa, narrowly rounded at both ends, verrucose or smooth-walled, surrounded by a mucilaginous sheath. **Asexual morph**: undetermined.

***Culture on PDA*:** Ascospores germinating on PDA at 25-28 ^{o}C in 12 h of light/12 h of dark, germ tubes appearing from most cells of the ascospores, hyaline to brown, and becoming brown to grayish. Colonies reaching 1 cm diam. after 7 days on PDA at 25-28 ^{o}C, colonies erumpent on the surface of media, surface smooth, velvety, slightly wavy at the margin, no asexual morph was produced in PDA after 60 days incubation.

Material examined*:* THAILAND, Chiang Rai Province, Mae Fah Luang University, on living leaves of Ficus sp. (Moraceae), 23 January 2015, S. Hongsanan AD01 (MFU 152252, holotype), (isotype MFU 15-2253; in KUN), ex-type living culture, MFLUCC 150228, MFLUCC 15-0229, and in BCC.

Notes: The genus *Ceramothyrium* was introduced by Batista and Maia (1957), with the type species *Ceramothyrium paiveae* Bat. & H. Maia in the family *Phaeosaccardinulaceae*. Species of *Ceramothyrium* are referred to as sooty molds because of their morphological and ecological similarity with other sooty mold species in *Capnodiaceae* (Chomnunti et al., 2012a, 2014). Phylogenetic analyses place *Ceramothyrium* in the family *Chaetothyriaceae* (*Chaetothyriales*, Eurotiomycetes) with strong support (Winka et al., 1998; Lutzoni et al., 2004; Miadlikowska & Lutzoni, 2004; Reeb et al., 2004; Chomnunti et al., 2012a, 2012b). Thirty epithets are listed in *Ceramothyrium* in Index Fungorum (2015). *Ceramothyrium ficus* and *C. longisolcano* differ, as *C. ficus* was found on living leaves, and has small ascomata, which are pale brown to yellowish at the center when viewed in squash mounts, and smaller ascospores with 7-8 transverse septa and 1-2

longitudinal septa, *C. longisolcano* was found on dead leaves, produces larger ascomata which are dark brown to reddish in the center when viewed in squash mounts, and larger ascospores with 7 transverse septa and 1 longitudinal septum. *Ceramothyrium ficus* is also distinct from *C. thailandicum* Chomnunti & K.D. Hyde which has ascomata darkened at the center when viewed in squash mounts, and clavate ascospores with 7-9 transverse septa and lacking longitudinal septa. Molecular analyses using ITS and LSU sequence data demonstrate a close relationship between *C. ficus* and *C. longisolcano* (90% ML and 1.0 PP support), but support *C. ficus* as a distinct species with high bootstrap support (100% ML and 1.0 PP).

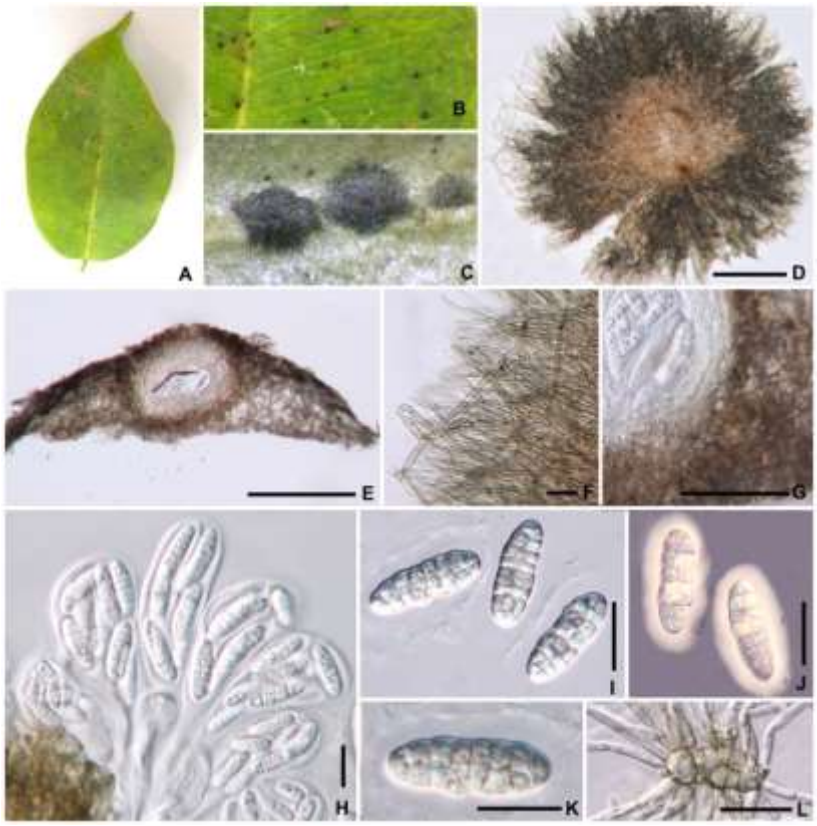

Fig. 13. *Ceramothyrium ficus* (**holotype**). A. Specimen. B, C. Ascomata developing on surface of leaves. D. Squash mount of ascomata covered by a subiculum. E. Section through ascoma. F. Margin of ascoma. G. Peridium of ascoma. H. Bitunicate asci. I. Ascospores with mucilaginous sheath. J, K. Ascospores with mucilaginous sheath in India Ik. L. Germinating ascospore. Scale bars: D, E = 100 μm, G = 20 μm, F, H-L = 20 μm.

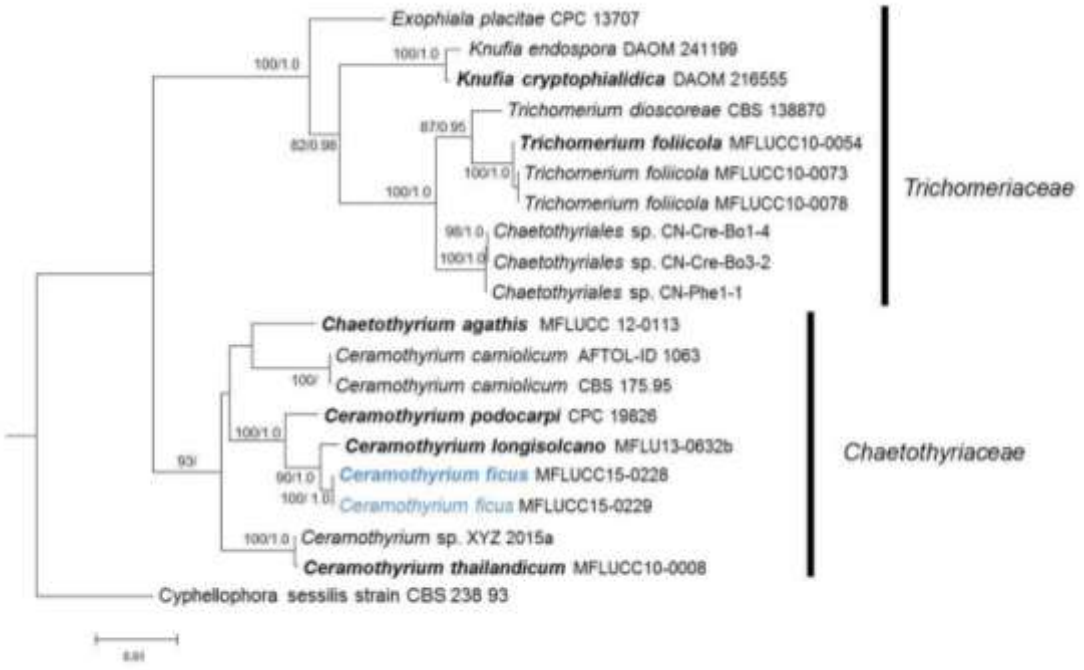

Fig. 14. The phylogenetic tree was obtained by RAxML maximum likelihood methods using ITS and LSU regions. The first set of numbers above the nodes are RAxML bootstrap value expressed values above 70% shown. The second set of numbers above the nodes are Bayesian posterior probabilities, with values above 0.95 shown. Strain numbers are indicated after species names. New sequence data are in blue bold, and other types are in black bold. The analysis included 10 strains from Trichomeriaceae, 8 strains from Chaetothyriaceae, and is rooted with Cyphellophora sessilis (Cyphellophoraceae) for the out-group; the alignment comprises 1,435 characters. The newly generated nucleotide sequences were compared against the GenBank database using the Mega BLAST program. Sequences that relate to Ceramothyrium were obtained from GenBank and were aligned using the multiple sequence alignment program, MAFFT (Katoh & Standley, 2013), checked manually using BioEdit software (Hall, 2014). Maximum-likelihood (ML) analysis was performed using raxmlGUIv.0.9b2 (Silvestro & Michalak, 2012), with 1,000 replicates. The model of evolution was estimated by using MrModeltest 2.2 (Nylander et al., 2008). Posterior probabilities were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001). Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation, and 10,000 trees were obtained. The first 2,000 trees, representing the burn-in phase were discarded (Cai et al., 2006, 2008). Phylogenetic trees were drawn by using Treeview v. 1.6.6 (Page, 2001).

Scorias mangiferae Hongsanan, P. Chomnunti, J.C. XU & K.D. Hyde, **sp. nov.**

GenBank: KT588604 (ITS), KT588603 (LSU).

Epiphytic, saprobic on sugary exudates from insects growing on the surface of branches and living leaves. *Superficial hyphae* 4-6 *μm* wide ($\bar{x}$ = 5 *μm*, n = 10), branched, septate, slightly constricted at the septa, brown to dark brown dark brown towards the edge, forming as thallus cover the surface of plants. **Asexual morph**: *Pycnidia* 295-385 high × 4655 *μm* diam. ($\bar{x}$ = 370 × 53 *μm*, n = 5), gregarious, arising from the hyphae, long stalked, flask-shaped, brown to pale brown at the base, brown to dark brown towards the tapering apex. *Ostiole* surrounded by septate, hyaline hyphae, with rounded tips. *Pycnidial wall* helical twisting, synnemata-like. *Conidiogenous* cells formed in the inner cells of the oval part. *Conidia* 6-7 × 2-3 *μm* ($\bar{x}$ = 6.7 × 2.5 μm, n = 10), ellipsoidal, unicellular, hyaline to greenish, with rounded ends.

Sexual morph: Undetermined.

***Culture on PDA*:** Conidia germinating on PDA at 27 ^{o}C for 12 h of light/12 h of dark, germ tubes appearing from conidia, hyaline, and becoming darkened after 10 days. Colonies reaching 1 cm diam. after 5 days on PDA at 25-28 ^{o}C, colonies superficial to erumpent, velvety, thin at the margin, water droplets forming on the surface of colonies, white to ivory, darkened and greenish at the margin after 14 days incubation, produce abundant pycnidia and red pigment on PDA after 5 days incubation.

***Material examined**:* THAILAND, Chiang Rai Province, Bandu, on branch of Mangifera sp. (Anacardiaceae), 4 February 2015, S. Hongsanan BJ01 (MFLU 15-2254, holotype), (isotype in KUN), ex-type living culture, MFLUCC 15-0230, in BCC.

Notes: The genus Scorias was established by Fries (1832), with the type species *Scorias spongiosa* (Schwein.) Fr. Species of *Scorias* grow on insect honeydew or sugary plant exudates (Hughes, 1976; Reynolds, 1978; Schoch et al., 2006; Chomnunti et al., 2011, 2014), and is characterized by subglobose to broadly ellipsoidal ascomata, bitunicate asci, and 3-4-trans-septate, hyaline to pale brown ascospores. The asexual morph *Scorias* is characterized by superficial hyphae covering the surface of hosts, long or short-stalked, flask shaped pycnidia, arising from hyphae, and hyaline, unicellular conidia tapering towards the apex (Chomnunti et al., 2011, 2014). Phylogenetic analyses indicated that the genus *Scorias* belongs in *Capnodiaceae* (Schoch et al., 2006, 2009; Crous et al., 2009; Chomnunti et al., 2011, 2014; Hyde et al., 2013). There are ten known species in this genus, but only two species have molecular data. *Scorias mangiferae* is most typical of *S. spongiosa* (Schwein.) Fr. in having hyphal networks covering the surface of hosts, and flask-shaped pycnidia arising from the hyphae, producing unicellular, hyaline conidia. However, our species differs from all other known species in the genus in its conidiomata having short stalks, which are brown to yellowish when immature, becoming brown at the base and

darkened in the upper part when mature, and in producing larger, hyaline conidia. Molecular analyses using combined LSU and ITS sequence data indicate *S. mangiferae* is a distinct species, which is related to *S. spongiosa* (90% ML and 0.95 PP support).

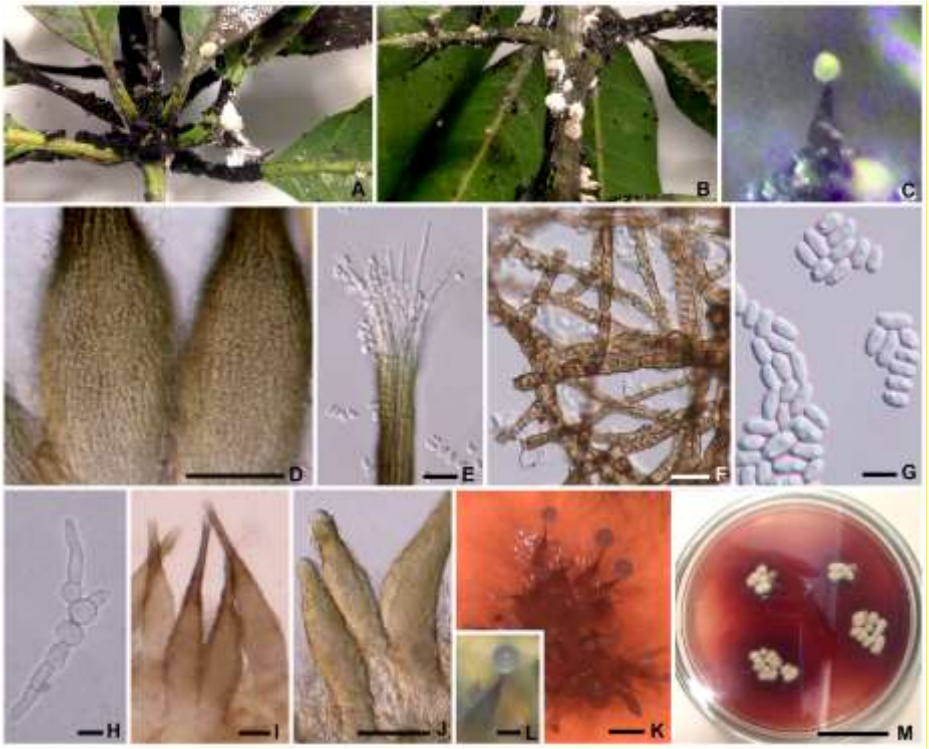

Fig. 15. ***Scorias mangiferae*** (**holotype**). A. Appearance of sooty mould on host. B. Insects (Mealy bugs) associated with Scorias mangiferae. C. Pycnidium. D. Pycnidia wall. E. Ostiole. F. Hyphal network. G. Conidia. H. Germinating conidium. I-L. Pycnidia developing on media. M. Colonies producing red pigmentation on PDA. Scale bars: D, I, J, L = 50 μm, K = 100 μm, E-H = 5 μm, M = 3 cm.

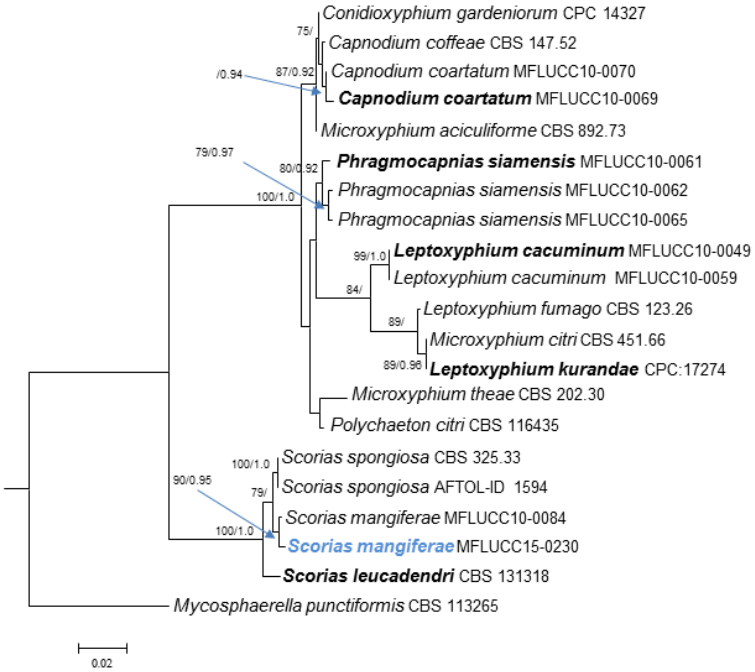

Fig. 16. The phylogenetic tree was obtained by RAxML maximum likelihood methods using sequences of ITS and LSU regions. The first set of numbers above the nodes are RAxML bootstrap value expressed expressed with values above 70% shown. The second set of numbers above the nodes are Bayesian posterior probabilities, with values above 0.95 shown. Strain numbers are indicated after species names. New sequence data are in blue bold, and other types are in black bold. The analyses included 5 strains from Scorias, 15 strains from others species in Capnodiaceae, and is rooted with Mycosphaerella punctiformis (Mycosphaerellaceae) for the out-group; the alignment comprises 1,325 characters. The newly generated nucleotide sequences were compared against the GenBank database using the Mega BLAST program. Sequences that relate to the Scorias were obtained from GenBank and were aligned using the multiple sequence alignment program, MAFFT (Katoh & Standley, 2013), checked manually using BioEdit software (Hall, 2014). Maximumlikelihood (ML) analysis was performed using raxmlGUIv.0.9b2 (Silvestro & Michalak, 2012), with 1,000 replicates. The model of evolution was estimated by using MrModeltest 2.2 (Nylander et al., 2008). Posterior probabilities were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001). Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation, and 10,000 trees were obtained. The first 2,000 trees, representing the burn-in phase were discarded (Cai et al., 2006, 2008). Phylogenetic trees were drawn by using Treeview v. 1.6.6 (Page, 2001).

Minimelanolocus R.F. Castañeda & Heredia, Cryptog. Mycol. 22(1): 7 (2001)

Notes: *Minimelanolocus* was introduced by Castañeda-Ruiz et al. (2001) and typified by *Minimelanolocus navicularis* (R.F. Castañeda) R.F. Castañeda based on the segregation of the genus *Pseudospiropes*. It is characterized by conspicuous, mononematous, solitary or fasciculate, septate, erect, straight or flexuous, smooth or verrucose, cylindrical, sinuate or geniculate, and brown to dark brown conidiophores with the melanised base and hyaline to brown, oblong, cylindrical, clavate to fusiform, euseptate, acropleurogenous conidia (Castañeda-Ruiz et al. 2001; Hern_andez-Restrepo et al. 2013; Xia et al. 2014). *Minimelanolocus* is morphologically well-studied genus which has been described from a wide range of hosts (Zhang et al. 2010; Ma et al. 2011a, b; Hernández-Restrepo et al. 2013; Xia et al. 2014), however, the phylogenetic analyses are lacking due to the absent of living cultures of the type species. Presently, there are 24 species listed in *Minimelanolocus* (Index Fungorum 2015). Liu et al. (2015) provided a backbone tree of *Chaetothyriales* and described four new species in *Minimelanolocus* which revealed LSU and SSU was not adequate to distinguish species within *Minimelanolocus* while the comparison of ITS region determined in accordance within the species. And thus, the species limits of *Minimelanolocus* was determined based on morphology and variability of ITS sequences.

***Minimelanolocus* sp.** Q. Tian & K.D. Hyde, **sp. nov**. Holotype: HKAS 84023.

Saprobic on decaying woody plant in aquatic habitats. **Asexual morph**: *Colonies* superficial, effuse, scattered, hairy, dark brown to black. *Mycelium* immersed, comprising septate, pale brown hyphae. *Conidiophores* mononematous, macronematous, unbranched, erect, straight or flexuous, smooth, dark brown, melanized at base, gradually paler and rounded towards the apex, septate. *Conidiogenous cells* holoblastic, integrated, sympodially proliferating, terminal and intercalary, pale brown or subhyaline. *Conidia* acrogenous, hyaline, cylindro-clavate, rounded at base and apex, solitary, 1–3-septate, smooth-walled. **Sexual morph**: undetermined.

Material examined: China, Yunnan Province, Canshang Mountain, on decaying wood, S-041 WHXM 24-1 (holotype, HKAS 84023), ex-type living culture MFLUCC.

Notes: *Minimelanolocus* sp. is morphologically similar to the superficial appearance of *Minimelanolocus melanicus*. It differs from *M. melanicus* in having hyaline, cylindro-clavate conidia and rounded at base and apex. Based on the molecular phylogeny and comparison of each ITS sequences, the new speices is defined here.

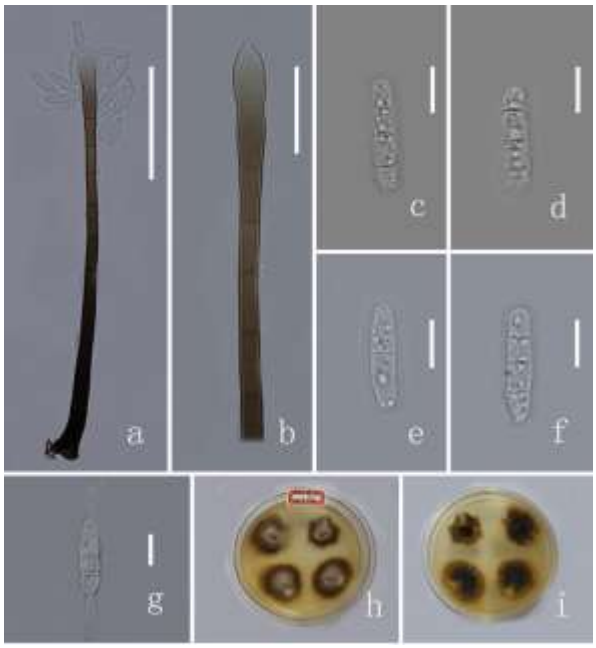

Fig 17. *Minimelanolocus* sp. (**Holotype**) **Scale bars:** a, b. Conidiophore. c-f. Conidia. g. Germinating conidium. h-i. colonies developing on media. Scale bars: a, b = 20 μm, c-g = 5 μm.

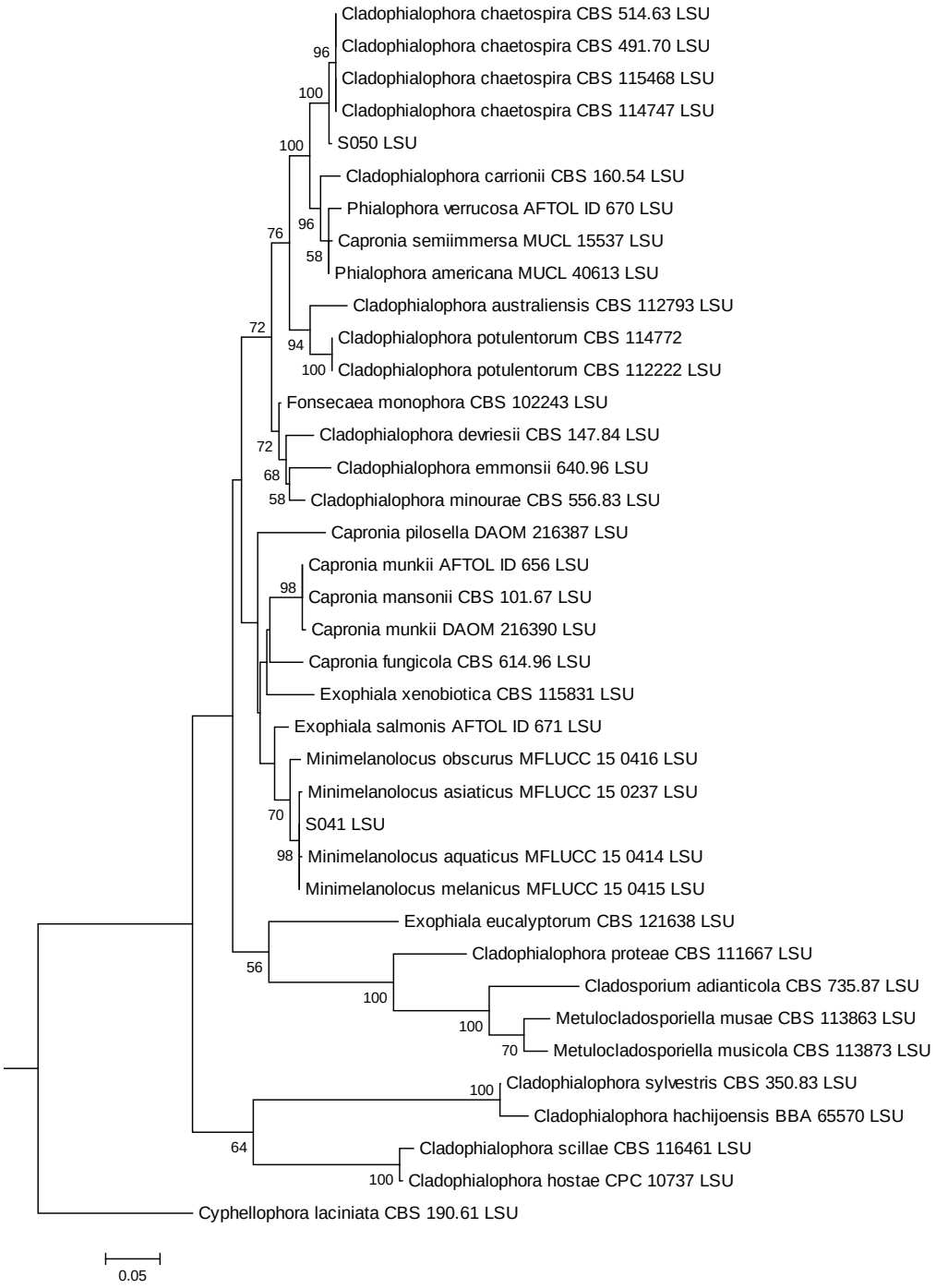

Fig. 18 Phylogram generated from Maximum likelihood (RAxML) analysis of combined LSU, SSU genes. Bootstrap support values for maximum likelihood equal or greater than 50 % are given above the nodes. The tree is rooted with *Cyphellophora laciniata* CBS 190.61.

Results of testing whether sooty moulds are xerophilic

Xerophilic is a term meaning “dry loving”. Xerophilic fungi were defined by Pitt (1975) as those capable of growth under at least one set of environmental conditions at a water activity (aw) less than 0.85. Many mould and yeast species are xerophilic. They may cause economic losses which spoil intermediate moisture and low water activity baked goods (Pitt and Hocking 1997). However, xerophilic organisms may have biotechnological potential in enzyme production or bioremediation, while any novel antibiotics will have huge medical potential (Pickard et al. 1991; Faull et al. 2002; Herath et al. 2012). Sooty moulds, genera in *Chaetothyriaceae* and species in *Trichomerium* occupy a unique habitat which is intermittently dry and wet, but they do not penetrate the host to obtain nutrients or moisture. In some cases, *Cladophialophora* and *Exophiala* also can endure extremotolerance conditions which evolved in high temperature and dryness (Sterflinger 1998; Ruibal 2004). Thus, it is essential to test whether the species in *Chaetothyriales*, especially in moulds group, are Xerophilic fungi and such knowledge will contribute to the future work of secondary metabolism. Sooty moulds appear to grow in extreme environments and it should be tested if they are xerophilic. The first simply test presented that sooty moulds should be xerophilic. Potentially, xerophilic organisms may have biotechnological potential in enzyme production or bioremediation.

In this study three sooty mould fungi; *Leptoxyphium cacuminum* (TS09), *Capnodium coffeae* (MFU07) and *Phragmocapnias siamensis* (S4) were chosen as a first group of experimental to test whether sooty mouolds are xerophilic. The basal medium comprised 0.3% malt extract, 0.3% yeast extract and 2.0% agar, we used NaOH and HCl to adjust pH, The final pH values of the media were 4.5, 5.5, 6.5 and 7.5. After transfering the culture onto the new plate we deposit the plates respectively in 15 °C, 25°C, 30°C incubator. Radial growth rates, expressed in mm/ day were calculated. Fig. 9 shows growth rates of *L. cacuminum* (TS09), *C. coffeae* (MFU07) and *P. siamensis* at 15, 25 and 30°C with pH = 6.5 at the aw = 0.90. For all selected fungi, pH=6.5 is best condition for growing and the influence of water activity on growth is ongoing. We added a mixture of glucose and fructose to reduce aw to 0.94, 0.88, 0.84, 0.80, 0.76 and 0.66. Fig. 9 indicates *C. coffeae* and *P. siamensis* are growing slowly under 15 and 30°C than under 25°C.For *P. siamensis*, under 30°C, it was stopped for growing. *L. cacuminum* is growing faster under 15°C

than under 25°C. All results will be completed and analyzed within next 6 months, axis table to present the interaction between pH, a_w and temperature will be completed as well.

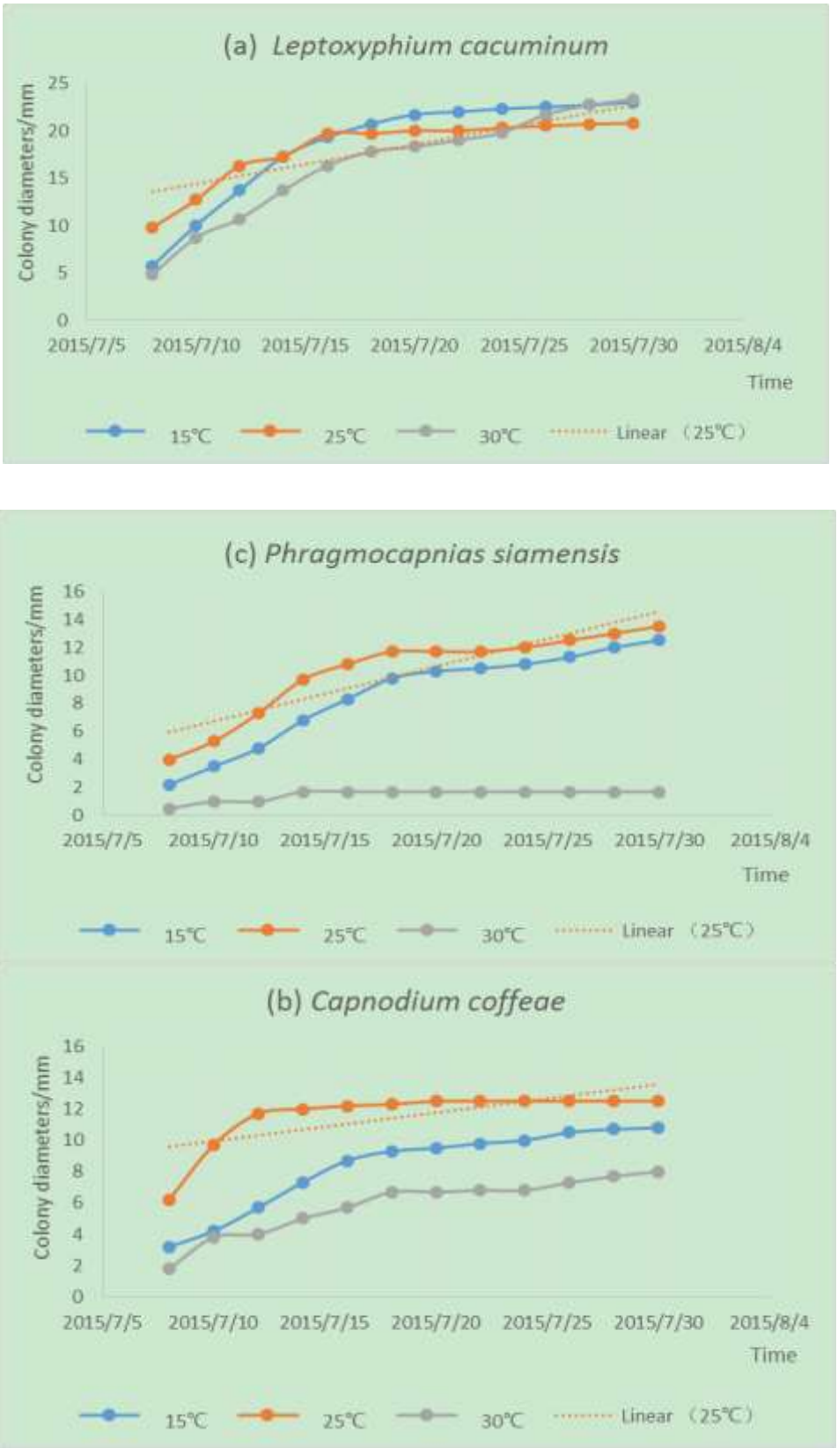

Fig. 19 Effect of water activity on radial growth rates of (a) *Leptoxyphium cacuminum* (b) *Capnodium coffeae* (c) *Phragmocapnias siamensis* at temperatures 15, 25 and 30°C**,** pH= 6.5, Aw=0.90.

เอกสารแนบหมายเลข 3

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.'

SCI paper

Qing Tian, Sinang Hongsanan, Dongqin Dai, Siti A. Alias, Kevin. D. Hyde and **Putarak Chomnunti*** (2015) Towards a natural classification of Dothideomycetes: clarification of Aldona, Aldonata and Viegasella (Parmulariaceae). Saudi Journal of Biological Sciences. (doi:10.1016/j.sjbs.2015.01.019)

Hongsanan S, Tian Q, Peršoh D, Zeng XY, Hyde KD, **Chomnunti P**, Boonmee S, Bahkali AH, Wen TC (2015) Meliolales. Fungal Diversity (1-51)DOI 10.1007/s13225-015-0344-7

Hongsanan S, Tian Q, Hyde KD, **Chomnunti P*** (2015) Two new species of sooty moulds, Capnodium coffeicola and Conidiocarpus plumeriae in Capnodiaceae. Mycosphere. Vol. 6(6), 814-824. Doi 10.5943/mycosphere/6/6/14.

Poster presentation

Putarak Chomnunti, Qing Tian, Sinang Hongsanan, Kevin Hyde. (2014) " Sooty moulds in Thailand" in The 10th International Mycological Congress, Queen Sirikit National Convention Centre Bangkok, Thailand 3-8 August 2014

Qing Tian, Sinang Hongsanan, Siti A. Alias, Peter E. Mortimer, Kevin D. Hyde, **Putarak Chomnunti.** (2014) "Taxonomy, phylogeny and biological activities of sooty moulds in Thailand" in The 10th International Mycological Congress, Queen Sirikit National Convention Centre Bangkok, Thailand 3-8 August 2014

Sinang Hongsanan, Ali H. Bahkali, Eric H.C. Mckenzie, Ekachai Chukeatirote, Kevin Hyde, **Putarak Chomnunti.** (2014) Trichopeltinaceae (Dothideomycetes) and earlier name for Brefeldiellaceae. in The 10th International Mycological Congress, Queen Sirikit National Convention Centre Bangkok, Thailand 3-8 August 2014

Invited speaker in workshop on "Diagnosis of Plant Disease (Ascomyctes fungi)", organized by ASEAN Regional Diagnostic Network Project, 22-26 June 2015, Chiang Rai, Thailand.

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Two new species of sooty moulds, *Capnodium coffeicola* and *Conidiocarpus plumeriae* in *Capnodiaceae*

Hongsanan S[1,2,3], Tian Q[1,2,3], Hyde KD[1,2,3] and Chomnunti P[3,4]

[1]*World Agroforestry Centre, East and Central Asia, Kunming 650201, Yunnan, China*
[2]*Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Lanhei Road No 132, Panlong District, Kunming, Yunnan Province, 650201, PR China*
[3]*Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, 57100, Thailand*
[4]*School of Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand*

Hongsanan S, Tian Q, Hyde KD, Chomnunti P. 2015 – Two new species of sooty moulds, *Capnodium coffeicola* and *Conidiocarpus plumeriae* in *Capnodiaceae*. Mycosphere 6(6), 814–824, Doi 10.5943/mycosphere/6/6/14

Abstract

Capnodiaceae is believed to be the largest family containing sooty mould species, the taxa of which can cause chlorosis, plant stunting disease, and marketability problems, due to black mycelium coating the surface of host. Presently, little molecular data are available for species of *Capnodiaceae* in GenBank, thus more collections and sequence data are needed to improve the understanding of genera and species boundaries in this family. "Sooty mould"-like taxa, appearing as black colonies on the surface of leaves, were collected in Chiang Rai Province, Thailand. Taxa were studied based on morphological characters and molecular analyses. A phylogenetic tree using combined LSU and ITS sequence data generated by Maximum likelihood analyses (LSU and ITS) indicated that the new species, *Capnodium coffeicola* and *Conidiocarpus plumeriae*, belong in *Capnodiaceae*. We introduce the two new species base on morphological characterization and phylogenetic analyses.

Key words – *Capnodiales* – Dothideomycetes – Phylogeny – Sooty moulds – Taxonomy

Introduction

Sooty moulds belong in seven families, which are *Antennulariellaceae* Woron., *Capnodiaceae* Höhn. ex Theiss., *Chaetothyriaceae* Hansf. ex M.E. Barr, *Coccodiniaceae* Höhn. ex O.E. Erikss., *Euantennariaceae* S. Hughes & Corlett ex S. Hughes, *Metacapnodiaceae* S. Hughes & Corlett and *Trichomeriaceae* Chomnunti & K.D. Hyde (Reynolds 1998, Winka et al. 1998, Hughes & Seifert 2012, Hyde et al. 2013, Chomnunti et al. 2014). They occur on various hosts, mainly forming a thin, superficial, network of dark mycelium on the surface of branches, flowers, fruits, leaves, and stems (Hughes 1976, Faull et al. 2002, Hyde et al. 2013, Chomnunti et al. 2014), and should not be confused with *Asterinales* M.E. Barr ex D. Hawksw. & O.E. Erikss. and *Meliolales* Gäum. ex D. Hawksw. & O.E. Erikss, which cause web-like, black colonies on leaves and cause minor damage to host plants by penetrating host cells for the uptake of nutrients (Ariyawansa et al. 2015, Hongsanan et al. 2014a, 2015a). Typically, sooty moulds reduce photosynthesis ability of plants through the mycelium coating; they can cause chlorosis under the mycelia, plant-stunting disease, low-yield, and marketability problems (Chomnunti et al. 2014).

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Corresponding Author: Chomnunti P – e-mail – putarak.cho@mfu.ac.th

Sooty moulds have a wide distribution, and are most common in tropical and subtropical regions (Chomnunti et al. 2014).

Capnodiaceae is the most common family of sooty moulds, which is placed in the order *Capnodiales*, class Dothideomycetes (Batista & Ciferri 1963, Hughes 1972, Crous et al. 2009a, b, Schoch et al. 2009, Chomnunti et al. 2011, 2014, Hyde et al. 2013, Wijayawardene et al. 2014, Liu et al. 2015). This family was introduced by Höhnel (1910), and later validated by Theissen (1916) and the generic type is *Capnodium* Mont. The family is characterized by superficial, septate, dark brown hyphae, forming a thin mycelial network on the surface of hosts, bitunicate asci, the sexual and asexual morphs can be found in the same or different hosts, however, for some the sexual morph is unknown (Chomnunti et al. 2011, 2014, Hyde et al. 2013). The asexual morphs form elongated pycnidia, with short or long narrow necks, have a conspicuous oval swelling near the base, middle or apex of the pycnidia, and produce hyaline conidia inside the swollen part (Chomnunti et al. 2011). Crous et al. (2009a, b) placed three genera in *Capnodiaceae* based on their phylogenetic analyses. They also noted that Capnodiales probably contains diverse lineages, and some of these might need to be established as new families. There are presently not enough sequence data to define a new family, thus more collections and sequence data are needed. Chomnunti et al. (2011) used LSU and SSU rRNA sequence data to classify genera and species in *Capnodiaceae*, and concluded, based on morphology and phylogeny, that this family contains four genera; *Capnodium*, *Leptoxyphium* Speg., *Phragmocapnias* Theiss. & Syd. and *Scorias* Fr. Liu et al. (2015) introduced a new genus in *Capnodiaceae* (*Chaetocapnodium* Hongsanan & K.D. Hyde), and a new species of *Phragmocapnias* (*Phragmocapnias philippinensis* Hongsanan & K.D. Hyde).

Conidiocarpus is the asexual morph of *Phragmocapnias* which was introduced by Woronichin in Jaczewski (1917); the type species is *Conidiocarpus caucasicus* Woron. However, Batista & Ciferri (1963) cited *C. penzigii* Woron. which was introduced in 1926, as the type species (Hughes 1976). There are several publications noting that the genus *Conidiocarpus* was introduced in 1926 based on the type species *C. penzigii*, thus it was synonymized under *Phragmocapnias* (sexual morph) in many publications. Bose et al. (2014) followed the discussion in Hughes (1976), and they transferred species in *Phragmocapnias* to *Conidiocarpus* based on the rules of nomenclatural priority. There are 11 species of, and 386 hits for *Phragmocapnias* in Index Fungorum (2016) and Google respectively and 10 species of, and 106 hits for *Conidiocarpus*. We therefore agree with Bose et al. (2014), that *Conidiocarpus* should be used over *Phragmocapnias*, for these linked genera. With the exception of *Phragmocapnias philippinensis*, all species in *Phragmocapnias* were transferred to *Conidiocarpus* by Bose et al. (2014). Thus herein we synonymize *Phragmocapnias philippinensis* under *Conidiocarpus philippinensis* (Hongsanan & K.D. Hyde) Hongsanan & K.D. Hyde.

In this study, we introduce two new species, *Capnodium coffeicola* and *Conidiocarpus plumeriae* in *Capnodiaceae*. The new taxa are compared morphologically with other species in *Capnodiales*. The introductions of *Capnodium coffeicola* and *Conidiocarpus plumeriae* are also supported by phylogenetic analyses of the LSU and ITS sequence data.

Materials & Methods

Collections, isolation and morphology

Specimens with “Sooty mould”-like taxa were collected in Chiang Rai Province, Thailand, and observed under a stereomicroscope. Ascomata were studied by free-hand section, and their morphology studied under a compound microscope (Nikon 80i), slides were preserved in lactoglycerol after photographing. Measurements were determined using Tarosoft (R) Image Frame Work v. 0.9.7. Single spore isolation was carried out using the methods in Chomnunti et al. (2014). Type specimens of the new species are deposited in the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand, and ex-type cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC), and in Kunming Institute of Botany (KIB). Faces of fungi numbers

and Index Fungorum numbers are provided as explained in Jayasiri et al. (2015) and Index Fungorum (2016).

DNA isolation, amplification and sequencing

DNA was extracted from mycelium using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China); following the instructions. The conditions for the polymerase chain reaction (PCR) were determined using the primer pairs LROR/LR5 to amplify the large subunit region (LSU), and ITS1/ITS4 to amplify the internal transcribed spacer region (ITS). The amplification was carried with setting times and temperatures for the initial denaturation and the final extension period following Hongsanan et al. (2014b, 2015b). PCR products were checked on 1% agarose electrophoresis gels, and sequenced by Majorbio Co., China. Sequences generated for the new species are deposited in GenBank.

Phylogenetic analysis

Thirty-six sequences were downloaded from GenBank to supplement the dataset. *Davidiella tassiana* was selected as outgroup taxon (Table 1). The data set, including the new species, *Capnodium coffeicola* and *Conidiocarpus plumeriae*, were aligned by using MAFFT (Katoh et al. 2009), and checked manually using Bioedit (Hall 1999). Maximum likelihood analysis was carried out in raxmlGUIv.0.9b2 (Silvestro & Michalak 2012). The search strategy was set to bootstrapping and the analysis performed using the GTRGAMMAI model. The number of replicates was inferred using the stopping criterion (Pattengale et al. 2009). The bootstrap values expressed from 1,000 repetitions by RAxML analysis which are equal or greater than 50% are given to the left of each node (Fig. 1). The model of evolution was performed in MrModeltest 2.2 (Nylander 2008). Posterior probabilities (PP) were set by MCMC sampling in MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001, Zhaxybayeva & Gogarten 2002), following the details in Cai et al. (2006, 2008) and Hongsanan et al. (2014a, b). Posterior probabilities values (PP) from Bayesian analysis which are equal or greater than 0.90 are given the right of each node (Fig. 1). Phylogenetic trees were viewed using MEGA v5.2.1 (Tamura et al. 2011).

Results

Phylogenetic analyses

The large subunit ribosomal (LSU) and Internal transcribed spacer (ITS) sequences from 38 isolates of *Capnodiaceae*, *Dissoconiaceae, Euantennariaceae* and *Mycosphaerellaceae,* were included in the phylogenetic analysis; *Davidiella tassiana* is used as the outgroup taxon (Fig. 1). In the tree, the family *Capnodiaceae* is placed within *Capnodiales* under Dothideomycetes. The *Capnodiaceae* clade comprised 29 strains which belong in *Antennariella* Bat. & Cif., *Capnodium* Mont., *Conidioxyphium* Bat. & Cif., *Leptoxyphium* Speg., *Microxiphium* (Harv. ex Berk. & Desm.) Thüm., *Phragmocapnias*, *Polychaeton* (Pers.) Lév., and *Scorias* Fr. (98% ML, 1.0 PP). The *Leptoxyphium* clade comprised three strains of *Leptoxyphium* including *Microxiphium citri* (100% ML, 1.0 PP), this result is similar to previous studies (Chomnunti et al. 2014, Liu et al. 2015). Four *Capnodium* strains clustered with species of *Conidioxyphium* and *Microxiphium* (97% ML and 1.0 PP), furthermore five strains of *Scorias* are basal. *Capnodium coffeicola* clustered in a moderately supported clade within the genus *Capnodium*, and is separated from the other species. The new taxon *Conidiocarpus plumeriae* is closely related to *C. betle* (Syd. et al.) T. Bose, with high bootstrap support, but is a distinct species (100% ML, 1.0 PP). Two representative strains of *Dissoconiaceae* clustered together with high bootstrap support (100% ML, 1.0 PP) and are related to *Capnodiaceae* (78% ML). Six strains of *Mycosphaerellaceae* grouped with high bootstrap support (100% ML, 1.0 PP) and are closely related to the two strains of *Euantennariaceae* (91% ML, 0.99 PP). These three families form a sister group to *Capnodiaceae* within the order *Capnodiales*.

Table 1 Taxa used in the phylogenetic analysis with GenBank accession numbers (LSU and ITS) and species voucher/culture numbers.

Species	Voucher/culture numbers	Accession numbers	
		LSU	ITS
Antennariella placitae	CBS 124785	GQ303299	GQ303268
Capnodium coartatum	MFLUCC10-0069	JN832614	-
Capnodium coartatum	MFLUCC10-0070	JN832615	-
Capnodium coffeae	CBS 147.52	GU214400	AJ244239
Capnodium coffeicola	MFLUCC15-0206	**KU358920**	**KU358921**
Chaetocapnodium siamensis	MFLUCC13-0778	KP744479	-
Conidioxyphium gardeniorum	CPC 14327	GU301807	-
Davidiella tassiana	CBS 399.80	-	AJ244227
Dissoconium aciculare	CBS 204.89	GU214419	AY725520
Leptoxyphium cacuminum	MFLUCC10-0049	JN832602	have
Leptoxyphium kurandae	CPC:17274	JF951170	JF951150
Leptoxyphium madagascariense	CBS 124766	GQ303308	GQ303277
Microxyphium aciculiforme	CBS 892.73	GU301847	-
Microxyphium citri	CBS 451.66	GU301848	-
Microxyphium theae	CBS 202.30	GU301849	GU296178
Mycosphaerella ellipsoidea	CBS:110843	GQ852602	AY725545
Mycosphaerella endophytica	CBS:114662	GQ852603	DQ302953
Mycosphaerella punctiformis	CBS 113265	NG027571	KF442502
Pallidocercospora irregulariramosa	CBS 111211	KF902053	KF901706
Conidiocarpus philippinensis	MFLUCC12-0110	KP744503	-
Conidiocarpus plumeriae	MFLUCC15-0205	**KU358918**	**KU358919**
Conidiocarpus siamensis	MFLUCC10-0053	JN832606	KU358922
Conidiocarpus siamensis	MFLUCC10-0061	JN832607	KU358923
Conidiocarpus siamensis	MFLUCC10-0062	JN832612	KU358924
Conidiocarpus siamensis	MFLUCC10-0063	JN832608	KU358925
Conidiocarpus siamensis	MFLUCC10-0064	JN832609	KU358926
Conidiocarpus siamensis	MFLUCC10-0065	JN832610	KU358927
Conidiocarpus siamensis	MFLUCC10-0074	JN832611	KU358928
Polychaeton citri	CBS 116435	GU214469	GU214649
Ramichloridium apiculatum	CBS 156.59	EU041848	EU041791
Rasutoria pseudotsugae	rapssd	EF114704	EF114687
Rasutoria tsugae	ratstk	EF114705	EF114688
Scorias leucadendri	CBS 131318	JQ044456	JQ044437
Scorias spongiosa	AFTOL-ID 1594	DQ678075	-
Scorias spongiosa	CBS 325.33	-	GU214696
Scorias mangiferae	MFLUCC15-0230	KT588603	KT588604

Conidiocarpus philippinensis (Hongsanan & K.D. Hyde) Hongsanan & K.D. Hyde, **comb. nov.**
Index Fungorum: IF551807
Facesoffungi number: FoF01771

≡ *Phragmocapnias philippinensis* Hongsanan & K.D. Hyde, in Liu et al., Fungal Diversity: 172:69 (2015)

Notes – This species was introduced in Liu et al. (2015) as *Phragmocapnias philippinensis*. Phylogenetic analyses indicated that it was placed in *Capnodiaceae*, however it did not cluster with others species in *Phragmocapnias sensu stricto* (Liu et al. 2015); the result is similar to our study. Based on morphology, Liu et al. (2015) stated that *P. philippinensis* is most similar to other species in *Phragmocapnias*, but differs in having 5-septate ascospores without a hyaline sheath, thus they introduced it as a new species. Bose et al. (2014) synonymized *Phragmocapnias* under *Conidiocarpus*, which was the oldest name of these linked genera. They transferred species of *Phragmocapnias* to *Conidiocarpus*, thus, herein we synonymize *P. philippinensis* under *C. philippinensis*. This may require new genus when more related species are found, but for the present we place it in *Conidiocarpus sensu lato.*

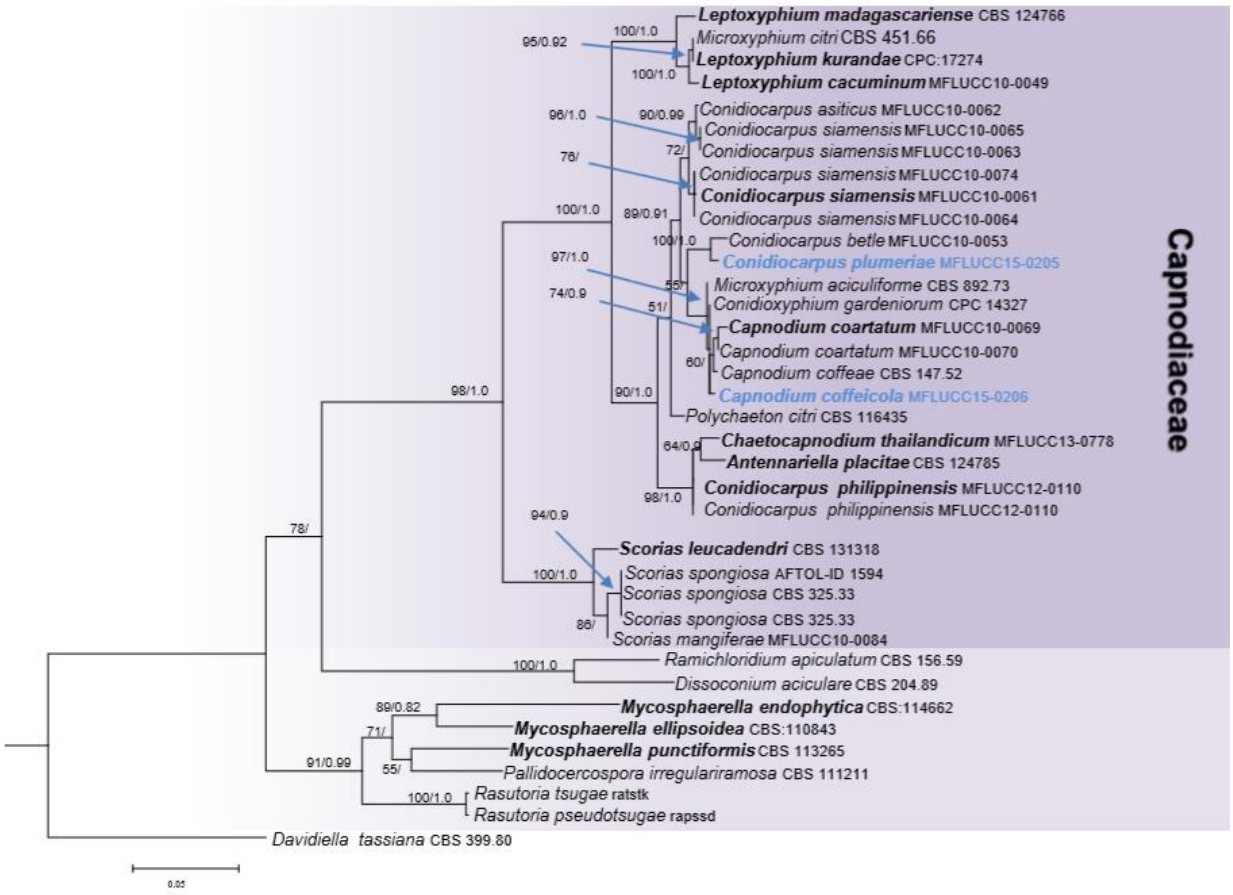

Fig. 1 – RAxML maximum likelihood phylogenetic tree generated from analysis of combined LSU and ITS sequence data. The first set of numbers above the nodes are RAxML bootstrap values equal or greater than 50%. The second set of numbers above the nodes are Bayesian posterior probabilities, with values above 0.9 shown. Strain numbers are indicated after species names. Ex-types strains are in black bold, new sequence data are in blue bold.

Capnodium coffeicola Hongsanan & K.D. Hyde, **sp. nov.** Fig. 2
Index Fungorum number: IF551802
Facesoffungi number: FoF01765

Etymology – *coffeicola* referring to the host on which the taxon was found.

Saprobic on sugary exudates from *Coccus* sp. (*Coccidae*, Insecta) growing on the surface of leaves, branches, and stems of *Coffea* sp. *Thallus* thin, dark brown, easily removed from the host surface, composed of cylindrical hyphae. *Superficial hyphae* 3–5 µm wide ($\overline{x}$=4 µm, n=20), septate, constricted at the septum, branched, brown to dark brown, with subcylindrical hyphal cells. **Sexual morph**: Undetermined. **Asexual morph**: *Pycnidia* 165–178 µm long ($\overline{x}$=170 µm, n=10), superficial, scattered or gregarious, blackish brown, cylindrical, swollen at the central part, 14–16 µm diam. ($\overline{x}$=34 µm, n=10), stalk black, 19–24 long × 18–23 µm diam. ($\overline{x}$=23 × 21 µm, n=20), wall comprising mostly cylindrical cells, the swollen part producing conidia inside. *Ostiole* 14–16 µm diam. ($\overline{x}$=15 µm, n=10), surrounded by hyaline hyphae, 23–26 × 2–3 µm ($\overline{x}$=25 × 2.5 µm, n=20). *Conidiogenous* cells formed on the inner cell walls of the swollen part. *Conidia* 5–7 × 1–3 µm ($\overline{x}$=6 × 2 µm, n=20), cylindrical to oblong, ends round, hyaline, smooth-walled.

Culture characters – Conidia germinating on PDA at 25–28°C for 12 h with dark, hyphae germinating from the conidia, septate, constricted at the septum, hyaline to grayish at the beginning, and become black to greenish later. Colonies slow growing, reaching 2 cm diam. after 5 days on PDA, colony superficial to erumpent, sometimes hyphae growing downwards and immersed into media, surface verrucose, velvety, branched at the margin, asexual structures produced in PDA after 3 days.

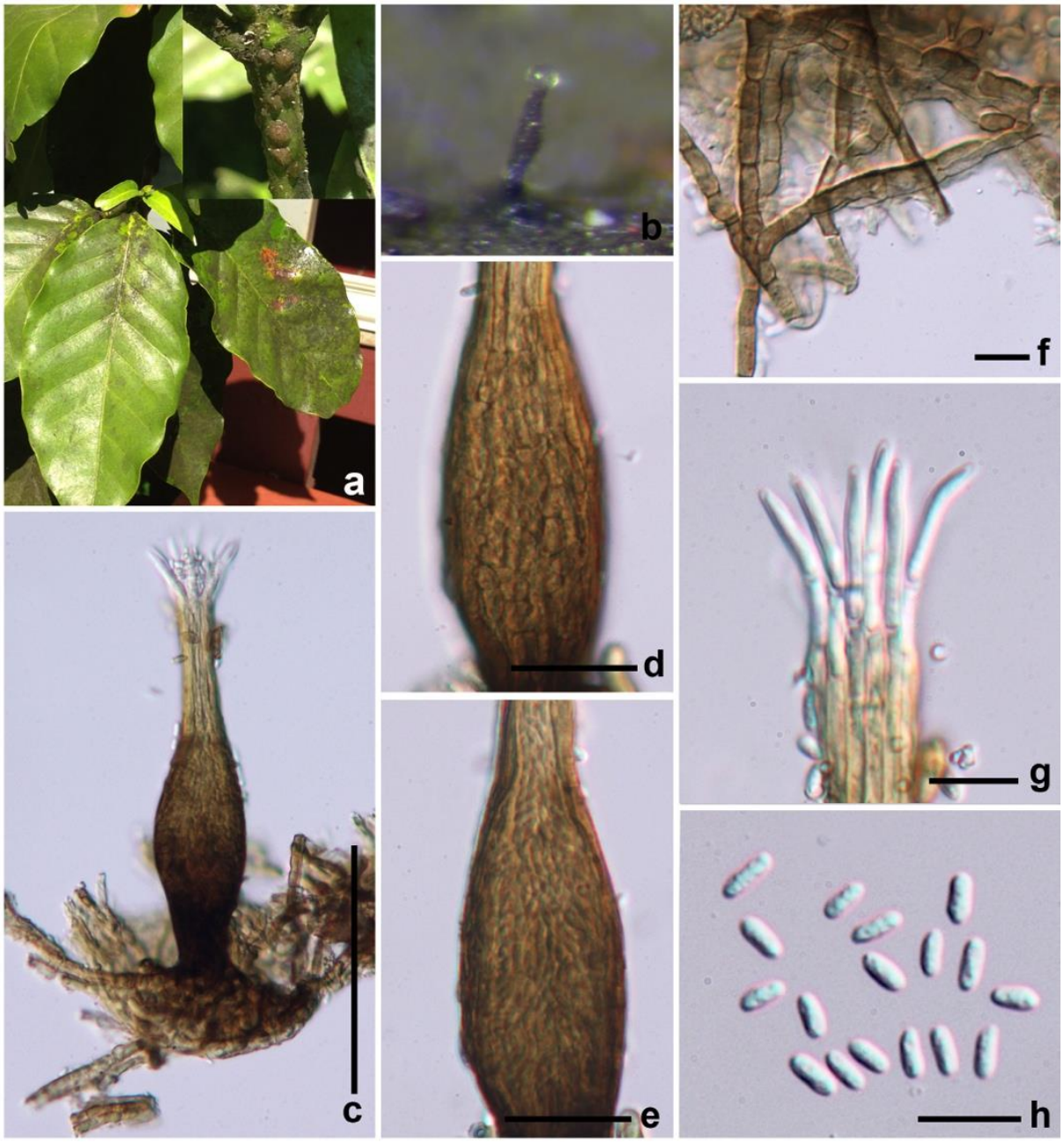

Fig. 2 – *Capnodium coffeicola* (holotype). a Substrate. b Pycnidia on surface of leaves. c Pycnidia when viewed in squash mount. d, e Cells wall of swollen part when viewed in squash mounts. f Septate hyphae constricted at the septum. g Ostiole surrounded by hyaline hyphae. k Unicellular conidia. – Bars c=100 µm, d, e=20 µm, f–h j=10 µm.

Material examined – Thailand, Chiang Rai, Tasud, Mae Fah Luang University, AD2 building on leaves of *Coffea* sp., 4 January 2015, S. Hongsanan, PST2-1 (MFLU 15-3565, **holotype**); *ibid.* (**isotype** in KIB) – ex-type living culture in MFLUCC 15-0206.

Notes – *Capnodium coffeicola* is most typical of *C. coartatum* Chomnunti & K.D. Hyde, but it has pycnidia with short and black stalks at the base, and is swollen at the central part, and cylindrical to oblong conidia, while *C. coartatum* has long and brown pycnidia, which are swollen at the base, lacks black and short stalks, and has ellipsoidal conidia. In addition, *C. coffeicola* is also similar to some species in the genus *Conidiocarpus*, based on its pycnidia being swollen at the central part of the pycnidium and a stalk in the lower swollen part. However, *Capnodium coffeicola* has a very short stalk and darkened lower swollen part. Phylogenetic analyses demonstrates that *C. coffeicola* belongs in *Capnodium*, within the family *Capnodiaceae.*

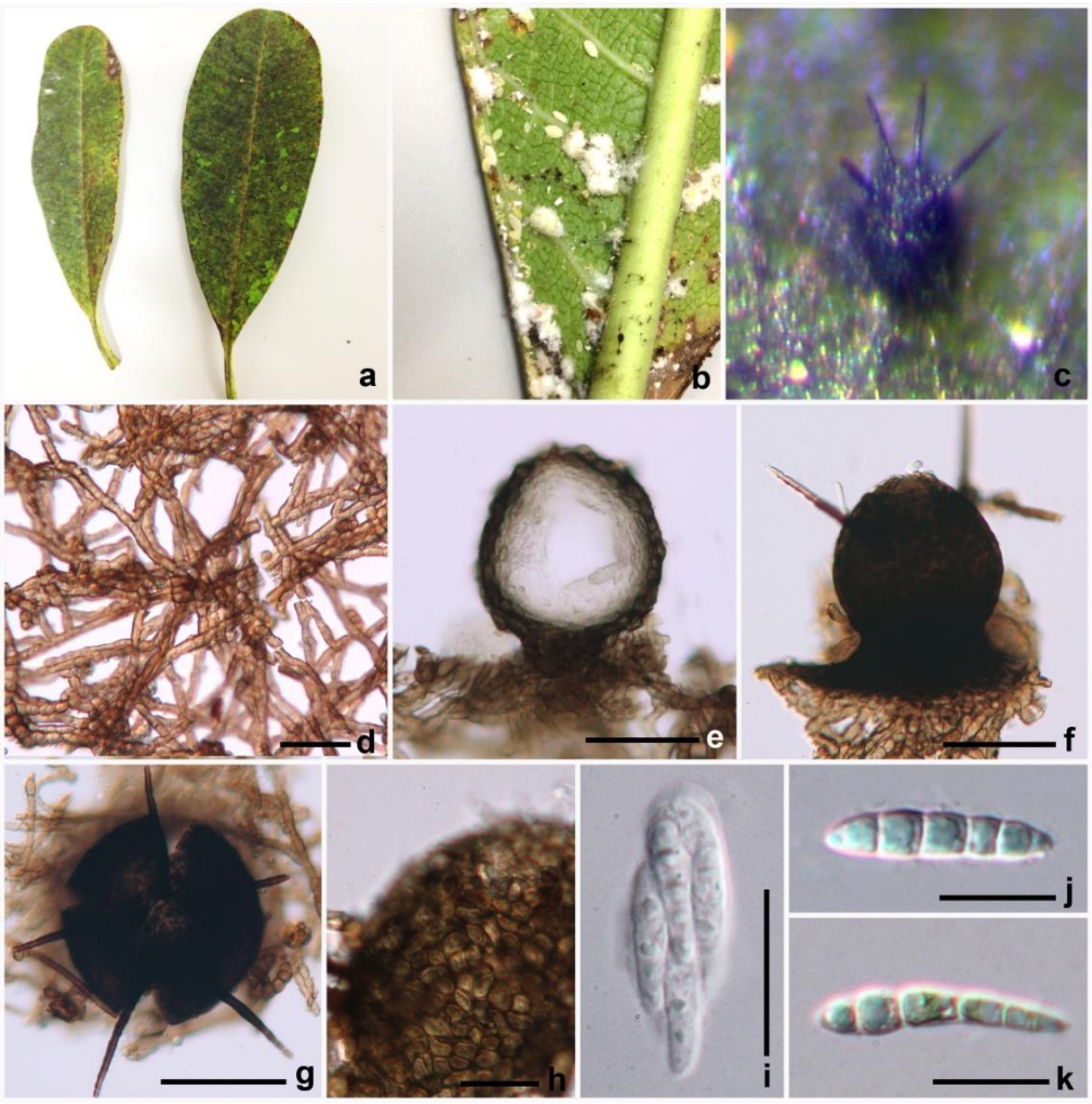

Fig. 3 – *Conidiocarpus plumeriae* (holotype). a Substrate. b Mealy bug on the lower surface of leaves. c Solitary ascomata on the surface of leaves. d Hyphal networks of *C. plumeriae*. e Section through ascoma. f, g Ascomata when viewed in squash mounts. h Upper wall when viewed in squash mounts. i Asci with 8-spores. j Ascospores. k Ascospores in Melzer's reagent. – Bars e-g=50 µm, d, h, i=20 µm, k, j=10 µm.

Conidiocarpus plumeriae Hongsanan & K.D. Hyde, **sp. nov.** Fig. 3
Index Fungorum IF551805
Facesoffungi number FoF01766

Etymology – *plumeriae* referring to the host on which the taxon was found.

Saprobic on sugary exudates from *Pseudococcus* sp. (*Pseudococcidae*, Insecta), growing on the upper surface of *Plumeria* sp. *Thallus* thin, dark brown, easily removed from the host surface, composed of cylindrical hyphae. *Superficial hyphae* 5 *µm* wide, branched, septate, slightly constricted and dark at the septum, pale brown to brown **Sexual morph**: *Ascomata* 90–95 µm diam. ($\bar{x}$=94 µm, n=10), superficial, solitary, subglobose, narrowly rounded above, constricted at the base, dark brown to black, ostiole present at maturity, thin-walled, with 3–4 ascomatal setae at the upper part of ascomata. *Ascomatal setae* 87–91 × 4–6 µm ($\bar{x}$=89 × 5 µm, n=10), aseptate, dark brown to reddish brown, but pale brown to hyaline at the apex. *Peridium* 10–13 µm ($\bar{x}$=11 µm,

n=10), comprising cells of *textura angularis*, inner layer hyaline, outer layer dark brown to reddish brown. *Hamathecium* lacking pseudoparaphyses. *Asci* 37–42 × 13–16 µm ($\bar{x}$=39 × 14 µm, n=10), 8-spored, bitunicate, fissitunicate, subcylindrical to obovoid, short pedicellate or sometimes apedicellate, ocular chamber not observed. *Ascospores* 19–25 × 4–6 µm ($\bar{x}$=22 × 5 µm, n=10), bi to tri-seriate, cylindrical to clavate, 5-septate, slightly constricted at the septum, with narrow ends, somewhat tapering towards the base, hyaline, smooth-walled. **Asexual morph**: Undetermined.

Culture characters – Ascospores germinating on PDA at 25–28°C for 12 h with dark, hyphae germinating at each cells of the ascospores, septate, constricted at the septum, hyaline to brown at the beginning, and become black to greenish, darker at the margin. Colonies slow growing reaching 2 cm diam. after 7 days on PDA, colony superficial to erumpent, surface verrucose, velvety.

Material examined – Thailand, Chiang Rai, Tasud, Mae Fah Luang University, AD2 building, on leaves of *Plumeria* sp., 10 January 2015, C. Singhapop, PST1-2 (MFLU 15-3564, **holotype**); *ibid.* (**isotype** in KIB) – ex-type culture in MFLUCC 15-0205.

Notes – *Conidiocarpus plumeriae* is most typical of *C. imperspicua* (Sacc.) Cif. & Bat., but differs in having long and hyaline ascospores, which are 5-septate at maturity, while *C. imperspicua* has short and brownish ascospores, with 4 septa at maturity. *Conidiocarpus plumeriae* is also similar to *C. betle*. It however differs in having subcylindrical to obovoid asci, and 5-septate ascospores, while *C. betle* has broadly clavate asci, and 4-septate ascospores, surrounded by a hyaline sheath. Phylogenetic analyses based on LSU and ITS sequence data indicate that *C. plumeriae* is closely related to *C. betle*, but is morphologically distinct.

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References

Ariyawansa HA, Hyde KD, Jayasiri1 SC, Buyck B, Chethana KWT, Cui YY, Dai DQ, Dai YC, Daranagama DA, Jayawardena RS, Lücking R, Ghobad-Nejhad M, Niskanen T, Thambugala KM, Voigt K, Zhao RL, Boonmee S, Bahkali AH, Chen J, Cui BK, Dayarathne MC, Dissanayake AJ, Ekanayaka AH, Hashimoto A, Hongsanan S, Jones EBG, Larsson E, Lewis D, Li WJ, Li QR, Liu JK, Luo ZL, Maharachchikumbura SSN, Mapook A, McKenzie EHC, Norphanphoun C, Pang KL, Perera RH, Phookamsak R, Phukhamsakda C, Randrianjohany E, Senanayake IC, Singtripop C, Shang Q, Tanaka K, Tian Q, Tian CM, Tibpromma S, Verbeken A, Abdel-Wahab MA, Wanasinghe D, Wijayawardene NN, Zhang JF, Zhang H, Abdel-Aziz FA, Adamčík S, Ammirati JF, Bulgakov T, Cabral AL, Callaghan TM, Callac P, Chang CH, Coca LF, Dal-Forno M, Dollhofer V, Fliegerová K, Greiner K, Griffith GW, Ho HM, Hofstetter V, Jeewon R, Kang JC, Kirk PM, Kytövuori I, Lawrey JD, Li JHX, Liu ZY, Liu XZ, Liimatainen K, Lumbsch HT, Matumura M, Moncada B, Nuankaew S, Parnmen S, Santiago MA, Sato G, Sommai S, Song Y, Souza CAF, Souza-Motta CM, Su HY, Suetrong S, Wang Y, Wei SF, Wen TC, Yuan HS, Zhou LW, Reblova M, Fournier J, Camporesi E. 2015 – Fungal Diversity Notes 111–246 - Taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 75, 27−274.

Batista AC, Ciferri R. 1963 – The sooty-molds of the family *Asbolisiaceae*. Quaderno del Laboratorio Crittogamico del Istituto Botanico dell'Università di Pavia 31, 1–229.

Bose T, Reynolds DR, Berbee MA. 2014 – Common, unsightly and until now undescribed: *Fumiglobus pieridicola* sp. nov., a sooty mold infesting *Pieris japonica* from western North America. Mycologia 106(4), 746–756.

Cai L, Guo XY, Hyde KD. 2008 – Morphological and molecular characterization of a new anamorphic genus *Cheirosporium*, from freshwater in China. Persoonia 20, 53−58.
Cai L, Jeewon R, Hyde KD. 2006 – Phylogenetic investigations of *Sordariaceae* based on multiple gene sequences and morphology. Mycological Research 110, 137−150.
Chomnunti P, Hongsanan S, Hudson BA, Tian Q, Peršoh D, Dhami MK, Alias AS, Xu J, Liu X, Stadler M, Hyde KD. 2014 – The sooty moulds. Fungal Divers 66, 1–36.
Chomnunti P, Schoch CL, Aguirre-Hudson B, KoKo TW, Hongsanan S, Jones EBG, Kodsub R, Chukeatirote E, Bahkali AH, Hyde KD. 2011 – *Capnodiaceae*. Fungal Diversity 51, 103–134.
Crous PW, Schoch CL, Hyde KD, Wood AR, Gueidan C, de Hoog GS, Groenewald JZ. 2009a – Phylogenetic lineages in the Capnodiales. Study of Mycology 64, 17–47.
Crous PW, Verkley GJM, Groenewald JZ, Samson RA (eds). 2009b – Fungal biodiversity. CBS Laboratory Manual Series Centra albureau voor Schimmelcultures, Utrecht.
Faull JL, Olejnik I, Ingrouille M, Reynolds D. 2002 – A reassessment of the taxonomy of some tropical sooty moulds. Tropical Mycology 2, 33–40.
Hall TA. 1999 – BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.
Höhnel F. 1910 – Fragmente zur Mykologie (Xi Mitteilung, Nr. 527 bis 573) Sitsungsber, Kaiserl. Akad Wiss Math-Naturwiss ClA bt 1 119, 618–679.
Hongsanan S, Chomnunti P, Crous PW, Chukeatirote E, Hyde KD. 2014b – Introducing *Chaetothyriothecium*, a new genus of *Microthyriales*. Phytotaxa 161(2), 157−164.
Hongsanan S, Li YM, Liu JK, Hofmann T, Piepenbring M, Bhat JD, Boonmee S, Doilom M, Singtripop C, Tian Q, Mapook A, Zeng XY, Bahkali AH, Xu JC, Mortimer PE, Wu XH, Yang JB, Hyde KD. 2014a – Revision of genera in *Asterinales*. Fungal Diversity 68, 1–68.
Hongsanan S, Tian Q, Bahkali AH, Yang JB, McKenzie EHC, D. Hyde KD. 2015b – *Zeloasperisporiales* ord. nov., and two new species of *Zeloasperisporium*. Cryptogamie Mycologie 36(3), 301–317.
Hongsanan S, Tian Q, Peršoh D, Zeng XY, Hyde KD, Chomnunti P, Boonmee S, Bahkali AH, Wen TC. 2015a – *Meliolales*. Fungal Diversity 74, 1–51.
Huelsenbeck JP, Ronquist F. 2001 – MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17(8), 754−755.
Hughes SJ. 1972 – New Zealand fungi 17, pleomorphism in *Euantennariaceae* and *Metacapnodiaceae*, two families of sooty moulds. New Zealand Journal of Botany 10, 225–242.
Hughes SJ. 1976 – Sooty moulds. Mycologia 68(4), 693–820.
Hughes SJ, Seifert KA. 2012 – Taxonomic and nomenclatural notes on sooty mould name based on species mixtures: *Hormiscium handelii* and *Torula lecheriana*. Mycoscience 53, 17–24.
Hyde KD, Jones EBG, Liu JK, Ariyawansha H, Boehm E, Boonmee S, Braun U, Chomnunti P, Crous P, Dai DQ, DiederichP, Dissanayake A, Doilom M, DoveriF, Hongsanan S, JayawardenaR, Lawrey JD, Li YM, Liu YX, Lücking R, Monkai J, Nelsen MP, Phookamsak R, Muggia L, Pang KL, Senanayake I, Shearer CA, WijayawardeneN, Wu HX, Thambugala M, Suetrong S, Tanaka K, Wikee S, Zhang Y, Hudson BA, Alias SA, Aptroot A, Bahkali AH, Bezerra LJ, Bhat JD, Camporesi E, Chukeatirote E, Hoog SD, Gueidan C, Hawksworth DL, Hirayama K, Kang JC, Knudsen K, Li WJ, Liu ZY, McKenzie EHC, Miller AN, Nadeeshan D, Phillip AJL, Mapook A, Raja HA, Tian Q, Zhang M, Scheuer C, Schumm F, Taylor J, Yacharoen S, Tibpromma S, Wang Y, Yan J, Li X. 2013 – Families of Dothideomycetes. Fungal Diversity 63, 1–313.
Index Fungorum. 2016 – http://www.indexfungorum.org/Names/Names.asp.
Jaczewski AA. 1917 – Key to Fungi. Vol. 2. (Fungi imperfecti). Petrograd 743.
Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat JD, Buyck B, Cai L, Dai YC, Abd-Elsalam KA, Ertz D, Hidayat I, Jeewon R, Jones EBG, Bahkali AH, Karunarathna SC, Liu JK, Luangsa-ard JJ, Lumbsch HT, Maharachchikumbura SSN, McKenzie EHC, Moncalvo JM, Ghobad-

Nejhad M, Nilsson H, Pang KL, Pereira OL, Phillips AJL, Raspé O, Rollins AW, Romero AI, Etayo J, Selçuk F, Stephenson SL, Suetrong S, Taylor TE, Tsui CKM, Vizzini A, Abdel-Wahab MA, Wen TC, Boonmee S, Dai DQ, Daranagama DA, Dissanayake AJ, Ekanayaka AH, Fryar SC, Hongsanan S, Jayawardena RS, Li WJ, Perera RH, Phookamsak R, de Silva NI, Thambugala KM, Tian Q, Wijayawardene NN, Zhao RL, Zhao Q, Kang JC, Promputtha I. 2015 – The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. Fungal Diversity 74, 3–18

Katoh K, Asimenos G, Toh H. 2009 – Multiple alignment of DNA sequences with MAFFT. Methods in Molecular Biology 537, 39–64.

Liu JK, Hyde KD, Jones EBG, Ariyawansa HA, Bhat DJ, Boonmee S, Maharachchikumbura SSN, Mckenzie EHC., Phookamsak R, Phukhamsakda C, Shenoy BD, Abdel-Wahab MA, Buyck B, Chen J, Chethana KWT, Singtripop C, Dai DQ, Dai YC, Daranagama DA, Dissanayake AJ, Doilom M, D'souza MJ, Fan XL, Goonasekara ID, Hirayama K, Hongsanan S, Jayasiri SC, Jayawardena RS, Karunarathna SC, Li WJ, Mapook A, Norphanphoun C, Pang KL, Perera RH, Peršoh D, Pinruan U, Senanayake IC, Somrithipol S, Suetrong S, Tanaka K, Thambugala KM, Tian Q, Tibpromma S, Udayanga D, Wijayawardene NN, Wanasinghe DN, Wisitrassameewong K, Zeng XY, Abdel-Aziz FA, Adamčík S, Bahkali AH, Boonyuen N, Bulgakov T, Callac P, Chomnunti P, Greiner K, Hashimoto A, Hofstetter V, Kang JC, Lewis D, Li XH, Liu XZ, Liu ZY, Matsumura M, Mortimer PE, Rambold G, Randrianjohany E, Sato G, Sri-Indrasutdhi V, Tian CM, Verbeken A, Von Brackel W, Wang Y, Wen TC, Xu JC, Yan JY, Zhao RL, Camporesi E. 2015 – Fungal diversity notes 1–110: taxonomic and phylogenetic contributions to fungal species. Fungal Diversity 72, 1–197.

Nylander JAA, Wilgenbusch JC, Warren DL, Swofford DL. 2008 – AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24, 581−583.

Page RDM. 2001 – Tree View: treed rawing software for Apple Macintosh and Windows. Available at http://taxonomy.zoology.gla.ac.uk/rod/ treeview.html

Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A. 2009 – How many bootstrap replicates are necessary? LNCS 5541, 184–200.

Reynolds DR. 1998 – *Capnodiaceous* sooty mold phylogeny. Canadian Journal of Botany 76, 2125–2130.

Schoch CL, Crous PW, Groenewald JZ, Boehm EW, Burgess TI, de Gruyter J, de Hoog GS, Dixon LJ, Grube M, Gueidan C, Harada Y, Hatakeyama S, Hirayama K, Hosoya T, Huhndorf SM, Hyde K D, Jones EB, Kohlmeyer J, Kruys A, Li YM, Lucking R, Lumbsch HT, Marvanova L, Mbatchou JS, McVay AH, Miller AN, Mugambi GK, Muggia L, Nelsen MP, Nelson P, Owensby CA, Phillips AJ, Phongpaichit S, Pointing SB, Pujade-Renaud V, Raja HA, Plata ER, Robbertse B, Ruibal C, Sakayaroj J, Sano T, Selbmann L, Shearer CA, Shirouzu T, Slippers B, Suetrong S, Tanaka K, Volkmann-Kohlmeyer B, Wingfield MJ, Wood AR, Woudenberg JH, Yonezawa H, Zhang Y, Spatafora JW. 2009 – A class-wide phylogenetic assessment of dothideomycetes. Studies in Mycology 64, 1–15.

Silvestro D, Michalak I, 2012 – RaxmlGUI: a graphical front-end for RAxML. Organisms Diversity & Evolution 12, 335–337.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011 – MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution 28(10), 2731–2739.

Theissen F, 1916 – Mykologische Abhandlungen. Verh. der Kaiserlich– Koniglichen Zoologisch– Botanischen Gesell. Wien 66, 296–400.

Wijayawardene NN, Crous PW, Kirk PM, Hawksworth DL, Boonmee S, Braun U, Chomnunti P, Dai DQ, D'souza MJ, Diederich P, Dissanayake A, Doilom M, Hongsanan S, Jones EBG, Groenewald JZ, Jayawardena R, Lawrey JD, Liu JK, Lücking R, Madrid H, Manamgoda DS, Muggia L, Nelson MP, Phookamsak R, Suetrong S, Tanaka K, Thambugala KM,

Wikee S, Zhang Y, Aptroot A, Ariyawansa HA, Bahkali AH, Bhat JD, Gueidan C, De Hoog GS, Knudsen K, McKenzie EHC, Miller AN, Mortimer PE, Wanasinghe DN, Phillips AJL, Raja HA, Slippers B, Shivas RS, Taylor JE, Wang Y, Woudenberg JHC, Piatek M, Cai L, Jaklitsch WM, Hyde KD. 2014 – Naming and outline of Dothideomycetes. Fungal Diversity 69 (1), 1−55.

Winka K, Eriksson O, Bång A. 1998 – Molecular evidence for recognizing the *Chaetothyriales*. Mycologia 90(5), 822–830.

Zhaxybayeva O, Gogarten JP, 2002 – Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. BMC Genomics 3, 4.

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Meliolales

Sinang Hongsanan[1,2,3,4] · Qing Tian[3,4] · Derek Peršoh[5] · Xiang-Yu Zeng[3,4] · Kevin D. Hyde[1,2,3,4,6] · Putarak Chomnunti[3,4] · Saranyaphat Boonmee[3,4] · Ali H. Bahkali[6] · Ting-Chi Wen[7]

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Abstract The order *Meliolales* comprises the families *Armatellaceae* and *Meliolaceae*. These are black mildews that grow on the surface of host plants, often regarded as minor plant pathogens. In this study, types or specimens of 17 genera of *Armatellaceae* and *Meliolaceae* were borrowed from herbaria and re-examined. *Armatella* is accepted in *Armatellaceae* and *Amazonia*, *Appendiculella*, *Asteridiella*, *Cryptomeliola*, *Endomeliola*, *Irenopsis* and *Meliola* are accepted in the family *Meliolaceae. Laeviomeliola* is synonymized under *Meliola. Ceratospermopsis*, *Ectendomeliola*, *Haraea*, *Hypasteridium*, *Leptascospora*, *Metasteridium*, *Ophiociliomyces*, *Ophioirenina*, *Ophiomeliola*, *Parasteridium*, *Pauahia*, *Pleomeliola*, *Pleomerium*, *Prataprajella*, *Ticomyces*, *Urupe* and *Xenostigme* are excluded from *Meliolaceae*, and are treated as doubtful genera or placed in ascomycetes genera incertae sedis. The type species of each genus is re-described and illustrated with photomicrographs. Notes are provided and comparisons made. Two new species of *Meliola* and one new species of *Irenopsis* are also introduced with molecular data and we provide the most populated phylogenetic tree of *Meliolomycetidae* to date. *Meliola thailandicum* was found on *Dimocarpus longan* (*Sapindales*) and *Acacia auriculiformis* (*Fabales*) and confirmed to be the same species in the molecular analyses. This has important implications as the several hundred *Meliola* species are recognized based on host associations. Thus the same species being recorded from two unrelated hosts sheds doubt on *Meliola* species being host-specific.

Keywords *Armatella* · *Armatellaceae* · Ascomycota · Black mildews · *Meliola* · *Meliolaceae* · *Meliolomycetidae* · *Sordariomycetes* · Morphology · Type · Species · Taxonomy

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✉ Ting-Chi Wen
tingchiwen@yahoo.com

[1] Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan, People's Republic of China

[2] World Agroforestry Centre, East and Central Asia, Kunming 650201, Yunnan, People's Republic of China

[3] Institute of Excellence in Fungal Research, Chiang Rai 57100, Thailand

[4] School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

[5] Ruhr-Universität Bochum, Geobotany, Universitätsstraße 150, 44780 Bochum, Germany

[6] Botany and Microbiology Department, College of Science, King Saud University, Riyadh, KSA 11442, Saudi Arabia

[7] The Engineering and Research Center of Southwest Bio-Pharmaceutical Resource, Ministry of Education, Guizhou University, Guiyang 550025, China

Introduction

Species of *Meliolales* are obligate, biotrophic, foliar pathogens, which occur as epiphytes on leaves, stems, branches, and sometimes on petioles of vascular plants (Hansford 1961; Hosagoudar 1994, 1996, 2008, 2013; Mibey and Hawksworth 1997; Old et al. 2003). They are commonly known as the "black mildews" or "dark mildews" based on their black colonies up to one cm diameter, produced on the surface of hosts, and are widely distributed especially in subtropical and tropical regions (Goos and Andersons 1974; Ainsworth 1963; Hosagoudar 1994, 1996, 2003a, 2008; Saenz and Taylor 1999; Mibey and Hawksworth 1997; Old

et al. 2003; Justavino et al. 2015; Maharachchikumbura et al. 2015). They are believed to show a high degree of host specificity, and therefore it is necessary to identify the host before determining the mildew (Hansford 1961; Hirata 1971; Hosagoudar and Goos 1989). The extent of host association, however, has not yet been determined, and molecular studies are needed to establish that species are host-specific. Species of *Meliolales* are not pathogens in a strict sense and do not cause extensive damage to host plants (Sabulal et al. 2006; Hosagoudar et al. 1997), however, they may increase the temperature and respiration in areas covered by the fungus, and also reduce photosynthesis as in the family *Asterinaceae* (Hongsanan et al. 2014). Taxa of the order obtain nutrients by producing haustoria in host cells, and heavy infections result in a dirty appearance on plants and economic products (Fig. 1) and may reduce their value (Hosagoudar et al. 1997, 2012). Wellman (1972) noted that this group produces significant affects on crops, but has never been seriously studied (Hosagoudar and Riju 2013).

Species of *Meliolales* are often confused with sooty moulds because they coat fruits and leaves superficially with black colonies. Sooty moulds are saprobes which develop on leaf surfaces feeding on honeydew (Chomnunti et al. 2011, 2014). Species of *Meliolales* produce a variety of structures which can penetrate host cells for the uptake nutrients (Mibey and Hawksworth 1997; Saenz and Taylor 1999). This order is different from powdery mildews in morphology, but can develop in the same ecological habitats (Saenz and Taylor 1999).

The order *Meliolales* comprises the families *Armatellaceae* and *Meliolaceae*. The family *Meliolaceae* was proposed by Martin (1941) based on the generic type *Meliola* (Martin 1941; Hosagoudar 1994, 1996, 2008; Mibey and Hawksworth 1997; Old et al. 2003; Justavino et al. 2015; Maharachchikumbura et al. 2015). Members of the family are characterized by a biotrophic habitat, superficial dark hyphae, although sometimes the hyphae may be immersed in plant cells, with lateral hyphopodia and/or phialides. Superficial ascomata develop in the black, web-like colonies and are flattened or globose to subglobose, and have a dark peridium. Setae or appendages cover the ascomata and/or black mycelia in some genera. Asci are unitunicate, usually 3–4-spored, with brown, 3–4-septate ascospores (Mibey and Hawksworth 1997; Hosagoudar and Riju 2013). The asexual states of *Meliolaceae* species have been reported as phialides on hyphae, however, they are morphologically relatively poorly studied. Many characters of the family, such as types of hyphopodia, nature of phialides and structure of asci, are unclear, because various studies have provided different interpretations. The family *Armatellaceae* was established by Hosagoudar (2003b), with generic type *Armatella* Theiss. & Syd. Members of the family are characterized by superficial hyphae with hyphopodia, lacking phialides, ascomata which develop in the black colonies, cylindrical to subcylindrical asci, with 4–8-spores, and ascospores hyaline to brown at maturity, and 1-septate (Hosagoudar 2003b). The asexual morph is undetermined.

Because of their biotrophic lifestyles, species of *Meliolales* have yet to be grown in culture. Molecular data for species of *Meliolales* have therefore been obtained by directly extracting DNA from ascomata. Thite (1975) and Goos (1978) succeeded in germinating the ascospores, but growth following germination was limited and later ceased. We therefore focused on obtaining DNA and sequence data directly from the ascomata.

Taxonomic review

Superficial hyphae of *Meliolales* species are characterized by web-like colony formation on the host surface, usually branched, septate, and brown. Hyphopodia and setae are present in the genera *Cryptomeliola*, *Irenopsis*, and *Meliola* (Hansford 1961; Hosagoudar 1994, 1996, 2008, 2013; Mibey and Hawksworth 1997; Old et al. 2003). *Endomeliola* have intercellular hyphae which extend into the mesophyll (Hughes and Pirozynski 1994).

The nature of the hyphopodia is thought to be extremely important in identifying species in *Meliolales* (Hansford 1961), but this has never been proven by molecular evidence. The term hyphopodia was used by Gaillard (1891). Goos and Gessner (1975) noted that the hyphopodia is one kind of appressoria. Both terms hyphopodia and appressoria have been used in the literature, so Goos and Gessner (1975) suggested retaining usage of either. However, hyphopodia and appressoria are different because appressoria are produced on germ tubes, while hyphopodia are produced on hyphae (Emmett and Parbery 1975). These terms have been used interchangeably in many studies. As hyphopodia are produced on hyphae we recommend use of the term “hyphopodia” for the structures in *Meliolales* species. The hyphopodia are characterized by two cells, a short basal stalk cell bearing a single capitate hyphopodium. Hyphopodia may be alternate, opposite, or mixed alternate and opposite on hyphae depending on the species. Two kinds of hyphopodia are recognized in *Meliolales*, capitate and mucronate (Gaillard 1891). Capitate hyphopodia comprise a short stalk cell, bearing a single hyphopodia, which is the attachment and absorption organ. This later produces a single haustorium in the epidermal layer of host plants (Hansford 1961). Different species have differently shaped hyphopodia ranging from ovate, oblong, or angular to lobate. The capitate hyphopodia on the lower surface of leaves are mostly irregular in shape, while species found on the upper surface of leaves are globose to subglobose (Hansford 1961).

Mucronate hyphopodia were illustrated by Hughes (1981) and Mueller et al. (1991). Mucronate hyphopodia were

Fig. 1 *Meliolaceae* on various hosts. **a**, **b** *Dimocarpus longan*. **c**, **d** *Citrus maxima*. **e** *Litchi chinensis*. **f** *Mangifera indica*. **g** *Citrus reticulata*

interpreted as phialides and are conoid or ampuliform on hyphae, however they vary in shape in the same colony (Hansford 1961). Phialides produce conidia or phialoconidia (Hughes 1981, Mueller et al. 1991). There is however, no report to confirm that phialoconidia can develop into robust hyphae (Hughes 1981). Most species of *Meliolaceae* produce opposite phialides, or occasionally they are alternate or mixed with capitate hyphopodia on the hyphae. Some species in *Meliolaceae* lack phialides, but the apparent lack is probably due to the difficulty of observing such structures. Phialides appear to lack penetration or function (Hansford 1961; Goos 1974; Goos and Gessner 1975; Mueller et al. 1991). Species in *Armatellaceae* are characterized by hyphae without phialides, and the asexual morph is undetermined (Hosagoudar 2003b).

Ascomata are solitary or scattered on colonies of superficial hyphae and mostly develop at the centre of the colony. The ascomata is first flat, cells are radially arranged, and then become globose to subglobose, however a few genera (e.g., *Amazonia*), have flattened ascomata even at maturity. The wall of the ascomata comprises two strata, the outer strata having dark brown walls, and inner strata hyaline walls. A rounded pore is present at the apex of ascomata and is filled with hyaline periphyses and is covered by hyphal layers, and

is thus hard to observe. The surface of ascomata is mostly verrucose. In *Asteridiella*, the surface has raised conoid cells, while conical appendages are present in *Appendiculella* species. The genus *Irenopsis* has setae on the surface of ascomata which are dark, smooth or rough, septate or aseptate, straight to coiled, with acute, rounded or hooked ends, and narrower than appendages in *Appendiculella*.

The asci of *Meliolales* have been reported differently in many studies. Müller and von Arx (1973) considered the asci of *Meliolales* species to be unitunicate. Eriksson (1981) introduced the term "Pseudoprototunicate" to describe the asci in *Meliolales*. A pseudoprototunicate ascus is characterized by thin walls, and is thought to have evolved from bitunicate asci, in which the endotunica is reduced. There is no opening mechanism, and they are evanescent at maturity (Eriksson 1981; Barr and Huhndorf 2001; Hofmann 2009). Hawksworth and Eriksson (1986) emended the description provided by Gäumann (1964) and concluded that *Meliolales* had bitunicate asci. However, Hosagoudar et al. (1997, 2012) used unitunicate to describe the asci of *Meliolales* species. We suggest using the term unitunicate asci to define the asci in *Meliolales* based on phylogeny, which place the species of *Meliolaceae* in *Sordariomycetes* (Maharachchikumbura et al. 2015).

The ascospores are very uniform in shape and septation in the family *Meliolaceae*. They are thick-walled, cylindrical to oblong, fusiform or obovoid, and slightly curved in some species. Some species have 3-septate ascospores, but in most they are 4-septate. In most species the central cells are widest and longest. The ascospores are more or less constricted at the septa, with smooth walls. The end cells are mostly rounded, but can rarely be conoid or apiculate (Hansford 1961; Mibey and Hawksworth 1997). All species in the family are hyaline when immature, and dark brown and thick-walled at maturity. However, some genera with two to multiseptate hyaline ascospores were included in *Meliolaceae* (Lumbsch and Huhndorf 2010). In this study, we only accept genera with 3–4-septate and brown ascospores in *Meliolaceae*. Ascospores in *Armatellaceae* are aseptate or 1-septate, hyaline to brown, and smooth or slightly rough-walled.

History of *Armatellaceae* and *Meliolaceae*

The family *Meliolaceae* was established by Martin (1941) without a Latin diagnosis but validated by Hansford (1946). Roger (1953) placed the *Meliolaceae* with nine other families in the order *Hypocreales*. This family has also been placed in the orders *Dothideales*, *Erysiphales*, *Meliolales*, *Myriangiales* and *Hypocreales* at various times (Martin 1941; Luttrell 1951, 1989; Roger 1953; Ainsworth et al. 1971; Müller and von Arx 1973; Yarwood 1973; Barr 1976; Eriksson 1981; Hawksworth et al. 1983). Monographs of the *Meliolaceae* were provided by Hansford (1961, 1963), with keys to the genera of the family based on morphology and host association (Hansford 1961). Ainsworth et al. (1971) and Eriksson and Hawksworth (1993) listed 50 genera in the family. This was reduced to 25 genera (Hawksworth et al. 1995) and later 22 genera (Kirk et al. 2008). A biogeographical distribution of *Meliolaceae* in India was provided by Hosagoudar (2006) and he included a key to the genera in the family. Lumbsch and Huhndorf (2010) placed *Meliolaceae* in the class *Sordariomycetes* with 26 genera. This was followed by Hyde et al. (2013) and Wijayawardene et al. (2014), who excluded *Meliolaceae* from *Dothideomycetes*. The family *Armatellaceae* was introduced by Hosagoudar (2003b). *Armatellaceae* contains a single monotypic genus (Table 1). Although *Basavamyces* Hosag. was included in *Armatellaceae* by Hosagoudar (2008), Hosagoudar et al. (2012) concluded that *Basavamyces* is not the member of *Armatellaceae* because of its 2-septate ascospores.

Saenz and Taylor (1999) directly sequenced DNA from ascomata and their phylogenetic tree indicated that *Meliolales* was a member of Pyrenomycetes, which are close to *Sordariales*. Pinho et al. (2012a) reported that the phylogenetic placement of members of *Meliolales* was uncertain because the phylogenetic analyses (28S rDNA) were not strongly supported, but this order is monophyletic within *Sordariomycetes*. Kirk et al. (2001) introduced a new subclass, *Meliolomycetidae* (*Sordariomycetes*) for members of *Meliolaceae*, but without a description or diagnosis. The placement of *Meliolomycetidae* in *Sordariomycetes* was confirmed by Justavino et al. (2015) based on their phylogenetic tree and Maharachchikumbura et al. (2015) validated the subclass. *Meliolales* species are biotrophic and cannot be cultured, thus there are few sequences for species in GenBank.

Ecology

The *Meliolales* are biotrophs, or pathogens of living leaves or occasionally dead leaves, and are common on scrubs in open park-lands and on inaccessible canopies of rainforest trees (Hansford 1961; Hosagoudar 2003b, 2008). Species of *Meliolales* are associated with a variety of plant substrates including living leaves, and occasionally petioles, twigs, and branches (Hansford 1961; Hosagoudar 1994, 1996, 2008; Hosagoudar and Riju 2013; Mibey and Hawksworth 1997; Old et al. 2003). Species of *Armatellaceae* and *Meliolaceae* have a worldwide distribution (Kirk et al. 2008). They are especially common in the tropics and have an extended distribution to sub-temperate to temperate regions, and are generally lacking in arid regions (Hansford 1961; Hosagoudar

Table 1 Genera included in *Armatellaceae* and *Meliolaceae* by different researchers

	Hosagoudar (2003b)	Hosagoudar (2008)	Hosagoudar et al. (2012)	This study		
Armatellaceae	*Armatella*	*Armatella* *Basavamyces*	*Armatella*	*Armatella*		
	Stevens 1925 (Meliolineae)	Hansford 1946 (Meliolineae)	Hansford (1961)	Boedijin (1961)	Lumbsch and Huhndorf (2010)	This study
	Actinodothis	*Actinodothis*	*Amazonia*	*Amazonia*	*Amazonia*	*Amazonia*
	Amazonia	*Amazonia*	*Appendiculella*	*Appendiculella*	*Appendiculella*	*Appendiculella*
Meliolaceae	*Irene*	*Appendiculella*	*Asteridiella*	*Armatella*	*Asteridiella*	*Asteridiella*
	Meliola	*Irene*	*Irenopsis*	*Asteridiella*	*Basavamyces*	*Cryptomeliola*
	Meliolina	*Irenina*	*Meliola*	*Irenopsis*	*Ceratospermopsis*	*Endomeliola*
	Meliolinopsis	*Irenopsis*		*Meliola*	*Cryptomeliola*	*Irenopsis*
		Meliola			*Ectendomeliola* *Endomeliola*	*Meliola*
					Haraea	
					Hypasteridium	
					Irenopsis	
					Laeviomeliola	
					Leptascospora	
					Meliola	
					Metasteridium	
					Ophiociliomyces	
					Ophioirenina	
					Ophiomeliola	
					Parasteridium	
					Pauahia	
					Pleomeliola	
					Pleomerium	
					Prataprajella	
					Ticomyces	
					Urupe	
					Xenostigme	

1994, 1996, 2008, 2013; Saenz and Taylor 1999; Mibey and Hawksworth 1997; Old et al. 2003; Justavino and Piepenbring 2007; Kirk et al. 2008; Piepenbring et al. 2011; Pinho et al. 2012b, 2013; Justavino et al. 2015). Studies on the dispersal of ascospores in *Meliolales* are lacking (Hansford 1961; Hosagoudar 1996; Nayar et al. 1998). Dispersal of species of *Meliolaceae* probably occurs by rain splash, insects, or air (Nayar et al. 1998). *Meliolaceae* species can survive after the leaves have been shed. When new leaves grow during the next season they are colonized by *Meliolaceae* with spores from leaves on the ground (Nayar et al. 1998). *Meliolaceae* in forests are more abundant during the cool season in tropical to subtropical regions. Even though forests are burnt leaving only the perennial root stocks; this group of fungi still occurs during the next season (Nayar et al. 1998). There is no report concerning the dispersal of ascospores in species of *Armatellaceae*.

Life cycle of *Armatellaceae* and *Meliolaceae*

Species of *Meliolales* have different infection mechanisms and the life cycle is illustrated in Fig. 2. Thimmaiah et al. (2013) stated that colonization starts when a mature ascospore attaches to the host substrate (Fig. 2a). A primitive stalk-cell bearing a single capitate, angular or lobate hyphopodium is mostly produced from the terminal cell of the ascospore; this is likely to be an intermediate step before mature hyphopodia development (Fig. 2b) (Tucker et al. 2010, Hongsanan et al. 2014). The hyphopodium then forms an apoplastic complex which is called an interaction apparatus, based on a study of *Asteridiella callista* (Justavino et al. 2014). At the contact zone between epidermal cells and hyphopodia, the apoplastic interaction apparatus forms a penetration pore so that the haustorium can develop into the cytoplasmic membrane and epidermal cells, and rarely in deeper tissues. Nutrient uptake

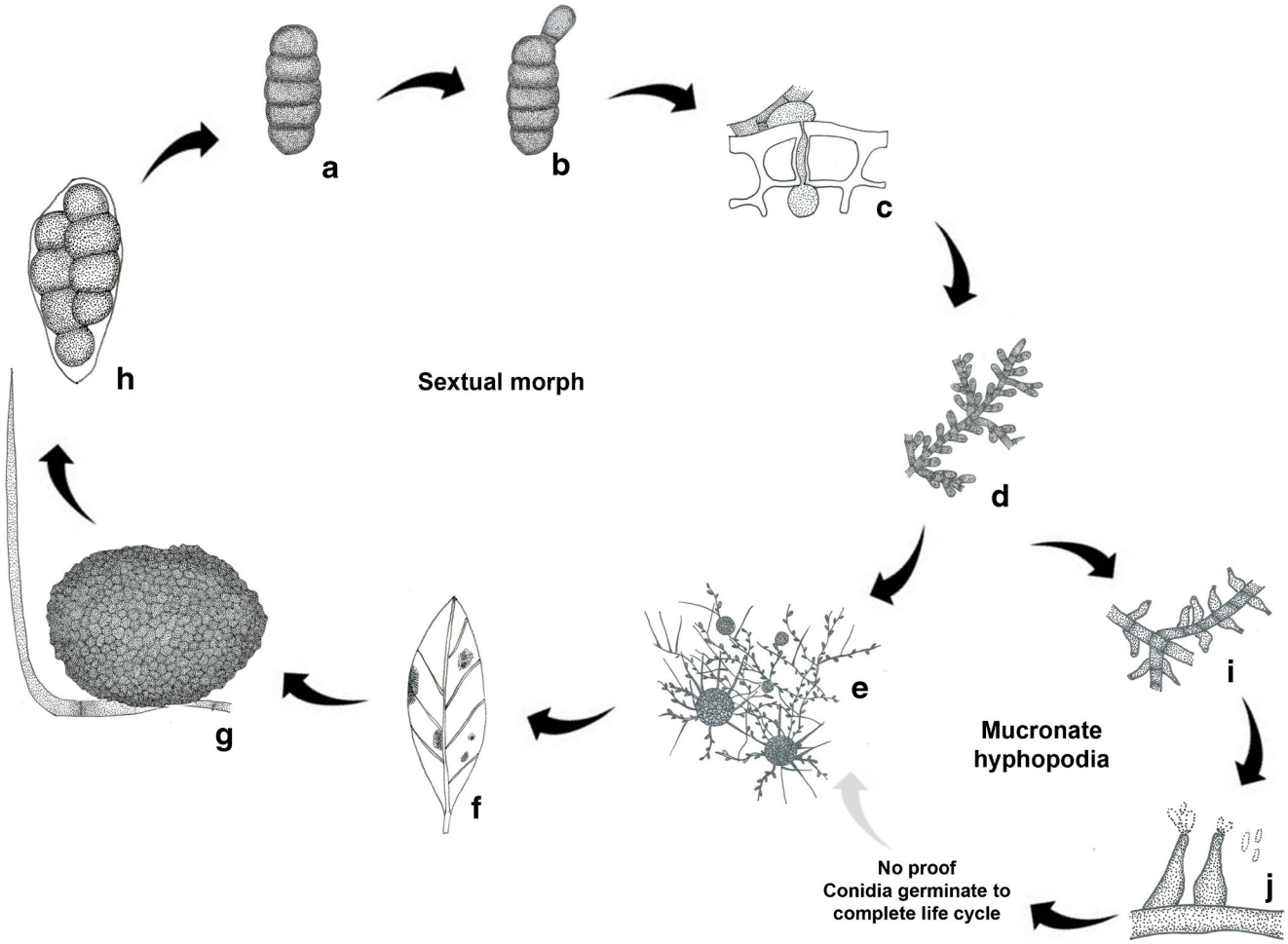


Fig. 2 Life cycle of *Meliola* sp. and its mucronate hyphopodia. **a** Ascospore. **b** Mature germinating ascospore. **c** Haustorium developing in epidermal cell. **d** Lateral hyphae forming superficially. **e** Colony formation. **f** Black spot of hyphae forming on the host surface. **g** Ascoma with hyphal setae. **h** Mature ascus. **i** Hyphopodia with phialides. **j** Phialoconidia

begins (Fig. 2c) and the other cells of the ascospores produce superficial hyphae with frequent branches, and then alternate or opposite lateral hyphopodia are formed on short stalks (Fig. 2d). Initial infection and web-like colony formation on the leaf surface follows (Fig. 2e, f). In *Meliola* species, superficial hyphae produce strong, dark setae. Ascomata are formed laterally on the hyphae, at first being flat, cells are radially arranged, and then becoming globose to subglobose at maturity (Fig. 2g). The genus *Amazonia* is the only genus with flattened ascomata. The ascomata are filled with sterile tissue and asci which are evanescent at maturity (Fig. 2h). Ascospores are produced within the 2–4-spored asci in *Meliolaceae* and 4–8-spored asci in *Armatellaceae*. The asexual morph of *Meliolaceae* develops from the hyphae, and form ampuliform hyphopodia which are called "phialides" (Fig. 2i). These structures developed on the same hyphae as the capitate hyphopodia or sometimes are found on separate hyphae, but lack penetration or apparent function (Mueller et al. 1991). Conidia, which are 1-celled, thin-walled and hyaline, are produced within the neck of phialides (Fig. 2j). The conidiogenous cells are probably present at the base of the phialides (Hughes 1981). It is unlikely that these small phialoconidia can develop into robust hyphae (Hughes 1981) and their function is unclear. There has been a lack of research to establish the asexual morph cycle. There is no reported asexual morph in *Armatellaceae*, and phialides are lacking.

Host specificity

Species in *Meliolales* are considered to have a high degree of host specificity because they are biotrophic on living leaves. Numerous studies have introduced new species based only on host association, even though taxa are morphologically identical (Hosagoudar 1987). Some species on different hosts are distinct in morphology and in penetration mechanisms (Fig. 3) (Hosagoudar 2003a; Justavino and Piepenbring 2007). Hence, most species of *Meliolales* species have been justified based on host association, and it is essential to establish the host

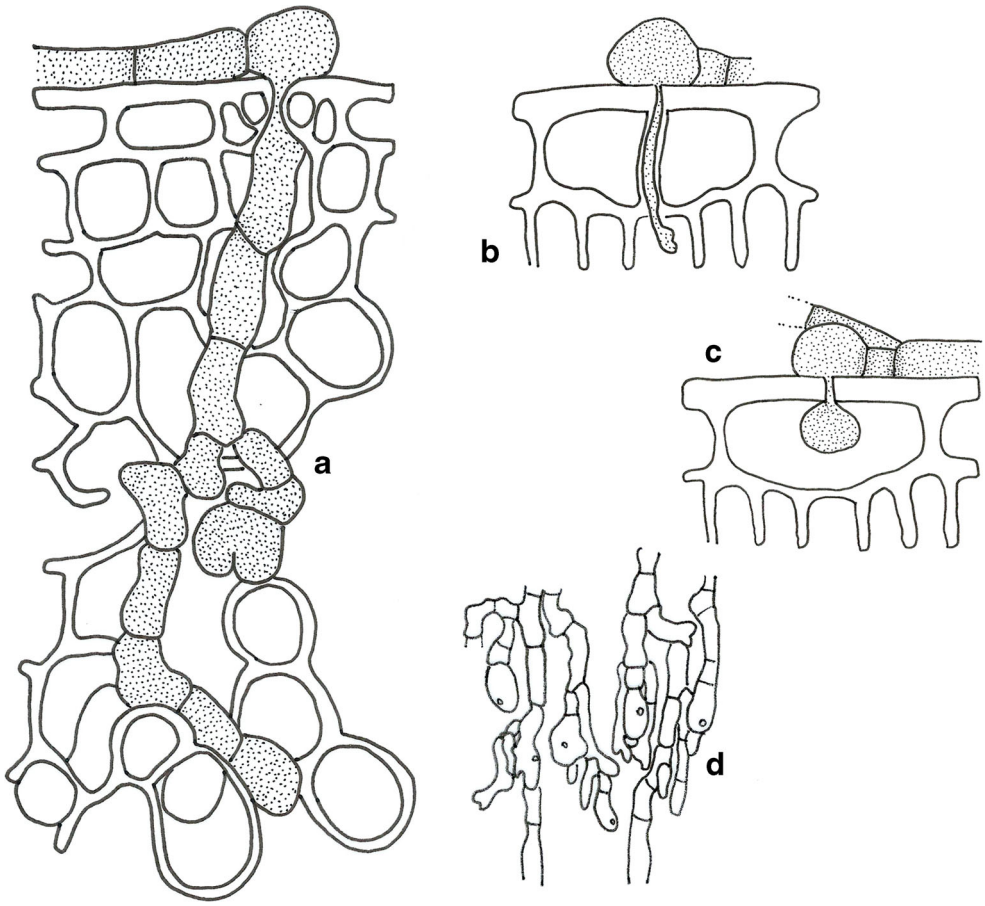


Fig. 3 Host penetration by *Meliolales* species (**a**–**c** redrawn and modified from Yamamoto 1954, **d** redrawn from Hughes and Pirozynski 1994). **a** Hyphopodium of *Meliolina octospora*. penetrating epidermis cells. **b** Hyphopodium of *Irenina rhaphiolepis* Yam. penetrating epidermis and mesophyll cells. **c** Hyphopodium of *Irenopsis coronata* var. *triumfettae* Stev. penetrating epidermis cell. **d** Hyphae with hyphopodia of *Endomeliola dingleyae* developing in epidermis and mesophyll cells

genus or family before identifying the fungal species. Whether *Meliolales* species are host specific needs verifying using molecular techniques.

Molecular phylogeny of *Meliolales*

The morphology of *Meliolales* species indicate that they belong to the class *Sordariomycetes* (Zhang et al. 2006). Pinho et al. (2012a) used sequence data of species in *Meliolaceae* and their phylogenetic tree confirmed the placement within *Sordariomycetes*. Justavino et al. (2015) mentioned a new subclass *Meliolomycetidae* based on their phylogenetic tree which was represented by a basal clade of *Meliolaceae* in *Sordariomycetes*. However, the phylogenetic placement of *Meliolaceae* members was uncertain within the clade because the phylogenetic analyses (28S rDNA) were not strongly supported, but monophyletic within *Sordariomycetes* (Pinho et al. 2012a). Maharachchikumbura et al. (2015) provided a backbone tree for *Sordariomycetes* based on LSU, SSU, TEF and RPB2 sequence data analysis, and their phylogenetic tree indicated that *Meliolomycetidae* is most closely related to *Sordariomycetidae* in *Sordariomycetes* as also found by Justavino et al. (2015). There are few sequences for the species of *Meliolaceae* as they are biotrophic and cannot be cultured (Maharachchikumbura et al. 2015). Most genera discussed here lack molecular data, however, the placements are based on morphological similarities and where possible phylogeny. No sequence data is available for any species of *Armatellaceae*.

Material and methods

Morphology

Type or other specimens of *Appendiculella*, *Asteridiella*, *Cryptomeliola*, *Endomeliola*, *Haraea*, *Irenopsis*, *Laeviomeliola*, *Leptascospora*, *Meliola*, *Ophiociliomyces*, *Ophioirenina*, *Ophiomeliola*, *Pauahia*, *Pleomeliola*, and *Pleomerium* were obtained from B, BPI, ILL, IMI, K(M), PDD, S, URM and ZT. Their morphology was observed under a stereomicroscope. Sections of ascomata were made freehand. Morphological characters were observed and photographed under a compound microscope (Nikon 80i).

Measurements were made using the Tarosoft (R) Image Frame Work v. 0.9.7.

Fresh specimens of *Meliolaceae* were collected in Thailand. Morphological characters were observed and photographed in the same way as herbarium specimens. Single spore isolation was performed following the method of Chomnunti et al. (2014) on PDA (potato dextrose agar) and MEA (malt extract agar), but was generally unsuccessful. Sequences were therefore obtained directly using dry fungal fruiting bodies.

Type specimens of the *Meliolaceae* are deposited in the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand. Faces of fungi numbers and Index fungorum numbers were obtained as in Jayasiri et al. (2015) and Index fungorum (2015).

DNA isolation, amplification and sequencing

DNA extraction was made directly from dry fungal fruiting bodies to obtain sequence data. Extraction was started by placing individual ascomata in 1.5 *ml* sterilized tubes and leaving overnight at −20 °C. DNA extraction was performed by E.Z.N.A® Forensic Genomic DNA Extraction Kit (OMEGA Bio-tek Norcross GA 2013), GoTaq® Hot start-Promega, Lysis Buffer for Microorganism to Direct PCR (TaKaRa); following the manufacturer's instructions.

For GoTaq® Hot start-Promega, amplification conditions were set up for initial denaturation of 3 min at 95 °C, followed by 35 cycles of 27 s at 94 °C, 60 s at 56 °C and 90 s at 72 °C, and a final extension period of 7 min at 72 °C. PCR-products were cleaned from excessive nucleotides and primers by adding 2 μl of 1:5 diluted ExoSAP-IT® (Affymetrix, ExoSAP-IT® For PCR Product Cleanup) to 5 μl of PCR-product. The mix was incubated in the thermocycler for 30 min at 37 °C, followed by 15 min at 80 °C, and sequencing was performed on a ABI 3130xl sequencer in the sequencing service in the faculty of biochemistry at the Ruhr-Universität Bochum. For the specimens which were performed by using Lysis Buffer for Microorganism to Direct PCR (TaKaRa) Kit and E.Z.N.A® Forensic Genomic DNA Extraction Kit, amplification conditions were set up for initial denaturation of 5 min at 95 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 52 °C and 90 s at 72 °C, and a final extension period of 10 min at 72 °C. PCR-products were checked on 1 % agarose electrophoresis gels stained with ethidium bromide. The purification and sequencing of PCR products were done by Majorbio Co., China.

Phylogenetic analysis

Sequences data were downloaded from GenBank to supplement the dataset (Supplementary Table 1). *Dothidea sambuci* was selected as outgroup taxon. The representative sequences included taxa from *Amplistromatales*, *Annulatascales*, *Boliniales*, *Chaetosphaeriales*, *Coniochaetales*, *Meliolales* and *Sordariales*, as well as some from tentatively placed lineages downloaded from GenBank (Supplementary Table 1), and aligned by using Bioedit v.7.0.1 (Hall 2004) and Clustal X 2.0.11 (Thompson et al. 1997).

RAxML (Maximum likelihood) analysis was performed by using raxmlGUIv.0.9b2 (Silvestro and Michalak 2012). The search strategy was set to rapid bootstrapping and the analysis using the GTRGAMMAI model. The number of replicates was inferred using the stopping criterion (Pattengale et al. 2009). Maximum likelihood bootstrap values equal or greater than 50 % are given as the first set of numbers above the nodes (Figs. 4 and 5). The model of evolution was performed by MrModeltest 2.2 (Nylander et al. 2008). Posterior probabilities (PP) were determined by Markov Chain Monte Carlo sampling (BMCMC) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) in MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation and 10, 000 trees were obtained. The first 2000 trees, representing the burn-in phase were discarded, the remaining 8000 trees were used for calculating posterior probabilities (Cai et al. 2006, 2008). Bayesian posterior probabilities (BYPP) equal or greater than 0.80 are given as second set of numbers above the nodes (Figs. 4 and 5).

Results and discussion

Molecular phylogeny

Phylogenetic analyses used LSU (Fig. 4) and ITS sequence data (Fig. 5) and indicate that the *Meliolales* clade clusters in the class *Sordariomycetes*, subclass *Meliolomycetidae*. The *Meliolaceae* clade includes species of the family *Meliolaceae* with 100 % ML support and 1.0 PP support, and is sister to *Rimaconus coronatus* and *R. jamaicensis*; this is similar to the results of Maharachchikumbura et al. (2015). However, we are unable to clarify the placement of *Armatellaceae* because there is no sequence data from this family. *Irenopsis walsurae* clustered in the *Meliolaceae* clade and was closely related to other species of *Irenopsis* with relatively high bootstrap support (99 % ML, 1.0 PP support). *Meliola mucunicola* is placed within the genus *Meliola* in the clade of *Meliolaceae* and closely related to *M. centellae* (55 % ML support). Five strains of *Meliola thailandicum* clustered in the clade of *Meliolaceae* within the genus *Meliola* and are sister to *M. variaseta* (84 % ML, 0.9 PP support). Four strains of *Meliola thailandicum* which were found on *Dimocarpus longan* (MFLU15-0377,

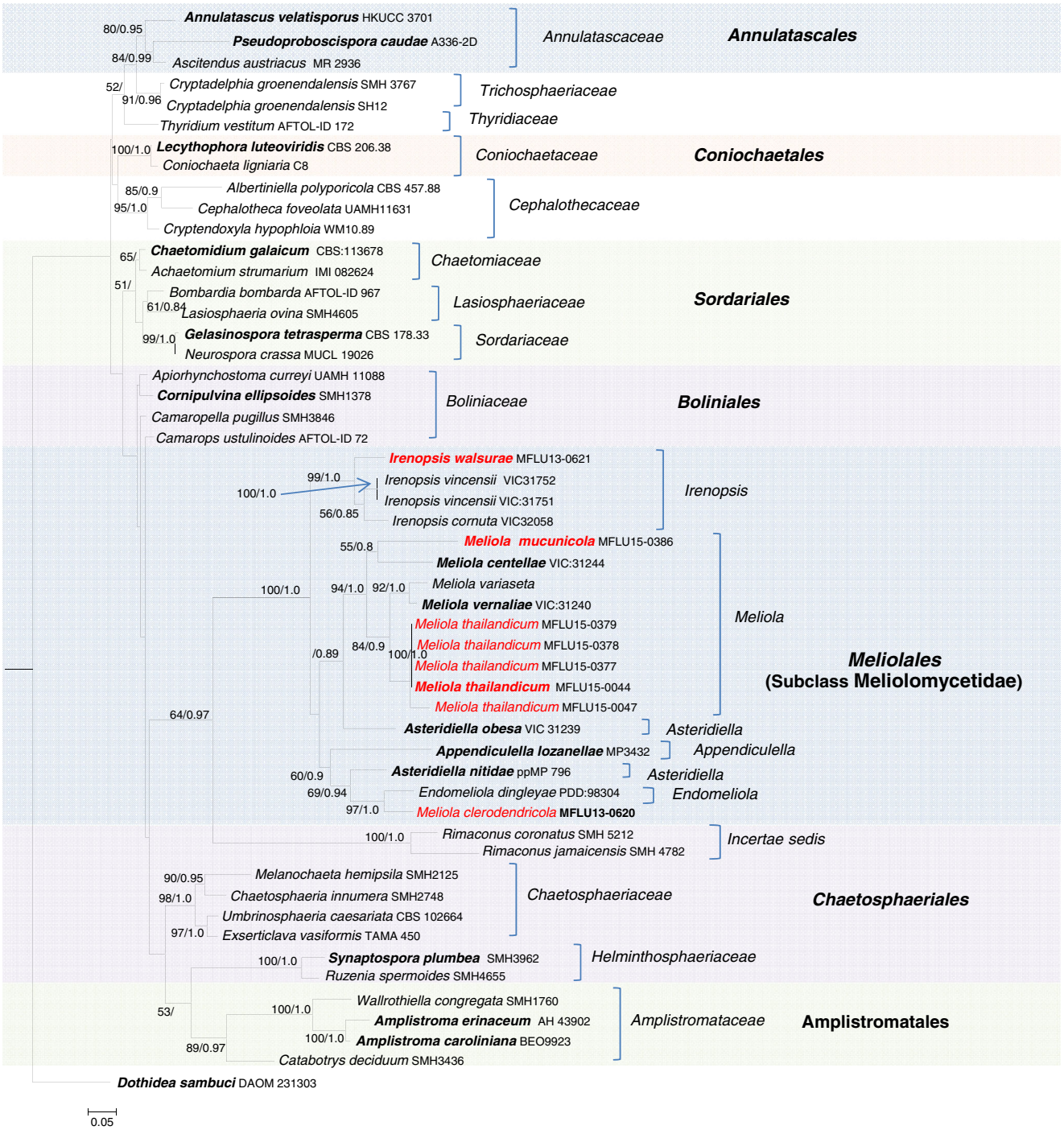


Fig. 4 RAxML maximum likelihood phylogenetic tree (LSU). The first set of numbers above the nodes are RAxML value expressed from 1000 repetitions with values above 50 % shown. The second set of numbers above the nodes are Bayesian posterior probabilities, with values above 0.8 shown. Strain numbers are indicated after species names. New sequence data are in red, types of the new species are in red bold, and other types are in black bold

MFLU15-0379, MFLU 15-0044, MFLU 15-0047) and one from *Acacia auriculiformis* (MFLU15-0378), are the same species in the LSU tree (100 % ML, 0.1 PP support), and in the ITS tree (100 % ML, 0.1 PP support). Polymorphic nucleotides from sequence data of the ITS show a few base pair differences between the four strains of *Meliola thailandicum* from *Dimocarpus longan* and one strain from *Acacia auriculiformis*, and are not significant to separate them (Table 2). Hence, we conclude that this species is not host-specific. *Appendiculella* was not closely related to *Asteridiella* contradicting the phylogenetic tree of Justavino et al. (2015), however, these authors noted that the two

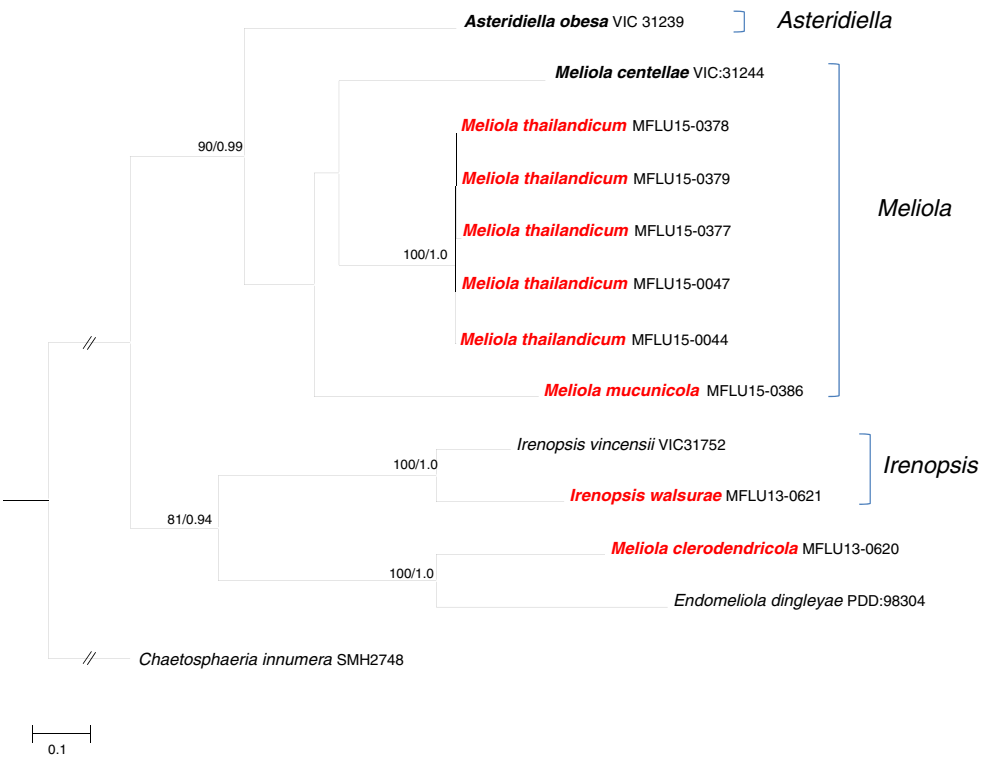


Fig. 5 RAxML maximum likelihood phylogenetic tree (ITS). The first set of numbers above the nodes are RAxML values expressed from 1000 repetitions with values above 50 % shown. The second set of numbers above the nodes are Bayesian posterior probabilities, with values above 0.8 shown. Strain numbers are indicated after species names. New sequence data of *Meliolaceae* are in red, types of the new species are in red bold, and other types are in black bold

species might be closely related as the conical appendages of *Appendiculella* could be homologous to the projecting conical cells on the ascoma wall in *Asteridiella*. Justavino et al. (2015), used LSU rDNA with 417 positions, and this may have resulted in a distinct separation of *Appendiculella* from *Asteridiella*. In the present study, the backbone tree used LSU sequence data with 635 positions and indicated that *Appendiculella lozanellae* is related to *Asteridiella nitidae* with 60 % ML and 0.9 PP support. *Asteridiella obese*, however, formed a distinct clade with *Meliola* species, and clustered in different subclades with *Asteridiella nitidae*, thus, more sequence data for *Appendiculella* and *Asteridiella* are needed to clarify their relationships. *Meliola clerodendricola* is sister to *Endomeliola dingleyae* (97 % ML, 1.0 PP support), however, these two stains are different in morphology. We suspect this may be wrong and needs to be verified in future, although host plants of *Endomeliola dingleyae* and *Meliola clerodendricola* belong to closely related orders in the euasterids (Jansen et al. 2007; Justavino et al. 2015). Some species have an uncertain placement, therefore, more gene and sequence data are needed to clarity the placement of the genera and species.

Table 2 Polymorphic nucleotides from sequence data of the ITS show a few base pair differences between the five strains of *Meliola thailandicum*. Polymorphisms unique to *Meliola thailandicum* are highlighted

Codes	Hosts	ITS											
		15	215	266	273	299	348	349	350	392	430	504	607
MFLU 15-0377	*Dimocarpus longan*	T	T	C	A	G	A	G	G	T	T	G	T
MFLU 15-0378	*Acacia auriculiformis*	G	G	C	A	G	A	G	G	T	T	G	T
MFLU 15-0379	*Dimocarpus longan*	G	G	C	A	G	A	G	G	T	T	G	T
MFLU 15-0044	*Dimocarpus longan*	G	G	C	A	G	A	G	G	T	T	G	A
MFLU 15-0047	*Dimocarpus longan*	G	G	C	A	G	A	G	G	T	T	G	A

Taxonomy

Key to genera of *Meliolales*

1. Hyphae with capitate hyphopodia, but without phialides	***Armatella*** (*Armatella*ceae)
1. Hyphae with capitate hyphopodia and phialides	2 (*Meliolaceae*)
2. Hyphae intercellular	***Endomeliola***
2. Hyphae superficial	3
3. Ascomata flattened	***Amazonia***
3. Ascomata globose to subglobose	4
4. Hyphae with dark brown to black setae	5
4. Hyphae without setae (setae may be present on ascomata)	6
5. Hyphal setae with bulbous tips, apical part curved, covering ascomata	***Cryptomeliola***
5. Hyphal setae simple, straight or sometimes slightly curved	***Meliola***
6. Ascomata with appendages or setae	7
6. Ascomata without appendages and setae, with raised conical cells on the ascoma wall	***Asteridiella***
7. Ascomata with larviform to cylindrical appendages	***Appendiculella***
7. Ascomata with long setae, mostly curved at the apex	***Irenopsis***

Armatellaceae Hosag., Sydowia 55(2): 165 (2003)

Facesoffunginumber: FoF00723

Epiphytes on the surface of leaves. *Superficial hyphae* dense, septate, brown, with hyphopodia, hyphal setae lacking. *Hyphopodia* capitate, alternate or opposite on hyphae, 2-celled, brown. **Sexual morph**: *Ascomata* superficial on the surface of hosts, globose to subglobose, flattened when immature, developing on hyphae, the surface verrucose, covered with tuberculate projections, ascomatal setae and appendages lacking. *Peridium* thick, comprising two strata, outer stratum amorphous and black, inner stratum thick, comprising reddish to brown, scleroparenchymatous cells of *textura angularis* to *globulosa*. *Hamathecium* with evanescent paraphyses. *Asci* 4–8-spored, unitunicate, subcylindrical to ovoid, or clavate, evanescent. *Ascospores* hyaline to brown, ellipsoidal to oblong, aseptate when immature, 1-septate at maturity, constricted at the septum, with rounded ends. **Asexual morph**: *Phialides* absent (Hansford 1946; Hosagoudar 2003b).

Notes: The family *Armatellaceae* differs from *Meliolaceae* by having aseptate to 1-septate, hyaline ascospores and a different peridial wall type. Phialides are lacking on the hyphae in *Armatellaceae*, but are found in *Meliolaceae*.

Type: ***Armatella*** Theiss. & Syd., Annls mycol. 13(3/4): 235 (1915)

= *Artallendea* Bat. & H. Maia, Atas Inst. Micol. Univ. Recife 1: 221 (1960)

Facesoffunginumber: FoF00722

Epiphytes on the surface of leaves. *Superficial hyphae* dense, septate, brown, with hyphopodia, hyphal setae lacking. *Hyphopodia* irregularly capitate, alternate or opposite on hyphae, 2-celled, brown. **Sexual morph**: *Ascomata* superficial on the surface of hosts, globose to subglobose, flattened when immature, developing on hyphae, surface verrucose, covered with tuberculate projections, ascomatal setae and appendages lacking. *Peridium* thick, comprising two strata, outer stratum amorphous and black, inner stratum thick, comprising reddish to brown, scleroparenchymatous cells of *textura angularis* to *globulosa*. *Hamathecium* with evanescent paraphyses. *Asci* 4–8-spored, unitunicate, subcylindrical to ovoid, or clavate, evanescent. *Ascospores* hyaline to brown, ellipsoidal to oblong, aseptate when immature, 1-septate at maturity, constricted at the septum, with rounded ends. **Asexual morph**: *Phialides* absent (Hansford 1946; Hosagoudar 2003b).

Notes: There is no sequence data for any species of this genus. Direct sequencing from fresh specimens is needed to clarify the phylogenetic placement.

Type species: ***Armatella litseae*** (Henn.) Theiss. & Syd., Annls mycol. 13(3/4): 235 (1915)

Facesoffunginumber: FoF00721

Epiphytes on the surface of leaves. *Superficial hyphae* 5–7 μm diam., ($\overline{x} = 7\ \mu m$, $n=10$), dense, branched, septate, brown to reddish, with hyphopodia, hyphal setae lacking. *Hyphopodia* 15–17 μm diam. ($\overline{x} = 16\ \mu m$, $n=10$), single stellate to sublobate on stalk cells, alternate on hyphae, 2-celled, brown to reddish. **Sexual morph**: *Ascomata* 210–235×174–192 μm ($\overline{x} = 226 \times 180\ \mu m$, $n=10$), superficial on surface of hosts, scattered, initially flattened, and becoming globose to subglobose, flattened when immature, developing on hyphae, surface verrucose, covered with tuberculate projections, ascomatal setae and appendages lacking. *Peridium* 43–59 *um*, thick, comprising two strata, outer stratum amorphous and black, inner stratum 29–37 *um*, thick, comprising reddish to brown, scleroparenchymatous cells of *textura angularis* to *globulosa*. *Hamathecium* with evanescent paraphyses. *Asci* 51–61×26–29 μm ($\overline{x} = 54 \times 27\ \mu m$, $n=10$), 4–8-spored, unitunicate, ovoid to clavate. *Ascospores* 33–35×10–11 μm ($\overline{x} = 34 \times 10\ \mu m$, $n=10$), 2–3-seriate, hyaline to light brown, ellipsoidal to oblong, aseptate when immature, 1-septate at maturity, constricted at the septum, rounded ends. **Asexual morph**: Undetermined.

Material examined JAPAN, Province Awa, Tokushima, Kigasumi, on *Litsea glauca* (Thunb.) Siebold (*Lauraceae*), 25 December 1897, S. Kusano (SF70331, **holotype**).

Notes: We re-examined the type specimen of *Armatella litseae*, and accept this as a separate family from *Meliolaceae* characterized by aggregated colonies on the surface of host, stellate hyphopodia, and ascomata covered with

tuberculate projections which are amorphous and black in section, and a thick inner stratum, comprising reddish to brown scleroparenchymatous cells of *textura angularis* to *globulosa*, aseptate to 1-septate ascospores, and lacking phialides. However, the phylogenetic placement needs clarification (Fig. 6).

Meliolaceae W. Martin ex Hansf., Mycol. Pap. 15: 23 (1946)

Facesoffunginumber: FoF00741

Epiphytes or *pathogens* on leaves, occasionally on stems or branches, often forming web-like colonies. *Superficial hyphae* branched, septate, brown to dark brown, hyphal setae present or lacking, with hyphopodia. *Hyphal setae* developing from hyphae in *Cryptomeliola* and *Meliola*, septate, branched or unbranched at apex, or with bulbous apices or apical part in *Cryptomeliola*, brown to dark brown. *Hyphopodia* capitate on hyphae, variously shaped. **Sexual morph**: *Ascomata* superficial on surface of black, web-like colonies on host, mostly globose to subglobose, or flattened in the genus *Amazonia*. *Peridium* comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum of brown to dark brown cells, or with raised conoid cells, appendages or setae, inner stratum of hyaline to pale brown cells. *Hamathecium* with evanescent paraphyses. *Asci* 2–4-

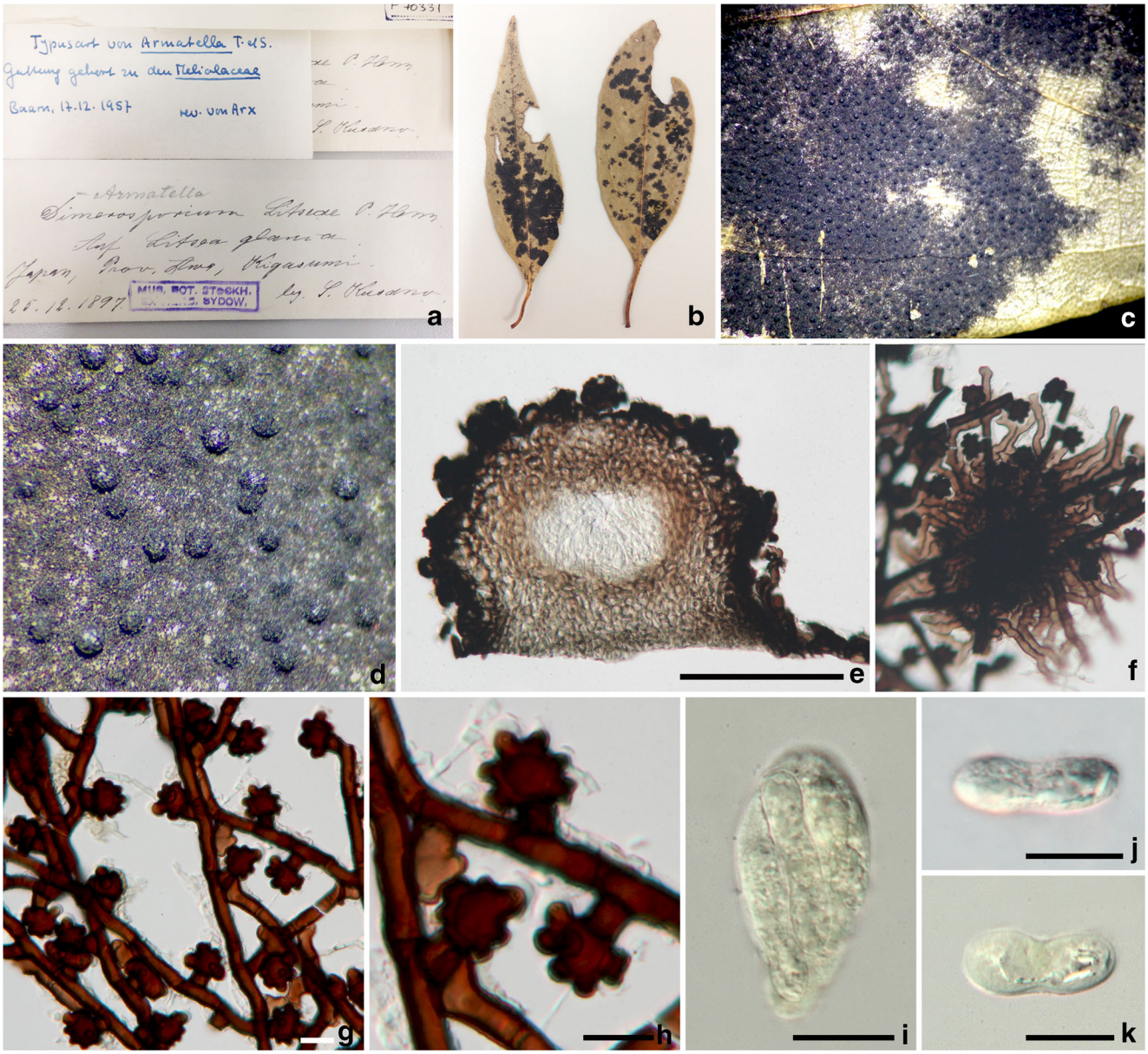


Fig. 6 *Armatella litseae* (holotype). **a** Herbarium packet. **b**–**d** Colonies on leaves. **e** Section through ascoma. **f** Upper cell walls of ascomata when viewed in squash mounts. **g**, **h** Hyphopodia on hyphae. **i** Immature ascus in Melzers' reagent. **j**, **k** Mature 1-septate ascospores in Melzer's reagent. Scale bars: **e**=100 μm, **i**–**k**=20 μm, **g**, **h**=10 μm

spored, unitunicate, subglobose to broadly clavate, lacking an opening mechanism. *Ascospores* 2–3-seriate, hyaline when immature, brown to dark brown at maturity, ellipsoid or cylindrical to ovoid, 3–4-septate. **Asexual morph**: *Phialides*, ampuliform or flask-shaped on hyphae. *Conidiogenous* cells formed directly from vegetative hyphae. *Conidia* unicellular, small and hyaline (from Cannon and Kirk 2007).

Type species: ***Meliola*** Fr., Syst. orb. veg. (Lundae) 1: 111 (1825)

Notes: Phylogenetic analyses place *Meliolaceae* species in the class *Sordariomycetes*, and they were therefore accommodated in a new subclass *Meliolomycetidae* (Liu et al. 2015; Justavino et al. 2015; Maharachchikumbura et al. 2015). In our phylogenies (Fig. 4), the subclass *Meliolomycetidae* is clearly supported.

Genera included in *Meliolaceae* are *Amazonia*, *Appendiculella*, *Asteridiella*, *Cryptomeliola*, *Endomeliola*, *Irenopsis* and *Meliola*. These genera are characterized by superficial hyphae with hyphopodia or intracellular hyphae with hyphopodia in *Endomeliola*, and forming lateral haustoria that may penetrate the epidermis or mesophyll cells. Hyphal setae are found in *Cryptomeliola* and *Meliola*. Ascomata are globose to subglobose except in *Amazonia* where they are flattened. Ascomatal setae are found in *Irenopsis* and ascomatal appendages are found in *Appendiculella*. Asci are unitunicate and ascospores brown to dark brown at maturity with 3–4 septa. *Basavamyces* was transferred to *Armatellaceae* based on having superficial hyphae without phialides, cylindrical to subcylindrical asci and 1–2-septa ascospores (Hosagoudar et al. 2012), but we treat the genus in *Sordariomycetes* genera, *incertae sedis* base on its morphology. *Ceratospermopsis*, *Ectendomeliola*, *Hypasteridium*, *Leptascospora*, *Metasteridium*, *Ophiociliomyces*, *Ophioirenina*, *Ophiomeliola*, *Prataprajella*, *Parasteridium*, *Pauahia*, *Pleomeliola*, *Pleomerium*, *Ticomyces*, *Urupe* and *Xenostigme* are excluded from *Meliolaceae*. This is based on morphology or because taxa are doubtful due to lack of good type specimens. *Hypasteridium*, *Metasteridium* and *Parasteridium* were introduced without species name and their application is uncertain (Eriksson and Hawksworth 1988). *Laeviomeliola* is synonymized under *Meliola* based on morphology.

Amazonia Theiss., Annls mycol. 11(6): 499 (1913)

Facesoffunginumber: FoF00718

Epiphytes on the surface of leaves. *Superficial hyphae* branched, septate, darker at the septa, brown, with hyphopodia, hyphal setae lacking. *Hyphopodia* capitate, alternate on hyphae, near to hyphal septa, 2-celled, brown to dark brown. **Sexual morph**: *Ascomata* superficial on surface of host, mostly gregarious, rarely solitary, circular, flattened, borne under radiating hyphae, branching at the rim, producing lateral hyphopodia, ascomatal setae and appendages absent. *Peridium* arranged radially when viewed in squash mounts, poorly developed at the base, with three strata, outer stratum comprising a dark brown to black amorphous layer, central stratum comprising flattened, thick-walled, brown cells of *textura angularis* and inner stratum of hyaline to reddish brown flattened cells. *Hamathecium* with evanescent paraphyses. *Asci* 2-spored, unitunicate, ellipsoid to obovoid, lacking an opening mechanism, short pedicellate or apedicellate, evanescent. *Ascospores* 2-seriate, hyaline to brown, ellipsoid to subcylindrical, 4-septate, constricted and darker at the septa, smooth-walled. **Asexual morph**: *Phialides* ampuliform, sometimes curved, alternate or opposite on hyphae, mixed with capitate hyphopodia, pale brown to brown. *Phialoconidia* rarely observed, hyaline.

Notes: The genus *Amazonia* was introduced by Theissen (1913), with the type species *Amazonia psychotriae* (Henn.) Theiss. *Amazonia* is typical of other members of *Meliolaceae* based on its unitunicate asci, and brown to dark brown, 4-septate ascospores. Höhnel (1918) noted this genus as transitional between *Meliola* and *Microthyriaceae* based on its flattened ascomata typical of species in *Microthyriaceae* (Wu et al. 2011), while asci and ascospores are typical of species in *Meliolaceae*. Molecular analyses place *Meliolaceae* in the class *Sordariomycetes*, while the family *Microthyriaceae* is placed in *Dothideomycetes* (Hyde et al. 2013; Maharachchikumbura et al. 2015).

Type species: ***Amazonia psychotriae*** (Henn.) Theiss., Annls mycol. 11(6): 499 (1913)

≡ *Meliola asterinoides* var. *psychotriae* Henn., Hedwigia 43: 361 (1904)

≡ *Amazonia psychotriae* (Henn.) Theiss., Annls mycol. 11(6): 499 (1913) var. *psychotriae*

= *Amazonia psychotriae* var. *major* Hansf., Reinwardtia 3: 102 (1954)

Facesoffunginumber: FoF00716

Epiphytes on the surface of leaves. *Superficial hyphae* 4–5 μm diam., branched, septate, darker at the septa, brown, with hyphopodia, hyphal setae lacking. *Hyphopodia* 10–11 μm diam. ($\bar{x}$ = 10μm, n=10), capitate, alternate on hyphae, near to hyphal septa, 2-celled, brown to dark brown. **Sexual morph**: *Ascomata* 440–495 μm diam. ($\bar{x}$ = 455 μm, n=5), superficial on surface of host, mostly gregarious, rarely solitary, circular, flattened, borne under radiating hyphae, branching at the rim, producing lateral hyphopodia, ascomatal setae and appendages absent. *Peridium* 20–26 μm thick, arranged radially when viewed in squash mounts, poorly developed at the base, with three strata, outer stratum comprising a dark brown to black amorphous layer, central stratum comprising flattened, thick-walled, brown cells of *textura angularis* and inner stratum of hyaline to reddish brown flattened cells. *Hamathecium* with evanescent paraphyses. *Asci*

45–50×23–28 μm ($\bar{x}$ = 47 × 26 μm, *n*=10), 2-spored, unitunicate, ellipsoid to obovoid, lacking an opening mechanism, short pedicellate or apedicellate, evanescent. *Ascospores* 37–40×15–20 μm ($\bar{x}$ = 39 × 19 μm, *n*=10), 2-seriate, hyaline to brown, ellipsoid to subcylindrical, 4-septate, constricted and darker at the septa, smooth-walled, with an evanescent sheath. **Asexual morph**: *Phialides* 7–8 μm ($\bar{x}$ = 8 μm, *n*=5), ampuliform, sometimes curved, alternate or opposite on hyphae, mixed with capitate hyphopodia, sometimes occurring on separate hyphae, pale brown to brown. *Phialoconidia* rarely observed, hyaline.

Material examined BRAZIL, Amazonas, Rio Negro, Manaus, on leaves of *Phychotria* sp. (*Rubiaceae*), March 1901, E. Ule No. 3152 (B700014752, **syntype**); BRAZIL, Amazonas, Rio Negro, Manaus, on leave of *Phychotria* sp. (*Rubiaceae*), 1901, E. Ule (B70001475).

Notes: *Amazonia* was introduced based on its flattened ascomata, with the type species as *A. psychotriae. Meliola asterinoides* var. *psychotriae* Henn. is a synonym of *A. psychotriae* Theissen (1913). We examined and illustrated the syntype of *A. psychotriae* from B (Figs. 7 and 8). Molecular data is lacking for this genus.

Appendiculella Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 128: 556 (1919)

Possibly synonyms (from Index Fungorum 2015):

≡ *Irenina* F. Stevens, Annls mycol. 25(5/6): 411 (1927)

≡ *Meliola* subgen. *Irenina* (F. Stevens) Cif., Annls mycol. 36(2/3): 203 (1938)

Facesoffunginumber: FoF00720

Epiphytes on the surface of leaves or stems. *Superficial hyphae* branched, septate, darker at the septa, with hyphopodia, hyphal setae lacking. *Hyphopodia* capitate, angular or lobate, alternate or opposite on hyphae, 2-celled, brown. **Sexual morph**: *Ascomata* superficial on surface of host, mostly gregarious on superficial hyphae, subglobose to globose, thick-walled, lacking ascomatal setae, but with raised conoid cells, which may extend to form larviform to cylindrical appendages. *Peridium* comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum of brown to dark brown cells of *textura angularis*, inner stratum of hyaline to pale brown flattened cells. *Hamathecium* with evanescent paraphyses. *Asci* 2–4-spored, unitunicate, oblong to obovoid, lacking an opening mechanism, short pedicellate or apedicellate, evanescent at maturity. *Ascospores* 2–3-seriate, hyaline to brown, fusiform to ellipsoid, 3–4-septate, slightly constricted and darker at the septa, smooth-walled. **Asexual morph**: *Phialides* ampuliform, alternate or opposite, mixed with capitate hyphopodia on hyphae, sometimes curved, brown. *Phialoconidia* not seen (Justavino and Piepenbring 2007).

Notes: The genus *Appendiculella* was introduced by Höhnel (1919) with the type species *A. calostroma* (Desm.) Höhn. and is characterized by conical appendages with transversely striate walls developing on the surface of the ascomata. This feature is similar to the conical ascomatal cells of *Asteridiella* (Justavino et al. 2015). *Appendiculella* species are distributed widely in the tropics (Kirk et al. 2008), with an 87 species epithets listed in Index Fungorum (2015). Most species were described based on host association. Justavino et al. (2015) provided the first sequence data for *Appendiculella lozanellae* (DQ508302), which grouped in the *Meliolaceae* clade in their phylogenetic tree. However, this relationship was weakly supported in the phylogenetic tree of Justavino et al. (2015). In this study, the phylogenies indicate a close relationship between *Appendiculella lozanellae* and *Asteridiella nitidae* (60 % ML support). However, the morphology of these two genera differs, and therefore we maintain these genera as distinct.

Type species: ***Appendiculella calostroma*** (Desm.) Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 128: 556 (1919)

≡ *Sphaeria calostroma* Desm., Bull. Soc. bot. Fr. 4: 22 (1857)

= *Chaetosphaeria calostroma* (Desm.) Sacc., Syll. fung. (Abellini) 2: 95 (1883)

= *Meliola calostroma* (Desm.) Höhn., Annls mycol. 15(5): 363 (1917)

Facesoffunginumber: FoF00719

Epiphytes on the surface of leaves. *Superficial hyphae* 5–6 μm diam. ($\bar{x}$ = 5 μm, *n*=10), branched, septate, darker at the septa, with hyphopodia, hyphal setae lacking. *Hyphopodia* 13–15 μm diam. ($\bar{x}$ = 14 μm, *n*=10), capitate, angular or lobate, alternate or opposite on hyphae, 2-celled, brown. **Sexual morph**: *Ascomata* 125–140 high×140–150 μm diam. ($\bar{x}$ = 125 × 149 μm, *n*=5), superficial on surface of host, mostly gregarious on superficial hyphae, subglobose to globose, thick-walled, lacking ascomatal setae, but with raised conoid cells, which may extend to form larviform to cylindrical appendages. *Peridium* 28–34 μm ($\bar{x}$ = 30μm, *n*=5), comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum of brown to dark brown cells of *textura angularis*, inner stratum of hyaline to pale brown flattened cells. *Hamathecium* with evanescent paraphyses. *Asci* 42–57×24–33 μm ($\bar{x}$ = 45 × 28μm, *n*=10), 2–4-spored, unitunicate, oblong to obovoid, lacking an opening mechanism, with short pedicellate or apedicellate, evanescent. *Ascospores* 35–37×12–13 μm ($\bar{x}$ = 36 × 12 μm, *n*=10), 2–3-seriate, hyaline to brown, fusiform to ellipsoid, 3-septate, slightly constricted and darker at the septa, smooth-walled. **Asexual morph**: Undetermined.

Material examined CHILE, Bío-Bío, Concepción, on surface of leaves of *Geum chilense* Balb. (*Rosaceae*), 5 June 1895, F.W. Neger (SF5748).

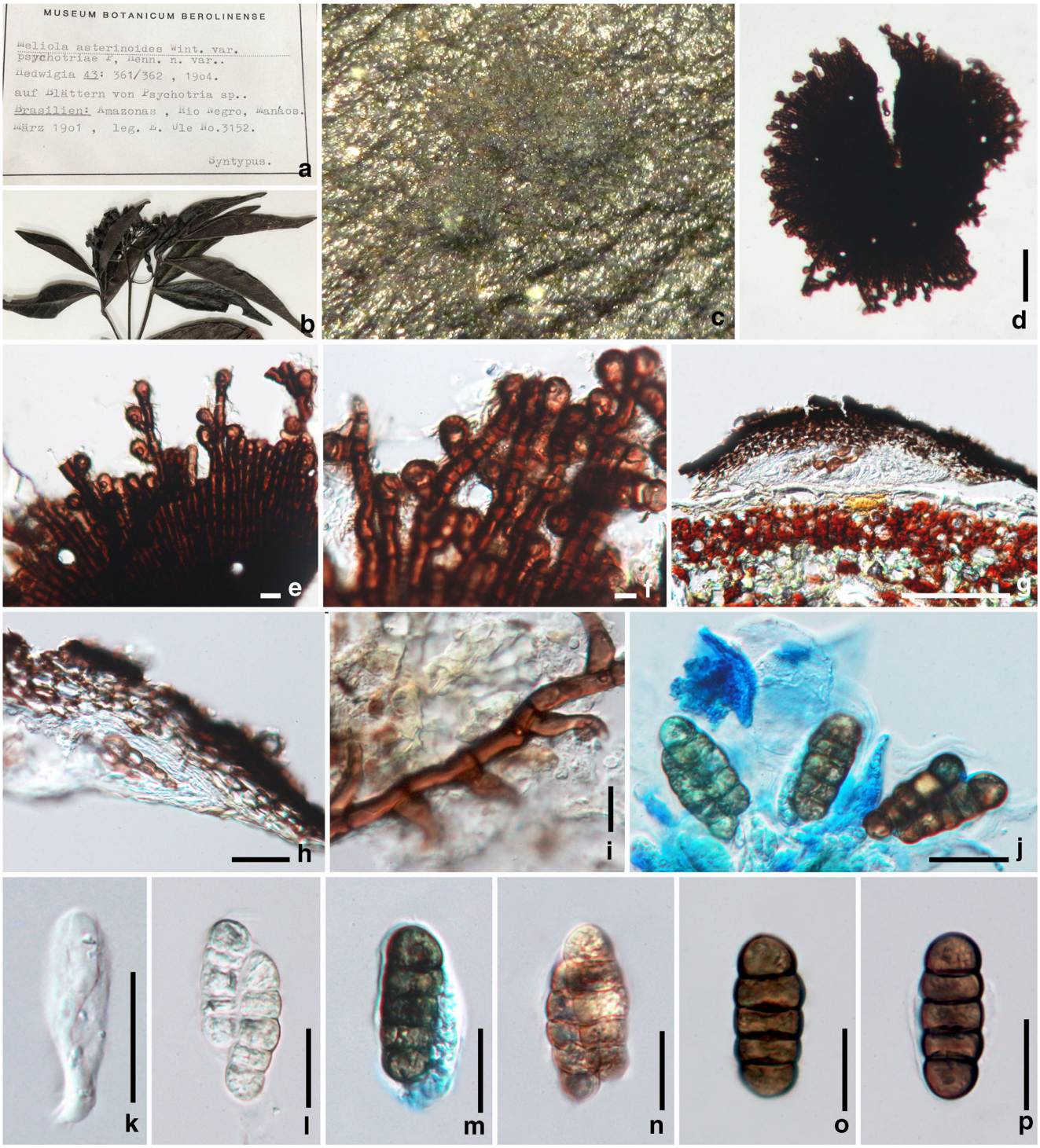


Fig. 7 *Amazonia psychotriae* (syntype). **a**, **b** Herbarium packet and specimens. **c** Colony on substrate. **d** Ascoma viewed in squash mount. **e**, **f** Hyphopodia at rim of ascoma. **g** Section through ascoma. **h** Peridium. **i** Phialides. **j** Ascus in cotton blue reagent. **k**, **l** Immature asci. **m**, **n** Mature asci. **o**, **p** Ascospores with 4 septa. Note the thin sheath in p. Scale bars: **d**, **g**=100 μm, **e**, **f**, **i**=10 μm, **h**, **j**, **l**–**p**=20 μm, **k**=50 μm

Notes: *Appendiculella calostroma* was established by Höhnel (1919) and infects plant species in *Rosaceae* (Hansford 1961; Justavino et al 2015) (Figs. 9 and 10).

Asteridiella McAlpine, Proc. Linn. Soc. N.S.W. 22(1): 38 (1897)

Possible synonymy (from Index Fungorum 2015):

= *Irene* Theiss et al., Annls mycol. 15(3/4): 194 (1917)

= *Meliola* subgen. *Irene* (Theiss et al.) Cif., Annls mycol. 36(2/3): 203 (1938)

Fig. 8 *Amazonia psychotriae* (B70001475). **a**, **b** Herbarium packet and specimen. **c** Colony on substrate. **d** Ascoma borne under radiating hyphae viewed in squash mounts. **e** Wall comprising radiating cells. **f** Hyphae with capitate hyphopodia. **g** Hyphopodia at rim of ascoma. **h**, **i** Immature asci. **j** Mature ascus. **k**–**m** Mature ascospores. Scale bars: **d**=100 μm, **e**=50 μm, **f**, **g**=10 μm, **h**–**m**=20 μm

= *Parasteridiella* H. Maia, Publicações Inst. Micol. Recife 267: 25 (1960)

Facesoffunginumber: FoF00724

Epiphytes on the surface of leaves and stems. *Superficial hyphae* branched, septate, darker at the septa, brown, with hyphopodia, hyphal setae lacking. *Hyphopodia* capitate, alternate or opposite on hyphae, near to hyphal septa, 2-celled, brown. **Sexual morph**: *Ascomata* superficial on surface of host, mostly gregarious, rarely solitary on superficial hyphae, subglobose to globose, thick-walled, ascomatal setae and appendages absent. *Peridium* comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum of dark brown cells of *textura angularis*, with raised conical cells, acute or rounded at the apex, and inner stratum of hyaline cells of *textura porrecta*. *Hamathecium* with evanescent paraphyses. *Asci* 2–4-spored, unitunicate, oblong to cylindrical. *Ascospores* 2–4-seriate, hyaline to brown, subcylindrical to oblong, 4-septate, slightly constricted and darker at the septa, not constricted in some species, smooth-walled (description modified from Hansford 1961, 1963; Mibey and Hawksworth 1997 and own observations). **Asexual morph**: *Phialides* rarely observed on hyphae, ampuliform, alternate or opposite, sometimes curved, pale brown to brown. *Conidia* hyaline (Hansford 1961; Hosagoudar 2013).

Notes: *Asteridiella* was introduced by McAlpine (1897) and differs from *Meliola* species in lacking hyphal setae. Most of the genera in *Meliolaceae* have been separated previously by setae and appendages. These characters seem hardly sufficient to separate *Asteridiella* from other genera, molecular data however, show *Asteridiella* as a distinct clade in *Meliolaceae*, sister to *Appendiculella*. *Asteridiella* may well

Fig. 9 *Appendiculella calostroma* (S-F5748). **a**, **b** Herbarium packet and specimen. **c** Ascomata on surface of leaves. **d** Transverse section through ascoma. **e** Ascoma viewed in squash mount. **f** Peridium comprising cells of *textura angularis*. **g** Conical appendage on ascomata. **h** Hyphae with hyphopodia. **i** Immature ascus in Melzer's reagent. **j** Mature ascus. **k**, **l** Mature ascospores with 3 septa. Scale bars: **d**, **e**=50 μm, **f**, **g**, **i**–**l**=20 μm, **h**=10 μm

be a distinct genus in having conical cells raised from the ascomata and in lacking ascomatal setae or appendages (Justavino et al. 2015). Species in *Asteridiella* are differentiated based on host as they are thought to be host-specific (Hansford 1961; Hosagoudar 2013). Less significant differences can be seen in the size of ascomata, hyphopodia shape and arrangement, however this needs testing at the molecular level (Hansford 1961; Hosagoudar 2013). *Asteridiella* species are commonly found in the tropics (Mibey and Hawksworth 1997; Kirk et al. 2008), with around 300 species estimated in Kirk et al. (2008), and almost 450 species epithets listed in Index Fungorum (2015). Molecular evidence placed *Asteridiella nitidae* Rodr. Just. (EF094839) and *A. obesa* (Speg.) Hansf. (DQ508302) in *Meliolaceae* as a distinct clade, however, the two species clustered in different subclades (Justavino et al. 2015). Therefore, molecular data is needed to clarify the distinctiveness of this and other genera.

Type species: ***Asteridiella solani*** McAlpine, Proc. Linn. Soc. N.S.W. 22(1): 38 (1897)

= *Asteridiella solani* var. *kodaikanalensis* Hosagoudar et al., in Nithyatharani et al., Scientific Transactions in Environment and Technovation 4(4): 165 (2011)

Facesoffunginumber: FoF00725

Epiphytes on the surface of leaves and stems. *Superficial hyphae* branched, septate, darker at the septa, brown, with hyphopodia, hyphal setae lacking. *Hyphopodia* 10–14 μm

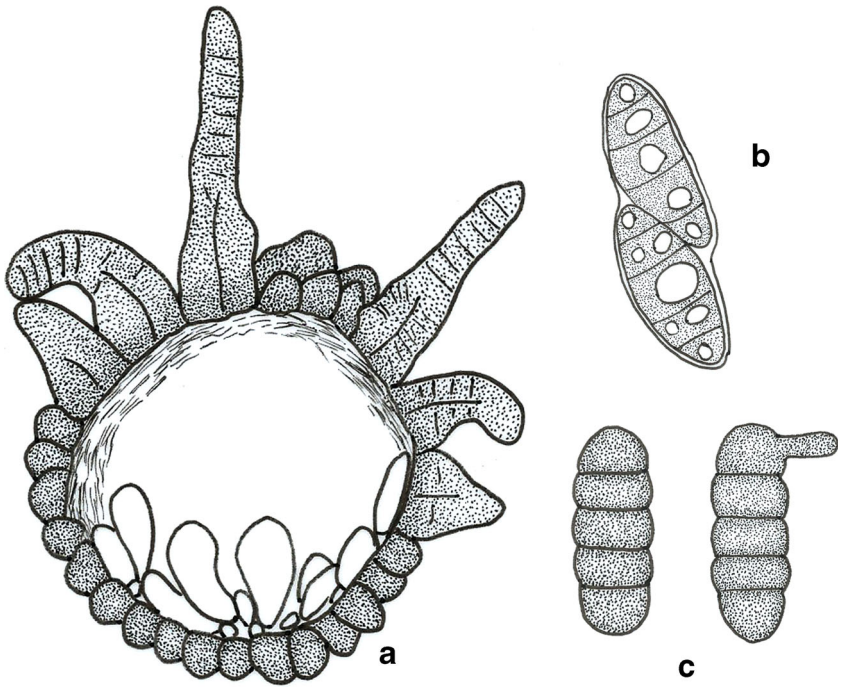


Fig. 10 *Appendiculella lozanellae* (redrawn from Justavino and Piepenbring 2007). **a** Transverse section through ascoma showing appendages on ascoma. **b** Ascus. **c** Ascospores with 4 septa

diam. ($\bar{x}$ = 13 μm, n=10), capitate, alternate or opposite on hyphae, near to hyphal septa, 2-celled, brown. **Sexual morph**: *Ascomata* 120–207 μm diam. ($\bar{x}$ = 198 μm, n=3), superficial on surface of host, mostly gregarious, rarely solitary on superficial hyphae, subglobose to globose, thick-walled, ascomatal setae and appendages absent. *Peridium* 35–46 μm thick, comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum of dark brown cells of *textura angularis*, with raised conical cells 35–70 μm long, acute or rounded at the apex, and inner stratum of hyaline cells of *textura porrecta. Hamathecium* with evanescent paraphyses. *Asci* 45–50×21–26 μm ($\bar{x}$ = 46 × 23 μm, n=3), 2–4-spored, unitunicate, oblong to cylindrical. *Ascospores* 37–42×15–19 μm ($\bar{x}$ = 38 × 18 μm, n=3), 2–4-seriate, hyaline to brown, oblong, 4-septate, slightly constricted and darker at the septa, sometimes not constricted, smooth-walled. **Asexual morph**: *Phialides* rarely observed on hyphae, ampuliform, alternate or opposite, sometimes curved, pale brown to brown. *Conidia* not seen (Hansford 1961; Hosagoudar 2013).

Material examined AUSTRALIA, on *Solanum viride* G. Forst. ex Spreng. (*Solanaceae*), 1896, V.H. Maiden (IMI 73840, glass slide from **ex-holotype**); PHILIPPINES, Benguet Province, Luzon, Mt. Santo Tomas, on leaf of *Solanum inaequilaterale* Merrill (*Solanaceae*), February 1925, Mary Strong Clemens 6410 (BPI 697764)

Notes: Part of the holotype specimen of this species is preserved as a glass slide at K (as IMI 73840), but we could not observe many characters (Fig. 11). However, a specimen (BPI 697764), and a redrawing of *Asteridiella solani* var. *kodaikanalensis* Hosag. et al. published by Hosagoudar (2013), and a redrawing from Hansford (1963) are provided here (Figs. 12, 13 and 14). Molecular data is lacking for *A. solani*.

Cryptomeliola S. Hughes & Piroz., Mycol. Pap. 174: 14 (1997)

Facesoffunginumber: FoF00727

Epiphytes on the surface of leaves, twigs, or petioles. *Superficial hyphae* branched, brown or dark-brown, with or without hyphopodia, hyphal setae present. *Hyphopodia* capitate, alternate or opposite on hyphae, near to hyphal septa, 2-celled, brown. *Hyphal setae* arising from hyphae, dense, septate, dark brown, curved, rounded or hooked at the apex. **Sexual morph**: *Ascomata* superficial on surface of host, solitary or gregarious, sometimes hidden amongst setae, subglobose to globose, carbonaceous, verrucose, ascomatal setae and appendages absent. *Peridium* comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum with dark brown to reddish cells of *textura globulosa* to *angularis*, and inner stratum with hyaline, flattened cells. *Hamathecium* with evanescent paraphyses. *Asci* 2–4-spored, unitunicate, ellipsoid to oval, evanescent. *Ascospores* 2-seriate, hyaline when immature and brown to dark brown at maturity, cylindrical to oblong, 4-septate, constricted and dark at septa, smooth-walled. **Asexual morph**: *Phialides* rarely seen, ampliform, interspersed between hyphal setae on hyphae (Mibey and Hawksworth 1997). *Conidia* unicellular, hyaline.

Notes: *Cryptomeliola* was raised to genus by Hughes & Pirozynski in Mibey and Hawksworth (1997). The genus

Fig. 11 *Asteridiella solani* (holotype). **a**, **b** Herbarium packet and extype slide. **c** Ascomata viewed in squash mount. **d** Hyphae with capitate hyphopodia. **e** Immature ascomata viewed in squash mount. **f**–**g** Ascospores with 4 septa. Scale bars: **c**=100 μm, **e**=50 μm, **f**, **g** =20 μm, **d**=5 μm

contains three species (Index Fungorum 2015). There is no sequence data for any species in *Cryptomeliola*.

Type species: ***Cryptomeliola orbicularis*** (Berk. & M.A. Curtis) S. Hughes & Piroz., in Mibey & Hawksworth, Mycol. Pap. 174: 15 (1997)

= *Meliola orbicularis* Berk. & M.A. Curtis, in Berkeley, J. Linn. Soc., Bot. 10(no. 46): 392 (1868) [1869]

= *Englerulaster orbicularis* (Berk. & M.A. Curtis) Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 119: 454 [62 repr.] (1910)

= *Meliolina orbicularis* (Berk. & M.A. Curtis) F. Stevens, Annls mycol. 25(5/6): 417 (1927)

Facesoffunginumber: FoF00726

Epiphytes on the surface of host. *Superficial hyphae* branched, brown or dark brown, with or without hyphopodia, hyphal setae present. *Hyphopodia* not seen. *Hyphal setae* 92–320 long×4–5 μm wide ($\bar{x}$ = 265 × 5, n=10), dense, septate, dark brown, curved and rounded, or hooked at the apex. **Sexual morph**: *Ascomata* 280–295 μm diam. ($\bar{x}$ = 288 μm, n=5), superficial on upper surface of host, solitary or gregarious, hidden amongst hyphae, subglobose to globose, carbonaceous, verrucose, ascomatal setae and appendages absent. *Peridium* 51–65 μm ($\bar{x}$ = 56μm, n=5), comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum with thick-walled, dark brown to reddish cells of *textura globulosa* to *angularis*, and inner stratum with hyaline, flattened cells. *Hamathecium* with evanescent paraphyses. *Asci* 60–71×37–49 μm ($\bar{x}$ = 67–43μm, n=10), 2−4-spored, unitunicate, ellipsoid to oval, evanescent. *Ascospores* 57–63×17–20 μm ($\bar{x}$ = 58 × 19 μm, n=10), 2-seriate, hyaline when immature and brown to dark brown at maturity, cylindrical to oblong, 4-septate, constricted and dark at the septa, rounded ends, smooth-walled. **Asexual morph**: Undetermined.

Notes: *Cryptomeliola orbicularis*, the type species of *Cryptomeliola*, was introduced based on *Meliola orbicularis* Berk. & Curtis (Mibey and Hawksworth 1997). *Cryptomeliola orbicularis* differs from *Meliola* species as the hyphae are immersed in the outer layers of host tissue or sometimes superficial, and hyphal setae are crowded, curly, and apically rounded. We re-examined the holotype specimen and could not find ascomatal setae, although the original description illustrated the species with ascomatal setae (Fig. 15). However, other species in the genus *Cryptomeliola* do not have an

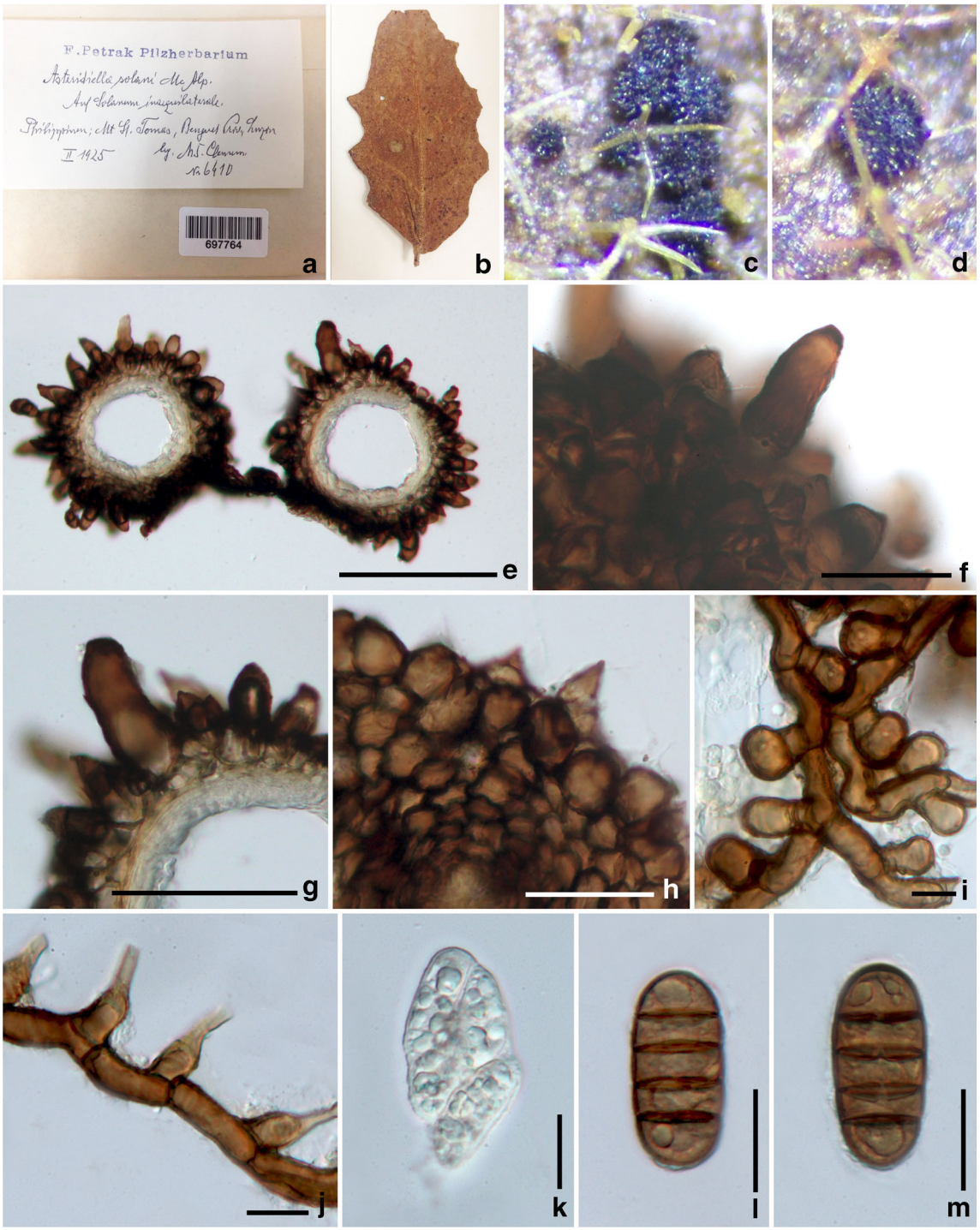


Fig. 12 *Asteridiella solani* (BPI 697764). **a**, **b** Herbarium packet and specimen. **c**, **d** Ascomata on host surface. **e** Section through ascomata. **f**, **g** Raised conical projections on ascomata. **h** Upper walled of ascoma viewed in squash mount. **i** Hyphae with capitate hyphopodia. **j** Hyphae with phialides. **k** Ascus when immature. **l**, **m** Ascospores with 4 septa. Scale bars: **e**=100 μm, **f**–h=50 μm, **i**, **j** =10 μm, **k**–**m**=20 μm

ascomatal setae, thus we illustrate *Cryptomeliola orbicularis* as lacking ascomatal setae base on the holotype specimen.

Material examined On bark of indet. host, C. Wright 557 comm, Curtis; ex herb. Berkeley (IMI 193125, **holotype**).

Endomeliola S. Hughes & Piroz., N.Z. Jl Bot. 32(1): 53 (1994)

Facesoffunginumber: FoF00729

Epiphytes or *pathogens* on surface of leaves, developing on reddish-brown necrotic areas. *Intercellular hyphae* branched, septate, cylindrical, smooth, brown to pale

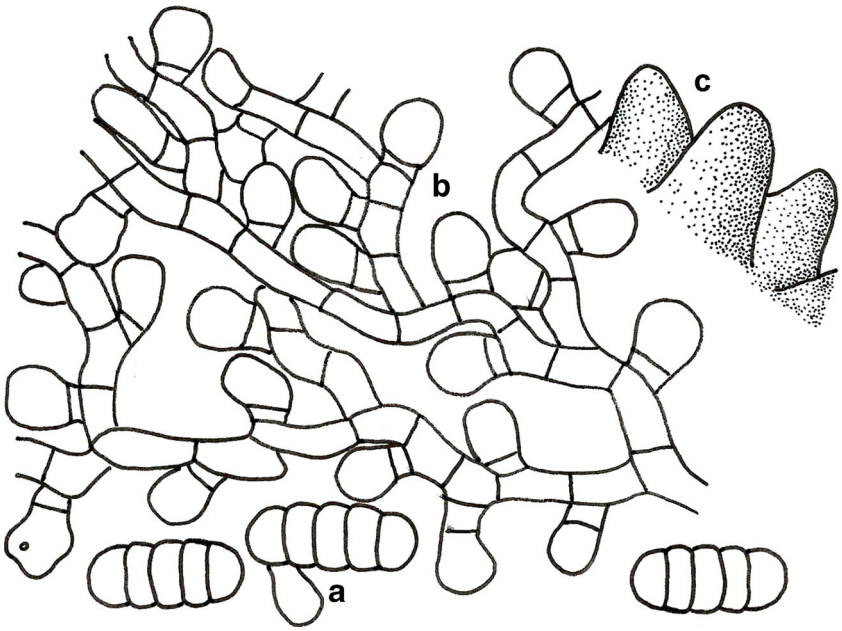


Fig. 13 *Asteridiella solani* (redrawn from Hansford 1963). **a** Ascospores. **b** Capitate hyphopodia on hyphae. **c** Raised conical projections on ascomata

brown, irregular, extending into the mesophyll, also penetrating between cells of the 2-layered palisade, with a single terminal hyphopodium. *Hyphopodia* irregular, mostly ellipsoidal to subglobose to ovoid to obovoid, occasionally angular or lobed in the mesophyll, generally with a short stalk cell, with hyaline pore at the centre of the head cell. **Sexual morph**: *Stromata* superficial on surface of host, solitary or gregarious, often discrete and then subglobose, somewhat flattened with a constricted base seated on the stromatic crust, black, comprising dark brown cells of *textura angularis* in transverse section, surface verrucose, with raised conical protuberant or ampuliform cells. *Peridium* comprising dark brown cells of *textura angularis* in transverse section, two strata, outer stratum of thick-walled, dark brown to reddish cells of *textura angularis*, and inner stratum of hyaline flattened

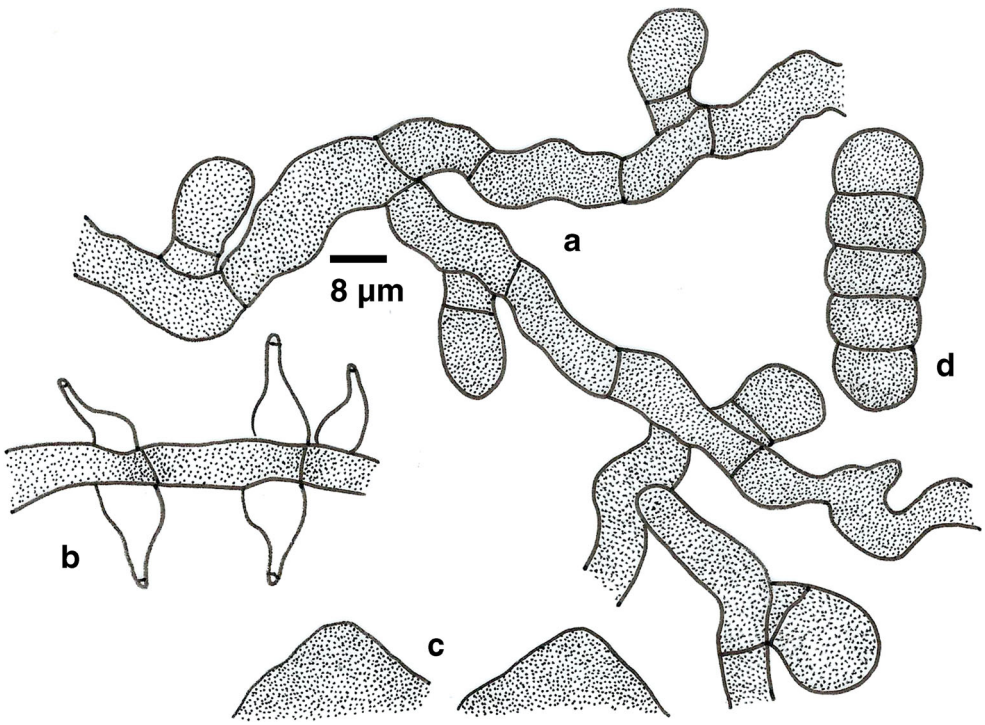
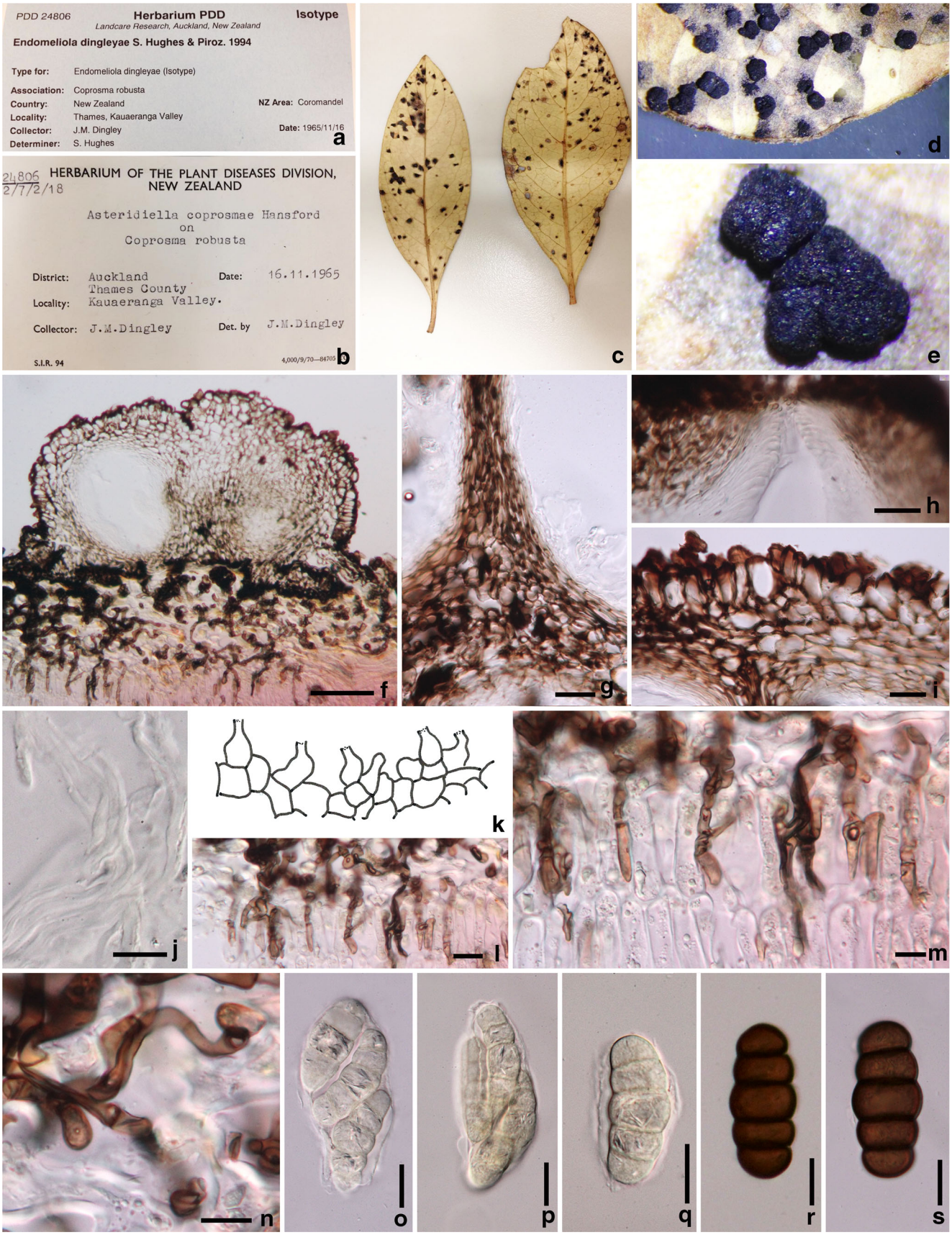


Fig. 14 *Asteridiella solani* var. *kodaikanalensis* (redrawn from Hosagoudar 2013). **a** Capitate hyphopodia on hyphae. **b** Phialides on hyphae. **c** Raised conical projections on ascoma. **d** Ascospore. Scale Bar: **a**=8 μm

Fig. 15 *Cryptomeliola orbicularis* (holotype). **a**, **b** Herbarium packet, specimen and drawing. **c** Colony on host surface. **d** Ascomata on substrate. **e** Section through ascoma. **f** Projecting cells on outside of peridium. **g**, **i** Setae arising on hyphae. **h** Upper wall of ascoma in squash mount. **j** Immature ascus. **k**, **l** Mature ascospores. Scale bars: **e**, **f**=50 μm, **h**, **k**, **l**=20 μm, **g**, **i**, **j**=10 μm

cells. *Ascomatal locules* 1–4-locules in a pulvinate stroma, with central ostioles lined with periphyses. *Hamathecium* comprising cylindrical, hyaline, aseptate paraphyses. *Asci* 4-spored, unitunicate, ellipsoidal to clavate. *Ascospores* 2–3-seriate, hyaline when immature and brown to dark brown at maturity, broadly ellipsoidal to subcylindrical, 4-septate, slightly constricted and darker at the septa, the central cell sometimes longer than the others, smooth-walled. **Asexual morph**: *Phialides* solitary or gregarious on superficial ascostromata, occasionally on ascomatal walls, ampuliform or flask-shaped, brown to dark brown. *Conidia* ellipsoidal, hyaline (asexual morph redescribed from Hughes and Pirozynski 1994).

Notes: *Endomeliola* was introduced by Hughes and Pirozynski (1994), with type species *E. dingleyae*. The genus is distinguished by intercellular hyphae formed in the mesophyll layer of the plant. *Endomeliola* is a monotypic genus, lacking molecular data. However, the morphology is distinct from other genera in *Meliolaceae* (Fig. 16) and thus, we accept the genus in *Meliolaceae*.


PDD 24806
Herbarium PDD
Landcare Research, Auckland, New Zealand
Isotype
Endomeliola dingleyae S. Hughes & Piroz. 1994
Type for: Endomeliola dingleyae (Isotype)
Association: Coprosma robusta
Country: New Zealand
NZ Area: Coromandel
Locality: Thames, Kauaeranga Valley
Collector: J.M. Dingley
Date: 1965/11/16
Determiner: S. Hughes
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HERBARIUM OF THE PLANT DISEASES DIVISION, NEW ZEALAND
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Coprosma robusta
District: Auckland
Thames County
Date: 16.11.1965
Locality: Kauaeranga Valley.
Collector: J.M.Dingley
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◀ **Fig. 16** *Endomeliola dingleyae* (isotype). **a**–**c** Herbarium packet and specimen. **d**–**e** Ascostromata on substrate. **f** Section through ascostromata. **g** Peridium between locules. **h** Periphyses. **i** Wall cells. **j** Paraphyses. **k** Phialides on surface of ascomata. **l**, **m** Intercellular hyphae. **n** Hyphopodia. **o**, **p** Asci. **q** Immature ascospore. **r**, **s** Mature ascospores. Scale bars: **e**, **f**=50 μm, **h**, **l**–**s**=20 μm, **g**, **i**, **j**=10 μm

Type species: ***Endomeliola dingleyae*** S. Hughes & Piroz., N.Z. Jl Bot. 32(1): 54 (1994)

Facesoffunginumber: FoF00728

Epiphytes, or *pathogens* on surface of leaves, developing on reddish brown necrotic areas. *Intercellular hyphae* 5–6 μm diam. ($\overline{x}$ = 5 μm, n=10), branched, septate, cylindrical, smooth, brown to pale brown, irregular, extending into the mesophyll, also penetrating between cells of the 2-layered palisade, with a single terminal hyphopodium. *Hyphopodia* 6–8 μm diam. ($\overline{x}$ = 7 μm, n=10), irregular, mostly ellipsoidal, subglobose, ovoid or obovoid, occasionally angular or lobed in the mesophyll, generally with a short stalk cell, with hyaline pore around the centre of the head cell. **Sexual morph**: *Stromata* 490–785 diam. × 255–575 μm high ($\overline{x}$ = 552 × 345 μm, n=10), superficial on surface of host, solitary or gregarious, often discrete and then subglobose, somewhat flattened with a constricted base seated on the stromatic crust, black, surface verrucose, with raised conical protuberant or ampuliform cells. *Peridium* 45–63 μm ($\overline{x}$ = 48 μm, n=5), comprising dark brown cells of *textura angularis* in transverse section, with two strata, outer stratum of thick-walled, dark brown to reddish cells of *textura angularis*, and inner stratum of hyaline flattened cells. *Ascomatal locules* 1–4-locules in a pulvinate stroma, with central ostioles lined with periphyses. *Hamathecium* comprising cylindrical, hyaline, aseptate paraphyses. *Asci* 74–94×38–44 μm ($\overline{x}$ = 92 × 41 μm, n=10), 4-spored, unitunicate, ellipsoidal to clavate. *Ascospores* 65–67×29–32 μm ($\overline{x}$ = 66 × 30 μm, n=10), 2–3-seriate, hyaline when immature and brown to dark brown at maturity, broadly ellipsoidal to subcylindrical, 4-septate, slightly constricted and darker at the septa, the central cell sometimes longer than the others, smooth-walled. **Asexual morph**: Undetermined.

Notes: Molecular data indicate that *Endomeliola dingleyae* is a member of family *Meliolaceae*. The species is closely related to *Asteridiella intidae* with high bootstrap support (Justavino et al. 2015), but differs considerably in morphology and it is possible that the data is incorrect. Therefore, further gene and sequence data are needed to clarity the placement of the genus and species.

Material examined NEW ZEALAND, Auckland Province, Thames County, Kauaeranga Valley, on living leaves of *Coprosma robusta* Raoul. (*Rubiaceae*), 16 November 1965, J.M. Dingley (PDD 24806, **isotype**).

Irenopsis F. Stevens, Annls mycol. 25(5/6): 411 (1927)

≡ *Meliola* subgen. *Irenopsis* (F. Stevens) Cif., Annls mycol. 36(2/3): 203 (1938)

Facesoffunginumber: FoF00734

Epiphytes on the surface of leaves and stems. *Superficial hyphae* branched, septate, darker at the septa, brown, with hyphopodia, hyphal setae lacking. *Hyphopodia* capitate, angular or lobate, alternate or opposite on hyphae, near to hyphal septa, 2-celled, brown. **Sexual morph**: *Ascomata* superficial on the surface of hosts, solitary or gregarious on superficial hyphae, globose to subglobose, thick-walled, with raised long setae on the surface. *Ascomatal setae* curved or hooked at the apex, developing on the ascomata. *Peridium* comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum thick-walled, dark brown cells of irregular *textura angularis*, and inner stratum of flattened, hyaline cells. *Hamathecium* with evanescent paraphyses. *Asci* 2–4-spored, unitunicate, obovoid to ovoid, evanescent. *Ascospores* 2-seriate, hyaline to brown, oblong to ovoid, 4-septate, constricted and darker at the septa, rounded ends, smooth-walled. **Asexual morph**: *Phialides* ampuliform, mixed with capitate hyphopodia, opposite or alternate, pale brown to brown. *Conidia* rarely observed, hyaline.

Notes: The genus *Irenopsis* was established by Stevens (1927). This genus is distinguished by having true setae on ascomata and lacking hyphal setae. *Irenopsis* comprises 180 species epithets in Index Fungorum (2015). Species were mainly introduced based on host association. Sequence data for *I. vincensii* places *Irenopsis* in *Meliolaceae* (Justavino et al. 2015).

Type species: ***Irenopsis tortuosa*** (G. Winter) F. Stevens, Annls mycol. 25(5/6): 439 (1927)

≡ *Meliola tortuosa* G. Winter, in Gaillard, Le Genre Meliola: 67 (1892)

≡ *Irenopsis tortuosa* (G. Winter) F. Stevens, Annls mycol. 25(5/6): 439 (1927) var. *tortuosa*

Possible synonym (from Index Fungorum 2015)

= *Meliola tonkinensis* var. *potomorphes* Cif., Annls mycol. 31(3): 149 (1933)

Facesoffunginumber: FoF00732

Epiphytes on the surface of leaves and stems. *Superficial hyphae* branched, septate, darker at the septa, brown, with hyphopodia, hyphal setae lacking. *Hyphopodia* capitate, curved, alternate or opposite on hyphae, near to hyphal septa, 2-celled, brown. **Sexual morph**: *Ascomata* 175–215 μm diam. × 95–120 μm high ($\overline{x}$ = 208 × 113 μm, n=5), superficial on surface of hosts, solitary or gregarious on superficial hyphae, globose to subglobose, thick-walled, with raised long setae on the surface. *Ascomatal setae* 8–10×92–107 μm ($\overline{x}$ = 9 × 102 μm, n=5), curved or hooked at the apices, developing on the ascomata. *Peridium* 20–26 μm ($\overline{x}$ = 24 μm,

n=5), comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum thick-walled, dark brown cells of irregular *textura angularis*, and inner stratum of flattened, hyaline cells. *Hamathecium* with evanescent paraphyses. *Asci* 54–58×22–25 μm ($\bar{x}$ = 55 × 23 μm, n=5), 2–4-spored, unitunicate, obovoid to ovoid, evanescent. *Ascospores* 37–40×14–15 μm ($\bar{x}$ = 37 × 15 μm, n=10), 2-seriate, hyaline to brown, oblong to ovoid, 4-septate, or with 4 transverse septa and 2 longitudinal septa, constricted and darker at the septa, rounded ends, smooth-walled. **Asexual morph**: *Phialides* 16–17×7–8 μm ($\bar{x}$ = 16 × 7 μm, n=10), ampliform, mixed with capitate hyphopodia, opposite or alternate, pale brown to brown. *Conidia* not seen.

Material examined DOMINICAN REPUBLIC, on leaves of *Potomorpha umbellata* Mich. (*Piperaceae*), August 1929, R. Ciferri 2426 (SF5890, **holotype**); VENEZUELA, El Limon bei Puerto La Cruz, on leaves of *Piperis marginati* Jacq. (*Piperaceae*), 16 January 1928, H. Sydow (SF77640).

Notes: The type specimen was collected in Brazil (Stevens 1927), but we could not locate and examine the type. Therefore a collection of *Meliola tonkinensis* var. *potomorphes* from S was studied (Figs. 17 and 18). One ascospore was found with 4 transverse septa and 2 longitudinal septa in *Irenopsis tortuosa* (S-F5890).

Irenopsis walsurae X.Z. Zeng & K.D. Hyde, **sp. nov**.

Facesoffunginumber: FoF00733

Index Fungorum: IF551220

Etymology:— *walsurae* referring to the host on which the taxon was found.

Holotype: MFLU13-0621

Epiphytes on the surface of living leaves. *Superficial hyphae* 7 μm diam., radiating outwardly, branched, septate, darker at the septa, brown, with hyphopodia, hyphal setae absent. *Hyphopodia* 14–19×9–13 μm ($\bar{x}$ = 16 × 11 μm, n=20), capitate, alternate on hyphae, near to hyphal septa, 2-celled, brown. **Sexual morph**: *Ascomata* up to 160 μm, superficial on surface of hosts, scattered, globose to subglobose, thick-walled, with long ascomatal setae. *Ascomatal setae* up to 170 μm long, raised on ascomata, and rounded at the apex. *Peridium* comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum a single layer of large, thick-walled, dark brown cells of irregular *textura angularis*, and inner stratum of flattened, hyaline cells. *Hamathecium* with evanescent paraphyses. *Asci* 2–3-spored, unitunicate, obovoid to ovoid, with short pedicel or apedicel, evanescent. *Ascospores* 33–39×12–18 μm ($\bar{x}$ = 36 × 14 μm, n=20), 2–3-seriate, hyaline to brown, oblong to ovoid, 3–4-septate, slightly constricted and darker at the septa, rounded ends, apical cell sometimes slightly longer, smooth-walled. **Asexual morph**: *Phialides* 16–21×8–10 μm ($\bar{x}$ = 18 × 9 μm, n=10), ampuliform, alternate to opposite, formed on separate hyphae, rarely mixed with capitate hyphopodia. *Conidia* undetermined.

Material examined THAILAND, Chiang Mai, Mae Taeng, Pa Pae, Bahn Pa Dheng, 128 Moo 3, Mushroom Research Centre, on the living leaves of *Walsura tubulata* Hiern. (*Meliaceae*), 22 November 2013, Xiangyu Zeng (MFLU 13-0621, **holotype**; **isotype**, KUN).

Notes: *Irenopsis walsurae* was found on living leaves of *Walsura tubulata* (*Meliaceae*). *Irenopsis* species known from this host family are *I. trichiliae* Hosag. & Riju, *I. chukrasiae* Hosag., *I. dysoxyli* Jana et al. and *I. indica* (Anahosur) Hosag. *Irenopsis walsurae* is most similar to *I. dysoxyli*, but differs in having longer ascospores and ascomatal setae, with smaller ascomata in *Irenopsis walsurae*. The new species is also similar to *I. trichiliae*, but differs in having opposite to unilateral phialides, separated from the hyphopodia in *Irenopsis walsurae*, while alternate to unilateral phialides, mixed with hyphopodia in *I. trichiliae*. There are no previous records of *Irenopsis* species reported on *Walsura*. Other genera in *Meliolaceae* known from *Walsura* are *Ectendomeliola walsurae* Hosag. & D.K. Agarwal, *Meliola walsurae* Hansf. and *M. walsuricola* Bagool & H. Biju, however, ascomatal setae are absent in these three species, while ascomatal setae are present in *Irenopsis walsurae*. *Ectendomeliola* is excluded from *Meliolaceae* in this study (Fig. 19).

Meliola Fr., Syst. orb. veg. (Lundae) 1: 111 (1825)

Possible synonyms (from Index Fungorum 2015):

Myxothecium Kunze, Syst. mycol. (Lundae) 3(1): 231 (1829)

Asterina subgen. *Asteridium* Sacc., Syll. fung. (Abellini) 1: 49 (1882)

Asteridium (Sacc.) Speg. ex Sacc., Syll. fung. (Abellini) 9: 435 (1891)

Facesoffunginumber: FoF00740

Epiphytes on the surface of leaves, stems or branches. *Superficial hyphae* branched, septate, darker at the septa, brown, with hyphopodia, hyphal setae present. *Hyphopodia* capitate, alternate or opposite on hyphae, near to hyphal septa, 2-celled, brown. *Hyphal setae* arising from hyphae, forming around the base of the ascomata in some species, straight or curly, rounded or acute at the apex, or branches, septate or aseptate, brown to dark brown, smooth-walled. **Sexual morph**: *Ascomata* superficial on the surface of hosts, solitary to gregarious on superficial hyphae, globose to subglobose, thick-walled, ascomatal setae and appendages absent, surface of ascomata verrucose. *Peridium* comprising dark brown

Fig. 17 *Irenopsis tortuosa* (holotype). **a**–**c** Herbarium packet and specimen. **d** Ascomata on substrate. **e**, **f** Ascomata when viewed in squash mounts. **g** Section through ascoma. **h** Upper wall of ascomata. **i** Hyphae with phialides. **j** Hyphae with capitate hyphopodia. **k** Seta on ascoma. **l** Hamathecium. **m**, **n** Immature asci. **o**, **p** Ascospores with 4 septa. **q** Ascospore with 4 transverse septa and 2 longitudinal septa. Scale bars: **e**=200 μm, **f**, g=100 μm, **h**, **m**–**q**=20 μm, **i**, **j**=5 μm, **k**, **l**=10 μm

cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum thick-walled, dark brown cells of irregular *textura angularis*, and inner stratum of flattened, hyaline cells. *Hamathecium* with evanescent paraphyses. *Asci* 2–4-spored, unitunicate, broadly clavate to oblong, evanescent. *Ascospores* 2–4-seriate, hyaline to brown, oblong to broadly cylindrical, 3–4-septate, constricted and darker at the septa, rounded ends, smooth-walled, verrucose when immature. **Asexual morph**: *Phialides* ampuliform, alternate or opposite on hyphae, sometimes curved, pale brown to brown. *Conidia* hyaline.

Notes: The genus *Meliola* was introduced by Fries (1825), and is the largest genus in the family *Meliolaceae*. *Meliola*

Fig. 18 *Irenopsis tortuosa* (S- F77640). **a**, **b** Herbarium packet and specimen. **c** Ascomata on host surface. **d** Ascoma when viewed in squash mounts. **e** Section through ascoma. **f** Peridium. **g** Upper wall of ascoma. **h** Phialides on hyphae. **i** Hyphae with capitate hyphopodia. **j** Young ascoma. **k** Seta on ascoma. **l**, **m** Immature asci. **n**–**p** Ascospores with 4 septa. Scale bars: **d**, **e**=50 μm, **f**, **g**, **j**–**p**=20 μm, **h**, **i**=10 μm

contains over 1200 species (Kirk et al. 2008). Most species were introduced based on host association. Molecular analysis of *Meliola* members were provided by Gregory and John (1999), Justavino et al. (2015) and Pinho et al. (2012a, 2014) and show their placement in *Meliolaceae*.

Type species: ***Meliola nidulans*** (Schwein.) Cooke, Grevillea 11(no. 57): 37 (1882)

≡ *Sphaeria nidulans* Schwein., Schr. naturf. Ges. Leipzig 1: 45 (1822)

≡ *Chaetosphaeria nidulans* (Schwein.) Rehm, Ascomyceten, fasc.: no. 287 (1875)

≡ *Meliola nidulans* (Schwein.) Cooke, Grevillea 11(no. 57): 37 (1882) var. *nidulans*

Facesoffunginumber: FoF00738

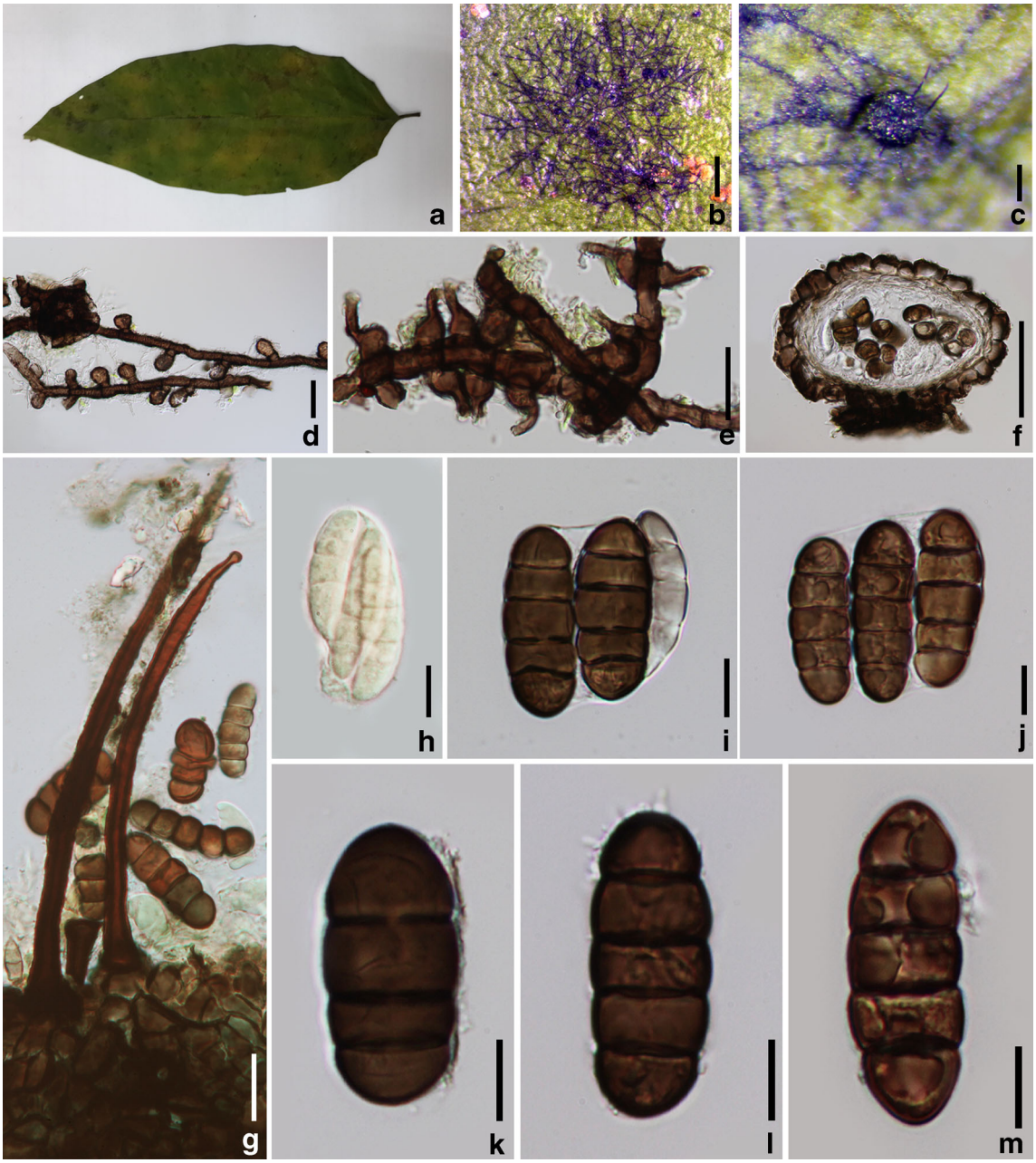


Fig. 19 *Irenopsis walsuri* (holotype). **a** Leaf specimen. **b** Colony on surface of leaves. **c** Ascoma on host substrate. **d** Hyphae with capitate hyphopodia. **e** Phialides on hyphae. **f** Section through ascoma. **g** Long setae forming on ascoma. **h** Immature ascus. **i–j** Mature asci. **k–m** Ascospores with 4 septa. Scale bars: **b**=500 μm, **c**=100 μm, **f**=50 μm, **d**, **e**, **g**=20 μm, **h–m**=10 μm

Epiphytes on the surface of leaves, stems or branches. *Superficial hyphae* branched, septate, darker at the septa, brown, with hyphopodia, hyphal setae present. *Hyphopodia* not seen. *Hyphal setae* up to 350 μm, forming around the base of the ascomata in some species, straight or curly, rounded or acute at the apex or branches, septate or aseptate, brown to dark brown, smooth-walled. **Sexual morph**: *Ascomata* 235–345 μm diam. ($\bar{x}$ = 312 μm, n=5), superficial on the surface of hosts, solitary to gregarious on superficial hyphae, globose to subglobose, thick-walled, ascomatal setae and appendages absent, surface of ascomata verrucose. *Peridium* 53–62 μm diam. ($\bar{x}$ = 56 μm, n=5), comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum a single layer or multi-layered when immature, of large, thick-walled, dark brown cells of irregular *textura angularis*, and inner stratum of flattened, hyaline cells. *Hamathecium* with evanescent paraphyses. *Asci* 58–63×32–36 μm ($\bar{x}$ = 60 × 31 μm, n= 5), 2–4-spored, unitunicate, broadly clavate to oblong, evanescent. *Ascospores* 45–54×15–19 μm ($\bar{x}$ = 51 × 18 μm,

n=5), 2–4-seriate, hyaline to brown, oblong to broadly cylindrical, 3–4-septate, constricted and darker at the septa, rounded ends, smooth-walled, verrucose when immature. **Asexual morph**: Undetermined.

Notes: The species *Meliola nidulans* was established by Cooke (1882), and was based on *Sphaeria nidulans* Schwein. We examined the type specimen of *M. nidulans* and could not obtain good photographs of the superficial hyphae, hyphopodia, and phialides because the specimen is not in good condition (Fig. 20).

Material examined USA, Pennsylvania, New Garden, on branches of *Cornus* sp. (*Cornaceae*), Michener Collection 711 (BPI 800359, holotype of *Sphaeria nidulans*).

Meliola clerodendricola Henn., Hedwigia 37: 288 (1898)

Facesoffunginumber: FoF00736

Epiphytes on surface of living leaves. *Superficial hyphae* 7 μm diam. radiating outwardly, straight to substraight, branched, septate, darker at the septa, brown, with hyphopodia, hyphal setae present. *Hyphopodia* 13–17 × 9–13 μm ($\bar{x}$ = 15 × 11 μm, n=20), capitate, unilateral on hyphae, near to hyphal septa, 2-celled, brown. *Hyphal setae* long, straight, acute at the apex, septate, brown, and smooth-walled. **Sexual morph**: *Ascomata* up to 160 μm diam., superficial on surface of host, dense, globose to subglobose, thick-walled, ascomatal setae and appendages absent. *Peridium* comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum a single layer of large, thick-walled, dark brown cells of irregular *textura angularis*, and inner stratum of flattened, hyaline cells. *Hamathecium* with evanescent paraphyses. *Asci* 2–4-spored, unitunicate, obovoid to ovoid, with short pedicel, evanescent. *Ascospores* 31–35 × 10–13 μm, ($\bar{x}$ = 33 × 12 μm, n=20), 2–4-seriate, hyaline to light brown, oblong to ellipsoid, 3–4-septate, constricted and darker at the septa, rounded ends, apical cell sometimes slightly larger, smooth-walled. **Asexual morph**: Undetermined.

Material examined THAILAND, Chiang Mai, Mushroom Research Centre, Mae Taeng, Pa Pae, Bahn Pa Dheng, 128 Moo 3, on the living leaves of *Clerodendrum* sp. (*Lamiaceae*), 22 November 2013, Xiangyu Zeng (MFLU 13-0620; KIB, **reference specimen designated here**).

Notes: *Meliola clerodendricola* (MFLU 13-0620) was found on living leaves of *Clerodendrum* sp. (*Lamiaceae*). The morphology of MFLU13-0620 is most typical of *Meliola clerodendricola* based on host association and size of hyphal setae, ascomata, and ascospores. We were unable to locate the holotype material of *Meliola clerodendricola*, but an isotype is preserved in S (F8150) (Fig. 21). However, DNA cannot be extracted from the type specimen, consequently, we designate this as a reference specimen (Ariyawansa et al. 2014). *Meliolaceae* species known from *Clerodendrum* sp. are *Asteridiella clerodendricola* Hosag. and *A. vivekananthanii* Hosag., however, hyphal setae are absent in these species, but are found in *M. clerodendricola*. In addition, *Asteridiella clerodendricola* can produce a pathogenic effect on the host, while there is no pathogenic effect with *Meliola clerodendricola*. Molecular analyses place *M. clerodendricola* in the *Meliolaceae* clade, with a putatively named specimen of *Endomeliola dingleyae* (97 % ML support and 1.0 PP support). This result is strange as *Meliola clerodendricola* has superficial hyphae and hyphal setae, while *Endomeliola dingleyae* has intercellular hyphae without hyphal setae. *Meliola clerodendricola* was found on a host in the order *Lamiales* and *Endomeliola dingleyae* was found on host in the order *Gentianales*; their hosts belong in closely related orders in the euasterids (Jansen et al. 2007; Justavino et al. 2015). The identification of *Endomeliola dingleyae* may be incorrect and although the sequence has been placed in GenBank it does not appear to be linked to a voucher specimen.

Meliola thailandicum Hongsanan & K.D. Hyde, **sp. nov**.

Facesoffunginumber: FoF00739

Index Fungorum: IF551218

Etymology:—from Latin *thailandicum* meaning Thailand, referring to the location where the fungus was found.

Holotype: MFLU15-0044

Epiphytes on the surface of living leaves. *Superficial hyphae* 6 μm diam., radiating outwardly, branched, septate, darker at the septa, brown to dark brown, with hyphopodia. *Hyphal setae* up to 300 μm long, septate, darker at the septa, 1–3 forked at the apex. *Hyphopodia* 7 μm diam., capitate, mostly alternate or sometimes opposite on hyphae, near to hyphal septum, 2-celled, brown. **Sexual morph**: *Ascomata* 185–200 μm diam. × 120–180 μm high ($\bar{x}$ = 190 × 134 μm, n=10), superficial on surface of host, mostly scattered or solitary, globose to subglobose, thick-walled, ascomatal setae and appendages absent. *Peridium* 18–20 μm ($\bar{x}$ = 20 μm, n=10), comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum a single layer of large, thick-walled, dark brown cells of irregular *textura angularis*, and inner stratum of flattened, hyaline cells. *Hamathecium* with evanescent paraphyses. *Asci* 59–60 × 26–28 μm ($\bar{x}$ = 60 × 27 μm, n=10), 2–4-spored, unitunicate, obovoid to ovoid, or broadly clavate, with short pedicel, sessile at maturity, evanescent. *Ascospores* 39–41 × 14–16 μm ($\bar{x}$ = 40 × 15 μm, n=20), 2–4-seriate, hyaline to brown, oblong to cylindrical, 4-septate, constricted and darker at the septa, rounded ends, with thin sheath when immature, smooth-walled. **Asexual morph**: *Phialides* 7–8 μm ($\bar{x}$ = 7 μm, n= 10), ampuliform, alternate to opposite, formed on separate hyphae, mixed with capitate hyphopodia. *Conidia* undetermined.

Material examined THAILAND, Chiang Rai, Amphoe Thoeng, on the living leaves of *Dimocarpus longan*

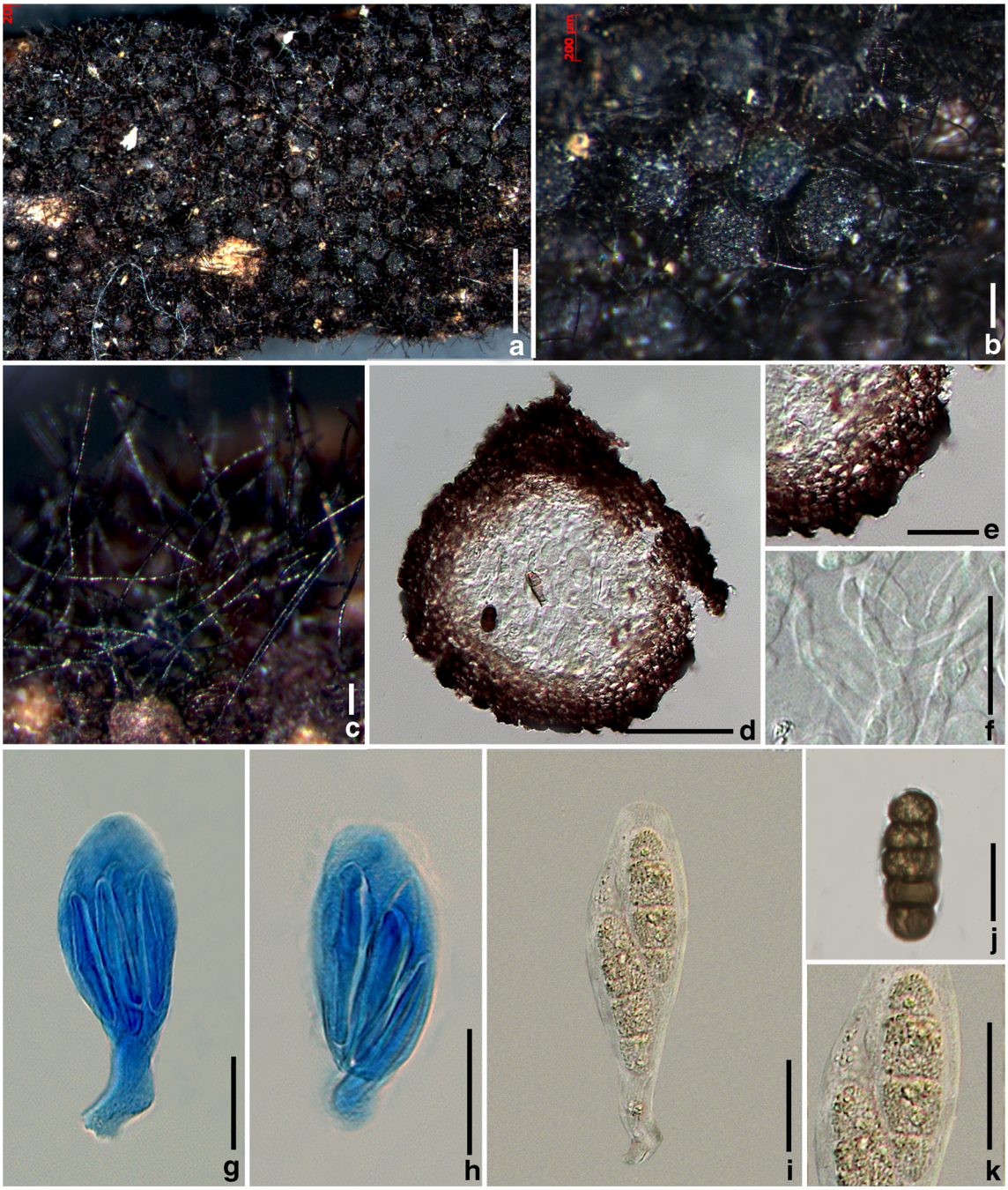


Fig. 20 *Meliola nidulans* (holotype) **a**, **b** Ascomata on host substrate **c** Long setae developed from hyphae. **d** Section through ascoma. **e** Peridium. **f** Paraphyses. **g**, **h** Immature asci in cotton blue reagent. **i** Immature ascus in Melzer's reagent. **j** Mature ascospore with 4 septa and rounded ends. **k** Immature ascospores in Melzer's reagent with verrucose surface. Scale bars: **a**=1000 μm, **b**=200 μm, **e**=50 μm, **c**, **f**–**k**=20 μm, **d**=100 μm

Lour. (*Sapindaceae*), 18 January 2015, S. Hongsanan (MFLU15-0044 **holotype**; KIB, **isotype**); Chiang Rai, Mueang, Rai Chun Tawan Meditation Centre, on the living leaves of *Dimocarpus longan* (*Sapindaceae*), 3 January 2015, S. Hongsanan (MFLU15-0047); Chiang Rai, Mueang, Tasud, on the living leaves of *Dimocarpus longan* (*Sapindaceae*), 11 February 2015, S. Hongsanan (MFLU15-0377); Chiang Rai, Mueang, Agricultural research center, on living leaves of *Acacia auriculiformis* A.Cunn. ex Benth. (*Fabaceae*), 23 January 2015, S. Hongsanan (MFLU15-0378); Chiang Rai, Mueang, Mae Fah Luang University, on the living leaves of *Dimocarpus longan* (*Sapindaceae*), 12 February 2015, S. Hongsanan (MFLU15-0379).

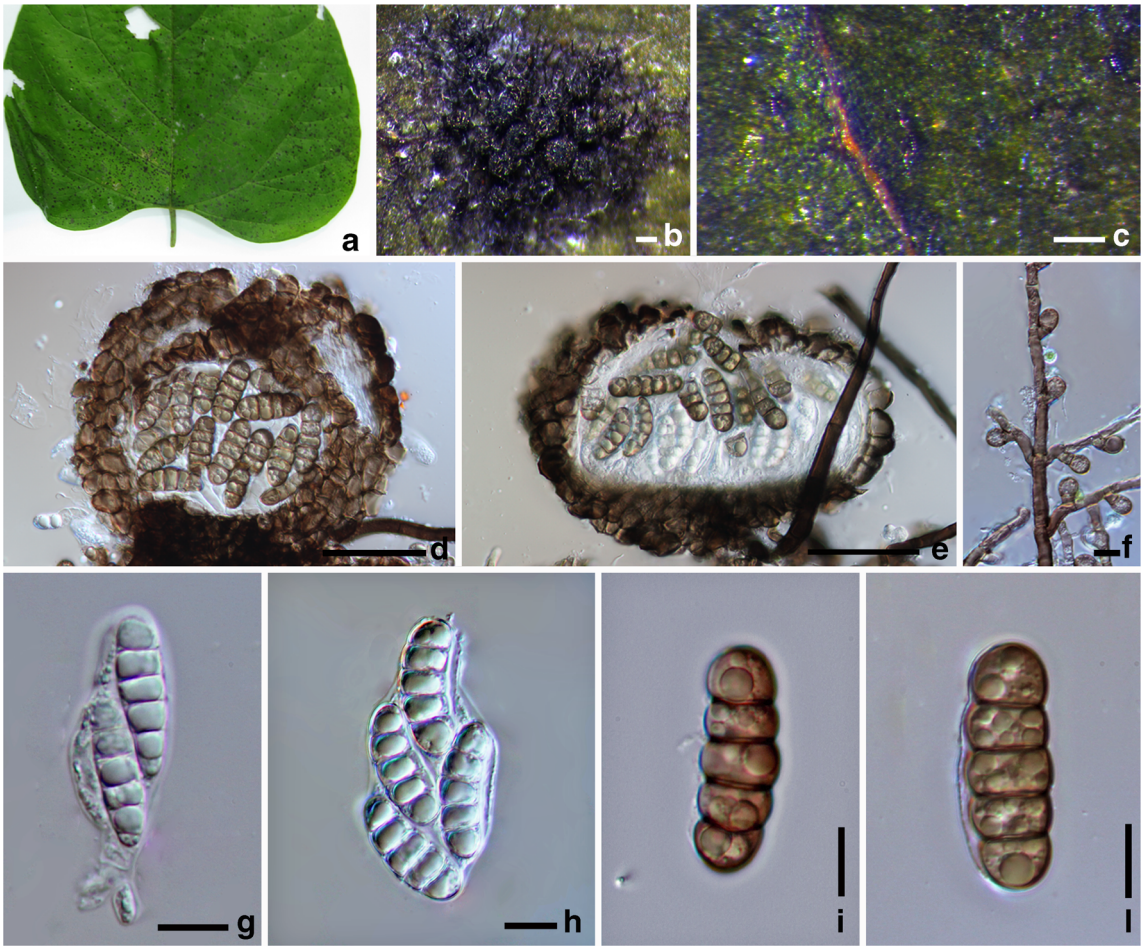


Fig. 21 *Meliola clerodendricola* (MFLU13-0620). **a** Leaf specimen. **b** Colony on surface of leaves. **c** Ascomata on host substrate. **d**, **e** Section through ascomata. **f** Hyphae with capitate hyphopodia. **g**, **h** Immature asci. **i**, **j** Ascospores with 4 septa. Scale bars: **b**, **c**=100 μm, **d**, **e**=50 μm, **f**–**l**=10 μm

Notes: *Meliola thailandicum* is common on living leaves of *Dimocarpus longan* in northern Thailand. *Meliola capensis* (Kalchbr. & Cooke) Theiss. and *M. dimocapi* Hosag. & T.K. Abraham are also known from *Dimocarpus longan*, however, they differ in having apically acute hyphal setae, and ascospores without a thin-sheath, while *M. thailandicum* has longer hyphal setae with 1 to many branches at the apex, and ascospores with thin-sheath when immature and that may be present at maturity (Figs. 22 and 23). *Meliola thailandicum* was also collected on *Acacia auriculiformis*. *Meliola* species known from this host genus are *M. acaciarum* Speg., *M. acaciae-binervatae* Hansf., *M. acaciae-confusae* Sawada, *M. acaciicola* Hansf. and *M. acaciorum* Speg., however, all species differ from *M. thailandicum* in having shorter hyphal setae, acute or rounded at the apex, and ascospores lacking a sheath. In *M. thailandicum* hyphal setae are longer and branched at the apex, and ascospores have thin sheaths when immature and sometimes when mature. Molecular analyses indicate that *M. thailandicum* from *D. longan* and *A. auriculiformis* are the same species which belong in *Meliolaceae* within the genus *Meliola* (100 % ML support and 1.0 PP support). Hence, we conclude that the species is not host-specific. This may have important implications on the numbers of *Meliolaceae* species, as most taxa were introduced based on host occurrence.

Meliola mucunicola Hongsanan & K.D. Hyde, **sp. nov**.

Facesoffunginumber: FoF00737

Index Fungorum: IF551219

Etymology:— *mucunicola* referring to the host on which the taxon was found.

Holotype: MFLU15-0386

Epiphytes on the surface of *Mucuna* living leaves. *Superficial hyphae* 5–8 μm ($\bar{x}$ = 6 μm, n=10), radiating outwardly, branched, septate, darker at the septa, brown to dark brown, with hyphopodia. *Hyphal setae* 167–195×8–10 μm ($\bar{x}$ = 185 × 9 μm, n=10), rounded to acute at the apex. *Hyphopodia* 10–13 μm ($\bar{x}$ = 12 μm, n=10), capitate, mostly alternate or sometimes opposite on hyphae, near to hyphal septa, 2-celled, brown. **Sexual morph**: *Ascomata* 120–145 μm diam. × 90–105 μm high ($\bar{x}$ = 122 × 96 μm, n=10), superficial on surface of hosts, mostly scattered or solitary, globose to subglobose, thick-walled, ascomatal hyphae or appendages

Fig. 22 *Meliola thailandicum* (holotype). **a** Leaf specimens. **b** Ascomata on host substrate. **c** Section through ascoma. **d** Hyphal seta. **e** Hyphal setae with forked apices. **f** Peridium. **g** Hyphae with capitate hyphopodia. **h** Terminal hyphae. **i** Upper wall of ascoma. **j** Phialides. **k** Immature ascospore in Melzer's reagent. **l**, **m** Mature ascospores. **n**, **o**, **q** Immature asci. **p** Immature ascus in Melzer's reagent. Scale bars: **c**, **d**= 100 μm, **e**, **f**=20 μm, **g**–**j**=10 μm, **k**–**q**=20 μm

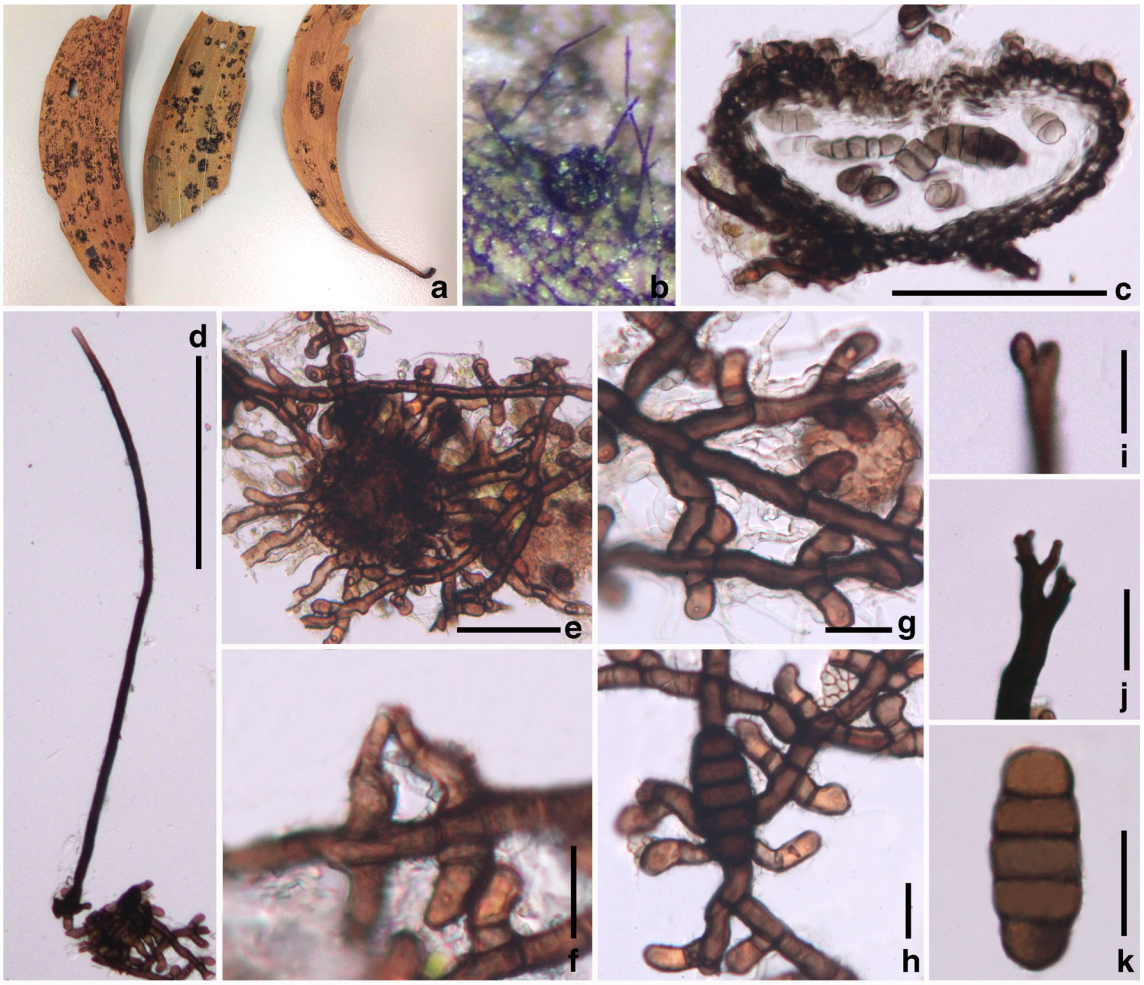


Fig. 23 *Meliola thailandicum* (from *Acacia auriculiformis*, MFLU 15-0378). **a** Leaf specimens. **b** Ascoma on host substrate. **c** Section through ascoma. **d** Hyphal seta. **e** Young ascoma. **f** Phialides on hyphae. **g** Capitate hyphopodia on hyphae. **h** Germinated ascospore. **i**, **j** Hyphal setae with branches at apex. **k** Mature ascospore with 4 septa. Scale bars: **c**=100 μm **d**=200 μm **e**=50 μm, **f**–**k**=20 μm

lacking. *Peridium* 15–19 μm ($\bar{x}$ = 17 μm, n=10), comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum a single layer of large, thick-walled, dark brown cells of irregular *textura angularis*, and inner stratum of flattened, hyaline cells. *Hamathecium* with evanescent paraphyses. *Asci* 44–53 × 22–23 μm ($\bar{x}$ = 48 × 23 μm, n=10), 2-spored, unitunicate, oblong to cylindrical, with short pedicel, sessile at maturity, evanescent. *Ascospores* 37–41 × 12–15 μm ($\bar{x}$ = 39 × 13 μm, n=20), 2-seriate, hyaline to brown, oblong to cylindrical, 4-septate, constricted and darker at the septa, rounded ends, with thin sheath when immature, smooth-walled. **Asexual morph**: *Phialides* 7–9 μm ($\bar{x}$ = 8 μm, n=10), ampuliform, alternate to opposite on hyphae, mixed with capitate hyphopodia. *Conidia* undetermined.

Material examined THAILAND, Chiang Rai, Mueang, Agricultural Research Center, on the living leaves of *Mucuna pruriens* (L.) DC. (*Fabaceae*), 23 March 2015, S. Hongsanan (MFLU15-0386, **holotype**; KIB, **isotype**).

Notes: *Meliola mucunicola* was found on living leaves of *Mucuna pruriens*. *Meliola* species known from this host genus are *M. mucunae* Hansf. and *M. mucunae-acuminatae* Hansf. The new species is most typical of *M. mucunae-acuminatae* in terms of morphology and host association, as *M. mucunae-acuminatae* can be found on the same host species. *Meliola mucunicola* differs from *M. mucunae-acuminatae* in having ascomata surrounded by many short setae (Fig. 24), while in *M. mucunae-acuminatae* ascomata are surrounded by a few long setae. Moreover, phialides of *Meliola mucunicola* are larger than those of *M. mucunae-acuminatae*. Molecular analyses place *Meliola mucunicola* in the *Meliolaceae* clade close to *Meliola centellae* Pinho & O.L. Pereira with 55 % ML support and 0.8 PP support.

Genera / species synonymized under *Meliola*

Laeviomeliola Bat., Atas Inst. Micol. Univ. Recife 1: 224 (1960)

Fig. 24 *Meliola mucunicola* (holotype). **a** Leaf specimen. **b** Ascomata and hyphal setae on host substrate. **c** Section through ascoma. **d** Hyphal setae. **e** Peridium. **f** Phialides on hyphae. **g** Hyphae with capitate hyphopodia. **h** Rounded apex of hyphal seta. **i** Upper wall of young ascoma. **j** Immature ascus. **k** Immature ascus in Melzer's reagent. **l** Upper wall of ascoma when viewed in squash mounts. **m**, **n** Mature ascospores with 4 septa. Scale bars: **c**, **d**=50 *μm*, **e**, **i**–**k**, **m**, **n**=20 *μm*, **f**–**h**, **l**=10 *μm*

≡ *Laeviomeliola psidii* Bat., Atas Inst. Micol. Univ. Recife 1: 225 (1960)

Meliola psidicola Hongsanan & K.D. Hyde, **nom. nov**.

Facesoffunginumber: FoF00847

Index Fungorum: IF551222

Epiphytes on the surface of leaves. *Superficial hyphae* 6–7 *μm* diam. ($\overline{x}$ = 6 *μm*, *n*=10), branched, septate, darker at the septa, brown, with hyphopodia. *Hyphal setae* 260–284×5–7 *μm* ($\overline{x}$ = 277 × 7 *μm*, *n*=10), rounded to acute at the apex. *Hyphopodia* 8–10 *μm* diam. ($\overline{x}$ = 9 *μm*, *n*=10), capitate, cylindrical to subcylindrical, alternate on hyphae, near to hyphal septa, brown, 2-celled. **Sexual morph**: *Ascomata* 158–182 *μm* diam. ($\overline{x}$ = 163, *n*=5), superficial on upper surface of leaves, solitary or gregarious on superficial hyphae, globose to subglobose, thick-walled, lacking ascomata setae and appendages. *Peridium* 22–25 *μm* ($\overline{x}$ = 23 *μm*, *n*=5), comprising dark brown cells of *textura angularis* when viewed in squash mounts, with two strata, outer stratum a single layer of large, thick-walled, dark brown cells of irregular *textura angularis*, and inner stratum of flattened, hyaline cells. *Hamathecium* with evanescent paraphyses (Batista and da Silva 1960). *Asci* 37–43×16–22 *μm* ($\overline{x}$ = 41 × 19 *μm*, *n*=3), 2-spored, unitunicate, clavate to obovoid, evanescent. *Ascospores* 44–52×15–16 *μm* ($\overline{x}$ = 46 × 15 *μm*, *n*=10), 2-seriate, hyaline when immature, brown at maturity, ellipsoid to fusiform, 4-septate, not constricted when immature, slightly constricted

and darker at the septa at maturity, acute ends, smooth-walled. **Asexual morph**: Undetermined.

Material examined BRAZIL, Pernambuco, Caruaru, on leaves of *Psidium guajavae* Linnaeus (*Myrtaceae*), 9 March 1958, Dr. Epaminondas de Barros Correia (URM 12749, **holotype**).

Notes: The genus *Laeviomeliola* is monotypic and was introduced by Batista and da Silva (1960), with the type species *L. psidii*. We re-examined the type specimen and concluded that the morphology of *Laeviomeliola* is quite similar to *Meliola* based on its hyphal setae. *Laeviomeliola psidii* differs in having fusiform ascospores with tapering ends, while in *Meliola* the ends are rounded (Fig. 25). Thus, *Laeviomeliola* should be synonymized under *Meliola*. As *Meliola psidii* is already in use, we provide a new name *Meliola psidiicola*. Fresh collection and molecular data are needed to confirm the position of the genus.

Doubtful genera within *Meliolales*

Ceratospermopsis Bat., Mycopath. Mycol. appl. 5: 165 (1951)

Notes: *Ceratospermopsis* was established by Batista (1951), and comprises two species, *C. cupaniae* Bat. and *C. xylopiae* Bat. The type species was not mentioned (Batista 1951; Index Fungorum 2015). We were unable to examine either species which are kept in URM, Brazil despite several requests. *Ceratospermopsis* is characterized by hyphal setae, lack of hyphopodia on the hyphae, globose to subglobose, membranous ascomata, 8-spored, subglobose asci, and muriform, slightly olivaceous, cylindrical to fusoid ascospores, with hyaline appendages (Batista 1951). We suggest that *C. xylopiae* should be the type species of the genus *Ceratospermopsis* based on its morphology. There is no sequence data to clarify the placement of the genus, which is poorly known and appears to lack herbarium material, thus we treat it as doubtful.

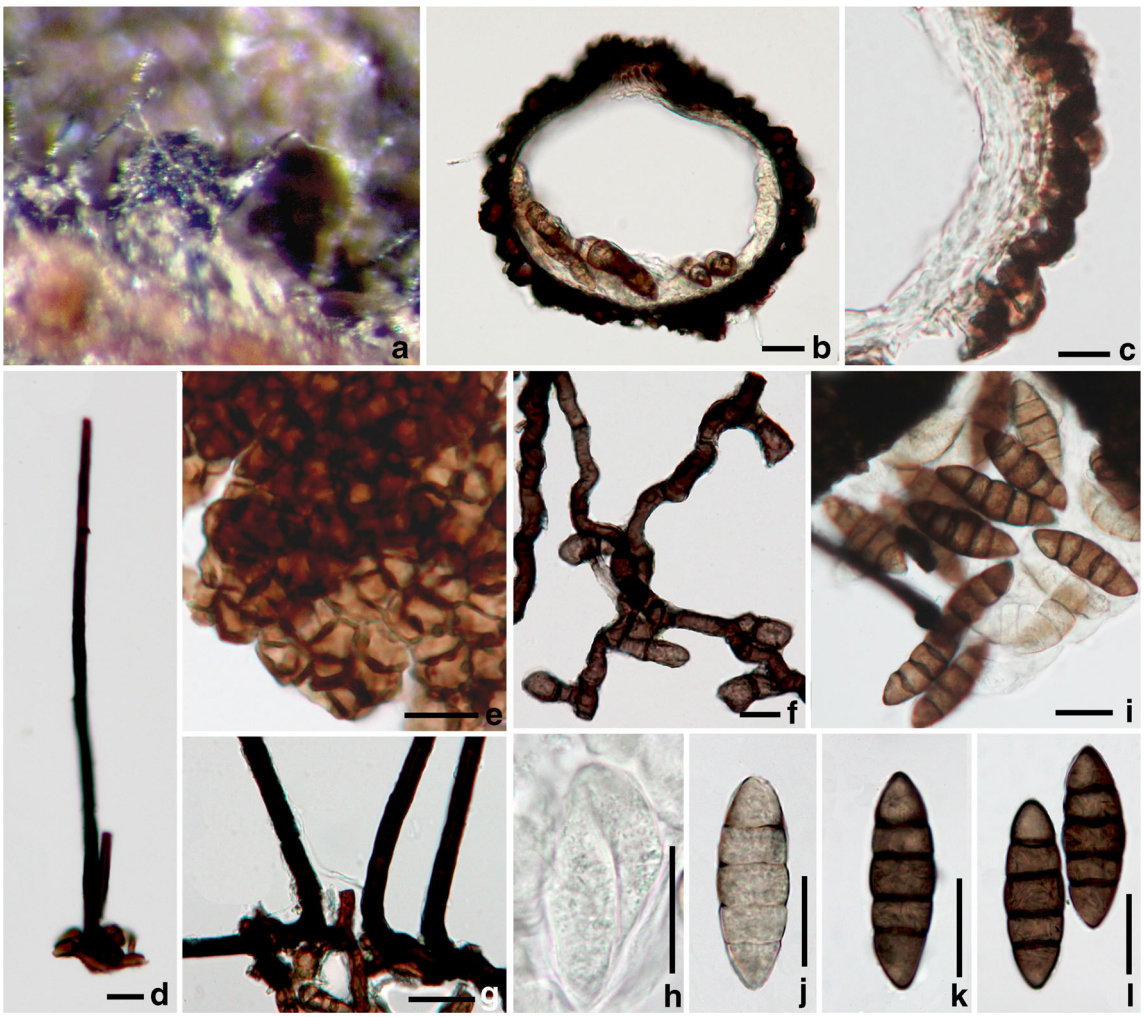


Fig. 25 *Meliola psidiicola* (holotype). **a** Ascoma on substrate. **b** Section through ascoma. **c** Section through peridium. **d** Hyphal seta. **e** Wall of ascoma in a squash mount. **f** Hyphae with capitate hyphopodia. **g** Hyphal setae on superficial hyphae. **h** Ascus when immature. **i–l** Ascospores with 4 septa. Scale bars: **b**, **d**, **e**, **g–l**=20 μm, **c**, **f**=10 μm

Type species: ***Ceratospermopsis xylopiae*** Bat., Mycopath. Mycol. appl. 5: 166 (1951)

Ectendomeliola Hosag. & D.K. Agarwal, Indian Phytopath. 59(1): 99 (2006)

≡ *Ectendomeliola walsurae* Hosag. & D.K. Agarwal, Indian Phytopath. 59(1): 99 (2006)

Notes: *Ectendomeliola* was established by Hosagoudar and Agarwal (2006) with the type species *E. walsurae*. This genus is characterized by 2-celled, ovate to subcylindrical hyphopodia on the hyphae, straight to curly hyphal setae, acute or rounded at the apex, and oblong to cylindrical ascospores, with 4 septa, and constricted at the septa (Figs. 26 and 27). *Ectendomeliola* is most typical of *Meliola* based on its hyphal setae, but differs in having hyphopodia immersed in host epidermal cells. This genus is also similar to *Endomeliola*, however the hyphopodia form in the epidermal layer, while hyphopodia extend into the mesophyll layer in *Endomeliola*. There are two species placed in the genus, *E. walsurae* from *Walsura trifolia* (A. Juss.) Harms. and *E. otonephelii* from *Otonephelium stipulaceum* (Bedd.) Radlk. (the latter species has longer hyphal setae than *E. walsurae*).

Haraea Sacc. & P. Syd., in Saccardo, Annls mycol. 11(3): 312 (1913)

Facesoffunginumber: FoF00731

Epiphytes on the surface of leaves, stems, and straw. *Superficial hyphae* branched, septate, aggregated, hyphopodia and hyphal setae lacking. **Sexual morph**: *Ascomata* superficial on surface of hosts, gregarious or solitary, globose to subglobose, flattened when immature, membranous to subcarbonaceous, brown to dark brown, and with aggregated hyphae at the margin, ascomatal setae and appendages absent. *Peridium* comprising dark brown of radially arranged cells when immature, with two strata, outer stratum of dark brown cells of *textura angularis*, and inner stratum of hyaline, flattened cells. *Hamathecium* with paraphyses. *Asci* 4–8-spored, rounded at the apex, with thick pedicel (Saccardo 1913). *Ascospores* 2–4-seriate, pale brown to brown, or reddish, oblong to fusoid, 3-septate, slightly constricted at the septa, smooth-walled. **Asexual morph**: Undetermined.

Notes: The genus *Haraea* was established by Saccardo (1913) with type species *H. japonica* Sacc. & P. Syd. It is a good genus but its placement is doubtful because hyphopodia are lacking, and asci were not illustrated. Hence, *Haraea* should be place as a doubtful genus until molecular data are available to verify the placement of this genus. Five species are listed in Index Fungorum (2015).

Type species: ***Haraea japonica*** Sacc. & P. Syd., Annls mycol. 11(3): 312 (1913)

Faces of fungi number: FoF00730

Epiphytes on the surface of leaves, stems, and straw. *Superficial hyphae* branched, septate, aggregate, hyphopodia and hyphal setae lacking. **Sexual morph**: *Ascomata* 100–140 μm diam. × 85–100 μm high ($\overline{x}$ = 135 × 95 μm, n=5), superficial on surface of hosts, gregarious or solitary, globose to subglobose, flattened when immature, membranous to subcarbonaceous, brown to dark brown, and aggregated hyphae at the margin, ascomatal setae and appendages absent. *Peridium* 25–34 μm diam. ($\overline{x}$ = 31 μm, n=5), comprising dark brown radially arranged cells when immature, with two strata, outer stratum of thick-walled, dark brown cells of *textura angularis*,

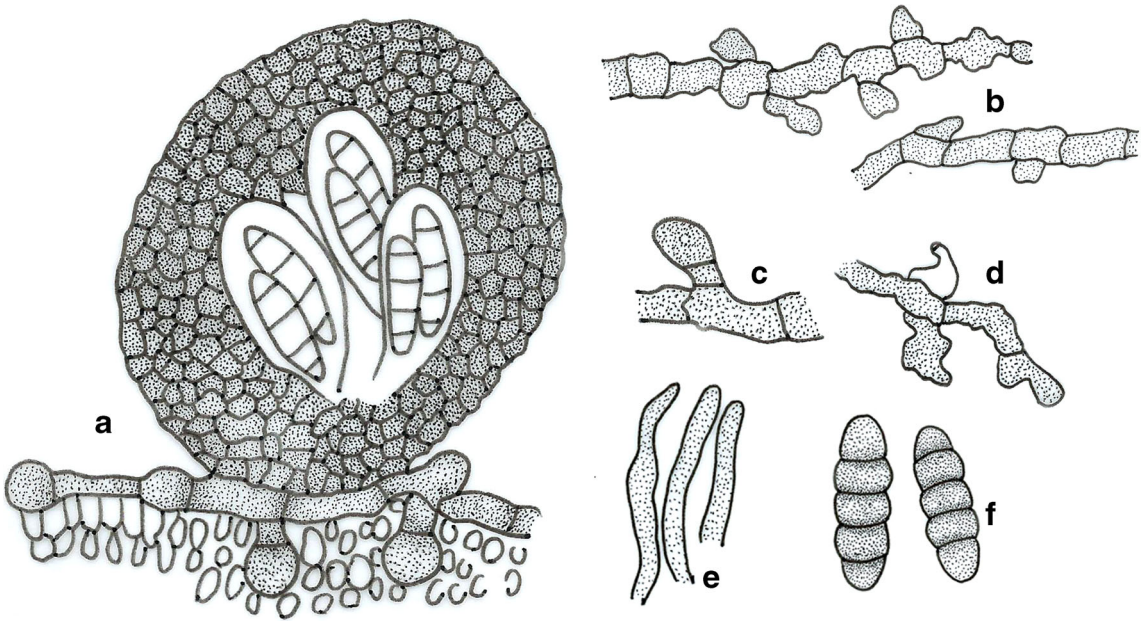


Fig. 26 *Ectendomeliola walsurae* (holotype, redrawn from Hosagoudar and Agarwal 2006). **a** Ascoma on the surface of host (notes: not sectioned and only showing ascoma shape and asci arrangement). **b** Septate hyphae. **c** Capitate hyphopodia on hyphae. **d** Phialides on hyphae. **e** Apex of the hyphal setae. **f** Ascospores with 4 septa

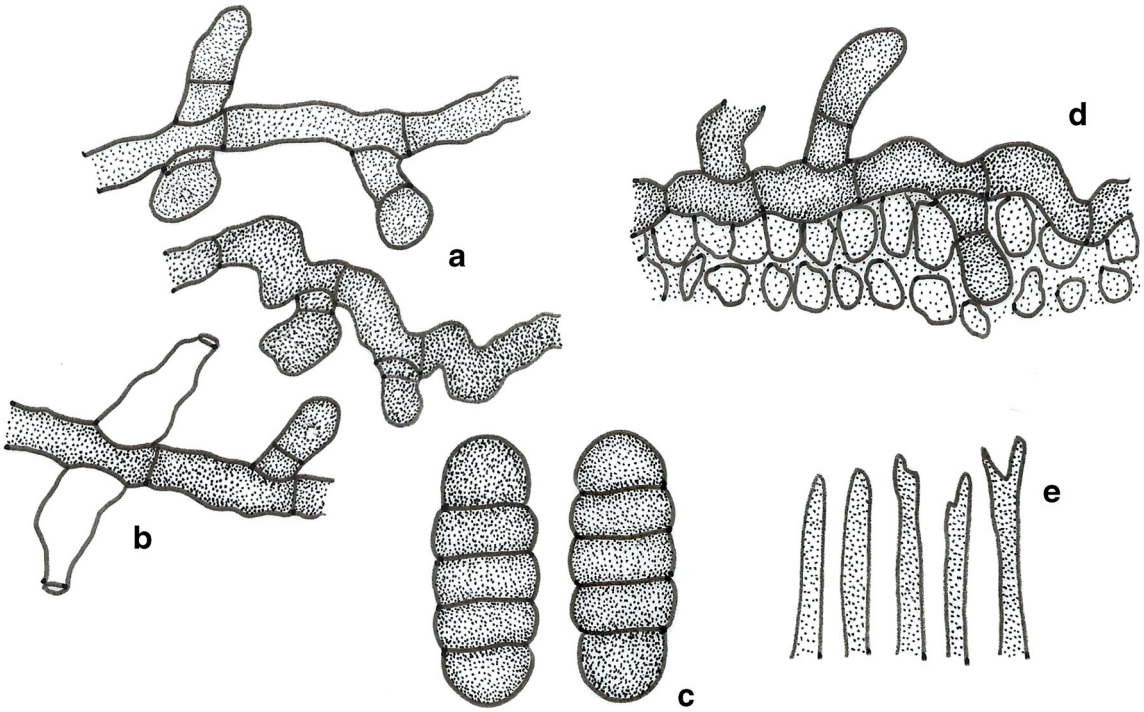


Fig. 27 *Ectendomeliola otonephelii* (holotype, redrawn from Hosagoudar and Archana 2013). **a** Capitate hyphopodia on hyphae. **b** Phialides on hyphae. **c** Ascospores with 4 septa. **d** Hyphopodia immersed in host epidermal cells. **e** Apex of the hyphal setae

and inner stratum of hyaline, flattened cells. *Hamathecium* with paraphyses. *Asci* 4–8-spored, rounded at the apex, with thick pedicel (Saccardo 1913). *Ascospores* 39–40×12–14 μm ($\bar{x} = 39 \times 14\ \mu m$, n=5), 2–4-seriate, pale brown to brown, somewhat reddish, oblong to fusoid, 3-septate, slightly constricted at the septa, smooth-walled. **Asexual morph**: Undetermined.

Material examined JAPAN, Prov. Mino, Kawauye-mura, on straw of *Sasa paniculata* (*Poaceae*), January 1913, K. Hara 97 (F10662, **holotype**).

Note: The holotype specimen of *Haraea japonica* is not in good condition. In this study, we could not find the characters to illustrate the asci and hamathecium (Fig. 28). This genus is also typical of *Iodosphaeria* Samuels et al. based on dark brown ascomatal setae, arising from cells at the ascomata surface with a stellate arrangement (Senanayake et al. 2015). Thus, *H. japonica* may be an over mature collection of *Iodosphaeria*, however, many characters were not seen in the type specimen. Hence, a new collection with sequence data are needed to illustrate its morphology and to clarify its placement in phylogenetic tree.

Hypasteridium Speg., Boln Acad. nac. Cienc. Córdoba 26(2-4): 349 (1921)

Notes: The genus was introduced with *Metasteridium* Speg. and *Parasteridium* Speg by Spegazzini (1921). However, no species name was mentioned, and the publication was noted as uncertain by Eriksson and Hawksworth (1988). Thus, we treat the three genera as doubtful until the original publication or herbarium specimens can be examined and emended.

Leptascospora Speg., Physis, Rev. Soc. Arg. Cienc. Nat. 4(no. 17): 284 (1918)

Facesoffunginumber: FoF00735

Epiphytes on the surface of leaves. *Superficial hyphae* branched at the base of ascomata. **Sexual morph**: *Ascomata* superficial, globose to subglobose, flattened above, with colorless appendages. *Hamathecium* with paraphyses. *Asci* 8-spored, cylindrical, rounded at the apex, narrowed at the base. *Ascospores* linear, multiseptate, rounded ends, hyaline, smooth-walled (Raciborski 1909). **Asexual morph**: Undetermined.

Notes: The monotypic genus *Leptascospora* was introduced by Spegazzini (1918), with the type species *L. uredinis*.

Type species: ***Leptascospora uredinis*** (Racib.) Speg., Physis, Rev. Soc. Arg. Cienc. Nat. 4(no. 17): 284 (1918)

Material examined INDONESIA, Java Bogor, on the lower surface of leaves of *Mucuna* sp. (*Fabaceae*), M. Raciborski (ZT Myc 24176, **holotype**).

Notes: *Leptascospora uredinis* was predated as *Hyaloderma uredinis* Racib. We re-examined the holotype material and it is in poor condition, thus, we treat

Fig. 28 *Haraea japonica* (holotype). **a**, **b** Herbarium packet and specimen. **c** Ascomata on host substrate. **d**, **e** Section through ascomata. **f** Hyphae at the base of ascomata. **g** Upper wall of ascoma. **h** Hyphae with septa. **i**–**k** Conidia. Scale bars: **e**=500 μm, **f**–**g**=100 μm, **h**–**m**=10 μm

Leptascospora as doubtful until it can be recollected and sequenced.

Metasteridium Speg.

Notes: *see* under *Hypasteridium* Speg.

Ophiociliomyces Bat. & I.H. Lima, Anais Soc. Biol. Pernambuco 13(2): 29 (1955)

Facesoffunginumber: FoF00743

Epiphytes or *saprobes* on leaves of *Bauhinia*. *Superficial hyphae* and *hyphopodia* lacking. **Sexual morph**: *Ascomata* superficial on the surface of leaves, solitary, globose, collapsing when dry. *Peridium* dark brown, comprising interwoven cells when viewed in squash mounts, darker at rim, with two strata, outer stratum of thick-walled, dark brown cells, and inner stratum of hyaline to pale brown cells. *Asci* 8-spored, bitunicate, clavate to ovoid, or subglobose, apedicellate, apically rounded, without ocular chamber. *Ascospores* fasciculate, hyaline to greyish, fusiform-clavate, 10–13-septate, not constricted at septa, straight to slightly curved, smooth-walled. **Asexual morph**: Undetermined.

Notes: *Ophiociliomyces bauhiniae* is the type species of *Ophiociliomyces* and has different morphology from the members of *Meliolaceae*. Thus, this genus is placed as doubtful. Four species are listed in Index Fungorum.

Type species: ***Ophiociliomyces bauhiniae*** Bat. & I.H. Lima, Anais Soc. Biol. Pernambuco 13(2): 30 (1955)

Facesoffunginumber: FoF00742

Epiphytes or *saprobic* on leaves of *Bauhinia raddiana*. *Superficial hyphae* and *hyphopodia* lacking. **Sexual morph**: *Ascomata* 185–240 μm diam. ($\bar{x}$ = 211 μm, n=5), superficial on the surface of leaves, solitary, globose, collapsing when dry. *Peridium* 18–27 μm, dark brown, comprising interwoven cells when viewed in squash mounts, darker at rim, with two strata, outer stratum of thick-walled, dark brown cells, and inner stratum of hyaline to pale brown cells. *Hamathecium* lacking pseudoparaphyses. *Asci* 46–67.5 × 13–32 μm ($\bar{x}$ = 55.5 × 21 μm n=10), 8-spored, bitunicate, clavate to ovoid, or subglobose, apedicellate, apically rounded, without ocular chamber. *Ascospores* 52–77 × 5–9.5 μm ($\bar{x}$ = 63.5 × 7 μm, n=20), fasciculate, hyaline to greyish,

fusiform-clavate, 10–13-septate, not constricted at septa, straight to slightly curved, smooth-walled. **Asexual morph**: Undetermined.

Material examined BRAZIL, Pernambuco; Recife, Dois Irmãos., on leaves of *Bauhinia raddiana* Bong. (*Fabaceae*), 5 February 1955, Osvaldo Soares da Silva, (URM 1235, **holotype**).

Notes: The type specimen was re-examined and lacked superficial hyphae, had bitunicate asci and multi-septate, hyaline ascospores (Fig. 29). We therefore treat this genus as doubtful within *Meliolales*.

Ophioirenina Sawada & W. Yamam., in Imazeki et al., Special Publication College of Agriculture, National Taiwan University 8: 35 (1959)

Facesoffunginumber: FoF00745

Epiphytes on the surface of leaves. *Superficial hyphae* branched, septate, with 1-celled hyphopodia. **Sexual morph**: *Ascomata* superficial on surface of leaves, solitary or gregarious, globose to subglobose, glabrous, dark brown to black, central ostiole at maturity. *Peridium* thick, comprising two strata, outer stratum of thick-walled, brown to dark brown cells of *textura angularis* to *globulosa*, inner stratum of hyaline to pale brown, scleroparenchymatous cells of *textura angularis* to *globulosa*. *Hamathecium* aparaphysate. *Asci* 8-spored, unitunicate, clavate, short pedicellate or sometimes apedicellate, ocular chamber absent. *Ascospores* fasciculate, hyaline to brown, clavate to narrowly fusiform, 4–6-septate, not constricted at septa, slightly darker at septa at maturity, smooth-walled. **Asexual morph**: Undetermined.

Notes: *Ophioirenina* was introduced and placed in *Meliolaceae* by Sawada and Yamamoto (in Sawada 1959) based on its superficial hyphae with hyphopodia, and globose ascomata developing in black colonies (Lumbsch and Huhndorf 2010; Patil and Mahamulkar 1999). *Ophioirenina* differs from *Meliolales* species in having 1-celled hyphopodia on the hyphae, papilla with a narrow pore or opening via a rupture, a peridial wall comprising two strata of scleroparenchymatous cells of *textura angularis* and hyaline ascospores. The genus is most typical of *Leptosphaeriaceae* species because of its scleroplectenchyma cells. We treat *Ophioirenina* as *Dothideomycetes* genera, *incertae sedis* until molecular data available.

Type species: ***Ophioirenina theae*** Sawada & W. Yamam., Special Publication College of Agriculture, National Taiwan University 8: 36 (1959)

Facesoffunginumber: FoF00744

Epiphytes on the surface of leaves. *Superficial hyphae* branched, septate, with 1-celled hyphopodia. **Sexual morph**: *Ascomata* 170–220 μm diam. ($\bar{x}$ = 197 μm, n=5), superficial on surface of leaves, solitary or gregarious, globose to subglobose, glabrous, dark brown to black, central ostiole at maturity. *Peridium* 43–52 μm ($\bar{x}$ = 46 μm, n=5), thick, comprising two strata, outer stratum of thick-walled, brown to dark brown cells of *textura angularis* to *globulosa*, inner stratum of hyaline to pale brown, scleroparenchymatous cells of *textura angularis* to *globulosa*. *Asci* 78–81 × 18–20 μm ($\bar{x}$ = 80 × 18 μm, n=10), 8-spored, unitunicate, clavate, short pedicellate or sometimes apedicellate, ocular chamber absent. *Ascospores* 50–62 × 5–6 μm ($\bar{x}$ = 58 × 5 μm, n=10), fasciculate, hyaline to brown, clavate to narrowly fusiform, 4–6-septate, not constricted at the septa, slightly darker at each septum at maturity, smooth-walled. **Asexual morph**: Undetermined.

Material examined TAIWAN, Shin-Chu, Formosa, on leave of *Camellia sinensis* (L.) Kuntze (*Theaceae*), 11 November 1925, K. Sawada (BPI 698821)

Notes: We found ascomata developing in the black colonies which are quite similar to *Meliolales* species, but differ based on their 1-celled hyphopodia, ascoma wall, and ascospore septation (Fig. 30).

Ophiomeliola Starbäck, Bih. K. svenska VetenskAkad. Handl., Afd. 3 25(no. 1): 22 (1899)

Facesoffunginumber: FoF00747

Epiphytes or *pathogens* on surface of living leaves. *Superficial hyphae* septate, dark brown, without hyphopodia. **Sexual morph**: *Ascomata* superficial on surface of leaves, solitary, subglobose to ovoid, covered with aerial hyphae, with apical, papillate ostiole. *Peridium* comprising two strata, outer stratum of thick-walled, dark brown to black cells of *textura globulosa*, and inner stratum of hyaline to pale brown cells, scleroparenchymatous cells of *textura globulosa* to *angularis*. *Hamathecium* comprising filiform paraphyses embedded in a gelatinous matrix. *Asci* 8-spored, unitunicate, clavate to cylindrical, with long pedicel. *Ascospores* fasciculate, hyaline, cylindrical to narrowly fusiform, 7-septate, not constricted at septa, slightly curved, tapering toward the ends, smooth-walled. **Asexual morph**: Undetermined.

Notes: *Ophiomeliola* was introduced by Starbäck (1899), with the type species *Ophiomeliola lindmanii*. Four species are listed for this genus in Index Fungorum and all lack molecular data. This genus cannot be placed in *Meliolaceae* based on its fusiform, 7-septate, hyaline ascospores, but it may belong to *Lasiosphaeriaceae* based on its ascomata wall. However, we treat this as *Sordariomycetes* genus *incertae sedis* until molecular data becomes available.

Type species: ***Ophiomeliola lindmanii*** Starbäck, Bih. K. svenska VetenskAkad. Handl., Afd. 3 25(no. 1): 22 (1899)

Facesoffunginumber: FoF00746

Epiphytes or *pathogens* on surface of living leaves. *Superficial hyphae* septate, dark brown, without hyphopodia. **Sexual morph**: *Ascomata* 310–345 μm high × 270–345 μm diam. ($\bar{x}$ = 334 × 295 μm, n=3), superficial on surface of leaves, solitary, subglobose to ovoid, covered with aerial

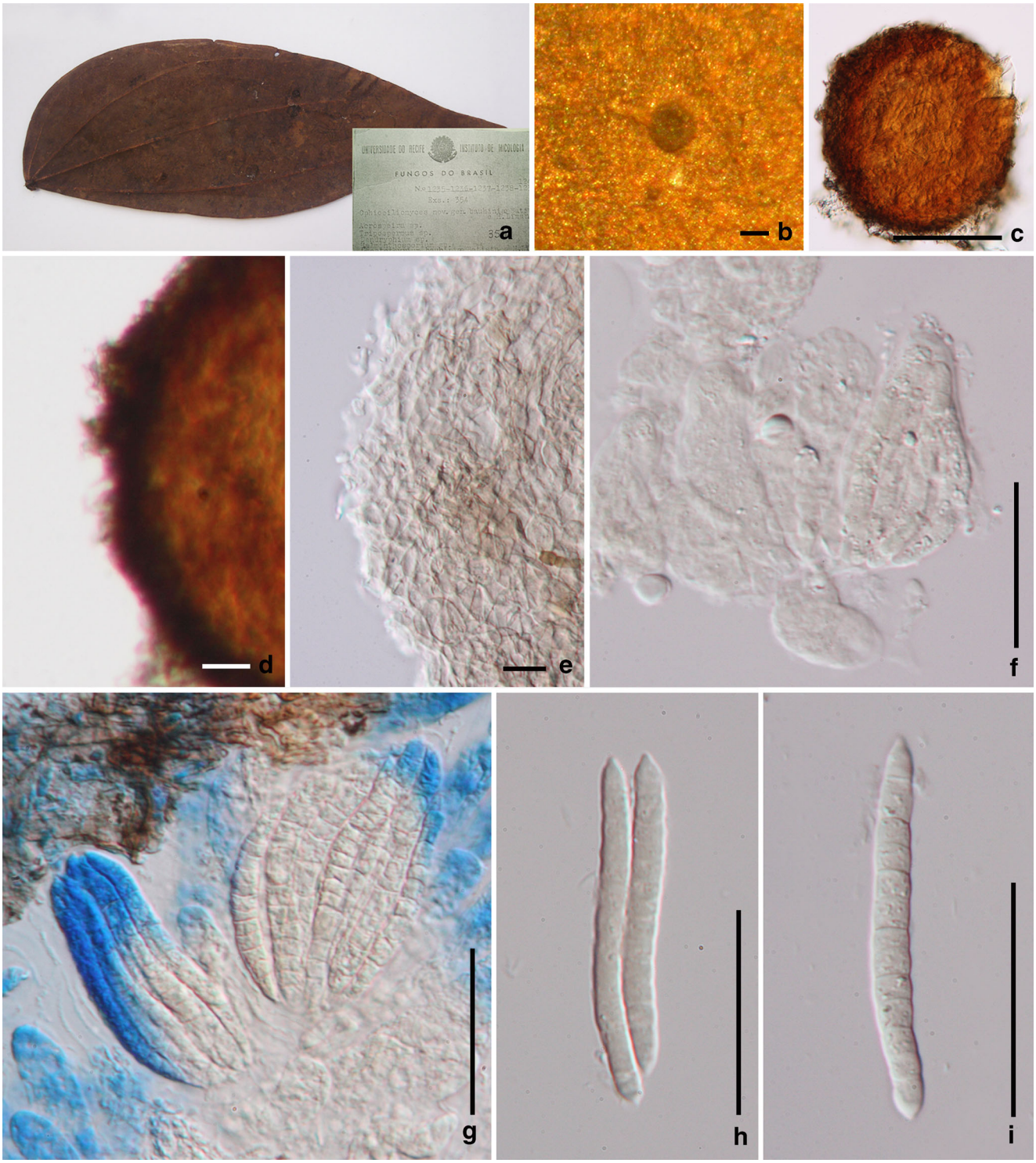


Fig. 29 *Ophiociliomyces bauhiniae* (holotype). **a** Herbarium material and specimen. **b** Ascoma on substrate. **c** Squash mount of ascoma. **d**, **e** Peridium and ascoma wall. **f**, **g** Asci at young and mature stages (**g** mounted in cotton blue reagent). **h**, **i** Ascospores. Scale bars: **b**, **c** =100 μm, **d**=20 μm, **e**=10 μm, **f**–**i**=40 μm

hyphae, with apical, papillate ostiole. *Peridium* 35–40 μm, comprising two strata, outer stratum of dark brown to black cells of *textura globulosa*, and inner stratum of hyaline to pale brown, scleroparenchymatous cells of *textura globulosa* to *angularis*. *Hamathecium* comprising filiform paraphyses embedded in a gelatinous matrix. *Asci* 121–141×17.5–20 μm ($\overline{x}$ = 135 × 19 μm, n=15), 8-spored, unitunicate, clavate to cylindrical, with long pedicel. *Ascospores* 88–125×3–6 μm

Fig. 30 *Ophioirenina theae* (holotype). **a**, **b** Herbarium packet and specimen. **c**, **d** Ascomata on substrate. **e** Section through ascomata. **f** Peridium and ascoma wall. **g** Ascoma when immature. **h** Hyphae with 1-celled appressoria. **i**, **j** Asci with 8-spores. **k**, **l** Ascospores. Scale bars: **e**=50 μm, **f**–**l**=20 μm

($\bar{x}$ = 109 × 5 μm, n=20), fasciculate, hyaline, cylindrical to narrowly fusiform, 7-septate, not constricted at septa, slightly curved, tapering toward the ends, smooth-walled. **Asexual morph**: Undetermined.

Material examined BRAZIL, Rio Grande do Sul, Hamburgerberg pr Porto Alegre, on leaves of *Myrtacea cujusdam* (*Myrtaceae*), September 1892, C.A.M. Lindman No. 37 (SF10033, **holotype**).

Notes: *Ophiomeliola lindmanii* is typical of species in *Lasiosphaeriaceae* in having aerial hyphae, a peridium comprising two strata, with the outer strata of dark brown to black cells of *textura globulosa* to *angularis*, unitunicate asci, and hyaline, multi-septate ascospores (Fig. 31). Molecular data is needed to clarify the placement.

Parasteridium Speg., Boln Acad. nac. Cienc. Córdoba 26(2-4): 349 (1921)

Notes: *see* under *Hypasteridium* Speg.

Pauahia F. Stevens, Bulletin of the Bernice P. Bishop Museum, Honolulu, Hawaii 19: 17 (1925)

Facesoffunginumber: FoF00749

Epiphytes on the surface of *Sideroxylon*. *Superficial hyphae* and *hyphopodia* absent. **Sexual morph**: *Ascostromata* superficial or erumpent at the base, pseudostromata, solitary, subglobose or irregular, thick-walled. *Locules* immersed in ascostromata, 1–multi-loculate. *Peridium of locules* comprising two strata, outer stratum of dark brown cells of *textura angularis*, and inner stratum of hyaline to pale brown, flattened cells. *Hamathecium* with paraphyses. *Asci* 2-spored, bitunicate, broadly clavate to oval, with short pedicel, evanescent. *Ascospores* 2-seriate, hyaline to brown, oblong to cylindrical, 3-septate, constricted and darker at the septa, terminal cells markedly smaller and slightly lighter than the others, thin sheath present at maturity, smooth-walled. **Asexual morph**: Undetermined.

Notes: The genus *Pauahia* was established within the family *Dothideaceae* in the section *Leveillelleae* by Stevens (1925). *Pauahia* is monotypic with *Pauahia sideroxyli* as its type species. We re-examined the type specimen of *P. sideroxyli* and found that the species differs from other genera in *Meliolaceae* in having pseudostromata, and lacking superficial hyphae.

Type species: ***Pauahia sideroxyli*** F. Stevens, Bulletin of the Bernice P. Bishop Museum, Honolulu, Hawaii 19: 17 (1925)

Facesoffunginumber: FoF00748

Epiphytes on the surface of *Sideroxylon*. *Superficial hyphae* and *hyphopodia* absent. **Sexual morph**: *Ascostromata* 700–1200 μm diam. × 353–410 μm high ($\bar{x}$ = 1000 × 365 μm, n=2), superficial or erumpent at the base, solitary, pseudostromata, subglobose or irregular, thick-walled. *Locules* 200–235 × 165–219 μm ($\bar{x}$ = 208 × 179 μm, n=5), immersed in ascostromata, 1–multi-loculate. *Peridium of locules* comprising two strata, outer stratum of dark brown cells of *textura angularis*, and inner stratum of hyaline to pale brown, flattened cells. *Hamathecium* with paraphyses. *Asci* 46–82 × 25–45 μm ($\bar{x}$ = 70 × 36 μm, n=5), 2-spored, bitunicate, broadly clavate to oval, with short pedicel, evanescent. *Ascospores* 20–23 × 62–77 μm ($\bar{x}$ = 22 × 65 μm, n=5), 2-seriate, hyaline to brown, oblong to cylindrical, 3-septate, constricted and darker at the septa, terminal cells markedly smaller and slightly lighter than the others, thin sheath present at maturity, smooth-walled. **Asexual morph**: Undetermined.

Notes: *Pauahia sideroxyli* is a single species in the genus *Pauahia*. We re-examined the type specimen which is very small and in poor condition, and found that *Pauahia sideroxyli* differs from *Meliolales* species in having a large pseudostromata, erumpent in host epidermal cells, and bitunicate asci (Fig. 32). Molecular data is lacking, so we cannot clarify the placement of this genus. Therefore, *Pauahia sideroxyli* is placed as *Dothideomycetes* genera, *incertae sedis* until it can be recollected and sequenced.

Material examined USA, Hawaii, Maui, Nahiku, on *Planchonella sandwicensis* (A.Gray) Pierre (*Sapotaceae*), January 1909, Lyon H. L. no. 61 (ILL00011484, **holotype**; BISH 499038, **isotype**).

Pleomeliola (Sacc.) Sacc., Syll. fung. (Abellini) 14(1): 17 (1899)

≡ *Meliola* subgen. *Pleomeliola* Sacc., Syll. fung. (Abellini) 1: 70 (1882)

Notes: *Pleomeliola* contains three species which are listed in Index Fungorum (2015) with the type species as *Pleomeliola fenestrata* (Cooke & Ellis) Sacc. The genus is characterized by subglobose, membranacous, reddish brown ascomata, 8-spored, ovoid asci, and cylindrical or clavate, muriform, 4–5-septate, hyaline to brown ascospores. We could not found any ascomata or hyphae on the holotype specimen. Hansford examined this specimen in 1952, and noted on the packet "This specimen is not *Meliola*" (Fig. 33) . We also exclude this genus from *Meliolales*. There is no molecular data available for this genus.

Type species: ***Pleomeliola fenestrata*** (Cooke & Ellis) Sacc., Syll. fung. (Abellini) 14(1): 17 (1899)

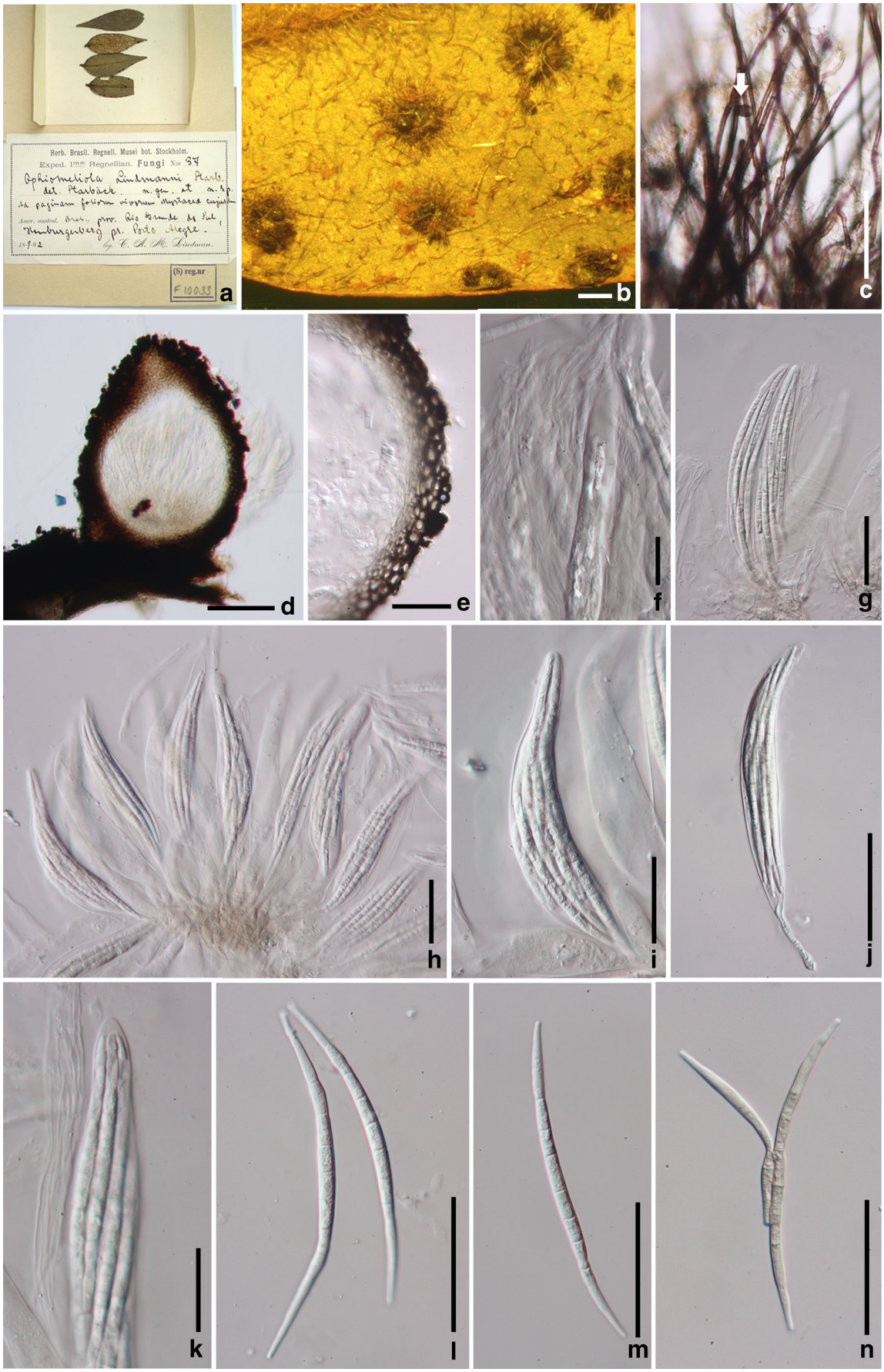
Material examined USA, New Jersey, on scales of pine cones (*Pinaceae*), MC. Cooke 2465 ex herb. (K(M) 193124, **holotype**).

Pleomerium Speg., Physis, Rev. Soc. Arg. Cienc. Nat. 4(no. 17): 284 (1918)

Facesoffunginumber: FoF00751

Epiphytes on the lower surface of leaves. *Superficial hyphae* branched, septate, darker at each septum, brown, lacking hyphopodia and hyphal setae. **Sexual morph**: *Ascomata* superficial, solitary on superficial hyphae, easily removed, subglobose to globose, surrounded and covered by hyphae,

Fig. 31 *Ophiomeliola lindmanii* (holotype). **a** Herbarium packet and material. **b**. Ascomata on substrate. **c** Aerial hyphae. **d** Vertical section of ascoma. **e** Peridium. **f** Paraphyses. **g**–**k** Unitunicate asci. **i**–**n** Ascospores. Scale bars: **b**=1 mm, **c**, **d**, **g**–**n**=50 μm, **e**=40 μm, **f**=20 μm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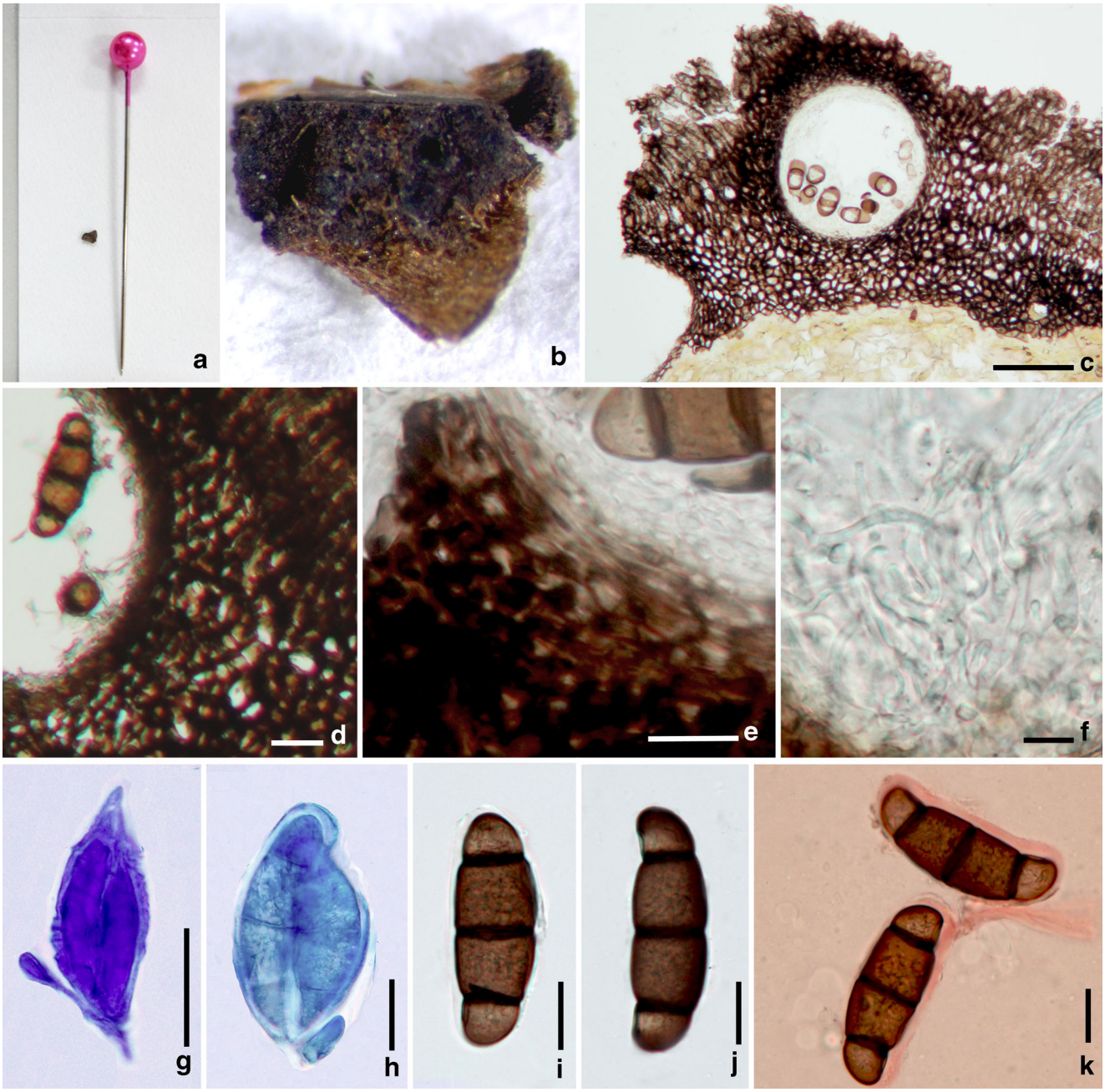


Fig. 32 *Pauahia sideroxyli* (holotype). **a** Specimen. **b** Ascostromata on substrate. **c** Section through ascostromata showing locule. **d**, **e** Peridium of ascostromata. **f** Hamathecium. **g**, **h** Asci with 2-spores in cotton blue reagent. **i**, **j** Ascospores with 3 septa and lighter end cells. **k** Ascospores in congo red reagent. Scale bars: **c**=100 μm, **d**, **e**, **g**–**k**=20 μm, **f**=10 μm

thin-walled. *Peridium* thin, membranous, comprising brownish to dark brown, interwoven cells when viewed in squash mounts. *Hamathecium* lacking pseudoparaphyses. *Asci* 8-spored, bitunicate, obovoid to subglobose, short pedicel or absent, ocular chamber not well developed. *Ascospores* 3–4-seriate, hyaline to brown, oblong to fusiform, muriform, 4–8 transverse septa, 1–2 longitudinal septa, not constricted but darker at septa, surrounded and covered by mucilage, smooth-walled. **Asexual morph**: *Conidia* oblong, 1-celled (Rehm 1901).

Notes: The monotypic genus *Pleomerium* was established within the family *Englerulaceae* by Spegazzini (1918). This genus differs from *Meliolales* species in having superficial hyphae without hyphopodia, bitunicate asci, and muriform ascospores. Molecular data is not available in GenBank. Hence, we suggest that the genus should be placed as *Dothideomycetes* genera, *incertae sedis*, until sequence data can clarify its placement.

Type species: ***Pleomerium fuscoviridescens*** (Rehm) Speg., Physis, Rev. Soc. Arg. Cienc. Nat. 4(no. 17): 284 (1918)

Fig. 33 *Pleomeliola fenestrata* (holotype). **a**–**d** Herbarium packet and specimen

≡ *Limacinia fuscoviridescens* Rehm, Hedwigia 40: 168 (1901)

≡ *Naetrocymbe fuscoviridescens* (Rehm) Bat. & Cif., Saccardoa 2: 159 (1963)

Facesoffunginumber: FoF00750

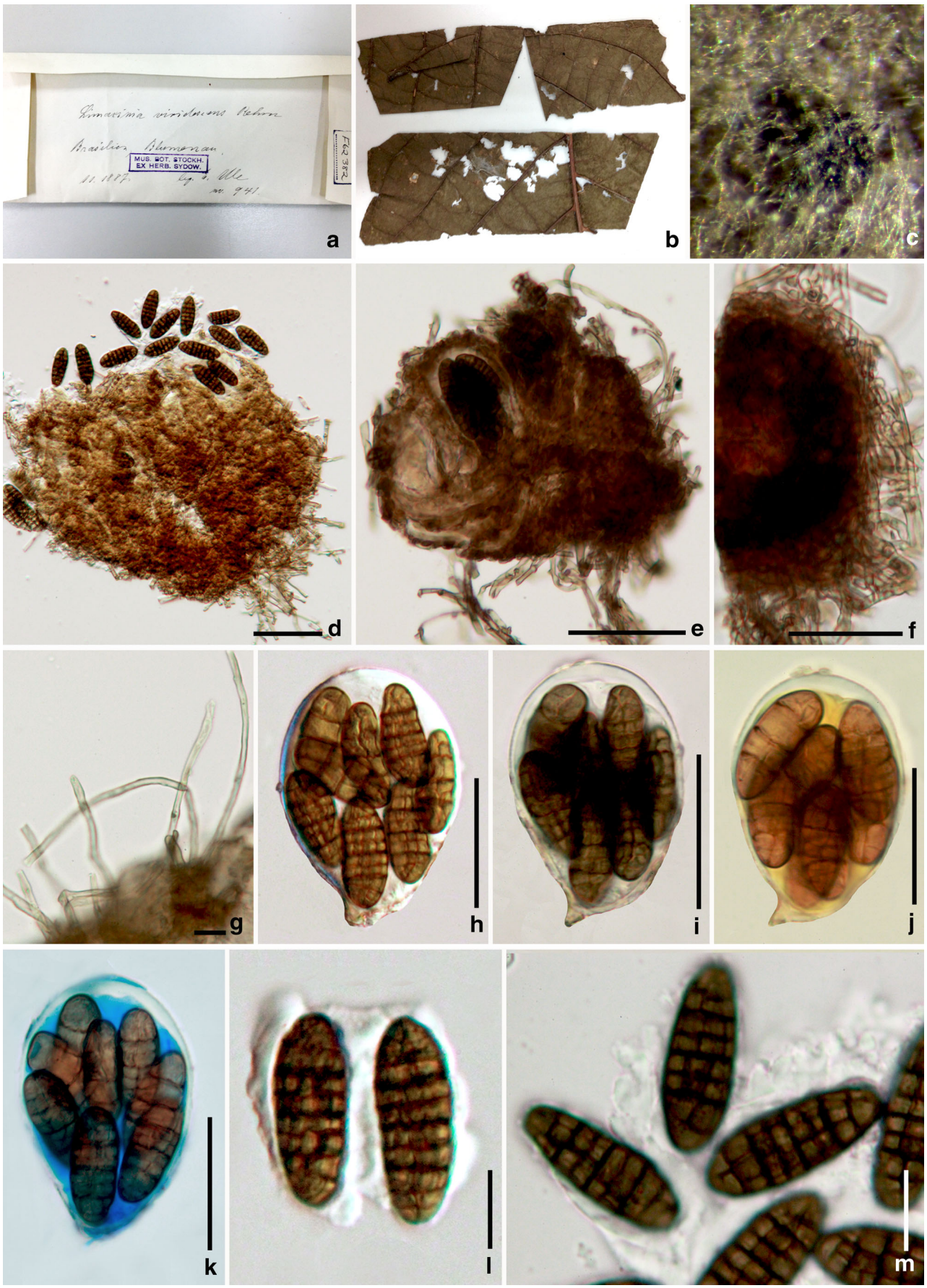
Epiphytes on the lower surface of leaves. *Superficial hyphae* branched, septate, darker at septa, brown, lacking hyphopodia and hyphal setae. **Sexual morph**: *Ascomata* 280–350 μm diam. ($\bar{x}$ = 312 μm, n=5), superficial, solitary on superficial hyphae, easily removed, subglobose to globose, surrounded and covered by hyphae, thin-walled. *Peridium* thin, membranous, comprising brownish to dark brown, interwoven cells when viewed in squash mounts. *Hamathecium* lacking pseudoparaphyses. *Asci* 84–96 × 58–63 μm ($\bar{x}$ = 87 × 60 μm, n=5), 8-spored, bitunicate, obovoid to subglobose, short pedicel or absent, ocular chamber not well-developed. *Ascospores* 44–47 × 16–17 μm ($\bar{x}$ = 45 × 16 μm, n=10), 3–4-seriate, hyaline to brown, oblong to fusiform, muriform, 4–8 transverse septa, 1–2 longitudinal septa, not constricted but darker at septa, surrounded and covered by mucilage, smooth-walled. **Asexual morph**: Undetermined.

Notes: *Pleomeliola fuscoviridescens* was introduced as *Limacinia fuscoviridescens* by Rehm (1901). We re-examined the holotype specimen of *P. fuscoviridescens* and found that it differs from other species in *Meliolales* (Fig. 34) This genus may belong in *Englerulaceae* because of its membranous ascomata, containing few asci, lack of ostiole, and dissolving at centre to release bitunicate asci. However, molecular data is needed to clarify its placement.

Material examined BRAZIL, Santa Catarina, Blumenau, on leaves of undetermined host, November 1887, E. Ule 941 b (SF7084, **holotype** of *Limacinia fuscoviridescens* Rehm).

Prataprajella Hosag., Nova Hedwigia 55(1-2): 224 (1992)

Notes: *Prataprajella* was introduced by Hosagoudar (1992). The type species was found on leaves of *Turpinia malabarica*, and was deposited in HCIO, which will not loan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◀ **Fig. 34** *Pleomerium fuscoviridescens* (holotype). **a**, **b** Herbarium packet and specimen. **c** Ascoma on substrate. **d** Ascoma viewed in squash mount. **e** Section through ascoma. **f** Upper wall cells of young ascoma. **g** Mycelium. **h** Ascus with 8-spores. **i** Ascus in 70 % lactic acid. **j** Ascus in Melzer's reagent. **k** Ascus in cotton blue reagent. **l**–**m** Ascospores at maturity. Scale bars: **d**, **e**=100 μm, **f**, **h**–**k**=50 μm, **g**, **l**=20 μm

specimens outside India. Therefore, a re-drawing from Hosagoudar (2006) is provided here (Fig. 35). *Prataprajella* is characterized by superficial hyphae with globose to stellate hyphopodia, hyphal setae lacking, globose to subglobose ascomata, with raised ascomatal setae, and conical appendages, brown, 3-septate ascospores (Hosagoudar 2006). We treat *Prataprajella* as a doubtful genus until the type specimen can be re-examined, or fresh collection can be collected and sequenced.

Type species : ***Prataprajella turpiniicola*** (Hosag.) Hosag., Nova Hedwigia 55(1-2): 225(1992)

Ticomyces Toro, J. Agric. Univ. Puerto Rico 36: 48 (1952)

Possible synonyms

= *Tonduzia* F. Stevens, Illinois Biol. Monogr. (Urbana) 11(2): 16 (1927)

= *Dontuzia* L.D. Gómez, Brenesia 2: 21 (1973)

Notes: *Ticomyces* was predated as *Tonduzia* Stevens in the family *Perisporiaceae* (Stevens 1927), with the type species *T. psychotriae* Stevens. Toro (1952) introduced *Ticomyces* in *Meliolaceae* (Kirk et al. 2008). However, the genus differs from other species in *Meliolales* in having superficial aseptate hyphae without hyphopodia. Ascospores are hyaline with several septa (Batista 1951). There is no molecular data available for this genus, thus we treat *Ticomyces* in *Sordariomycetes* genera, *incertae sedis*.

Type species: ***Ticomyces psychotriae*** (F. Stevens) Toro, J. Agric. Univ. P. Rico 36: 49 (1952)

Tonduzia psychotriae F. Stevens, Illinois Biol. Monogr. (Urbana) 11(2): 168 (1927)

Dontuzia psychotriae (F. Stevens) L.D. Gómez, Brenesia 2: 21 (1973)

Material examined COSTA RICA, Columbiana, on *Psychotria brachiate* Sw. (*Rubiaceae*), 19 July 1923, F.L. Stevens 570 (ILL00011485, glass slide from **holotype**).

Notes: We re-examined the type specimen of *Ticomyces psychotriae* but could not see asci and ascospores (Fig. 36). Therefore, new fresh collection and sequence data are needed to clarify placement of the genus.

Urupe Viégas, Bragantia 4(1-6): 125 (1944)

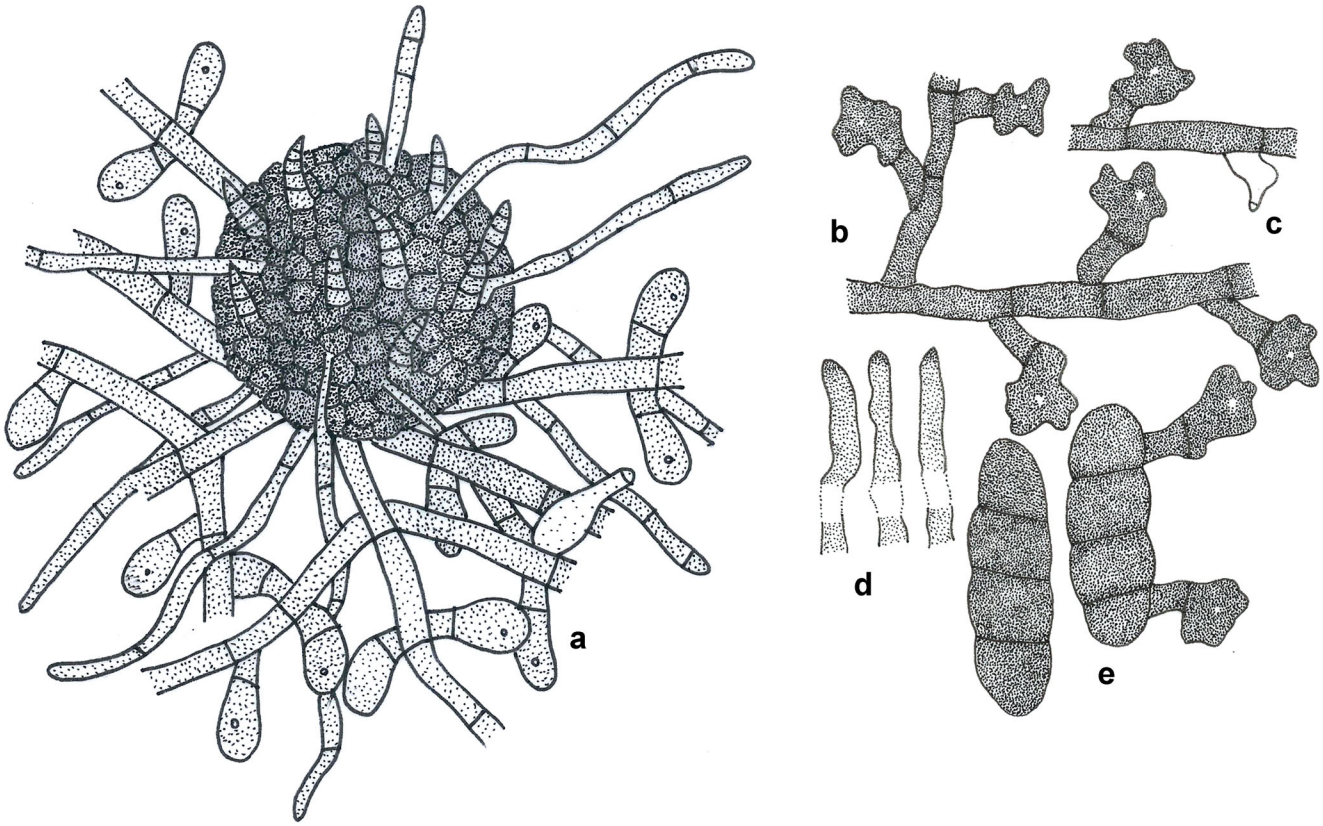

Fig. 35 *Prataprajella* sp. (**a** redrawn from Hosagoudar 2006), ***Prataprajella turpiniicola*** (**b**–**e** redrawn from Hosagoudar and Riju 2013). **a** Ascoma with appendages and setae surrounded by septate hyphae with capitate hyphopodia and phialides. **b** Stellate hyphopodia on hyphae. **c** Stellate hyphopodia mixed with phialides on hyphae. **d** Setae with rounded ends. **e** Ascospores with 3 septa and germinated ascospore

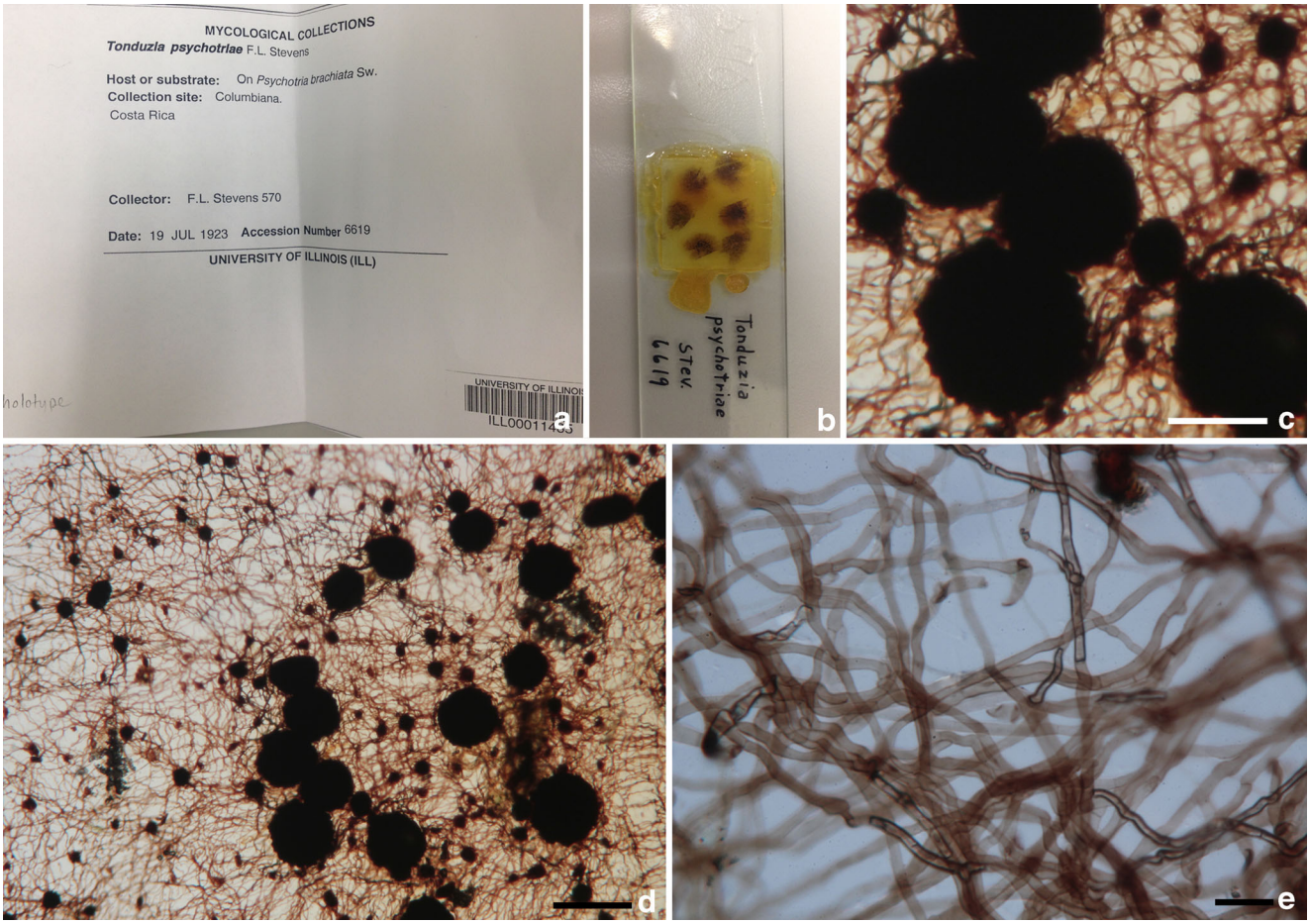

Fig. 36 *Ticomyces psychotriae* (holotype). **a** Herbarium packet. **b** Glass slide. **c**, **d** Ascomata developed on hyphae. **e** Hyphae without hyphopodia

Notes: *Urupe* was introduced by Viégas (1944) and is a monotypic genus. The genus is characterized by intercellular hyphae, reddish ascomata, clavate asci with 8-spores, and hyaline, 3-septate ascospores (Viégas 1944). We were unable to borrow the type species, therefore, we treat *Urupe* as *Sordariomycetes* genera, *incertae sedis* based on morphology as illustrated in Viégas (1944).

Type species: ***Urupe guaduae*** Viégas, Bragantia 4(1-6): 125 (1944)

Xenostigme Syd., Annls mycol. 28(5/6): 434 (1930)

Notes: *Xenostigma* was established by Sydow (1930) with the type species *X. trichophila* Syd. The genus is monotypic and differs from *Meliolales* species in having slimy ascomata at maturity, with light grey cells, asci with 8-spores, and dark brown to black, 1-septate ascospores (Sydow 1930). We were unable to locate type material of *Xenostigme trichophila*. Thus, we place *Xenostigme* as *Sordariomycetes* genera, *incertae sedis* until molecular data is available.

Type species: ***Xenostigme trichophila*** Syd., Annls mycol. 28(5/6): 434 (1930)

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References

Ainsworth GC (1963) Ainsworth and Bisby's dictionary of the fungi, 5th edn. Commonwealth Mycological Institute, Kew, p 547

Ainsworth GC, James PW, Hawksworth DL (1971) Ainsworth & bisby's dictionary of the fungi, 6th edn. CAB, Kew

Ariyawansa HA, Hawksworth DL, Hyde KD, Jones EBG, Maharachchikumbura SSN, Manamgoda DS, Thambugala KM, Udayanga D, Camporesi E, Daranagama A, Jayawardena R, Liu JK, McKenzie EHC, Phookamsak R, Senanayake IC, Shivas RG, Tian Q, Xu JC (2014) Epitypification and neotypification: guidelines with appropriate and inappropriate examples. Fungal Divers 69(1):57–91

Barr ME (1976) Perspectives in the ascomycotina. Mem New York Bot Gard 28:1–8
Barr ME, Huhndorf SM (2001) *Loculoascomycetes*. Mycota 7A:283–305
Batista AC (1951) Alguns fungos de fumagina de Pernambuco. Mycopathol Mycol Appl 5(2–3):147–172
Batista AC, da Silva MH (1960) Dois novos gêneros de fungos Meliolaceae. Atas do Inst de Micol da Univ do Recife 1:221–227
Boedijin KB (1961) Notes on the *Meliolales*. Persoonia 1(4):393–404
Cai L, Jeewon R, Hyde KD (2006) Phylogenetic investigations of *Sordariaceae* based on multiple gene sequences and morphology. Mycol Res 110:137–150
Cai L, Guo XY, Hyde KD (2008) Morphological and molecular characterization of a new anamorphic genus *Cheirosporium*, from freshwater in China. Persoonia 20:53–58
Cannon PF, Kirk PM (2007) Fungal families of the world. CABI Bioscience, Wallingford
Chomnunti P, Schoch CL, Aguirre-Hudson B, KoKo TW, Hongsanan S, Jones EBG, Kodsub R, Chukeatirote E, Bahkali AH, Hyde KD (2011) *Capnodiaceae*. Fungal Divers 51:103–134
Chomnunti P, Hongsanan S, Hudson BA, Tian Q, Peršoh D, Dhami MK, Alias AS, Xu J, Liu X, Stadler M, Hyde KD (2014) The sooty moulds. Fungal Divers 66:1–36
Cooke MC (1882) The *Perisporiaceae* of Saccardo's sylloge fungorum. Grevillea 11(57):35–38
Emmett RW, Parbery DG (1975) Appressoria. Ann Rev Phytopathol 13: 147–165
Eriksson OE (1981) The families of bitunicate ascomycetes. Nord J Bot 1(6):800–800
Eriksson OE, Hawksworth DL (1988) Notes on ascomycete systematics – nos 552–732. Syst Ascomycetum 7(1):59–101
Eriksson OE, Hawksworth DL (1993) Notes on ascomycete systematics – nos 1530–1610. Syst Ascomycetum 12(1–2):23–50
Fries E (1825) Systema orbis. Vegetabilis 2:1–373
Gaillard A (1891) Les hyphopodies myceliennes des Meliola. Bull Trimest Soc Mycol Fr 7:99–101
Gäumann E (1964) Die Pilze. Brikhäuser, Basel
Goos RD, Andersons JH (1974) The meliolaceae of Hawaii. Sydowia 26: 73–80
Goos RD, Gessner RV (1975) Hyphal modifications of *Sphaerulina pedicellata*: appressoria or hyphopodia? Mycologia 67:1035–1038
Goos RD (1974) A scanning electron microscope and in vitro study of *Meliola palmicola*. Proc Lowa Acad Sci 81:23–27
Goos RD (1978) Field and laboratory studies of meliolaceous fungi in Hawaii. Mycologia 70:996–1006
Gregory SS, John WT (1999) Phylogenetic relationships of *Meliola* and *Meliolina* inferred from nuclear small subunit rRNA sequences. Mycol Res 103(8):1049–1056
Hall TA (2004) BioEdit. Ibis Therapeutics, Carlsbad, CA, 92008, USA. (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). 18 March 2005
Hansford CG (1946) The foliicolous Ascomycetes and their parasites and associated fungi. Mycol Pap 15:1–240
Hansford CG (1961) The *Meliolaceae* a monograph. Sydowia Beih 2:1–806
Hansford CG (1963) Iconographia meliolinearum. Sydowia Beih 5:285
Hawksworth DL, Eriksson OE (1986) The names of accepted orders of ascomycetes. Syst Ascomycetum 5(1):175–184
Hawksworth DL, Sutton BC, Ainsworth GC (1983) Ainsworth and Bisby's dictionary of the fungi, 7th edn. Commonwealth Mycological Institute, Kew
Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1995) Ainsworth and Bisby's dictionary of the fungi, 8th edn. CAB International, Wallingford
Hirata K (1971) Host range and geographic distribution of *Erysiphaceae* as viewed from the families of Angiosperms, and comparison with *Meliolineae*. Sydowia 25:100–118
Hofmann TA (2009) Plant parasitic *Asterinaceae* and *Microthyriaceae* from the Neotropics (Panama). PhD thesis. 408pp. The faculty of biological sciences at the J.W.Goethe-University Frankfurt am Main, Germany
Hongsanan S, Li YM, Liu JK, Hofmann T, Piepenbring M, Bhat JD, Boonmee S, Doilom M, Singtripop C, Tian Q, Mapook A, Zeng XY, Bahkali AH, Xu JC, Mortimer PE, Wu XH, Yang JB, Hyde KD (2014) Revision of genera in asterinales. Fungal Divers 68:1–68
Hosagoudar VB (1987) *Meliolaceae* of S.India, 1. Eeon Tax Bot 11(1): 157–160
Hosagoudar VB (1992) *Prataprajella*, a new genus of the family *Meliolaceae*. Nova Hedwigia 55:223–226
Hosagoudar VB (1994) *Meliolales* of India. J Econ Taxon Bot 18(2):253–265
Hosagoudar VB (1996) *Meliolales* of India. Botanical Survey of India, Calcutta, p 363
Hosagoudar VB (2003a) *Meliolaceae* of Kerala, India– XVIII New species, varieties and records. Zoo's Print J 999–1002
Hosagoudar VB (2003b) *Armatellaceae*, a new family segregated from the *Meliolaceae*. Sydowia 55:162–167
Hosagoudar VB (2006) Additions to the fungi of Kerala II. Zoos Print J 21:2412–2416
Hosagoudar VB (2008) *Meliolales* of India. Vol. II. Botanical Survey of India, Calcutta, p 390
Hosagoudar VB (2013) *Meliolales* of India. vol. III. J Threatened Taxa 5(6):3993–4068
Hosagoudar VB, Agarwal DK (2006) *Ectendomeliola*, a new genus of the family *Meliolaceae*. Indian Phytopathol 59:98–100
Hosagoudar VB, Archana GR (2013) A new species of the genus *Ectendomeliola* (*Meliolaceae*) from Kerala, India. J Threatened Taxa 2(8):1092–1095
Hosagoudar VB, Goos RD (1989) *Meliolaceous* fungi from the state of Kerala, India I. Mycotaxon 36(1):221–247
Hosagoudar VB, Riju MC (2013) Foliicolous fungi of Silent Valley National Park, Kerala, India. J Threatened Taxa 5(3):3701–3788
Hosagoudar VB, Abraham TK, Pushpangadan P (1997) The *Meliolineae* a supplement. The Director TBG and RI, Pal ode, Thiruvananthapuram, 20
Hosagoudar VB, Dhivaharan V, Nithyatharani R (2012) Foliicolous fungi of kodaikanal in Tamil Nadu. Bull Basic Appl Plant Biol 2(1):94
Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17(8):754–755
Hughes SJ (1981) Mucronate hyphopodia of *Meliolaceae* are phialides. Can J Bot 59:1514–1517
Hughes SJ, Pirozynski KA (1994) *Endomeliola dingleyae*, a new genus and new species of *Meliolaceae*. New Zealand J Bot 32:53–59
Hyde KD, Jones EBG, Liu JK, Ariyawansa H, Boehm E, Boonmee S, Braun U, Chomnunti P, Crous PW, Dai DQ, Diederich P, Dissanayake A, Doilom M, Doveri F, Hongsanan S, Jayawardena R, Lawrey JD, Li YM, Liu YX, Lücking R, Monkai J, Muggia L, Nelsen MP, Pang KL, Phookamsak R, Senanayake I, Shearer CA, Suetrong S, Tanaka K, Thambugala KM, Wijayawardene NN, Wikee S, Wu HX, Zhang Y, Aguirre-Hudson B, Alias SA, Aptroot A, Bahkali AH, Bezerra JL, Bhat DJ, Camporesi E, Chukeatirote E, Gueidan C, Hawksworth DL, Hirayama K, Hoog SD, Kang JC, Knudsen K, Li WJ, Li XH, Liu ZY, Mapook A, McKenzie EHC, Miller AN, Mortimer PE, Phillips AJL, Raja HA, Scheuer C, Schumm F, Taylor JE, Tian Q, Tibpromma S, Wanasinghe DN, Wang Y, Xu JC, Yan JY, Yacharoen S, Zhang M (2013) Families of *Dothideomycetes*. Fungal Divers 63:1–313
Index Fungorum (2015) http://www.indexfungorum.org/Names/Names.asp
Jansen RK, Cai Z, Raubeson LA, Daniell H, dePamphilis CW, Leebens-Mack J, Müller KF, Guisinger-Bellian M, Haberle RC, Hansen AK, Chumley TW, Lee SB, Peery R, McNeal JR, Kuehl JV, Boore JL (2007) Analysis of 81 genes from 64 plastid genomes resolves

relationships in angiosperms and identifies genomes cale evolutionary patterns. PNAS 104:19369–19374
Jayasiri SC, Ariyawansa HA, Liu JK, Jones EBG, Hyde KD (2015) The faces of fungi database: fungal names linked with morphology, molecular and human attributes. Fungal Divers (In press)
Justavino DR, Piepenbring M (2007) Two new species of *Appendiculella* (*Meliolaceae*) from Panama. Mycologia 99(4):544–552
Justavino DR, Velásquez JC, Sánchez COM, Rincón R, Oberwinkler F, Bauer R (2014) The interaction apparatus of *Asteridiella callista* (*Meliolaceae*, Ascomycota). Mycologia 106(2):216–223
Justavino DR, Kirschner R, Piepenbring M (2015) New species and new records of *Meliolaceae* from Panama. Fungal Divers 70(1):73–84
Kirk PM, Cannon PF, David JC, Stalpers JA (2001) Ainsworth & Bisby's dictionary of the fungi, 9th edn. CABI, Wallingford
Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Dictionary of the fungi. 10th ed. CAB International, 784
Liu JK, Hyde KD, Jones EBG, Ariyawansa HA, Bhat DJ, Boonmee S, Maharachchikumbura SSN, McKenzie EHC, Phookamsak R, Phukhamsakda C, Shenoy BD, Abdel-Wahab MA, Buyck B, Chen J, Chethana KWT, Singtripop C, Dai DQ, Dai YC, Daranagama DA, Dissanayake AJ, Doilom M, D'souza MJ, Fan XL, Goonasekara ID, Hirayama K, Hongsanan S, Jayasiri SC, Jayawardena RS, Karunarathna SC, Li WJ, Mapook A, Norphanphoun C, Pang KL, Perera RH, Peršoh D, Pinruan U, Senanayake IC, Somrithipol S, Suetrong S, Tanaka K, Thambugala KM, Tian Q, Tibpromma S, Udayanga D, Wijayawardene NN, Wanasinghe D, Wisitrassameewong K, Zeng XY, Abdel-Aziz FA, Adamčík S, Bahkali AH, Boonyuen N, Bulgakov T, Callac P, Chomnunti P, Greiner K, Hashimoto A, Hofstetter V, Kang JC, Lewis D, Li XH, Liu XZ, Liu ZY, Matsumura M, Mortimer PE, Rambold G, Randrianjohany E, Sato G, Sri-Indrasutdhi V, Tian CM, Verbeken A, von Brackel W, Wang Y, Wen TC, Xu JC, Yan JY, Zhao RL, Camporesi E (2015) Fungal diversity notes 1–110: taxonomic and phylogenetic contributions to fungal species. Fungal Divers 72(1):1–197
Lumbsch HT, Huhndorf SM (2010) Life and earth sciences. Part one. Outline of Ascomycota 2009. Myconet volume 14. Field Museum of Natural History 1–40
Luttrell ES (1951) Taxonomy of the pyrenomycetes. Univ Missouri Stud 24:1–120
Luttrell ES (1989) Morphology of *Meliola floridensis*. Mycologia 81: 192–204
Maharachchikumbura SSN, Hyde KD, Jones EBG, McKenzie EHC, Huang SK, Abdel-Wahab MA, Daranagama DA, Dayarathne M, D'souza MJ, Goonasekara ID, Hongsanan S, Jayawardena RS, Kirk PM, Konta S, Liu JK, Liu ZY, Norphanphoun C, Shang KLQ, Shenoy BD, Xiao Y, Bahkali AH, Kang J, Somrothipol S, Suetrong S, Wen T, Xu J (2015) Towards a natural classification and backbone tree for Sordariomycetes. Fungal Divers 72:199–301
Martin GW (1941) Outline of the fungi. Stud Nat Hist Univ Iowa 18:192–204
McAlpine D (1897) New South Wales fungi. Proc Linn Soc New South Wales 22:38
Mibey RK, Hawksworth DL (1997) *Meliolaceae* and *Asterinaceae* of the Shimba Hills. Kenya Mycol Pap 174:1–108
Mueller WC, Goos RD, Quainoo J, Morgham AT (1991) The structure of the phialides (mucronate hyphopodia) of the Meliolaceae. Can J Bot 69:803–807
Müller E, von Arx J (1973) Pyrenomycetes: *Meliolales*, *Coronophorales*, *Sphaeriales*. In: Ainsworth G, Sparrow F, Sussman A (eds) The fungi IV. Academic, New York, pp 87–132
Nayar TS, Hosagoudar VB, Prakashkumar R, Jothish S, Thripthy KM (1998) The occurrence of *Meliolaceae* ascospores in the air of Kerala, India. Grana 37:253–254
Nylander JAA, Wilgenbusch JC, Warren DL, Swofford DL (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24: 581–583
Old KM, Wingfield MJ, Yuan ZQ (2003) A manual of diseases of eucalypts in South-East Asia, 106
Patil MS, Mahamulkar SH (1999) Studies on *Meliolaceae* of India-IV. Indian Phytopath 52(3):245–253
Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A (2009) How many bootstrap replicates are necessary? LNCS 5541:184–200
Piepenbring M, Hofmann TA, Kirschner R, Mangelsdorff R, Perdomo O, Justavino DR, Trampe T (2011) Diversity patterns neotropocal plant parasitic microfungi. Ecotropica 17:27–40
Pinho DB, Firmino AL, Ferreira-junior WG, Pereira OL (2012a) An efficient protocol for DNA extraction from *Meliolales* and the description of *Meliola centellae* sp. nov. Mycotaxon 122:333–345
Pinho DB, Pereira OL, Firmino AL, Silva M, Ferreira-Junior WG, Barreto RW (2012b) New *Meliolaceae* from the Brazilian Atlantic Forest 1. Species on hosts in the families *Asteraceae*, *Burseraceae*, *Euphorbiaceae*, *Fabaceae* and *Sapindaceae*. Mycologia 104:121–137
Pinho DB, Firmino AL, Ferreira-Júnior WG, Pereira OL (2013) New *Meliolaceae* from the Brazilian Atlantic Forest 2: species on host families *Annonaceae*, *Cecropiaceae*, Meliaceae, Piperaceae, Rubiaceae, Rutaceae and Tiliaceae. Mycologia 105:697–711
Pinho DB, Junior JH, Firmino AL, Junior BTH, Mizubuti ESG, Pereira OL (2014) Reappraisal of the black mildews (*Meliolales*) on *Hevea brasiliensis*. Trop Plant Pathol 39(1):89–94
Raciborski M (1909) Parasitische und epiphytische Pilze Javas. Bull Int de l'Acad des Sci de Cracovie Classe des Sci Math et Nat 3:346–394
Rannala B, Yang Z (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. J Mol Evol 43:304–311
Rehm H (1901) Beiträge zur Pilzflora von Südamerika. XIII. *Xylariaceae*. Hedwigia 40:141–170
Roger L (1953) Phytopathologie des pays Chauda. In: Encyclopedia mycologique 1638–1653
Sabulal B, Hosagoudar VB, Pradeep NS, Dan M, George V (2006) Chemical composition and antimicrobial activity of *Meliola toddaliae* infected leaf oil of *Pamburus missionis*. J Mycopathol Res 44:237–242
Saccardo PA (1913) Notae mycologicae. Series XVI. Ann Mycol 11(4): 312–325
Saenz GS, Taylor JW (1999) Phylogenetic relationships of *Meliola* and *Meliolina* inferred from nuclear small subunit rRNA sequences. Mycol Res 103:1049–1056
Sawada K (1959) Descriptive catalogue of Taiwan (Formosan) fungi. Part XI. Spec Publ Coll Agric Nat Taiwan Univ 8:1–268
Senanayake IC, Maharachchikumbura SSN, Hyde KD, Bhat JD, Dai DQ, Daranagama DA, Dayarathne MC, Konta S, Li WJ, Wijayawardene NN, Xiao YP, Wen TC, Norphanphoun C, Li QR, Liu XZ, Bahkali AH, Jones EBG, Kang JC, Wang Y, Camporesi E (2015) Towards unraveling relationships in *Xylariomycetidae* (Sordariomycetes). Fungal Divers 73(1):73–144
Silvestro D, Michalak I (2012) RaxmlGUI: a graphical front-end for RAxML. Org Divers Evol 12:335–337
Spegazzini C (1918) Notas micologicas. Physis 4:281–295
Spegazzini C (1921) Boln Acad nac Cienc Córdoba 26(2–4):349
Starbäck K (1899) Ascomyceten der ersten Regnell'schen expedition. I. Bihang till kungliga svenska. Vetenskaps-Akademiens Handlingar 25(1):1–68
Stevens FL (1925) Hawaiian fungi. Bernice P Bishop Mus Bull 19:1–189
Stevens FL (1927) The *Meliolineae*. I Ann Mycol 25:405–469
Sydow H (1930) Novae fungorum species - XX. Ann Mycologici 28(5–6):432–447
Theissen F (1913) Uber einige mikrothyriaceen. Ann Mycol 11:499
Thimmaiah CJ, Hosagoudar VB, Jayashankar M (2013) Black mildews of Kodagu, Karnataka. J Threatened Taxa 5(16):5021–5180

Thite AN (1975) Ascospore germination in *Meliola jasminicola*. Indian Phytopathol 28:94–96

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Toro RA (1952) Study of the tropical American black-mildews. J Agric Univ P R 36:24–87

Tucker SL, Besi MI, Galhano R, Franceschetti M, Goetz S, Lenhert S, Osbourn A, Sesmaa A (2010) Common genetic pathways regulate organ-specific infection-related development in the rice blast fungus. Plant Cell Prev, www.aspb.org

Viégas AP (1944) Alguns fungos do Brazil. II. Ascomicetos. Bragantia 4: 1–392

von Höhnel F (1918) Fragments Zur mycologie mitteil. Ibid 128:556

von Höhnel F (1919) Fragmente zur Mykologie. XXIII Mitteilung, Nr. 1154 bis 1188. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften Math.-naturw. Klasse Abt. I. 128:535–625

Wellman FL (1972) Tropical American plant disease. The Scarcccrow Press, Metuchen, p 989

Wijayawardene NN, Crous PW, Kirk PM, Hawksworth DL, Boonmee S, Braun U, Chomnunti P, Dai DQ, D'souza MJ, Diederich P, Dissanayake A, Doilom M, Hongsanan S, Jones EBG, Groenewald JZ, Jayawardena R, Lawrey JD, Liu JK, Lücking R, Madrid H, Manamgoda DS, Muggia L, Nelsen MP, Phookamsak R, Suetrong S, Tanaka K, Thambugala KM, Wikee S, Zhang Y, Aptroot A, Ariyawansa HA, Bahkali AH, Bhat JD, Gueidan C, De Hoog GS, Knudsen K, McKenzie EHC, Miller AN, Mortimer PE, Wanasinghe DN, Phillips AJL, Raja HA, Slippers B, Shivas RS, Taylor JE, Wang Y, Woudenberg JHC, Piątek M, Cai L, Jaklitsch WM, Hyde KD (2014) Naming and outline of *Dothideomycetes*–2014. Fungal Divers 69(1):1–55

Wu X, Schoch CL, Boonmee S, Bahkali AH, Chomnunti P, Hyde KD (2011) A reappraisal of *Microthyriaceae*. Fungal Divers 51(1):189–248

Yamamoto W (1954) Studies on the parasitism of sooty mould fungi. Phytopathol Soc Japan 18(3–4):102–106

Yarwood CE (1973) Pyrenomycetes: erysiphales. In: Ainsworth GC, Sparrow FK, Sussman AS (eds) The fungi: an advanced treatise. Vol. IVA. Academic, New York, pp 71–86

Zhang N, Castlebury LA, Miller AN, Hundorf SM, Schoch CL, Seifert KA, Rossman AY, Rogers JD, Kohlmeyer J, Volkmann-Kohlmeyer B, Sung G-H (2006) An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. Mycologia 98: 1076–1087

Zhaxybayeva O, Gogarten JP (2002) Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. BMC Genomics 3:4

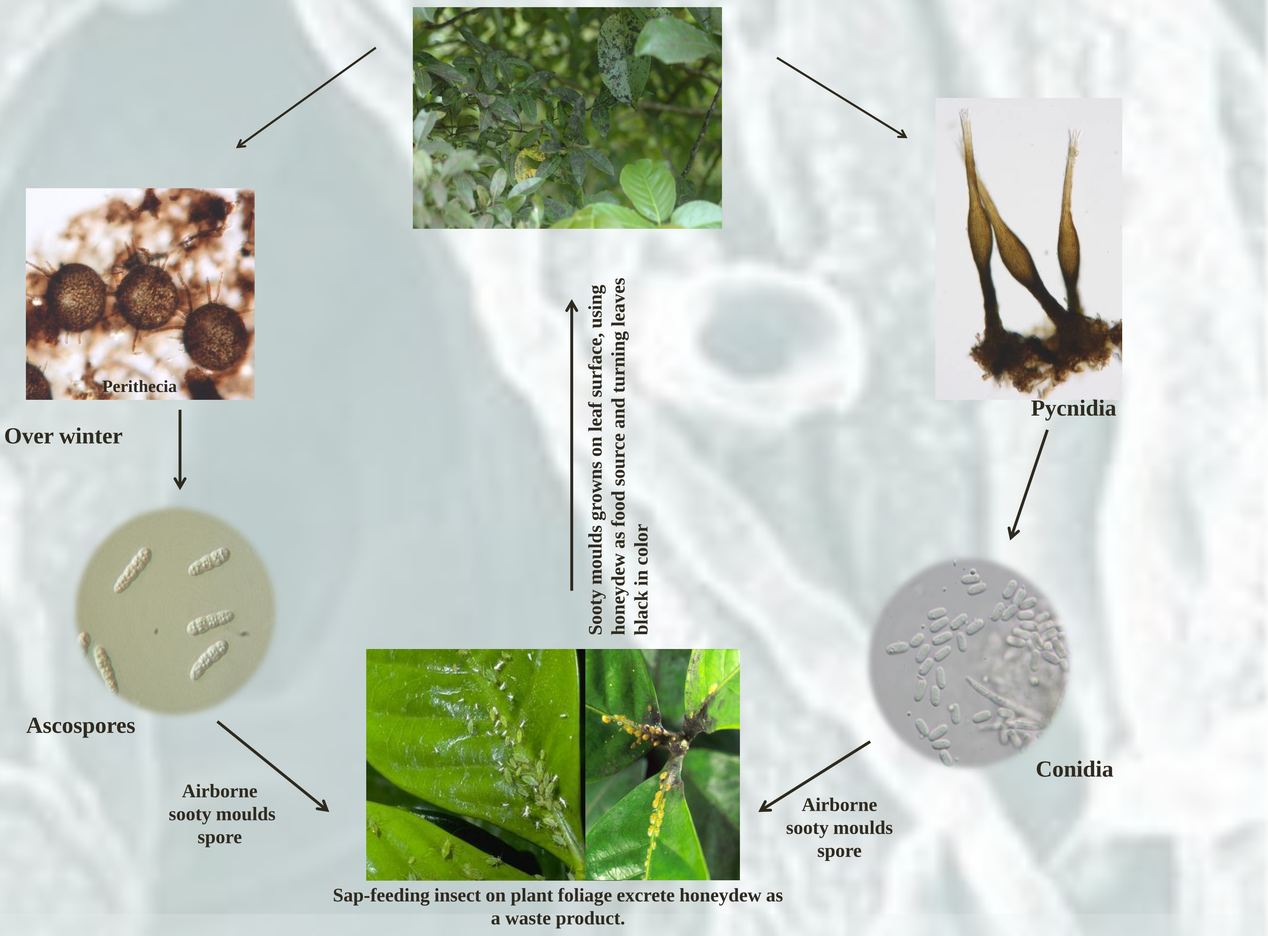
Sooty moulds in Thailand

Putarak Chomnunti[1]*, Qing Tian[1], Sinang Hongsanan[1], Kevin Hyde[1]

[1]School of Science, Mae Fah Luang University, THAILAND

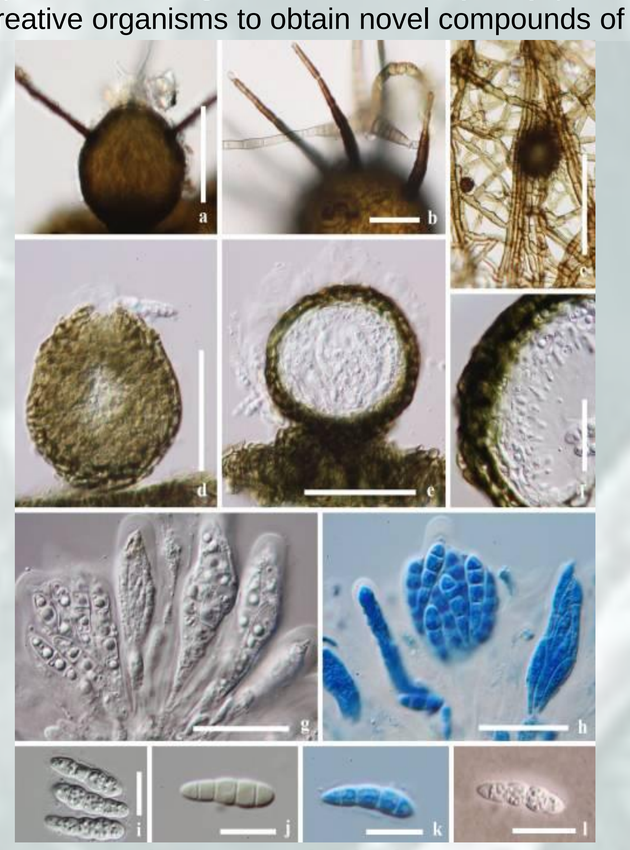
Sooty molds are hyper-diverse and we have discovered many new species in Thailand. Presently sooty moulds are found in seven families and several orphaned genera of Ascomycota, many of them lack connections between the asexual and sexual morphs, and numerous species remain in uncertain taxonomic placements. Sooty moulds have been reported in *Capnodiaceae*, *Euantennariaceae* and *Metacapnodiaceae* of Class Dothideomycetes, and *Chaetothyriaceae*, *Coccodiniaceae* and *Trichomeriaceae* of Class Eurotiomycetes. Various sooty moulds in Thailand are complex and recently three families in two classes of fungi have been clarified. We have studied the systematics of sooty moulds in Thailand, based on morphological characters using the type species as references and molecular analysis of DNA sequence data and found tropical sooty moulds to belong in *Capnodiaceae*, *Chaetothyriaceae* and *Trichomeriaceae*. However, this was the first serious study of this group of fungi in any tropical country and further studies are needed. By investigating a specific habitat where we know all plant species, we can establish whether sooty moulds are diverse, or if we are already aware of most species. By carrying molecular analysis of sooty mould collections we can establish if these poorly studied fungi are presently well-identified. We are finding new species and families of sooty moulds. There is a three-way relationship between the plant host, the insect sap feeder and the sooty moulds that grow on the excreted sugary sap. By analyzing the fungal species, plant hosts and insect species in a restricted habitat, it will be possible to establish whether there are any relationships and the result will be applied to control the sooty moulds in economic plants using bio-control.

Keywords: *Capnodiaceae*, *Chaetothyriaceae*, *Dothideomycetes*, *Trichomeriaceae*, Sooty moulds

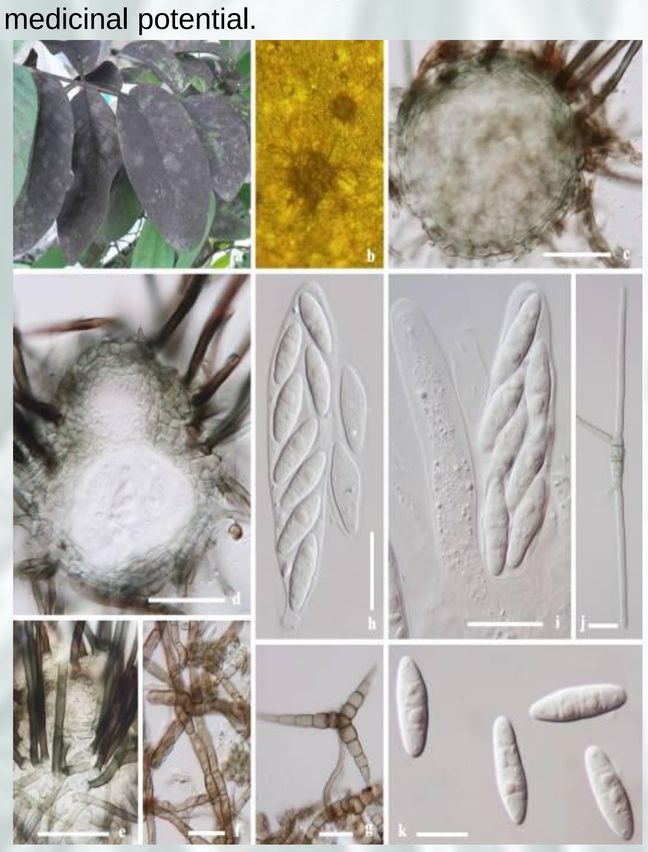


Sap-feeding insect on plant foliage excrete honeydew as a waste product.

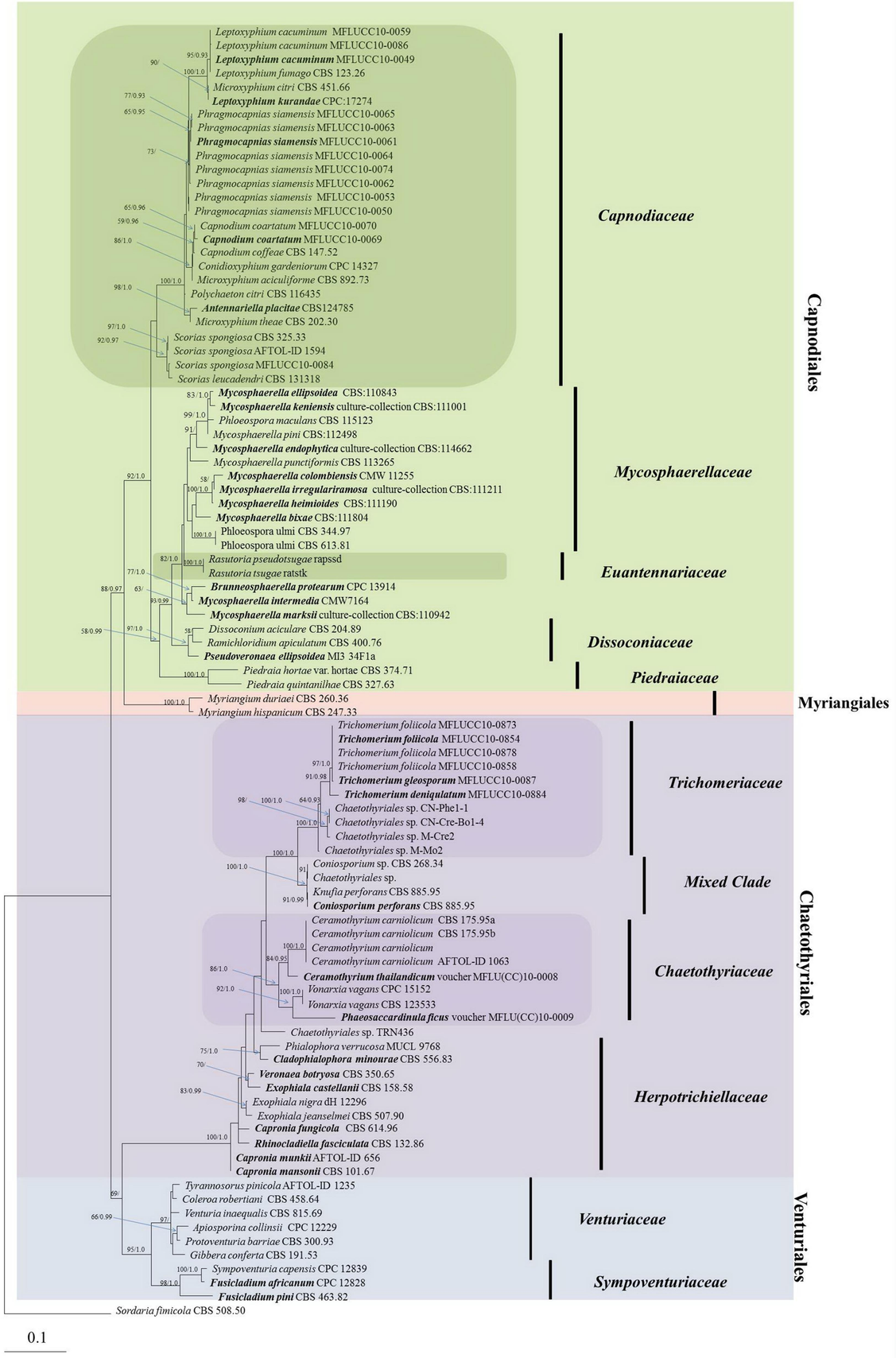
The Sooty Mould Life Cycle asexual and sexual genera have often been linked as the same biological species by association in the same colony. grow on sugars and appear to outcompete typical weed fungi and bacteria; they may produce antibiotics for this purpose and may be potential creative organisms to obtain novel compounds of medicinal potential.



***Phragmocapnias betle* (*Capnodiaceae*)**



***Trichomerium foliicola* (*Trichomeriaceae*)**



Randomized Axelerated Maximum likelihood (RAxML) most likely LSU tree for the analyzed sooty moulds and related taxa. Sordaria fimicola (CBS 508.50) is used as outgroup. RAxML bootstrap support values (BP>50) and Bayesian posterior probabilities (PP>90) are given at the nodes (BP/PP). Type strains are emphasized in bold. The culture collection numbers are indicated for each taxon.

References:

Batista, A. C. & Ciferri, R. (1963). Capnodiales. Saccardoa, 2, 1–296.

Dhami MK, Weir BS, Taylor MW, Beggs JR (2013) Diverse Honeydew-Consuming Fungal Communities Associated with Scale Insects. PLoS ONE 8 (7):e70316. doi:10.1371/journal.pone.0070316

Hughes, S. J. (1972). New Zealand fungi 17, Pleomorphism in *Euantennariaceae* and *Metacapnodiaceae*, two families of sooty moulds. New Zealand Journal of Botany, 10, 225–242.

Hughes, S. J. (1976). Sooty moulds. Mycologia, 68(4), 693–820

Hughes, S. J. & Seifert, K. A. (2012). Taxonomic and nomenclatural notes on sooty mould name based on species mixtures: *Hormiscium handelii* and *Torula lecheriana*. Mycoscience, **53,** 17–24.

Jouraeva VA, Johnson DL, Hassett JP, Nowak DJ, Shipunova NA, Barbarossa D (2006) Role of sooty mold fungi in accumulation of fine-particle-associated PAHs and metals on deciduous leaves. Environ Res 102:272–282

Trichopeltinaceae (Dothideomycetes), an earlier name for *Brefeldiellaceae*

SINANG HONGSANAN[1,2], ALI H. BAHKALI[3], ERIC H.C. MCKENZIE[4], EKACHAI CHUKEATIROTE [1,2] & KEVIN D. HYDE[1,2]*

1 Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, 57100, Thailand
2 School of Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand
3 Department of Botany and Microbiology, College of Sciences, King Saud University, Riyadh, KSA
4 Landcare Research, Private Bag 92170, Auckland, New Zealand
* email: kdhyde3@gmail.com

Abstract

The family *Trichopeltinaceae* is poorly known. This is due to an unclear history, few modern morphological studies and lack of sequence data in GenBank. *Trichopeltinaceae* introduced in 1914 as *Trichopeltaceae* to accommodate the subfamilies Trichopeltinae and Brefeldiineae. In 1958 the spelling of the family was corrected to *Trichopeltinaceae*, as it was presumably based on the genus *Trichopeltina*. Both *Brefeldiellaceae* and *Trichopeltinaceae* contain morphologically similar epiphytic foliar taxa, the only difference being that the thallus is linear in *Trichopeltinaceae* and rounded in *Brefeldiellaceae*. *Trichopeltinaceae* is the earliest name for these taxa which discussed here.

Trichopeltina **Theiss., *Beih. bot. Zbl.*, Abt. 2 32: 3 (1914), Index fungorum: 5566**

Foliar epiphytes, **particularly on leaves, mainly characters having thallus "root"-like darkened, very thin, superficial, comprising neatly or irregularly arranged, angular or cylindrical cells, dark brown, thyriothecia developing under thallus tissue, with central ostiole. *Asci* 8-spored, bitunicate, fissitunicate, clavate, usually with a small ocular chamber. *Ascospores* long clavate, 1–3-septate, constricted at each septa, upper cells wider and shorter than lower cells, hyaline, sometimes pale brown, smooth-walled.**

Fig 1. Morphology of thallus of *Trichopeltina* .

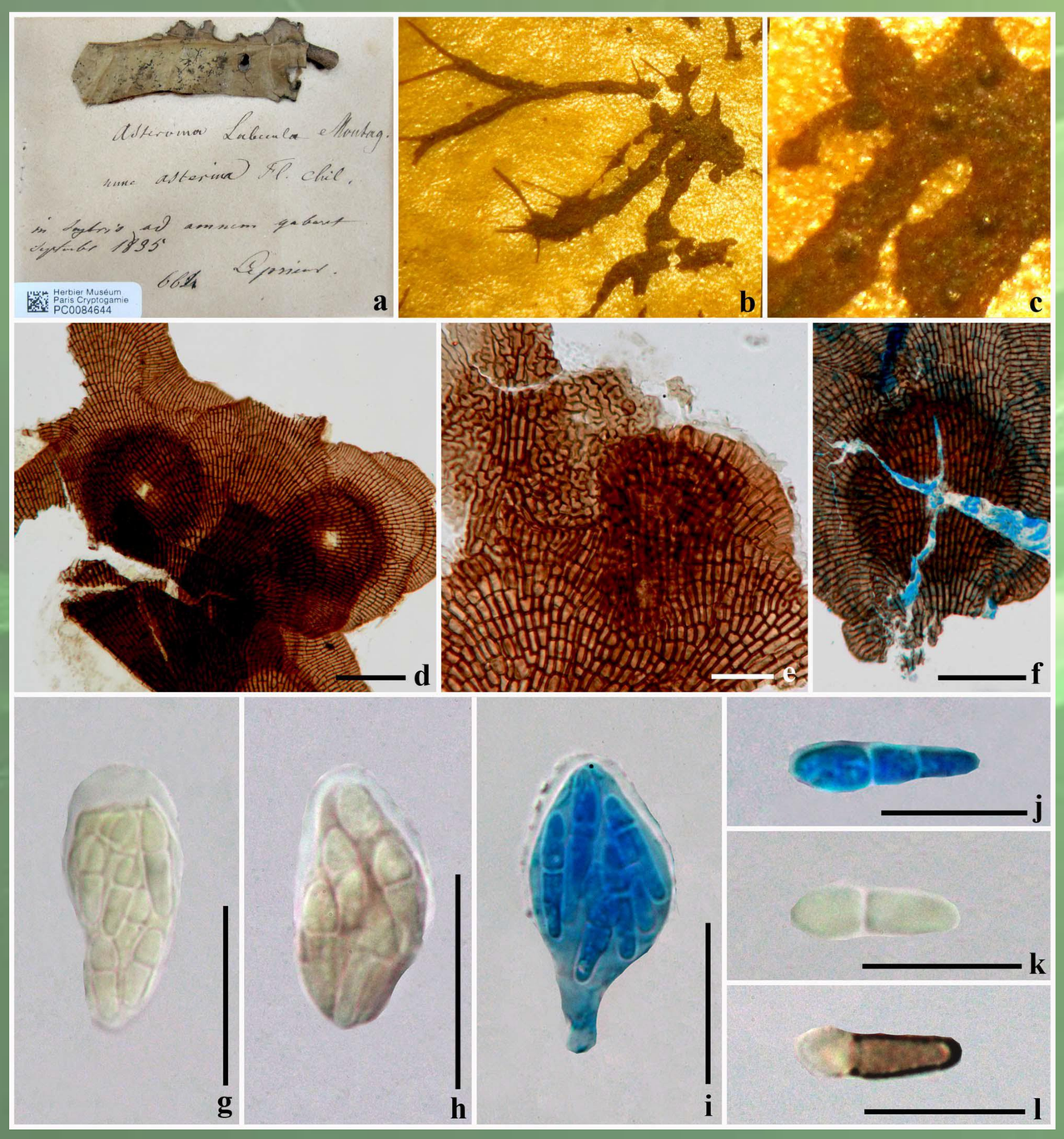


Fig 2. *Trichopeltina labecula* (lectotype).

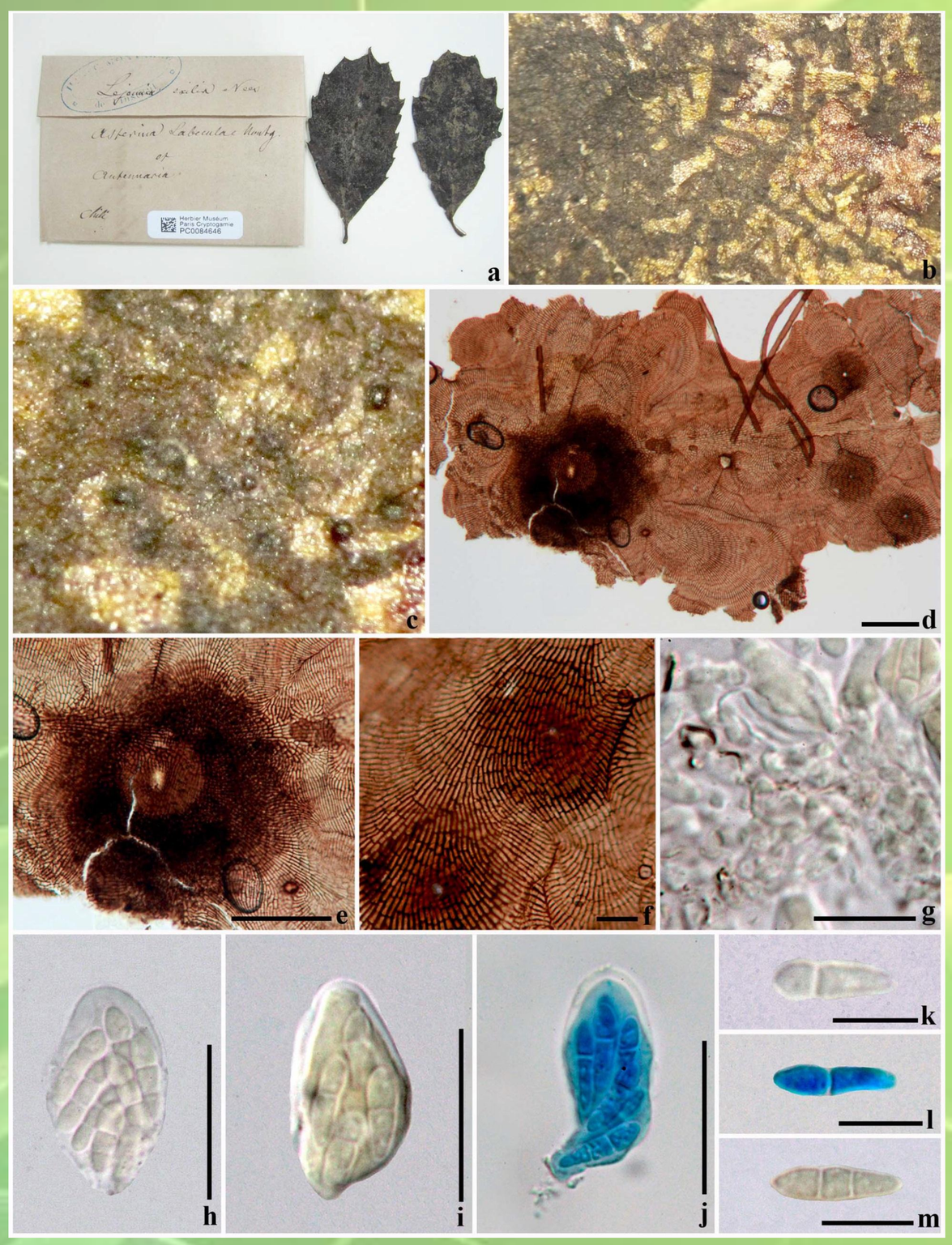


Fig 3. *Trichopeltina labecula* (PC 0084646)

Results and Discussions

The earliest family name for the fungal foliar epiphytes in this study is *Trichopeltinaceae*, based on the type *Trichopeltina labecula* (Batista et al. 1958) . This is similar to *Brefeldiella brasiliensis* (Spegazzini 1889), which is the type species of *Brefeldiellaceae*, a family introduced by Müller & von Arx (1962). We treat *Brefeldiellaceae* as a synonym of *Trichopeltinaceae*. The family differs from *Trichothyriaceae* in having thyiothecium under thallus, while thyriothecium on thallus in *Trichothyriaceae*. The holotype material of *Trichopeltina labecula* is illustrated in this study, although in most previous studies it had been reported that it could not be found. Further study is needs to clarify the concept and relationships.

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Referrences

Hyde, K.D., Jones, E.B.G., Liu, J.K., Ariyawansa, H., Boehm, E., Boonmee, S. (2013) Families of Dothideomycetes. Fungal Diversity 63: 1–313.
Stevens, F.L. (1925) Trichopeltaceae in Hawaiian Fungi. Bernice P. Bishop Museum Bulletin No. 19: 78–86.
Theissen, F. (1914) Trichopeltaceae n. fam. Hemisphaerialium. Zentralblatt für Bakteriologie und Parasitenkunde Abteilung 2, 39: 625–640.
Barr, M.E. (1987) Prodromus to class Loculoascomycetes. Published by the author, Amherst, Massachusetts Hamilton I, Newell.
Batista, A.C., Costa, A.A. & Ciferri, R. (1958) Orgânogênese e sistemática dos fungos *Trichopeltinaceae* (Theiss.) emend. nobis. Atti dell'Istituto Botanico dell'Università di Pavia Ser. 5, 15: 35–56.

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ORIGINAL ARTICLE

Towards a natural classification of Dothideomycetes: Clarification of *Aldona*, *Aldonata* and *Viegasella* (*Parmulariaceae*)

Qing Tian [a,b], Sinang Hongsanan [a,b], Dongqin Dai [a,b], Siti A. Alias [c], Kevin D. Hyde [a,b], Putarak Chomnunti [a,b,*]

[a] *Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[b] *School of Science, Mae Fah Luang University, 333 M 1 Thasud, Muang, Chiang Rai 57100, Thailand*
[c] *Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia*

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KEYWORDS

Foliar epiphytes;
Parmulariaceae;
Types

Abstract Foliar epiphytes in *Parmulariaceae* (Dothideomycetes) are groups of relatively poorly known taxa. Species of *Parmulariaceae* are biotrophic, plant-parasitic microfungi that develop on the surface of living plants. We collected *Aldona stella-nigra* during a survey of foliar epiphytes in the Philippines and thus we restudied this poorly known species and re-examined some similar taxa. In this paper we re-describe and illustrate the type species of some similar genera; *Aldona, Aldonata* and *Viegasella* in *Parmulariaceae* which are parasitic on the surface leaf spots and also provide details of the asexual state of these unusual fungi. By illustrating the genera we anticipate fresh collections of these genera to be obtained for further studies so that they can be epitypified and molecular data can be analyzed to obtain a natural classification.

1. Introduction

We are studying foliar epiphytes, which are groups of relatively poorly known fungi in Dothideomycetes, Sordariomycetes and Eurotiomycetes, mostly occurring in tropical regions (Batista and Ciferri, 1962, 1963; Chomnunti et al., 2011, 2012a,b, 2014; Hosagoudar, 2012; Hyde, 1996, 2001; Reynolds and Gilbert, 2005; von Arx and Müller, 1975; Wu et al., 2011; Hyde et al., 2013). They can be grouped into four or more distinct types based on their appearance on the host and their biology. Their occurrence may become more common place on fruit with global warming and the movement towards organic products (Stover, 1975).

The sooty moulds (e.g. *Antennulariaceae*, *Capnodiaceae*, *Chaetothyriaceae*, *Euantennariaceae*, *Meliolinaceae*, *Trichomeriaceae*, *Tripos poriopsidaceae*) form thick blackened mycelial layers on the surface of healthy green leaves and branches of

* Corresponding author at: School of Science, Mae Fah Luang University, 333 M 1 Thasud, Muang, Chiang Rai 57100, Thailand.
E-mail address: putarak.cho@mfu.ac.th (P. Chomnunti).
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trees, or even grasses, soil and rocks beneath trees, often with several species coexisting together (Chomnunti et al., 2011, 2012a,b, 2014; Hyde et al., 2013). They grow on the sugary exudates excreted on to the leaves by plant sap sucking insects and although they do not directly damage the host they can reduce yields by reducing photosynthesis (Chomnunti et al., 2011, 2014). They may also cause dirty black blemishes on fruits, thus reducing marketability.

The black moulds (*Asterinaceae*, *Microthyriaceae* and *Meliolaceae*) are generally biotrophic and produce sparse, superficial, web-like arrangements of blackened mycelium on the leaf surface, usually producing hyphopodia, while ascomata or pycnidia develop in the centre of the "webs" beneath or above the mycelia (Wu et al., 2011; Hosagoudar and Riji, 2011; Hongsanan et al., 2014a). The hyphopodia penetrate the host tissues and produce haustoria, which absorb nutrients from the host (Dean, 1997; Gregory and John, 1999; Hansford, 1946; Hughes, 1993; Mibey and Hawksworth, 1995; Yi and Valent, 2013; Hongsanan et al., 2014a). These fungi, however rarely cause significant damage to the host, although they may reduce marketability of fruits or leaves (Hofmann, 2010). Again it is likely their occurrence on fruit will become more commonplace with the occurrence as global warming and the movement towards organic products.

A third group named fly speck fungi, often cause blemishes on fruits which are mostly caused by genera of *Schizothyriaceae*. They form clusters as ascomata on fruits linked by superficial mycelia (Batzer et al., 2008; Gleason et al., 2011; Ivanović et al., 2010). Again these fungi, rarely cause primary damage to the host, however they may cause the economic losses. The fourth group, which is perhaps a subgroup of group 3, are inconspicuous and often only visible as single or groups of fruiting bodies, often on the lower surface of leaves of tropical trees. These secretive fungi appear to produce structures under the fruiting bodies that penetrate host cells and obtain nutrients, although in some cases they may cause leaf necrosis and probably feed on dead plant cells. The genera of *Aulographaceae*, *Micropeltidaceae*, *Microthyriaceae* and *Parmulariaceae* make up this group (Inácio and Cannon, 2008; Wu et al., 2011, 2014a,b; Hongsanan et al., 2014a,b).

Most of these foliar epiphytes are relatively poorly known and have rarely been subjected to sequence analysis (Wu et al., 2011; Hongsanan et al., 2014a,b; Wijayawardene et al., 2014). This is because it is impossible to isolate these biotrophs into culture or in case of sooty moulds because they comprise several species intermingled in a sooty mould colony (Chomnunti et al., 2014).

We are studying the foliar epiphytes in order to understand their role, as well as their phylogenetic relationships with other fungi (Chomnunti et al., 2011, 2012a,b, 2014; Hongsanan et al., 2014a; Wu et al., 2011, 2014b). Most species are Ascomycota, but it may be possible that some are Basidiomycota. These fungi may also be important as it is likely they produce unique novel chemicals as they occupy unusual niches, belong to chemically poorly studied genera and are members of poorly known families (Aly et al., 2011; Bills, 1996; Debbab et al., 2011, 2012, 2013). We collected a species of *Aldona* during a survey of foliar epiphytes in the Philippines which matches *A. stella-nigra* Racib. and thus have restudied this poorly known species and some similar taxa. In this paper we revisit there morphologically similar genera; *Aldona, Aldonata* and *Viegasella* in *Parmulariaceae*. We also provided details of the asexual state of these unusual fungi.

2. Materials and methods

2.1. Morphological study

Fresh specimens were collected from the Philippines, while type specimens were obtained from M, K and S (for full names of herbaria see http://sweetgum.nybg.org/ih/index.php). One or two fruiting bodies were rehydrated in 3–5% KOH and transferred by fine forceps to a drop of water on a slide. Microscope slide mount were prepared with water and lactophenol with cotton blue reagent. Sections of ascomata were made by free hand and mounted in lactic acid. Observations and hand sections were examined under a stereoscope (Nikon ECLIPSE 80i) and photographed by a Canon 550D digital camera fitted to the microscope. Measurements were made with Tarosoft (R) Image Frame Work and photographic plates processed and improved using Adobe Photoshop CS3 (Adobe Systems Inc., The United States).

2.2. Isolation and culture

Isolations were made via single ascospores in order to obtain pure culture following the methodology described in Chomnunti et al. (2011). Ascomata were removed from the substrate surface by a sterilized fine forceps or needle and were transferred to a drop of sterile water in a small glass container. The drop of water was examined under a stereoscope to establish that enough and the correct spores had been transferred. The drops of spore suspension were then placed on malt extract agar (MEA) or potato dextrose agar (PDA) in the centre of pre-marked squares in a grid on the Petri dish by a Pasteur pipette. The plates were incubated overnight in an incubator (25 °C) and then examined for single germinated spores under a microscope at high power after 12–24 h and any germinating spores were transferred singly to at least three new plates. Colonies were transferred to new Petri dishes with the appropriate media at 25–28 °C for 12 h of light/12 h of dark and then observed under a microscope and all characters were photographed after one month.

3. Results

Although we obtained a culture of our fresh collection of *Aldona stella-nigra* from single ascospores and sequenced this (ITS, SSU), a blast search showed it to be close to *Meira argovae* strain AS006 (GenBank No. AY15867). *Meira argovae* is accommodated in Exobasidiomycetes which causes leaf and flower gall disease (Basidiomycota, *Incertae sedis* order and family level). The two taxa cannot be confused as *Aldona stella-nigra* is an ascomycete with asci and ascospores, while *Meira argovae* belongs in Exobasidiomycetes with sori in leaves which produce basidiospores. We suspect that a basidiomycetous yeast was growing in association with the ascomycete and outgrew our fungus in culture.

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3.1. Taxonomy

Aldona Racib., Parasit. Alg. Pilze Java's (Jakarta) 1: 19 (1900).

MycoBank: MB 115, *Facesoffungi number*: FOF00309.

Parasitic on the upper and lower surface causing reddish–brown leaf spots visible from both sides of the leaf. *Leaf spots* solitary to gregarious, rounded, brown, black in centre, dark brown near ascomata margins. **Sexual state**: *Ascomata* gregarious, semi-immersed to erumpent, black, linear, radial or star-shaped, branching, coriaceous, shiny, opening by longitudinal slits. *Peridium* composed of amorphous black tissue, base thin composed of brown cells of *textura angularis*. *Hamathecium* of dark brown to hyaline, branching, pseudoparaphyses. *Asci* 8-spored, bitunicate, clavate to cylindrical, with a relatively long pedicel, apically rounded with an ocular chamber. *Ascospores* elongate-clavate, upper cells larger and wider, basal cells short and narrow, hyaline, trans-septate, constricted at the septa, especially between the second and third cells from the apex, wall smooth. **Asexual state**: *Conidiomata* solitary to gregarious, black, shiny, carbonaceous, globose to subglobose or irregular, between and beneath the ascomata, hard to remove from leaf surface. *Peridium* comprising 2 layers, outer layer thick and composed of darkly pigmented cells, inner layer composed of hyaline to pale brown cells of *textura angularis*. *Conidiophores* forming from the inner cell walls. *Conidiogenous cells* hyaline, integrated. *Conidia* aseptate, fusiform, hyaline, tapering at both ends, smooth-walled.

Notes: Aldona was described by Raciborski (1900) as parasite on living leaves of *Pterocarpus indicus* Willd. and placed in *Hysteriaceae* (Saccardo, 1904; Penzig and Saccardo, 1904). It was transferred to *Phacidiaceae* by Von Höhnel (1917), Bisby (1923) and Zogg (1962) also suggested that *Aldona* cannot be placed in *Hysteriaceae*. *Aldona* however has bitunicate asci, but species in *Phacidiaceae* have unitunicate asci and therefore Nannfeldt (1932) suggested this genus cannot be accommodated in *Phacidiaceae*. Teodora (1937) also listed this genus from living leaves of *Pterocarpus* sp. in the Philippines. Müller and von Arx (1962) provided a key and placed *Aldona* in *Dothioraceae*. Müller and Patil (1973) referred to *Aldona* with three species: *A. stella-nigra* Racib., *A. americana* Pert. & Cif. and *A. minima* E. Müller & Patil. All species are from living leaves of *Pterocarpus* species. Sivanesan and Sinha (1989) compared *Aldona* with *Aldonata* and synonymized them because of their morphologically indistinguishable characteristics. However, consideration of both genera indicated that there are differences based on ascospores and ascomata (*Aldonata* has much larger locules and muriform rather than transversely septate ascospores). At present the genus *Aldona* is placed in *Parmulariaceae* with three species (Inácio and Cannon, 2008; Hyde et al., 2013).

Key to genera discussed in this paper:

1. Ascomata superficial	*Viegasella*
1. Ascomata initially immersed and then becoming erumpent or superficial	2
2. Ascospores with only transverse septa	*Aldona*
2. Ascospores with transverse and longitudinal septa	*Aldonata*

Aldona stella-nigra Racib., Parasit. Alg. Pilze Java's (Jakarta) 1: 19 (1900).

MycoBank: MB 172102, *Facesoffungi number*: FOF00310. Figs. 1, 2 and 3.

Parasitic growing on the upper and lower surface of living leaves. *Leaf spots* hypophyllous, solitary to gregarious, orbiculare, brown, black in centre, yellowish swollen on leaf surface. **Sexual state**: *Ascomata* 132–392 × 83–120 μm diam ($\overline{X}$ = 352 × 100 μm, n = 5), semi-immersed to erumpent, opening by longitudinal slits 122–271 μm diam ($\overline{X}$ = 207 μm, n = 6), hard to remove from leaf surface, gel-like or soft when wet, brittle when dry. *Peridium* 34–53 μm diam ($\overline{X}$ = 43 μm, n = 10), thick-walled, 2-layers, outer layer thick and composed of darkly pigmented amorphous cells, inner layer of hyaline to pale brown cells of *textura angularis*. *Hamathecium* 51–74 μm long and 0.6–1.4 μm wide ($\overline{X}$ = 64 × 1 μm, n = 10), filamentous pseudoparaphyses, anastomosing in gelatinous matrix with asci embedded in mucilage. *Asci* 59–84 μm long × 14–31 μm wide ($\overline{X}$ = 68 × 21 μm, n = 10), 8-spored, bitunicate or fissitunicate, broadly fusiform to obovoid with thin-walls 1–2 μm diam ($\overline{X}$ = 2 μm, n = 10), thick at apex, 2–5 μm diam ($\overline{X}$ = 2 μm, n = 7), short and narrow pedicellate or occasionally with long and narrow pedicellate 21–7 μm × 8–25 μm diam ($\overline{X}$ = 4 × 12 μm, n = 10), with ocular chamber, forming from the base of the ascomata, asci vertically arranged and embedded in a gelatinous matrix. *Ascospores* 28–48 μm long × 7–10 μm wide ($\overline{X}$ = 37 × 8 μm, n = 10), 2–3-seriate, long clavate, hyaline, 4–5 septate, apical cells wider and shorter, basal cells longer and narrower, 3–5 × 7–20 μm diam ($\overline{X}$ = 4 × 13 μm, n = 10), both ends rounded, slightly constricted at the septa, smooth-walled. **Asexual state**: *Conidiomata* 120–335 μm long × 75–205 μm wide ($\overline{X}$ = 264 × 154 μm, n = 10), solitary to gregarious, black, shiny, carbonaceous, globose to subglobose or irregular, mostly growing on the spot, surrounded by ascomata, hard to remove from leaf surface. *Peridium* 25–45 μm diam ($\overline{X}$ = 33 μm, n = 7), comprising 2 layeres, outer layer thick and composed of darkly pigmented 15–35 μm diam ($\overline{X}$ = 23 μm, n = 7) cells, inner layer composed of hyaline to pale brown 10–20 μm diam ($\overline{X}$ = 15 μm, n = 7) cells of *textura angularis*. *Conidiophores* 15–20 × 0.5–2 μm diam ($\overline{X}$ = 17 × 1 μm, n = 10), forming from the inner cell walls, embedded in a gelatinous matrix with conidia groups, broadly at the basal of conidiophore and narrow at apex. *Conidiogenous cells* hyaline, integrated. *Conidia* 1–1.5 × 3–3.5 μm diam ($\overline{X}$ = 1 × 3 μm, n = 10), aseptate, fusiform, narrow at both ends broad at the centre, hyaline or sometime slightly greenish, smooth-walled, conidia in a gelatinous matrix.

Material examined: INDONESIA. Sumatra, on leaves of *Pterocarpus indicus* Willd. (*Fabaceae*), 20 February 1959, Raciborski (M! 176025, **isotype**).

Other specimen examined: PHILIPPINES. Los Baños: Mt Makiling, on living leaves of *Pterocarpus draco* L. (*Fabaceae*), February 2012, K.D. Hyde (MFU14_0011).

Aldonata Sivan. & A.R.P. Sinha, Mycol. Res. 92(2): 248 (1989).

MycoBank: MB25242, *Facesoffungi number*: FOF00311.

Parasitic on leaf spots on the upper leaf surface. *Spots* solitary, scattered, sometimes confluent, variable in shape, circular to irregular, greyish-white, edge diffuse. **Sexual state**: *Ascomata* semi-immersed, globose to subglobose, black, shiny,

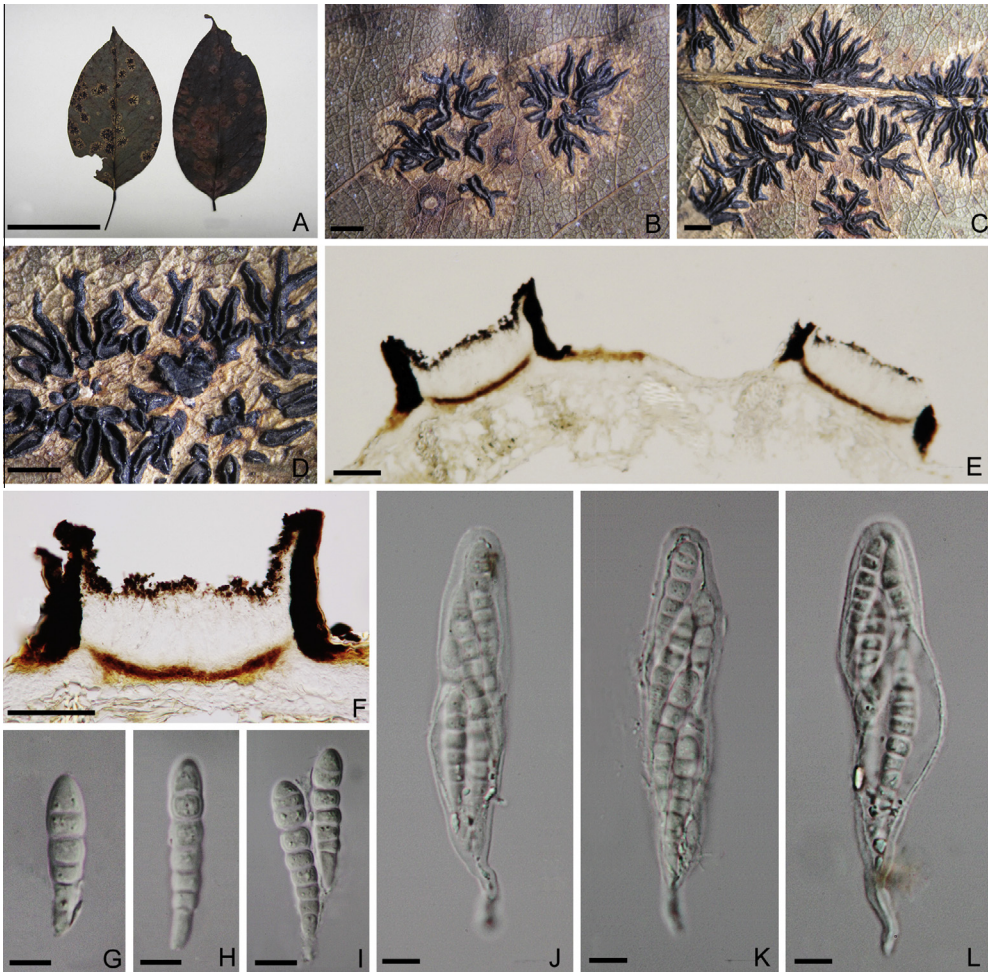

Figure 1 *Aldona stella-nigra* (isotype). (A) Spots on lower surface of leaves, (B–D) black ascomata with longitudinal openings, (E and F) section of ascomata, (G–I) hyaline ascospores with 6–8 septa, (J–L) clavate to cylindrical asci containing eight ascospores (scale bars: A = 50 mm, B–D = 1 mm, E and F = 100 μm, G–L = 10 μm).

appearing as flexuous lines on the leaf spot surface with a clearly-defined margin, hard remove from leaves surface. *Peridium* 1-layered, composed of poorly-defined brown to black cells. *Hamathecium* transverse septate, long, colourless, branched, pseudoparaphyses. *Asci* 8-spored, bitunicate, clavate to broadly clavate, pedicellate, thin-walled. *Ascospores* multiseriate, ellipsoid to fusiform, muriform, hyaline, with up to 8 transverse and longitudinal septa, lower cell narrow and longer, caudiform. **Asexual state**: *Conidiomata* solitary to gregarious, sub-immersed, black and shiny, carbonaceous, globose to irregular, mostly growing around the central of grayish-white spot. *Peridium* composed of 1 layer of thick-walled colourless cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells, arising from basal cells of inner peridium wall, *Conidiogenous cells* hyaline, integrated. *Conidia* aseptate, fusiform to cylindrical, hyaline, tapering at both ends, smooth-walled.

Notes: *Aldonata* was introduced by Sivanesan and Sinha (1989) as a monotypic genus, it contains the single species *Aldonata pterocarpi* Sivan. & A.R.P. Sinha. The genus is characterized by much larger locules than *Aldona* and muriform ascospores. *Aldonata* is presently placed in *Parmulariaceae* (Inácio and Cannon, 2008), and is similar to *Aldona*. *Aldona* and *Aldonata* are only genera of *Parmulariaceae* which have colourless ascospores and are occur on legumes (Inácio and Cannon, 2008). *A. pterocarpi* was described and illustrated in Inácio and Minter (2002) which has the grayish-white lines surrounding the colony and are similar to the cream-coloured lines in *Aldona stella-nigra* (Inácio and Minter, 2002). The asexual state of *Aldonata pterocarpi* has not previously been reported, but we found pycnidia surrounding the ascomata.

Aldonata pterocarpi Sivan. & A.R.P. Sinha, Mycol. Res. 92(2): 249 (1989).

MycoBank: MB134615, *Facesoffungi number*: FOF00312. Fig. 4.

Parasitic on leaf spots of *Pterocarpus draco*, on the upper leaf surface. Spots solitary, scattered, sometimes confluent, variable in shape, circular to irregular, greyish-white to light brown, edge diffuse. **Sexual state**: *Ascomata* 0.8–1.3 × 0.4–0.9 mm ($\overline{X}$ = 1 × 0.6 mm, n = 10), semi-immersed in leaves, globose to subglobose, black, shiny, appearing as flexuous lines on the leaf surface with a clearly defined margin. *Peridium* 570–690 × 220–280 μm ($\overline{X}$ = 612 × 240 μm, n = 6), apex brittle, often fragmenting during sectioning, 1-layered, thick at base and sides, up to 70 μm, composed of poorly-defined brown to black cells. *Hamathecium* of 1–2 μm broad with transverse

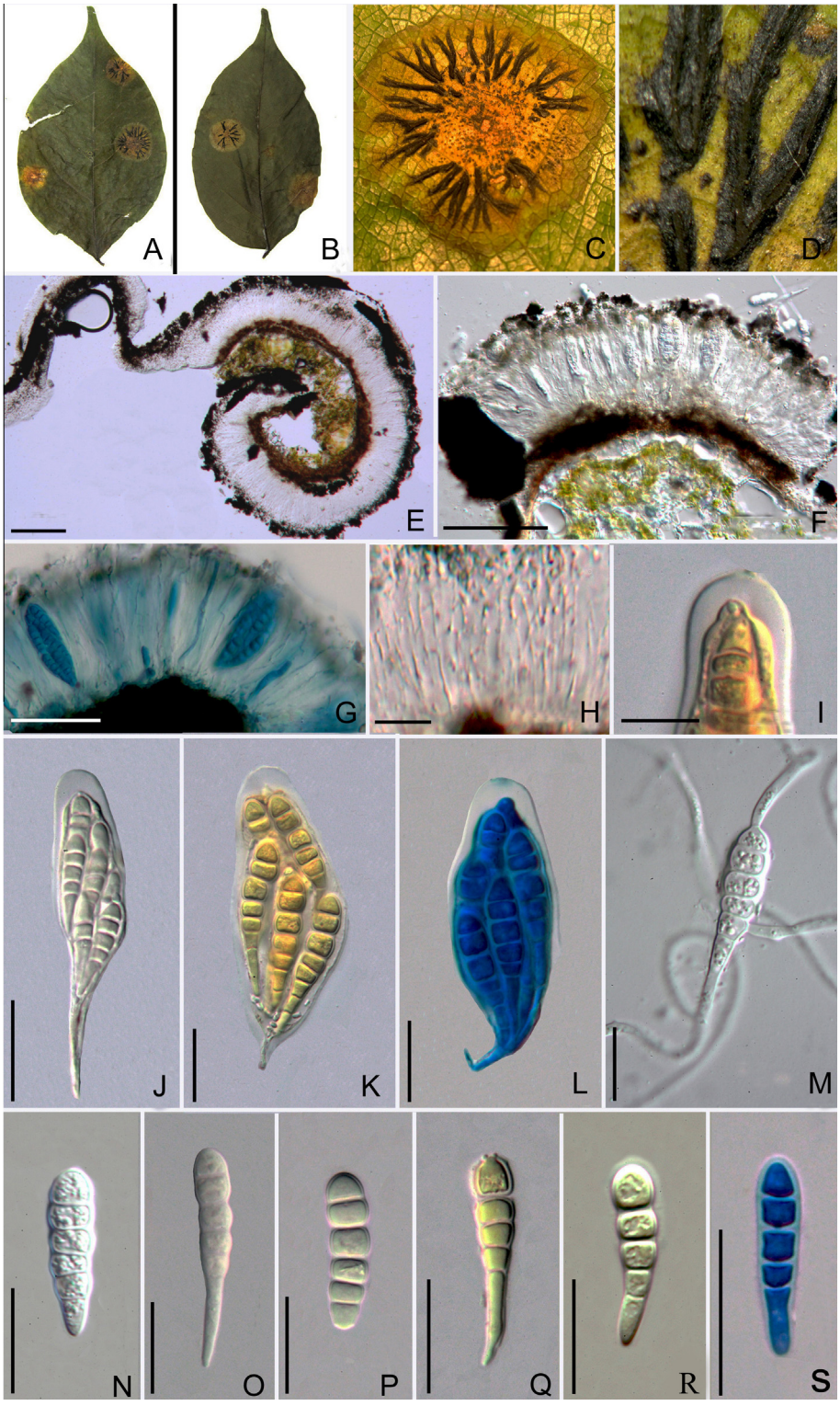

Figure 2 *Aldona stella-nigra*. (A and B) Colony on lower sides of living leaves, (C and D) ascomata on yellowish leaf spot, (E and F) vertical section of ascomata, (G) asci arrangement in gelatinous matrix, (H) Hamathecium, (I) ascus tip, note ocular chamber in Melzer's reagent, (J–L) asci with ascospores, note K mounted in Melzer's reagent and L mounted in cotton blue reagent, (M) germinating ascospore, (N–S) ascospores, note the long and narrow ends, Q and S mounted in Melzer's reagent, S mounted in cotton blue reagent (scale bars: E and F = 100 μm, G = 50 μm, I = 10 μm, H, J–S = 20 μm).

septate, long, colourless, branched pseudoparaphyses. *Asci* 65–95 × 15–30 μm ($\overline{X}$ = 78 × 22 μm, n = 6), 8-spored, bitunicate, clavate to broadly clavate, pedicellate up to 34 μm long, thin-walled, with ascospores arranged in a cluster. *Ascospores* 30–55 × 8–11 μm ($\overline{X}$ = 41 × 9 μm, n = 10), multiseriate, ellipsoid to fusiform, muriform, hyaline, with up to 8 transverse

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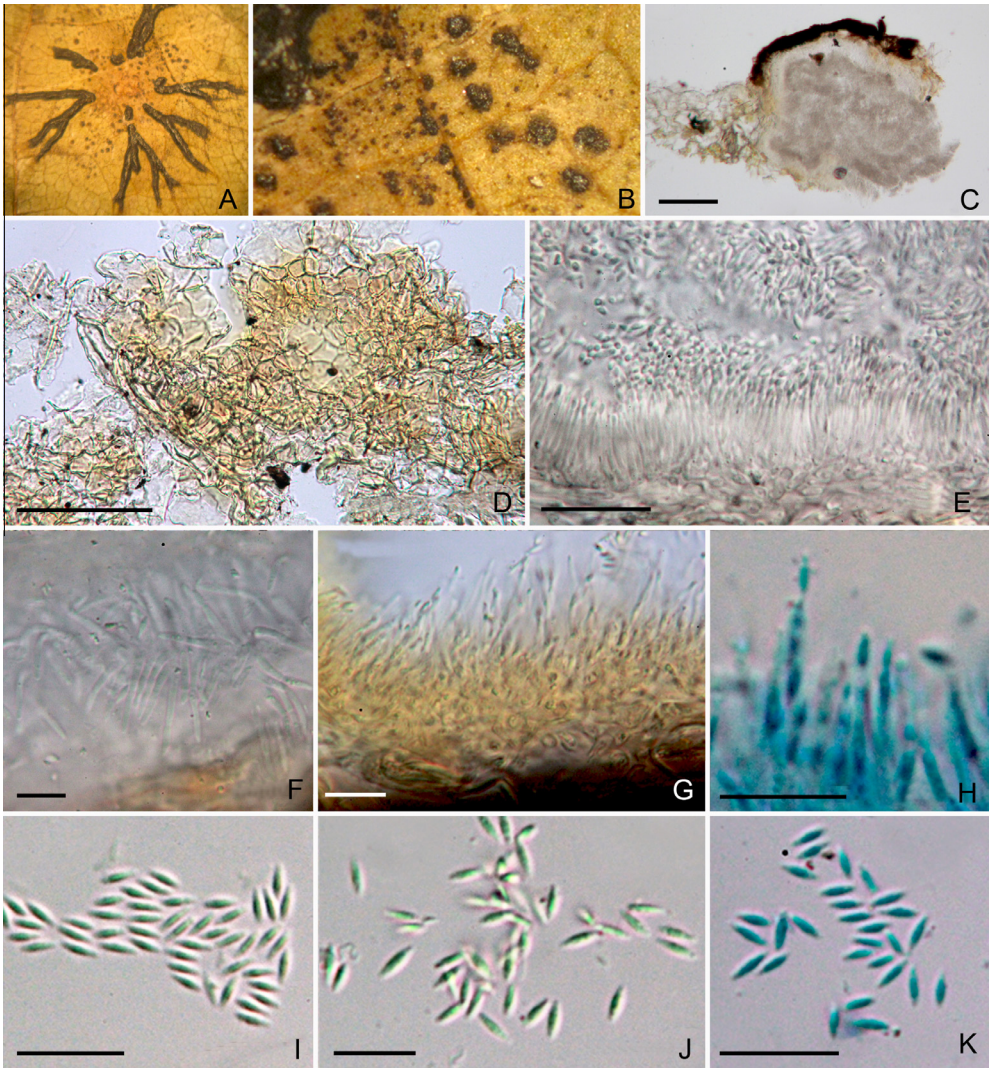

Figure 3 *Aldona stella-nigra*. (A and B) Yellow colony with conidiomata occurring on upper and lower surface of living leaves, (C) section through conidioma, (D) yellow tissues of conidioma, (E) conidia and conidiophores, (F–G) hyaline conidiophores, (H) conidiogenous cells, (I) aseptate conidia, (J) conidia in Melzer's reagent, (K) conidia in cotton blue reagent (scale bars: C and D = 100 μm, E = 20 μm, F–K = 10 μm).

and longitudinal septa, lower cell narrow and longer, caudiform. **Asexual state**: *Conidiomata* 80–155 × 75–135 μm diam ($\overline{X}$ = 120 × 100 μm, n = 10), solitary to gregarious, sub-immersed, black and shiny, carbonaceous, globose to irregular, mostly growing around the centre of grayish-white spot, *Peridium wall* up to 12 μm broad, composed of 1 layer of thick-walled colourless cells of *textura angularis*. *Conidiophores* 5–9 × 1–2 μm ($\overline{X}$ = 6 × 1.5 μm, n = 10), reduced to conidiogenous cells, arising from basal cells of inner peridium wall, *Conidiogenous cells* hyaline, integrated. *Conidia* 2–4 × 1–1.5 μm ($\overline{X}$ = 3 × 1 μm, n = 10), aseptate, fusiform to cylindrical, hyaline, tapering at both ends, smooth-walled.

Material examined: INDIA. Andaman Islands: Port Blair, on leaves of *Pterocarpus draco*, 1 November 1987, A.R.P. Sinha (K! IMI 322833, **holotype**).

Viegasella Inácio & P.F. Cannon, Mycol. Res. 107(1): 82 (2003).

MycoBank: MB 28709, *Facesoffungi number*: FOF00313.

Parasitic on the upper leaf surface. *Colonies* solitary, scattered, sometimes confluent, variable in shape, circular to irregular, light brown to reddish with a diffuse edge. *Ascomata* superficial, black, shiny, appearing as flexuous lines on the leaf surface, opening by longitudinal splits. *Ostiole* conspicuous. *Peridium* thick at the sides, composed of brown to black thick-walled cells of *textura angularis*, outer brown to black carbonaceous substance and internal hyaline cells, two-layered. Upper and lower wall thin, not well developed, sometimes absent. *Hamathecium* filamentous pseudoparaphyses, with transverse septa, long, colourless, branched, and verrucose at the tips. *Asci* 8-spored, bitunicate, cylindrical to clavate, short-pedicellate, thin-walled, embedded in mucilage. *Ascospores* biseriate or multiseriate, ellipsoid to fusiform or ellipsoid to narrowly ovoid, verrucose, usually two-celled, normally unequal, constricted at each transverse septum, upper cell wider, lower cell narrow and longer, hyaline, becoming pale brown when spores are senescent, each cell has an oil drop.

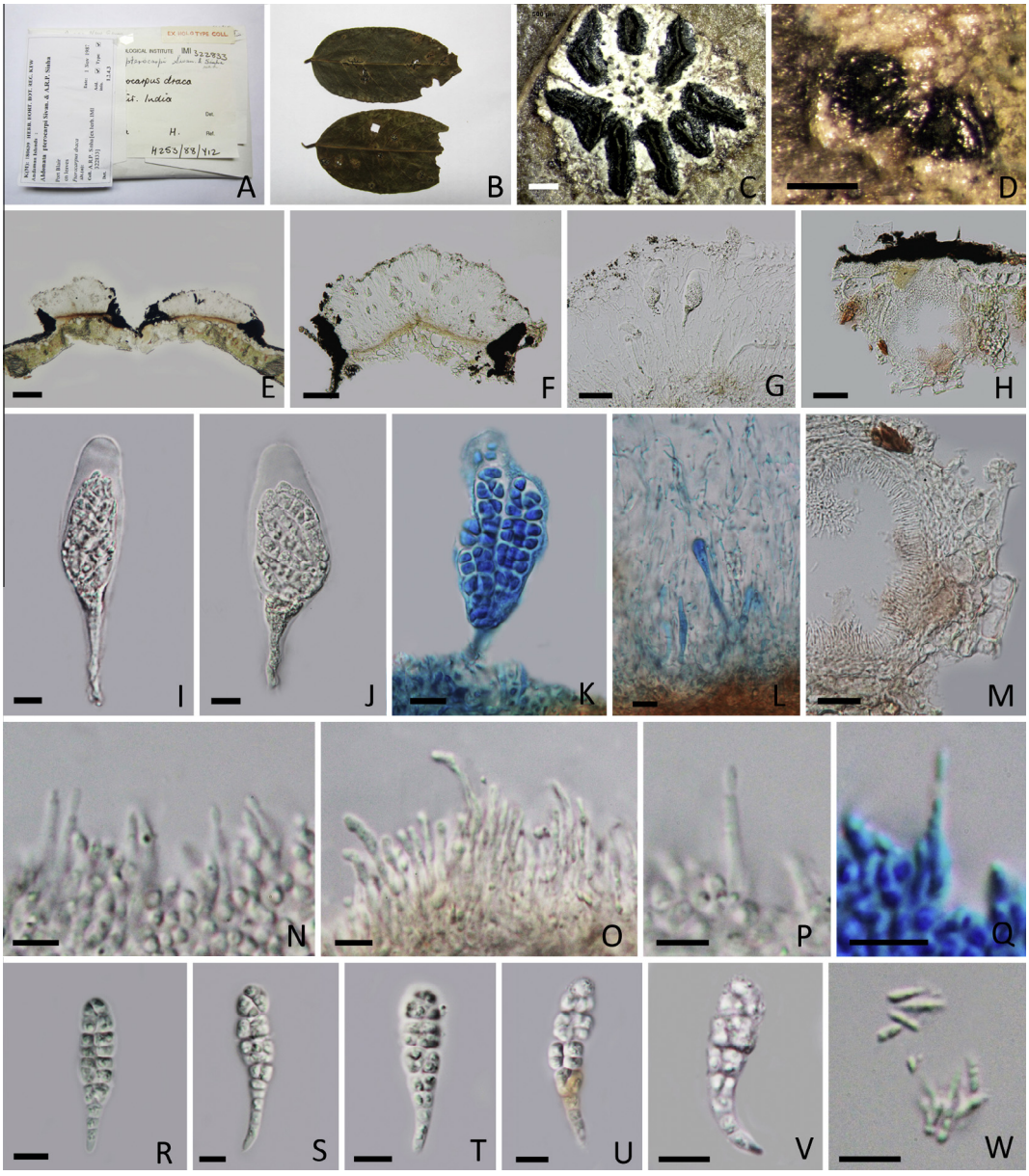

Figure 4 *Aldonata pterocarpi* (holotype). (A) Herbarium labels, (B) Herbarium material, (C) ascomata on host surface, (D) conidiomata on host surface, (E–G) section of ascomata, (H) section of conidiomata, (I–K) asci with ascospores, note K mounted in cotton blue reagent, (L) Hamathecium, (M) peridium of conidiomata wall, (N–Q) conidiogenous cells, (R–V) ascospores, note the long and narrow ends, (W) conidia (scale bars: C = 500 μm, D–E = 200 μm, F = 100 μm, G and H = 50 μm, M = 20 μm, I–L, R–V = 10 μm, N–Q, W = 5 μm).

Notes: *Viegasella* was introduced by Inácio and Cannon (2003) to accommodate *Schneepia pulchella* because of its internal and external stromata and haustoria and was placed in the family *Parmulariaceae*. Its superficial similarity to a lichen was mentioned by Spegazzini (1888). *Viegasella* was compared with *Parmularia*, *Symphaeophyma* and *Mintera* by Inácio and Cannon (2003). It is difficult to distinguish *Viegasella pulchella* and *Aldonata pterocarpi* based only on the macroscopic characters such as orientation of ascomata as both species have grayish-white to reddish lines surrounding the colony. *Viegasella pulchella* has tiny black spots around ascomata at the edge of leaf spots and 1-septate ascospores which differ from *Aldonata pterocarpi* which has the tiny black conidiomata at centre of leaf spots surrounded by the ascomata with muriform ascospores. The asexual state of *Viegasella pulchella* has not previously been reported. The pycnidia-like black tiny dots surrounding the ascomata lack contents.

Viegasella pulchella (Speg.) Inácio & P.F. Cannon, Mycol. Res. 107(1): 83 (2003).

MycoBank: M 373406, *Facesoffungi number*: FOF00314. Fig. 5.

≡ *Parmularia pulchella* (Speg.) Sacc. & P. Syd., Syll. fung. (Abellini) 14(2): 709 (1899).

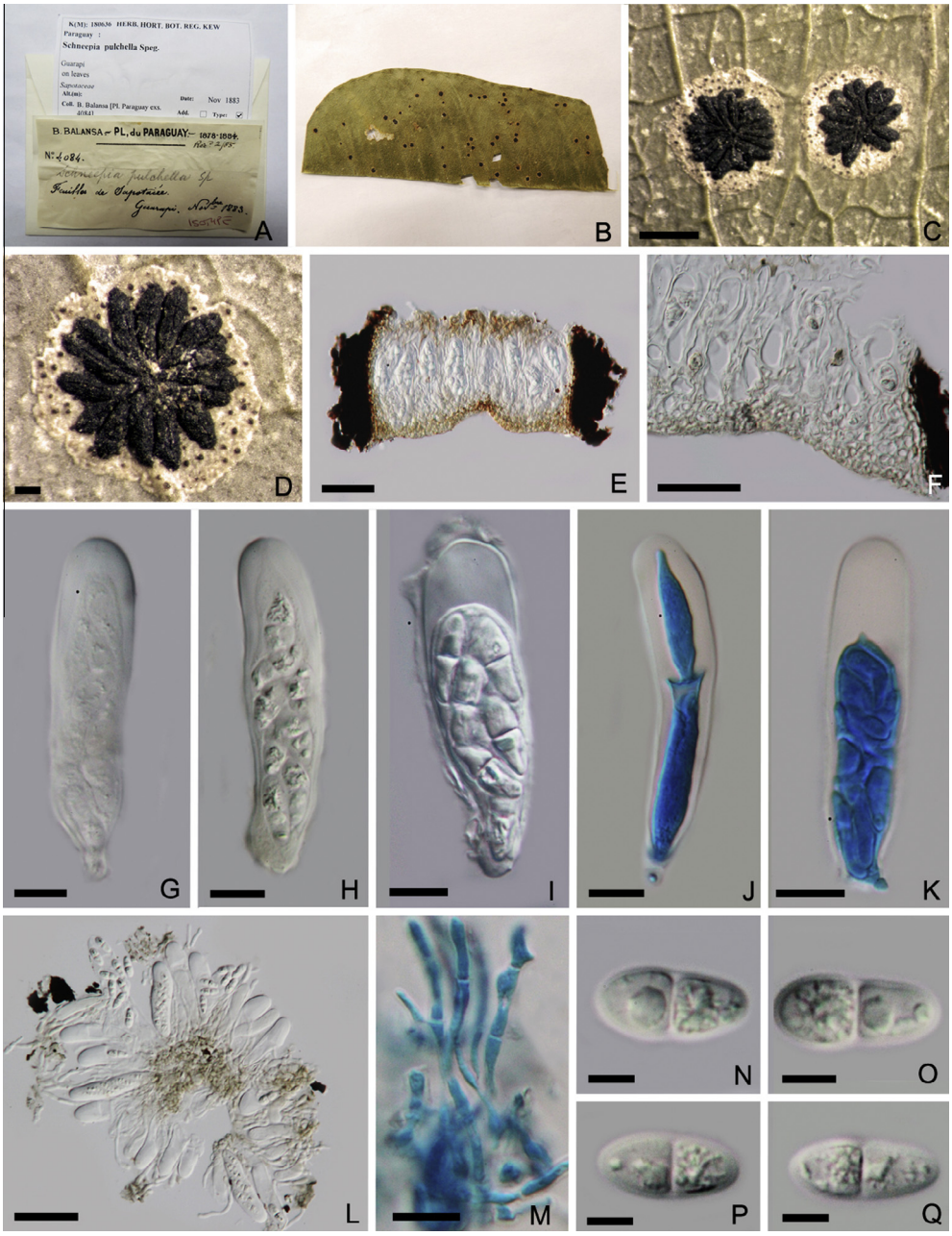

Figure 5 *Viegasella pulchella* (isotype). (A) Herbarium labels, (B) Herbarium material, (C and D) ascomata on host surface, (E and F) section of ascomata, (G–L) asci with ascospores, note J and K mounted in cotton blue reagent, (M) Hamathecium. (N–Q) ascospores (scale bars: C = 1000 μm, D = 200 μm, E and F, L = 50 μm, G–K, M = 10 μm, N–Q = 5 μm).

≡ *Schneepia pulchella* Speg., Anal. Soc. cient. argent. 26(1): 55 (1888).

Parasitic on the upper leaf surface. **Sexual state**: Spots solitary, scattered, sometimes confluent, variable in shape, circular to irregular, light brown to reddish with a diffuse edge. *Ascomata* 482–765 × 174–232 μm ($\overline{X}$ = 574 × 203 μm, n = 10), superficial on the leaves, black, shiny, appearing as flexuous lines on the leaf spot surface, opening by longitudinal splits. *Ostiole* conspicuous. *Peridium* thick at the sides, 27–34 μm ($\overline{X}$ = 31 μm, n = 10), composed of brown to black thick-walled cells of *textura angularis*, outer cells thick, brown to black, and internal hyaline cells, two-layered. Upper and lower wall thin, not well developed, sometimes absent. *Hamathecium* 2–5 μm broad with transverse septa, filamentous pseudoparaphyses, long, colourless, branched, and verrucose at the tips. *Young asci* variable in shape, cylindric-clavate to clavate, with a subapical chamber visible before spore delimitation. *Mature Asci* 52–79 × 11–19 μm ($\overline{X}$ = 63 × 14 μm, n = 10), 8-spored, bitunicate, cylindrical to clavate, thick-walled particularly in the upper part, short-pedicellate, thin-walled, embedded in mucilage. *Ascospores* 11–17 × 6–8 μm ($\overline{X}$ = 15 × 6 μm, n = 10), biseriate or multiseriate, ellipsoid to fusiform or narrowly ovoid, verrucose, usually two-celled, normally unequal, upper cell wider, lower

cell narrow and longer, transverse septa obviously shrink, hyaline and then becoming light brown when spores are senescent, with an oil drop, the apex rounded and the base obtuse, the mucilaginous sheath degenerating at maturity. **Asexual state**: Unknown.

Material examined: PARAGUAY. Guarapi, on leaves of *Sapotaceae* sp., November 1883, B. Balansa (PL. Paraguay exs. 4084, K (M): 180636, **isotype**).

Acknowledgments

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References

Aly, A., Debbab, A., Proksch, P., 2011. Fifty years of drug discovery from fungi. Fungal Divers. 50, 3–19.

Batista, A.C., Ciferri, R., 1962. The Chaetothyriales. Sydowia Beih. 3, 129.

Batista, A.C., Ciferri, R., 1963. Capnodiales. Saccardoa 2, 1–296.

Batzer, J.C., Arias, M.M.D., Harrington, T.C., Gleason, J.Z., Crous, P.W., 2008. Four species of *Zygophiala* (*Schizothyriaceae*, Capnodiales) are associated with the sooty blotch and flyspeck complex on apple. Mycologia 100 (2), 246–258.

Bills, G.F., 1996. Isolation and analysis of endophytic fungal communities from woody plants. In: Redlin, S.C., Carris, L.M. (Eds.), Endophytic Fungi in Grasses and Woody Plants: Systematics, Ecology and Evolution. APS Press, St. Paul, Minnesota, USA, pp. 31–65.

Bisby, G.R., 1923. The literature on the classification of the Hysteriales. Trans. Br. Mycol. Soc. 8, 176–189.

Chomnunti, P., Schoch, C.L., Aguirre-hudson, B., Ko, T.W.K., Hongsanan, S., Jones, E.B.G., Kodsueb, R., Phookamsak, R., Chukeatirote, E., Bahkali, A.H., Hyde, K.D., 2011. *Capnodiaceae*. Fungal Divers. 51, 103–134.

Chomnunti, P., Bhat, D.J., Jones, E.B.G., Chukeatirote, E., Bahkali, A.H., Hyde, K.D., 2012a. *Trichomeriaceae*, a new sooty mould family of Chaetothyriales. Fungal Divers. 56, 63–76.

Chomnunti, P., Ko, T.W.K., Chukeatirote, E., Cai, L., Jones, E.B.G., Kodsueb, R., Chen, H., Hassan, B.A., Hyde, K.D., 2012b. Phylogeny of *Chaetothyriaceae* in northern Thailand including three new species. Mycologia 104, 382–395.

Chomnunti, P., Hongsanan, S., Aguirre-hudson, B., Tian, Q., Peršoh, D., Dhami, M.K., Alias, A.S., Xu, J.C., Liu, X.Z., Stadler, M., Hyde, K.D., 2014. The sooty moulds. Fungal Divers. 66, 1–36.

Dean, R.A., 1997. Signal pathways and appressorium morphogensis. Annu. Rev. Phytopathol. 35, 211–234.

Debbab, A., Aly, A.H., Proksch, P., 2011. Bioactive secondary metabolites from terrestrial endophytes and associated marine derived fungi. Fungal Divers. 49, 1–12.

Debbab, A., Aly, A.H., Proksch, P., 2012. Endophytes and associated marine derived fungi – ecological and chemical perspectives. Fungal Divers. 57, 45–83.

Debbab, A., Aly, A.H., Proksch, P., 2013. Mangrove derived fungal endophytes – a chemical and biological perception. Fungal Divers. 61, 1–27.

Gleason, M.L., Batzer, J.C., Sun, G.Y., Zhang, R., Arias, M.M.D., Sutton, T.B., Crous, P.W., Ivanović, M., Mcmanus, P.S., Cooley, D.R., Mayr, U., Weber, R.W.S., Yoder, K.S., Ponte, E.M.D., Biggs, A.R., Oertel, B., 2011. A new view of sooty blotch and flyspeck. Plant Dis. 95 (4), 368–383.

Gregroy, S.S., John, W.T., 1999. Phylogenetic relationships of *Meliola* and *Meliolina* inferred from nuclear small subunit rRNA sequences. Mycol. Res. 103 (8), 1049–1056.

Hansford, C.G., 1946. The foliicolous Ascomycetes, their parasites and associated fungi. Mycol. Pap. 15, 1–240.

Hongsanan, S., Chomnunti, P., Crous, P.W., Chukeatirote, E., Hyde, K.D., 2014a. Introducing *Chaetothyriothecium*, a new genus of Microthyriales. Phytotaxa 161 (2), 157–164.

Hongsanan, S., Bahkali, A.H., McKenzie, E.H.C., Chukeatirote, E., Hyde, K.D., 2014b. *Trichopeltinaceae* (Dothideomycetes), an earlier name for *Brefeldiellaceae*, with a new species of *Trichopeltina*. Phytotaxa 176 (1), 270–282.

Hofmann, T.A., 2010. Plant parasitic *Asterinaceae* and *Microthyriaceae* from the Neotropics (Panama) (Ph.D. thesis, 408). The Faculty of Biological Sciences at the JW Goethe-University Frankfurt am Main, Germany.

Hosagoudar, V.B., Riju, M.C., 2011. Some interesting *Meliolaceae* members from Western Ghats Region of Kerala State. Plant Pathol. Quar. J. Fungal Biol. 1 (2), 121–129.

Hosagoudar, V.B., 2012. Asterinales of India. Mycosphere 2 (5), 617–852.

Hughes, S.J., 1993. *Meliolina* and its excluded species. Mycol. Pap. 66, 1–255.

Hyde, K.D., 1996. Biodiversity of fungi in North Queensland. Aust. Syst. Bot. 9, 261–271.

Hyde, K.D., 2001. Non-lichenised Australian Ascomycetes. Aust. Syst. Bot. 14, 357–375.

Hyde, K.D., Jones, E.B.G., Liu, J.K., Ariyawansa, H., Boehm, E., Boonmee, S., Braun, U., Chomnunti, P., Crous, P.W., Dai, D.Q., Diederich, P., Dissanayake, A., Doilom, M., Doveri, F., Hongsanan, S., Jayawardena, R., Lawrey, J.D., Li, Y.M., Liu, Y.X., Lücking, R., Monkai, J., Muggia, L., Nelsen, M.P., Pang, K.L., Phookamsak, R., Senanayake, I., Shearer, C.A., Suetrong, S., Tanaka, K., Thambugala, K.M., Wijayawardene, N.N., Wikee, S., Wu, H.X., Zhang, Y., Aguirre-hudson, B., Alias, S.A., Aptroot, A., Bahkali, A.H., Bezerra, J.L., Bhat, D.J., Camporesi, E., Chukeatirote, E., Gueidan, C., Hawksworth, D.L., Hirayama, K., Hoog, S.D., Kang, J.C., Knudsen, K., Li, W.J., Li, X.H., Liu, Z.Y., Mapook, A., McKenzie, E.H.C., Miller, A.N., Mortimer, P.E., Phillips, A.J.L., Raja, H.A., Scheuer, C., Schumm, F., Taylor, J.E., Tian, Q., Tibpromma, S., Wanasinghe, D.N., Wang, Y., Xu, J.C., Yan, J.Y., Yacharoen, S., Zhang, M., 2013. Families of Dothideomycetes. Fungal Divers. 63 (1), 1–313.

Inácio, C.A., Cannon, P.F., 2003. *Viegasella* and *Mintera*, two new genera of *Parmulariaceae* (Ascomycota), with notes on the species referred to *Schneepia*. Mycol. Res. 107 (1), 82–92.

Inácio, C.A., Minter, D.W., 2002. IMI descriptions of fungi and bacteria. 145 (1445).

Inácio, C.A., Cannon, P.F., 2008. The genera of Parmulariaceae. In: CBS Biodiversity Series 8. CBS Fungal Biodiversity Centre.

Ivanović, M.M., Ivanović, M.S., Batzer, J.C., Tatalović, N., Oertel, B., Latinović, J., Latinović, N., Gleason, M.L., 2010. Fungi in the apple sooty blotch and flyspeck complex from Serbia and Montenegro. Plant Path. 92 (1), 65–72.

Mibey, R.K., Hawksworth, D., 1995. *Diporothecaceae*, a new family of ascomycetes, and the term 'hyphopodium'. Syst. Ascomycetum 14, 25–31.

Müller, E., Patil, B.V., 1973. The genus *Aldona* Raciborski (Ascomycetes). Trans. Brit. Mycol. Soc. 60 (1), 117–121.

Müller, E., Von Arx, J.A., 1962. Die Gattungen der didymosporen Pyrenomyceten. Beitr. Kryptogamenfl. Schweiz. 11 (2), 1–922.
Nannfeldt, J.A., 1932. Studien uber die Morphologie und Systematik der nicht-lichenisierten, inoperkulaten Discomyceten. Nova Acta Regiae Soc. Sci. Upsal. IV 8, 1–368.
Penzig, O., Saccardo, P.A., 1904. Icones fungorum javanicorum. Otto Albert Julius Penzig, pp. 1–124.
Raciborski, M., 1900. Batavia, Indonesia, Staatsdruckerei. Parasitische Algen und Pilze Javas. 1, pp. 1–39.
Reynolds, D.R., Gilbert, G.S., 2005. Epifoliar fungi from Queensland Australia. Aust. Syst. Bot. 18, 265–289.
Saccardo, P.A., 1904. De Diagnostica et nomenclatura mycologica, Admonita quaedam. Ann. Mycol. 2, 195–198.
Sivanesan, A., Sinha, A.R.P., 1989. *Aldonata*, a new ascomycete genus in the *Parmulariaceae*. Mycol. Res. 92 (2), 246–249.
Spegazzini, C., 1888. Fungi Guaranitici. Pugillus II. Annales de la Socieda Científica Argentina 26, 5–74.
Stover, R.H., 1975. Sooty moulds of bananas. Trans. Brit. Mycol. Soc. 65, 328–330.
Teodora, N.G., 1937. An enumeration of Philippine fungi. Technical Bulletin. Department of Agriculture and Commerce, Philippine Islands, No. 4, pp. 1–585.
Wijayawardene, N.N., Crous, P.W., Kirk, P.M., Hawksworth, D.L., Boonmee, S., Braun, U., Dai, D.Q., D'souza, M.J., Diederich, P., Dissanayake, A., Doilom, M., Hongsanan, S., Jones, E.B.G., Groenewald, J.Z., Jayawardena, R., Lawrey, J.D., Liu, J.K., Lücking, R., Madrid, H., Manamgoda, D.S., Muggia, L., Nelsen, M.P., Phookamsak, R., Suetrong, S., Tanaka, K., Thambugala, K.M., Wanasinghe, D.N., Wikee, S., Zhang, Y., Aptroot, A., Ariyawansa, H.A., Bahkali, A.H., Bhat, J.D., Gueidan, C., Chomnunti, P., De Hoog, G.S., Knudsen, K., Li, W.J., McKenzie, E.H.C., Miller, A.N., Mortimer, P.E., Phillips, A.J.L., Piątek, M., Raja, H.A., Shivas, R.S., Slippers, B., Taylor, J.E., Tian, Q., Wang, Y., Woudenberg, J.H.C., Cai, L., Jaklitsch, W.M., Hyde, K.D., 2014. Naming and outline of Dothideomycetes–2014 including proposals for the protection or suppression of generic names. Fungal Divers. 69 (1), 1–55.
Wu, H.X., Schoch, C.L., Boonmee, S., Bahkali, A.H., Chomnunti, P., Hyde, K.D., 2011. A reappraisal of *Microthyriaceae*. Fungal Divers. 51, 189–248.
Wu, H.X., Li, Y.M., Ariyawansa, H.A., Li, W.J., Yang, H., Hyde, K.D., 2014a. A new species of *Microthyrium* from Yunnan, China. Phytotaxa 176 (1), 213–218.
Wu, H.X., Tian, Q., Li, W.J., Hyde, K.D., 2014b. A reappraisal of *Microthyriaceae*. Phytotaxa 176 (1), 201–212.
Von Arx, J.A., Müller, E., 1975. A reevaluation of the bitunicate ascomycetes with keys to families and genera. Stud. Mycol. 9, 1–159.
Von Höhnel, F.X.R., 1917. System der Phacidiales v. H [M]. Borntraeger.
Yi, M., Valent, B., 2013. Communication between filamentous pathogens and plants at the biotrophic interface. Ann. Rev. Phytopathol. 51, 587–611.
Zogg, H., 1962. Die Hysteriaceae s. str. und Lophiaceae, unter besonderer Berücksichtigung der mitteleuropäischen Formen. Beitr Kryptogamenflora Schweiz 11, 1–190.

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