



# Microfungi associated with *Clematis* (Ranunculaceae) with an integrated approach to delimiting species boundaries

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## Abstract

The cosmopolitan plant genus *Clematis* contains many climbing species that can be found worldwide. The genus occurs in the wild and is grown commercially for horticulture. Microfungi on *Clematis* were collected from Belgium, China, Italy, Thailand and the UK. They are characterized by morphology and analyses of gene sequence data using an integrated species concept to validate identifications. The study revealed two new families, 12 new genera, 50 new species, 26 new host records with one dimorphic character report, and ten species are transferred to other genera. The new families revealed by multigene phylogeny are Longiostiolaceae and Pseudomassarinae in Pleosporales (Dothideomycetes). New genera are *Anthodidymella* (Didymellaceae), *Anthosulcatispora* and *Parasulcatispora* (Sulcatisporaceae), *Fusiformispora* (Amniculicolaceae), *Longispora* (Phaeosphaeriaceae), *Neobyssosphaeria* (Melanommataceae), *Neoleptospora* (*Chaetosphaeriales*, genera *incertae sedis*), *Neostictis* (Stictidaceae), *Pseudohelminthosporium* (Neomassarinae), *Pseudomassarina* (Pseudomassarinae), *Sclerenchymomyces* (Leptosphaeriaceae) and *Xenoplectosphaerella* (Plectosphaerellaceae). The newly described species are *Alloleptosphaeria clematidis*, *Anthodidymella ranunculacearum*, *Anthosulcatispora subglobosa*, *Aquadictyospora clematidis*, *Brunneofusispora clematidis*, *Chaetosphaeronema clematidicola*, *C. clematidis*, *Chromolaenica clematidis*, *Diaporthe clematidina*, *Dictyocheirospora clematidis*, *Distoseptispora clematidis*, *Floricola clematidis*, *Fusiformispora clematidis*, *Hermatomyces clematidis*, *Leptospora clematidis*, *Longispora clematidis*, *Massariosphaeria clematidis*, *Melomastia clematidis*, *M. fulvicomae*, *Neobyssosphaeria clematidis*, *Neoleptospora clematidis*, *Neoroussouella clematidis*, *N. fulvicomae*, *Neostictis nigricans*, *Neovaginatisspora clematidis*, *Parasulcatispora clematidis*, *Parathyridaria clematidis*, *P. serratifoliae*, *P. virginianae*, *Periconia verrucosa*, *Phomatospora uniseriata*, *Pleopunctum clematidis*, *Pseudocapulatispora clematidis*, *Pseudocoleophoma clematidis*, *Pseudohelminthosporium clematidis*, *Pseudolophiostoma chiangraiense*, *P. clematidis*, *Pseudomassarina clematidis*, *Ramusculicola clematidis*, *Sarocladium clematidis*, *Sclerenchymomyces clematidis*, *Sigarispora clematidicola*, *S. clematidis*, *S. montanae*, *Sordaria clematidis*, *Stemphylium clematidis*, *Wojnowiciella clematidis*, *Xenodidymella clematidis*, *Xenomassariosphaeria clematidis* and *Xenoplectosphaerella clematidis*. The following fungi are recorded on *Clematis* species for the first time: *Angustimassarina rosarum*, *Dendryphion europaeum*, *Dermatopleospora mariae*, *Diaporthe ravennica*, *D. rudis*, *Dichotomopilus ramosissimum*, *Dictyocheirospora xishuangbannaensis*, *Didymosphaeria rubi-ulmifolii*, *Fitzroyomyces cyperacearum*, *Fusarium celtidicola*, *Leptospora thailandica*, *Memnoniella oblongispora*, *Neodidymelliopsis longicolla*, *Neoeutypella baoshanensis*, *Neoroussouella heveae*, *Nigrograna chromolaenae*, *N. obliqua*, *Pestalotiopsis verruculosa*, *Pseudoberkleasmiium chiangmaiense*, *Pseudoophiobolus rosae*, *Pseudoroussouella chromolaenae*, *P. elaeicola*, *Ramusculicola thailandica*, *Stemphylium vesicarium* and *Torula chromolaenae*. The new combinations are *Anthodidymella clematidis* ( $\equiv$  *Didymella clematidis*), *A. vitalbina* ( $\equiv$  *Didymella vitalbina*), *Anthosulcatispora brunnea* ( $\equiv$  *Neobambusicola brunnea*), *Fuscohypha kunmingensis* ( $\equiv$  *Plectosphaerella kunmingensis*), *Magnibotryascoma rubriostiolata* ( $\equiv$  *Teichospora rubriostiolata*), *Pararoussouella mangrovei* ( $\equiv$  *Roussouella mangrovei*), *Pseudoneoconiothyrium euonymi* ( $\equiv$  *Roussouella euonymi*), *Sclerenchymomyces jonesii* ( $\equiv$  *Neoleptosphaeria jonesii*), *Stemphylium rosae* ( $\equiv$  *Pleospora rosae*), and *S. rosae-caninae* ( $\equiv$  *Pleospora rosae-caninae*). The microfungi on *Clematis* is distributed in several classes of

Ascomycota. The analyses are based on morphological examination of specimens, coupled with phylogenetic sequence data. To the best of our knowledge, the consolidated species concept approach is recommended in validating species.

**Keywords** 73 new taxa · Ascomycota · Belgium · China · Dothideomycetes · *Incertae sedis* · Italy · Lecanoromycetes · Phylogeny · Sordariomycetes · Taxonomy · Thailand · UK

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**Introduction**

*Clematis* (Ranunculaceae) is a flowering climber which has become a popular plant in horticulture (Linnaeus 1753; Yuan et al. 2010). The genus contains between 250 and 350 species and hybrids (Grey-Wilson 2000; Lehtonen et al. 2016; He et al. 2019). *Clematis* is widespread in warm-temperate or montane ecosystems and is native to most areas of China, Europe, Korea, and Russia (Tamura 1956; Ziman and Keener 1989; Yuan and Yang 2020). *Clematis vitalba* (old man's beard), the type species of *Clematis* is an invasive weed broadly distributed in Europe, and also expanding to New Zealand and South America (Ogle et al. 2000; Leuschner and Ellenberg 2017; Redmond and Stout 2018). *Clematis vitalba* can influence the biodiversity dynamics of native plants (Ogle et al. 2000; Ashton and Lerdau 2008). In Thailand, *Clematis* species are mostly found in the northern provinces of Chiang Mai, Chiang Rai and Nan, which are mountainous areas with a tropical savanna climate (Tamura 1997, 2000). Many *Clematis* species are grown as ornamental plants. *Clematis* species are also used in Traditional Chinese Medicine and some secondary metabolites isolated from *Clematis* have been tested in vitro, but there are no reports of successful clinical tests or if it is safe to consume the plant parts (Ding et al. 2009; Fu et al. 2010; Feng et al. 2011; Hawaze et al. 2012; Lu et al. 2014; Zhao et al. 2016). *Clematis* species are herbaceous vines, with

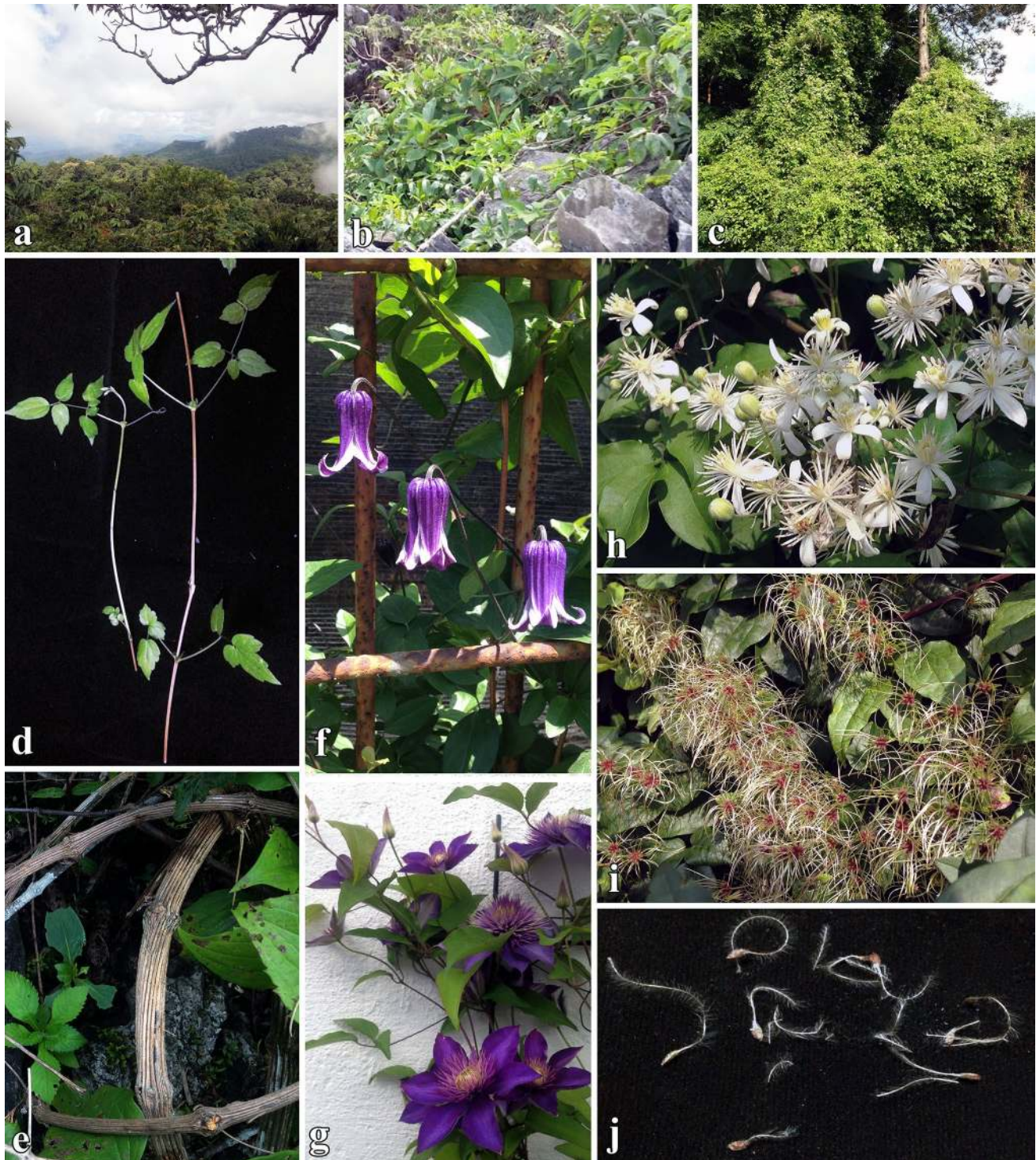
opposite compound, bipinnate to tripinnate leaves, and leather-like flowers with feather achenes (Johnson 2001) (Fig. 1). Section-level phylogenetic classification of *Clematis* by Lehtonen et al. (2016) includes specific characteristics and geographic distribution for each section. The estimated divergence time of the available sequences for *Clematis* have shown that the stem age was in the Oligocene (25.99 million years ago; Lehtonen et al. 2016).

Fungal species associated with *Clematis* have been documented since the late eighteenth century (Lamarck 1805; Saccardo 1892; Farr and Rossman 2020; Index Fungorum 2020). Index Fungorum and the U.S. National Fungus Collections Fungal Database lists over 500 records, mainly as saprobes, or pathogens that can cause leaf lesions and wilt in *Clematis* species (Baylis 1954; Braun 1992; Ahn and Shearer 1998; Wanasinghe et al. 2014; Chen et al. 2015; Crous et al. 2019).

In this study, *Clematis* samples were collected in Belgium, China, Italy, Thailand, and the UK to establish the microfungi associated with this host and to analyze their host-specificity. In addition, fungal isolates were evaluated for their antagonistic activity against selected microorganism (Phukhamsakda et al. 2018; Hyde et al. 2019b; Macabeo et al. 2020). The delineation of new species introduced in this study relies on a polyphasic approach based on morphological traits (MSC), molecular data (PSC), and application of Genealogical Concordance Phylogenetic Species Recognition (GCPSR) (Taylor et al. 2000). GCPSR model relies on performing a pairwise homoplasy index coupled with phylogenetic relatedness in a multi-locus dataset and the interpretation of nucleotide differences (Turner et al. 2013; Quaedvlieg et al. 2014; Jeewon and Hyde 2016). We also compared the morphology of our new collections with documented fungal taxa recorded in public databases and discuss their ecological species concepts.

**Materials and methods****Sample collection, morphological study and isolation**

Fresh *Clematis* specimens were collected or received from Belgium, China, Italy, Thailand and England (the UK). Some specimens have single collection because this study mainly focused on the diversity of fungi associated with *Clematis*. One collection is defined as a sample of fungus that can be identified with a single collecting trip which was used to cast the number of species. Thus, the plant materials were mainly collected and received from a single trip of the aforementioned countries. The specimens were maintained



**Fig. 1** **a–c** Habitats of *Clematis* species. **d** Opposite compound with bi-pinnate to tri-pinnate leaves. **e** Woody climbing stem. **f** Inflorescence of *C. pitcheri*. **g** Inflorescence of *C.* “Crystal Fountain”.

**h** Inflorescence of *C. vitalba*. **i** Achenes of *C. vitalba*. **j** Enlarged achenes of *C. subumbellata* with plumose style

in paper bags for transport to the laboratory. The specimens were examined using a Motic SMZ 168 Series stereo-microscope. Thereafter, vertical free-hand sections were made by a razor blade and placed on a droplet of sterilized water on a glass slide. A Nikon ECLIPSE 80i compound microscope was used to examine the samples and a Canon 600D digital camera fitted to the microscope was used to photograph the samples. Tarosoft (R) Image Frame Work program was used for measurements and photo-plate were made by using Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, United States).

Pure cultures were obtained from single ascospores isolation on malt extract agar (MEA: 33.6 g/L, malt extract Difco™) or potato dextrose agar (PDA: 39 g/L, potato dextrose media Difco™) as described by Chomnunti et al. (2014) which were incubated at 16–25 °C with the standard light cycles, 12 h in the light followed by 12 h in the dark for about four up to eight weeks. Asexual reproduction was induced by placing agar squares with mycelia on water agar or MEA placed with additional substances such as sterile pine needles or rice straws. Authentic type specimens are deposited in Mae Fah Luang University (MFLU) herbarium and ex-type living cultures are deposited at the Mae Fah Luang Culture Collection (MFLUCC). Faces of fungi numbers (Jayasiri et al. 2015) and Index Fungorum numbers (2020) are provided.

### DNA extraction, amplification and sequencing

The Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) (Hangzhou, P. R. China) and gene extraction kit (Bio Basic Inc., Canada) were used for DNA extraction from mycelium. The fruiting bodies DNA was extracted by using Forensic DNA Kit–D3591-01 (OMEGA bio-tek) following the manufacturer's instructions. Polymerase chain reaction (PCR) was used to amplify partial gene regions with primer pairs as described in Tibpromma et al. (2018). The PCR amplifications were performed in a total volume of 25 µL solution containing 10–20 ng of DNA template, Easy Taq PCR Super Mix (mixture of Easy Taq™ DNA Polymerase, dNTPs, and optimized buffer) and 10 picomolar forward and reverse primers. Amplification reactions were performed following Phukhamsakda et al. (2016) and Tibpromma et al. (2018). Genomic DNA and PCR amplification products were checked on 1% agarose gel. PCR products were purified as described in the manufacturer's instructions (EZ-10 PCR Products Purification Kit, Bio basic Canada INC.). Sequences were generated by Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China) and the sequencing service at Helmholtz Centre For Infection Research (HZI, Braunschweig, Germany).

### Sequence alignment and phylogenetic analysis

Consensus sequences were assembled using SeqMan v. 7.0.0 (DNASTAR, Madison, WI). Sequences of closely related strains were retrieved using BLAST searches against GenBank (<http://www.ncbi.nlm.nih.gov>). Sequences were aligned with MAFFT version 7 (Katoh et al. 2019) (<http://mafft.cbrc.jp/alignment/server>), with minimal adjustment of the ambiguous nucleotides by visual examination and manually corrected in AliView program (Larsson 2014). Leading or trailing gaps exceeding the primer binding site were trimmed from the alignments prior to tree building and alignment gaps were treated as missing data. The concatenation of the multigene datasets was created in Sequence Matrix (Vaidya et al. 2011).

Phylogenetic analyses of the single gene and combined gene were based on maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference posterior probabilities (BYPP). PAUP program was used for MP bootstrap analyses, with 1000 bootstrap replicates using 10 rounds of the heuristic search replicates to estimate the homoplasy yield. The random addition of sequences and subsequent TBR branch swapping during each bootstrap replicate, with each replicate was limited to 1000 rearrangements. Gaps were treated as missing data; all characters were unordered and given equal weight. The statistics for parsimony were described under the phylogenetic legend with the values of Tree Length (TL), Consistency Index (CI), Retention Index (RI), Relative Consistency Index (RC) and Homoplasy Index (HI) calculated for trees generated under different optimality criteria. The best fitting substitution model for each single gene partition and the concatenated data set was determined in MrModeltest 2.3 (Nylander 2004) for Bayesian inference posterior probabilities and ML. Maximum likelihood analyses, including 1000 bootstrap replicates, were performed using the RAXML-HPC2 on XSEDE (8.2.12) in the CIPRES Science Gateway (Stamatakis 2014; Miller et al. 2017). The general time reversible (GTR) model was used for nucleotide substitution with a discrete gamma distribution plus invariant site (GTR + I + G). The bootstrap replicates were summarized onto the best scoring tree (Miller et al. 2017). The Bayesian inference posterior probabilities (PP) distribution (Zhaxybayeva and Gogarten 2002) was estimated by Markov Chain Monte Carlo sampling (MCMC) in MrBayes 3.2.2 on XSEDE (Ronquist and Huelsenbeck 2003). Six simultaneous Markov chains were run for 1,000,000 to 10,000,000 generations, depending on individual settings for the fungal group. The resulted trees were sampled at one tree every 100th or 1000th generation. The first 10–25% of burn-in phase of the analyses were discarded based on suitable burn-in phases determined by using Tracer version 1.7 (Rambaut et al. 2018). The remaining trees were used to calculate

posterior probabilities in the majority-rule consensus (MRC) trees (50%) with critical value for the topological convergence diagnostic set to 0.01.

FigTree v. 1.4 (Rambaut 2014) was used to visualize phylogenetic trees and data files and the phylogram was edited using Adobe Illustrator CS v. 6 (Adobe Systems, USA). All sequences generated in this study were submitted to GenBank. All entries are represented using phylogenetic tree and relevant description.

### Genealogical concordance phylogenetic species recognition analysis

The closely related strains that resulted from morphology and phylogeny evidence of recombination were prospectively analyzed using the genetic distances by performing a pairwise homoplasy index test ( $\Phi_w$ ) (Taylor et al. 2000; Bruen et al. 2006). A pairwise homoplasy index (PHI) test was performed in SplitsTree (version 4.1.4.4) using the Kimura's two parameter (K2P) models for low genetic distance datasets. LogDet transformation were applied for the average of nucleotide frequencies and splits decomposition graph options (Gu and Li 1996a, b; Taylor et al. 2000; Bruen et al. 2006; Huson and Bryant 2006; Gioan and Paul 2012; Nishimaki and Sato 2019). The standard deviation of split frequencies PHI test results ( $\Phi_w$ ) < 0.05 indicate significant recombination within the dataset.

### Taxonomy

#### Phylum Ascomycota R.H. Whittaker

The taxa are arranged as in the Outline of Fungi and fungus-like organisms (Wijayawardene et al. 2016, 2020).

#### Subphylum Pezizomycotina Erikss. & K. Winka

#### Class Dothideomycetes sensu O.E. Erikss & Winka

For the classification of Dothideomycetes we follow Hyde et al. (2013), Liu et al. (2017) and Hongsanan et al. (2020).

#### Subclass Pleosporomycetidae C.L. Schoch et al.

#### Pleosporales Luttrell ex M.E. Barr

Pleosporales is the largest and most diverse order in Dothideomycetes with over 75 families (Hongsanan et al. 2020).

#### Amniculicolaceae Y. Zhang, C.L. Schoch, J. Fourn., Crous & K.D. Hyde

Amniculicolaceae was introduced for freshwater-associated ascomycetes. This family is characterized by solitary ascomata with a rough black surface. The members usually stain the surface of the substrate purple and have short pedicellate asci, with hyaline or pale brown or brown, 1- to

multi-septate or muriform ascospores (Hyde et al. 2013). The family comprises *Amniculicola*, *Murispora*, *Neomasariosphaeria*, *Pseudomassariosphaeria* and *Vargamyces* (Zhang et al. 2009; Hyde et al. 2013; Ariyawansa et al. 2015a; Hernández-Restrepo et al. 2017). We introduce a novel saprobic genus, *Fusiformispora* from *Clematis* collections in Thailand. Maximum likelihood and Bayesian analyses of the combined dataset (LSU, SSU, ITS, *tef1* and *rpb2*) is shown in Fig. 2.

#### *Fusiformispora* Phukhams. & K.D. Hyde, gen. nov.

*Index Fungorum* number: IF557106; *Facesoffungi* number: FoF 07242, Fig. 3.

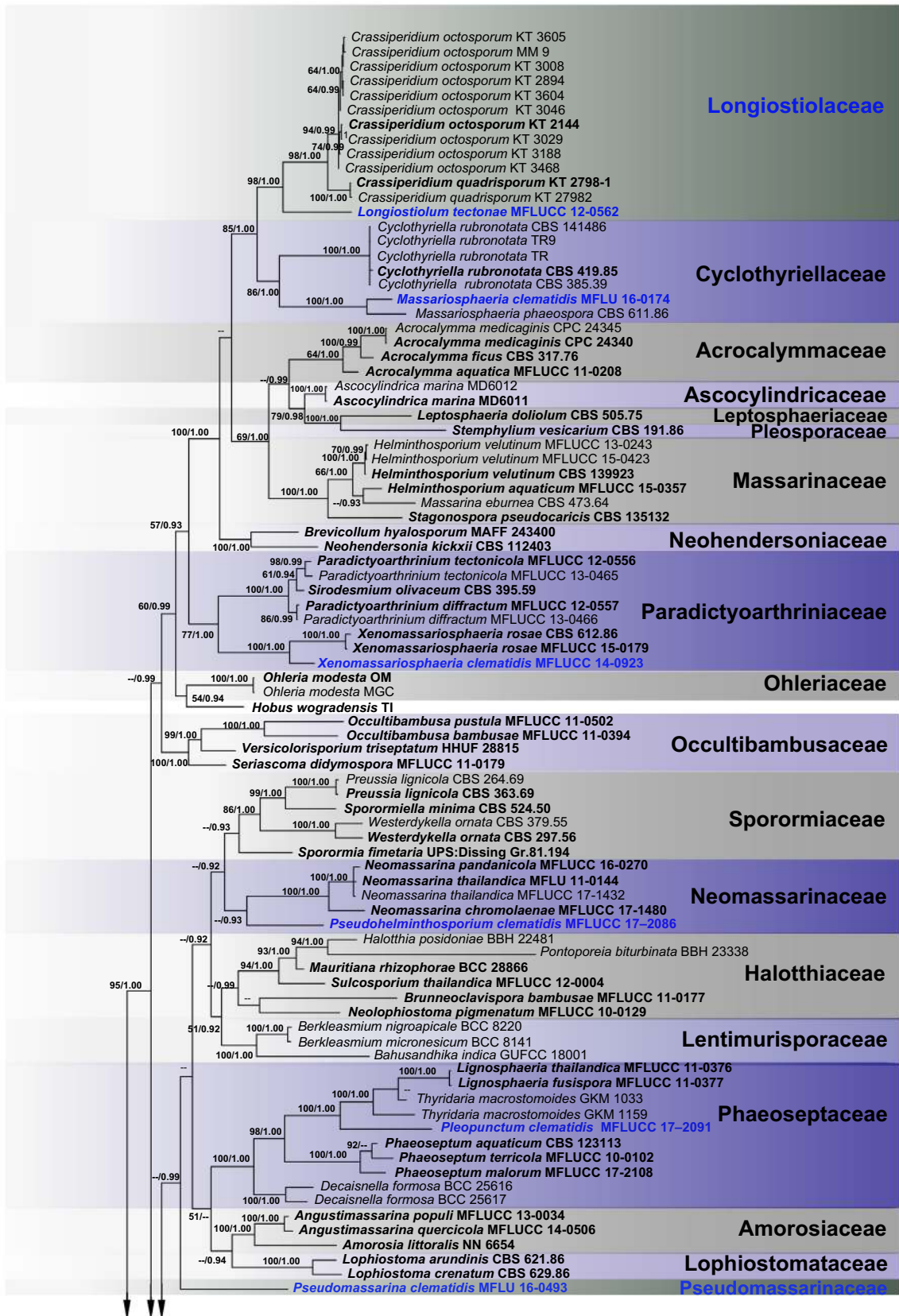
*Etymology*: Genus name reflects the fusiform shape of its ascospores.

*Saprobic* on decaying wood or herbaceous plant material in terrestrial habitats. **Sexual morph**: *Ascomata* on surface of the host, covered by a pseudoclypeus, visible as black spots, solitary, scattered, uniloculate, obpyriform to compressed globose, coriaceous, brown to dark brown, ostiolate. *Ostioles* central, brown to dark brown, papillate. *Peridium* multilayered, cells of *textura angularis*, inner layers comprising thin, hyaline cells. *Hamathecium* composed of dense, filiform, branched, transversely septate, trabecular pseudo-paraphyses anastomosing above asci. *Asci* 8-spored, bitunicate, fissitunicate, thick-walled, cylindrical-clavate, apically rounded, short, with a furcate pedicel, with ocular chamber. *Ascospores* biserial, partially overlapping, broad fusiform, tapering towards the acute ends, hyaline, with guttules in each cell, constricted at the septa, smooth-walled, with a thin mucilaginous sheath. **Asexual morph**: Undetermined.

*Type species*: *Fusiformispora clematidis* Phukhams., M.V. de Bult & K.D. Hyde

**Fig. 2** The Bayesian 50% majority-rule consensus phylogram based on combined LSU, SSU, ITS, *tef1* and *rpb2* sequence data of related families in Pleosporales. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted with species of Hysteriales. One hundred and fifty-three strains were included in the DNA analyses which comprised 4394 characters (848 characters for LSU, 1044 characters for SSU, 556 characters for ITS, 910 characters for *tef1*, and 1036 characters for *rpb2*, including gap regions). The tree from the maximum likelihood analysis had similar topology to the Bayesian analyses. The best scoring RAxML tree had a final likelihood value of  $-73089.933914$ . The matrix had 2676 distinct alignment patterns, with 40.81% undetermined characters and gaps. Estimated base frequencies were as follows; A=0.246412, C=0.245743, G=0.272077, T=0.235768; substitution rates AC=1.617324, AG=3.695355, AT=1.662826, CG=1.183453, CT=8.283938, GT=1.000000; gamma distribution shape parameter  $\alpha=0.635095$ . The GTR+I+G model was selected for every partition in Bayesian analysis. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. The type sequences are in bold and the species determined in this study are indicated in blue





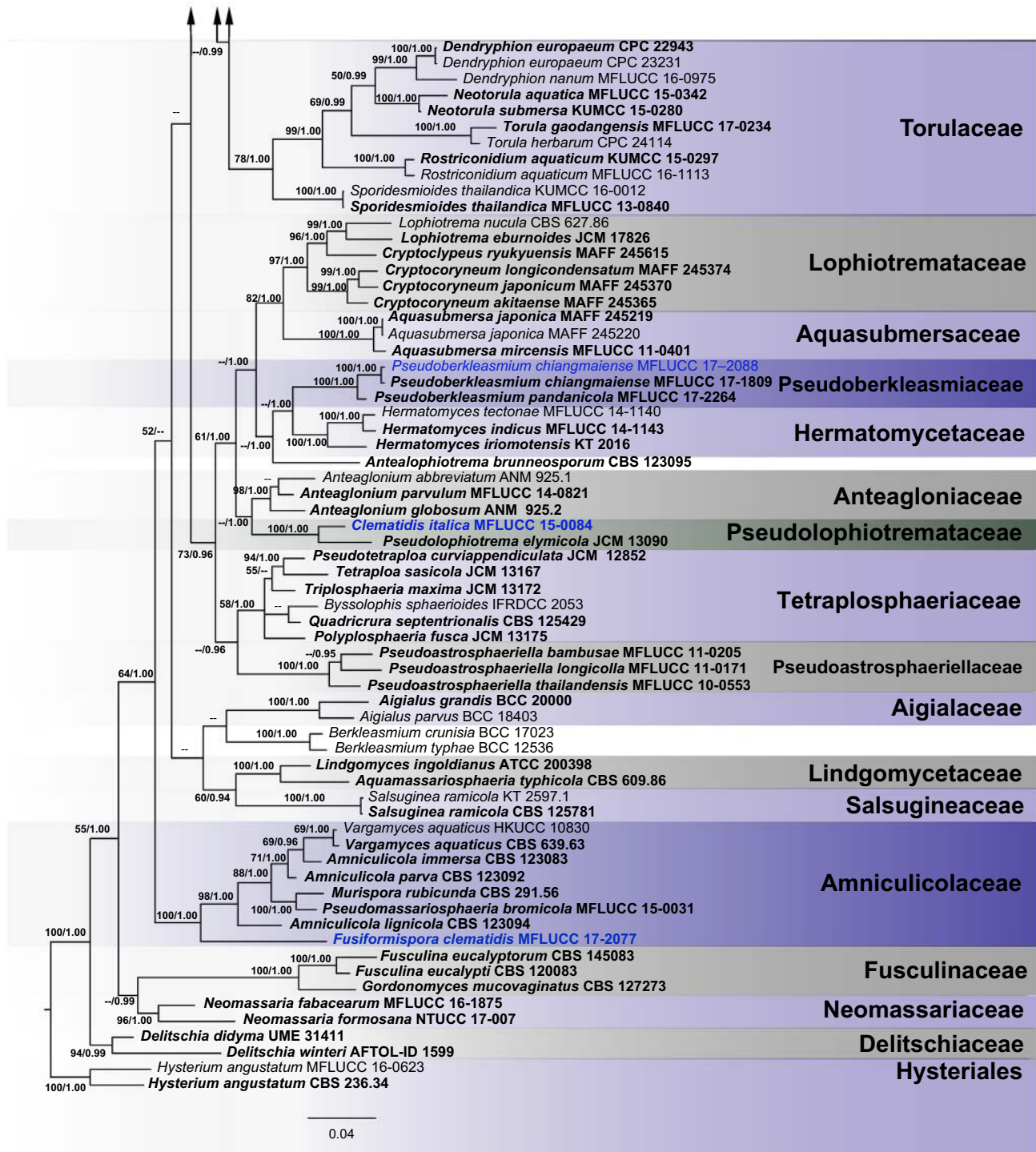
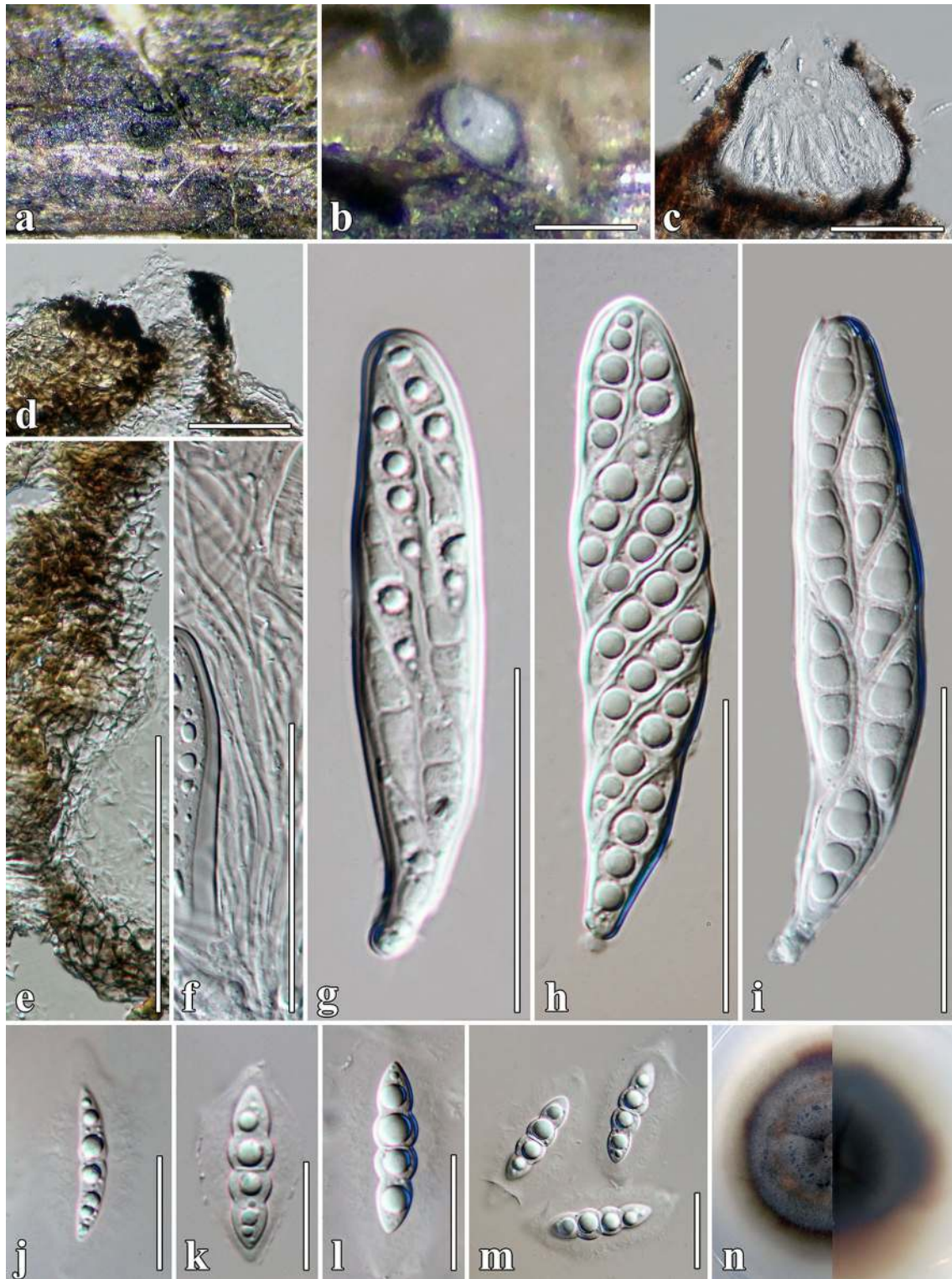


Fig. 2 (continued)

Notes: *Fusiformispora* is established as a monotypic genus. In the multi-gene phylogenetic analyses, the isolate MFLUCC 17-2077 formed a basal lineage with other genera in Amniculicolaceae (Fig. 2) with strong support (100% ML/1.00 BYPP). The genus is compatible with the

concept of Amniculicolaceae in having compressed globose, coriaceous, brown to dark brown ascomata, ostiolate, with trabeculate, anastomosed pseudoparaphyses (sensu Liew et al. 2000), and fusiform ascospores that are hyaline and septate with mucilaginous appendages (Zhang et al. 2009).



**Fig. 3** *Fusiformispora clematidis* (MFLU 17–1485, holotype). **a** Appearance of ascoma on host surface. **b** Close up of ascoma on host substrate. **c** Vertical section of ascoma. **d** Ostiolar canal. **e** Section

of peridium. **f** Pseudoparaphyses. **g–i** Asci. **j–m** Ascospores. **n** Culture characteristics on MEA. Scale bars: **b**=200  $\mu$ m, **c**=100  $\mu$ m, **d**, **j–m**=20  $\mu$ m, **e–i**=50  $\mu$ m

*Fusiformispora* is similar to *Amniculicola* Zhang & K.D. Hyde, however, the genus differs by having thinner peridium walls with sub-carbonaceous ascomatal type. *Amniculicola* is an aquatic genus and its species have cylindrical asci and uniseriate arrangement of ascospores, while *Fusiformispora* has cylindric-clavate asci and biseriate arrangement of ascospores and is from a terrestrial habitat. We therefore, introduce a new genus based on morphological and phylogenetic evidence for a fungal collection on *Clematis fulvicoma*.

***Fusiformispora clematidis*** Phukhams., M.V. de Bult & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557107; *Facesoffungi number*: FoF 07243, Fig. 3.

*Etymology*: Epithet reflects the host *Clematis*.

*Holotype*: MFLU 17–1485.

*Saprobic* on dead stems of *Clematis fulvicoma*. **Sexual morph**: *Ascomata* 165–190 × 200–275 µm ( $\bar{x}$  = 175 × 225 µm, n = 5), on surface of host, covered by a pseudoclypeus, visible as black spots, immersed to superficial, solitary, scattered, uniloculate, obpyriform to compressed globose, base flattened, brown to dark brown, partially carbonaceous, rough-walled, with apical ostioles. *Ostioles* central, 55 × 35 µm, brown to dark brown, papillate, with easily opening by a pore, filled with periphyses. *Peridium* 10–18 µm wide, multilayered, comprising 4–5 layers of brown to dark brown cells of *textura angularis*, inner layers comprising thin, hyaline cells. *Hamathecium* composed of dense, 0.5–1.5 µm wide ( $\bar{x}$  = 1.3 µm, n = 50), filiform, branched, trabeculate pseudoparaphyses, anastomosing above the asci, reaching the ostiole, transversely septate. *Asci* 86–127 × 18–24 µm ( $\bar{x}$  = 100 × 25 µm, n = 40), 8-spored, bitunicate, fissitunicate, thick-walled, cylindric-clavate, apically rounded, short, with furcate pedicel, ocular chamber clearly visible when immature. *Ascospores* 24–36 × 5–10 µm ( $\bar{x}$  = 30 × 8 µm, n = 50), biseriate, partially overlapping, broad fusiform, tapering towards the ends, acute at both ends, hyaline, with (1–)3–4 transverse septa, with large guttules in each cell, constricted at the septa, deeply constricted at the median septum, cell above median septum slightly wider than below, smooth-walled, with 4–12 µm wide mucilaginous sheath. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA reaching 50 mm diam. after 4 weeks at 25 °C. Cultures from above, grey-brown, with reddish brown mixed in the mycelium, dense, colonies circular, flat, umbonate, raised from the agar in the centre, dull, covered with aerial mycelium, white mycelium at the edge; reverse dark brown, dense, circular, with irregular, fimbriate margin, pinkish mycelium radiating outwardly.

*Material examined*: Thailand, Chiang Rai Province, on dead stems of *Clematis fulvicoma* Rehder & E.H. Wilson, 20 March 2017, C. Phukhamsakda, CMTH22 (MFLU 17–1485, **holotype**); ex-type living culture, MFLUCC 17–2077.

*Host*: *Clematis fulvicoma*—(This study).

*Distribution*: Thailand—(This study).

*GenBank accession numbers*: LSU: MT214542; SSU: MT226661; ITS: MT310589; *tefl*: MT394725; *rpb2*: MT394677.

*Notes*: In a BLASTn search of GenBank, the LSU sequence of *Fusiformispora clematidis* MFLUCC 17–2077 showed 96% similarity to *Lindgomyces pseudomadisonensis* KT 2742 (LC149916), while the ITS sequence had 91% similarity to *Vargamyces aquaticus* CBS 636.91 (NR\_154471). *Fusiformispora clematidis* is phylogenetically distinct, therefore we introduce the collection as a new species.

#### **Amorosiaceae** Thambug. & K.D. Hyde

Amorosiaceae was introduced for *Amorosa* Mantle & D. Hawksw. and *Angustimassarina* Thambugala, Tanaka & K.D. Hyde (Thambugala et al. 2015). Amorosiaceae is characterized by immersed or semi-immersed ascomata with a short crest-like papilla, and hyaline ascospores with a mucilaginous sheath. Asexual morphs of this family were described as sporodochia (Mantle et al. 2006; Thambugala et al. 2015). Jayasiri et al. (2019) reported *Amorocoelophoma* from a decaying pod of *Cassia* species. Phylogeny combined with morphological observations confirm the placement of *Amorocoelophoma* as the first coelomycetous species in Amorosiaceae (Fig. 4).

#### **Angustimassarina** Thambug., Kaz. Tanaka & K.D. Hyde

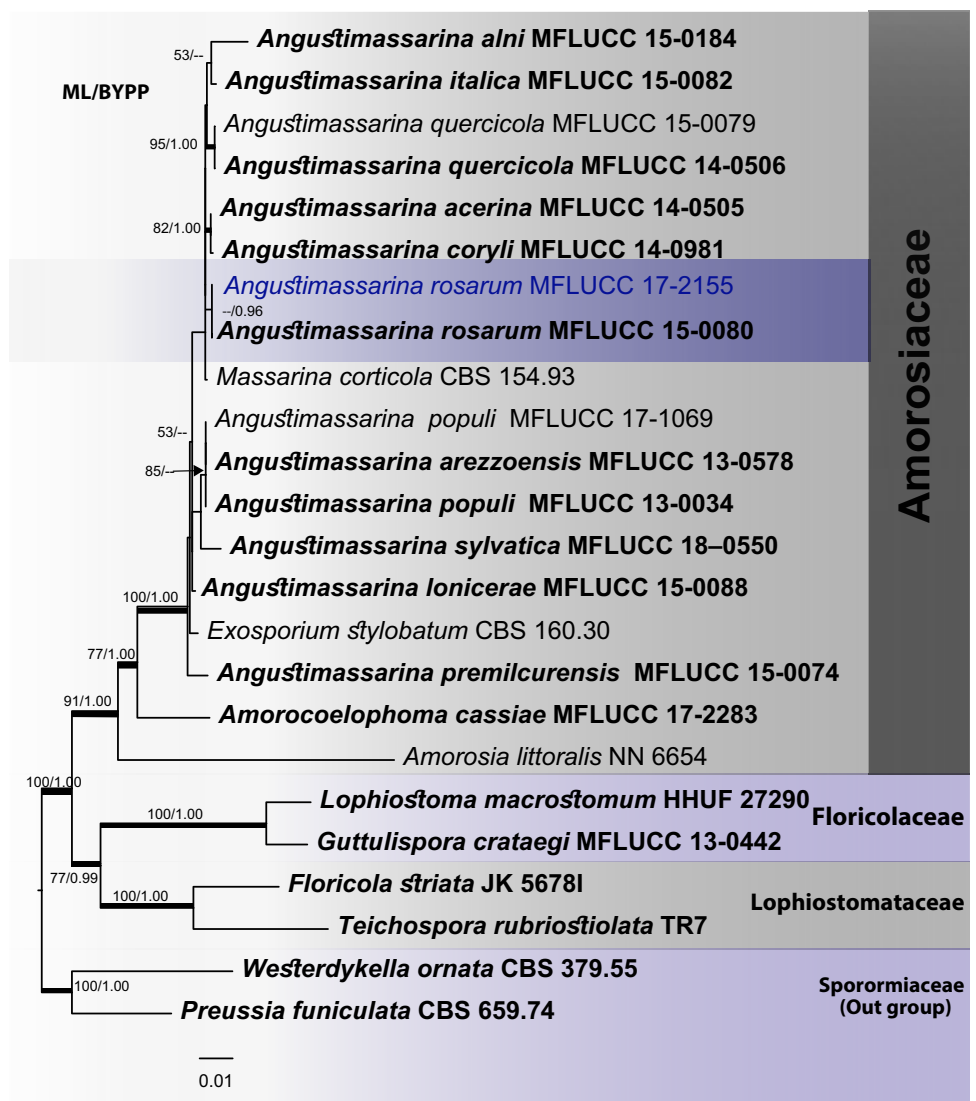
*Angustimassarina* was described for fungal species that have ascospores resembling *Massarina* while being narrowly fusiform in shape. The genus has immersed to semi-immersed ascomata, coriaceous, dark brown to black, globose to subglobose, ostiolate, cylindrical to cylindric-clavate asci and fusiform to cylindrical or ellipsoidal-fusiform ascospores surrounded by a mucilaginous sheath. The asexual morph of this genus is hyphomycetous. Twelve species are listed in Index Fungorum for *Angustimassarina* (Thambugala et al. 2015; Wanasinghe et al. 2018; Hyde et al. 2019a). In this study, *Angustimassarina rosarum* was isolated from *Clematis viticella* and identification was based on multigene phylogenetic analysis of LSU, SSU, ITS, and *tefl* sequence data (Fig. 4) and its compatible morphology (Fig. 5).

***Angustimassarina rosarum*** Tibpromma, Camporesi & K.D. Hyde, Fungal Diversity 89: [21] (2018), new host record.

*Index Fungorum number*: IF553939; *Facesoffungi number*: FoF 03964, Fig. 5.

*Saprobic* on dead stems of *Clematis viticella*. **Sexual morph**: *Ascomata* 221–306 × 267–400 µm ( $\bar{x}$  = 265 × 340 µm, n = 5), scattered, gregarious, immersed, coriaceous, dark globose to subglobose, brown to black, ostiolate. *Ostioles* 57 × 134 µm, central, rounded, papillate, with opening by a pore. *Peridium* 14–40(–60) µm ( $\bar{x}$  = 20 µm, n = 10), thick at

**Fig. 4** The best scoring RAxML tree with a final likelihood value of  $-10209.184183$  based on combined LSU, SSU, ITS and *tefl* sequence data for Amorosiaceae. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted with species of Sporormiaceae. The tree from the maximum likelihood analysis had similar topology to the Bayesian 50% majority-rule consensus phylogram. The matrix had 809 distinct alignment patterns with 21.55% undetermined characters and gaps. Estimated base frequencies were as follows; A=0.246053, C=0.243449, G=0.269921, T=0.240577; substitution rates AC=1.221779, AG=2.152972, AT=1.558241, CG=0.998233, CT=7.007203, GT=1.000000; gamma distribution shape parameter  $\alpha=2.180328$ . The species determined in this study is indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than (BS  $\geq 50\%$ /BYPP  $\geq 0.90$ )



the sides, broad at the apex and thinner at the base, comprising brown to dark brown cells of *textura angularis*, fusing at the outside with the host tissues. *Hamathecium* composed of dense, 1.5–2.5  $\mu\text{m}$  wide, septate, long, cellular pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 77–85  $\times$  10–16  $\mu\text{m}$  ( $\bar{x}=78 \times 15 \mu\text{m}$ ,  $n=30$ ), 8-spored, bitunicate, fissitunicate, broad cylindrical to cylindrical-clavate, with bulbous pedicel, rounded at the apex, with a minute ocular chamber. *Ascospores* 17–23  $\times$  4–4.5  $\mu\text{m}$  ( $\bar{x}=20 \times 4 \mu\text{m}$ ,  $n=15$ ), biseriate, partially overlapping, broad fusiform, hyaline, 1(–3) septate, deeply constricted at the primary septum, widest at the centre and tapering towards the ends, straight, smooth-walled, 1(–3)-guttulate, surrounded by a 5–10  $\mu\text{m}$  wide mucilaginous sheath. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Culture black in the middle, radiating white, dense, circular, umbonate, entries edge,

shiny, dull, undulate, radially furrowed, reverse black, radiating outwardly, white.

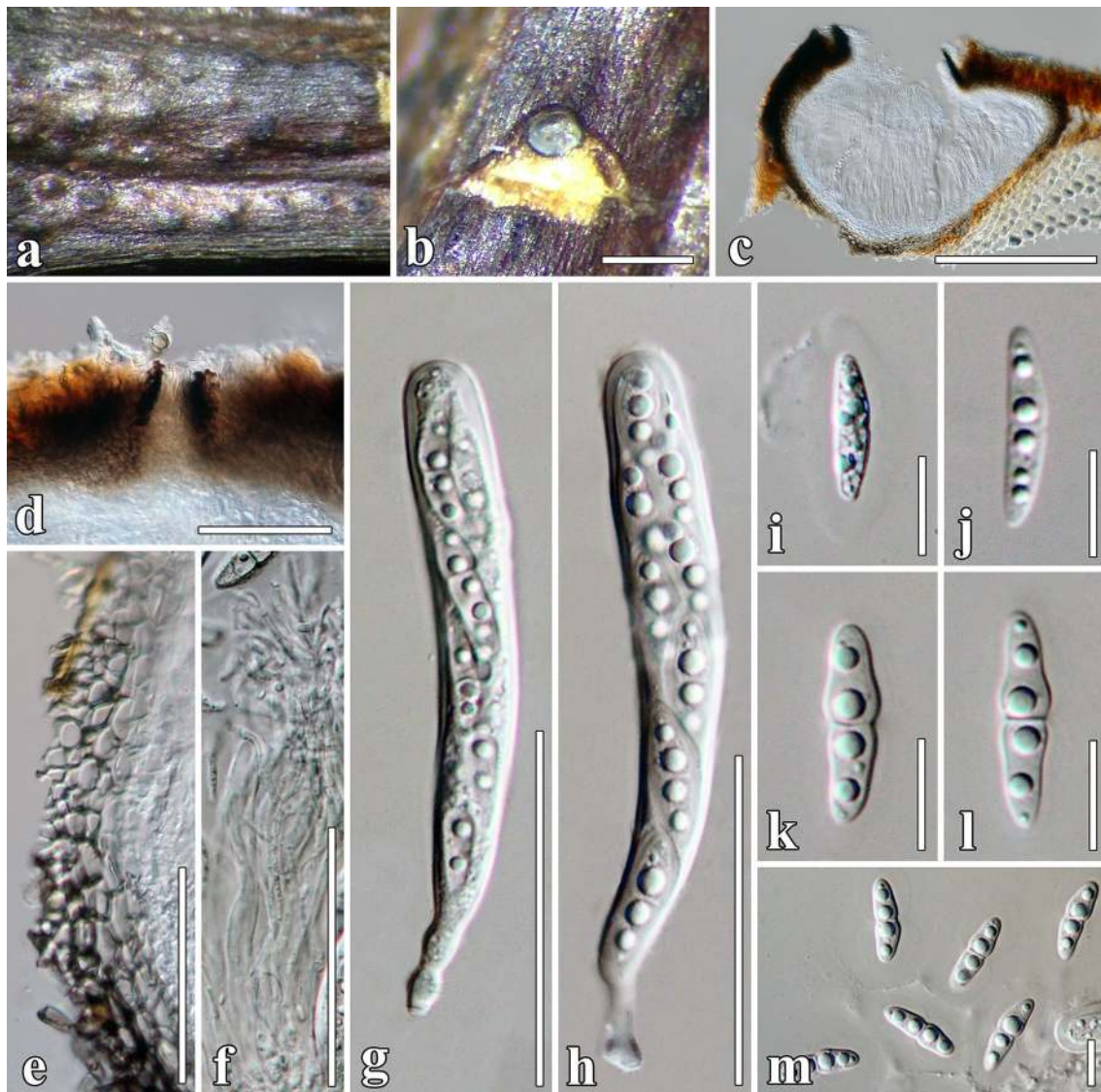
**Material examined:** Belgium, Flemish Brabant, Meise Botanic Garden, Bouchout Domain, dead stems of *Clematis viticella* L., 13 June 2017, D. Ertz & C. Gerstmanns, BRCV1 (MFLU 17–1513); living culture, MFLUCC 17–2155.

**Hosts:** *Clematis viticella*, *Rosa canina*—(Wanasinghe et al. 2018; this study).

**Distribution:** Belgium, Italy—(Wanasinghe et al. 2018, this study).

**GenBank accession numbers:** LSU: MT214543; SSU: MT226662; ITS: MT310590; *tefl*: MT394726; *rpb2*: MT394678.

**Notes:** *Angustimassarina rosarum* (MFLUCC 15–0080) was described from *Rosa canina* in Italy (Wanasinghe et al. 2018). We compared our collection with *A. rosarum* and both have similar morphology in terms of ascomata, asci, and ascospores. *Angustimassarina rosarum* (MFLUCC



**Fig. 5** *Angustimassarina rosarum* (MFLU 17–1513). **a** Appearance of ascomata on host surface. **b** Close up of ascoma on host substrate. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Section of

peridium. **f** Pseudoparaphyses. **g–h** Asci. **i–m** Ascospores. Scale bars: **b** = 500  $\mu$ m, **c** = 200  $\mu$ m, **d–f** = 50  $\mu$ m, **g, h** = 20  $\mu$ m, **i–m** = 10  $\mu$ m

15–0080) has globose to subglobose, cylindric-clavate asci (124  $\times$  143  $\mu$ m), and club-shaped pedicel (70  $\times$  10  $\mu$ m). It has a minute ocular chamber, with fusiform to ellipsoidal ascospores (19  $\times$  5  $\mu$ m) and a hyaline, 1 septate at the centre, with two large guttules in each cell. Our collection has larger ascomata (265  $\times$  340  $\mu$ m), but is similar in overall morphology (Fig. 5). In molecular analysis, the new strain forms a close relationship with *A. rosarum* (MFLUCC 15–0080). Comparison of the ITS sequence data reveals no significant difference (one base pair difference) between our new collection and *A. rosarum* (MFLUCC 15–0080). However, the *tef1* sequence is not available for *A. rosarum* (MFLUCC 15–0080) for comparison. Therefore, we introduce a new host record of *A. rosarum* on *Clematis* species herein.

#### Cyclothyriellaceae Jaklitsch & Voglmayr

Cyclothyriellaceae was introduced for *Cyclothyriella rubronotata* (= *Thyridaria rubronotata*) and *Massariosphaeria phaeospora* as revealed by molecular phylogeny (Jaklitsch and Voglmayr 2016). Cyclothyriellaceae is characterized by scattered, immersed-erumpent ascomata, with occasional purple stain on plant tissue, and narrow, anastomosing, trabeculate pseudoparaphyses (sensu Liew et al. 2000). Asci are cylindrical to clavate, bitunicate and 8-spored. Ascospores are brown, ellipsoid to fusoid, with several eusepta. The asexual morph is pycnidial with hyaline to brown conidia (Zhang et al. 2012; Jaklitsch and Voglmayr 2016). We describe a novel species of *Massariosphaeria* recorded on *Clematis vitalba* from Italy (Fig. 2).

**Massariosphaeria** (E. Müll.) Crivelli

*Massariosphaeria* was introduced for species with reddish brown to brown, multi-septate, phragmosporous to dictyosporous and usually with colouration on the surface of the substrate (Crivelli 1983; Leuchtman 1984; Zhang et al. 2012). Several studies have proved the polyphyletic placement of *Massariosphaeria*, classifying them into distinct genera (Zhang et al. 2012; Ariyawansa et al. 2015a; Phukhamsakda et al. 2016). *Massariosphaeria* has comparable peridium and phragmosporous characters with *Chaetomastia* (Teichosporaceae) (Barr 1989). However, the characteristic of ascomata position and the ascospores characters of *Chaetomastia* and *Massariosphaeria* are distinct. The type species *Massariosphaeria phaeospora* (CBS 611.86) is currently placed in Cyclothyriellaceae (Fig. 2). Twenty-one epithets are listed under *Massariosphaeria* in Index Fungorum (2020). Based on phylogenetic analysis including Cyclothyriellaceae, our collection (MFLU 16–0174) clustered with *M. phaeospora* (CBS 611.86) with strong support (100% MLBS/1.00 BYPP). A new *Massariosphaeria* species on *Clematis vitalba* is introduced herein (Fig. 6).

***Massariosphaeria clematidis*** Phukhams., Wanas., Camporesi & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557108; *Facesoffungi number*: FoF 07248, Fig. 6.

*Etymology*: Epithet reflects the host *Clematis*.

*Holotype*: MFLU 16–0174.

*Saprobic* on dead stems of *Clematis vitalba*. **Sexual morph**: *Ascomata* 340–430 × 215–300 µm ( $\bar{x}$  = 280 × 255 µm, n = 5), with only black shiny ostioles present on the surface of host, solitary, scattered, immersed, globose to compressed globose, sub-carbonaceous to coriaceous, dark brown to black, rough-walled, with short hyphae projecting from peridium, ostiolate. *Ostioles* centrally located, 125–175 × 110–130 µm ( $\bar{x}$  = 140 × 120 µm, n = 10), carbonaceous, papillate, periphysoids. *Peridium* 22–33 µm wide, composed of 6–8 (–12 at apex) layers of dark brown to black cells of *textura angularis*, inner layer composed of hyaline gelatinous cells. *Hamathecium* composed of numerous, dense, long, 1.6–3.5 µm wide ( $\bar{x}$  = 2.5 µm, n = 50), filiform, transversely septate, branched, anastomosing, cellular pseudoparaphyses. *Asci* 150–225 × 20–30 µm ( $\bar{x}$  = 180 × 25 µm, n = 20), 8-spored, bitunicate, fissionate, cylindric-clavate to broad cylindrical, with furcate pedicel, with ocular chamber visible when immature. *Ascospores* 35–45 × 10–15 µm ( $\bar{x}$  = 40 × 12 µm, n = 50), biseriolate or overlapping, broad fusiform, narrow towards the apex, initially hyaline, becoming yellowish to brown at maturity, 6–8-transversely euseptate, constricted at the septa, third cell from apex usually enlarged, smooth-walled, guttulate and indentations present, surrounded by a 8–20 µm wide, mucilaginous sheath. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA reaching 20 mm diam. after 4 weeks at 18 °C. Culture from above brown, yellowish towards the edge, dense, circular, flat, dull, fimbriate, radially furrowed, and slightly covered with white aerial mycelium; reverse black with radiating cream mycelium.

*Material examined*: Italy, Forlì-Cesena Province, Strada San Zeno—Galeata, dead aerial stems of *Clematis vitalba* L., 7 November 2013, E. Camporesi, IT1509 (MFLU 16–0174, **holotype**).

*Host*: *Clematis vitalba*—(This study).

*Distribution*: Italy—(This study).

*GenBank accession numbers*: LSU: MT214544; SSU: MT226663; ITS: MT310591.

*Notes*: Our new collection MFLU 16–0174 from Italy formed a close relationship with the type species of *Massariosphaeria*, *M. phaeospora* (CBS 611.86) with strong support (100% ML/1.00 BYPP). The strain is compatible with the concept of *Massariosphaeria* in having scattered, immersed, papillate ascomata with thick ostiolar wall, dense pseudoparaphyses, cylindro-clavate asci and broad fusiform, reddish brown to brown, multi-septate ascospores with a mucilaginous sheath (Müller 1950; Zhang et al. 2012). MFLU 16–0174 is distinguishable from *M. phaeospora* by its partial carbonaceous ostioles, narrower, 6–8-transversely euseptate ascospores which are swollen at the third cell. *Massariosphaeria vitalbae* ( $\equiv$  *Leptosphaeria vitalbae*) was described from *Clematis vitalba* in Switzerland (Ahn and Shearer 1998). Characters of MFLU 16–0174 include a long neck ( $\bar{x}$  = 140 × 120 µm), with sub-carbonaceous to coriaceous peridium types and ascospores enlarged at the third cell. *Massariosphaeria vitalbae* has sub-parenchymatous cells type with short ostiolar necks and 9–10-septate and ascospores enlarged at the third cell (Müller 1950). *Massariosphaeria vitalbae* closely resembles *Paramassariosphaeria clematidicola*, however, fresh collections are required to clarify its taxonomic placement (Wanasinghe et al. 2016b).

In a BLASTn search of GenBank, the LSU sequence of *Massariosphaeria clematidis* (MFLU 16–0174) was found to be 96% similar to *M. phaeospora* strain CBS 611.86 (FJ795503). We introduce a novel species of *Massariosphaeria*, *M. clematidis* based on the morphological (Fig. 6) and phylogenetic evidence (Fig. 2).

**Dictyosporiaceae** Boonmee & K.D. Hyde

Dictyosporiaceae was introduced with *Dictyosporium* as the type genus in Dothideomycetes (Boonmee et al. 2016). The sexual morph of Dictyosporiaceae has immersed to erumpent or superficial, globose to subglobose and dark brown to black ascomata including bitunicate, cylindric-clavate asci with septate and hyaline ascospores with a thick mucilaginous sheath (Tanaka et al. 2015; Boonmee et al. 2016). Most members of Dictyosporiaceae have a hyphomycetous asexual morph with cheirosporous or dictyosporous





**Fig. 6** *Massariosphaeria clematidis* (MFLU 16–0174, **holotype**). **a** Appearance of ascomata on host surface. **b** Vertical section of ascoma. **c** Ostiolar canal. **d** Section of peridium. **e** Pseudoparaphyses. **f–i** Asci. **j–m** Ascospores. **n** Ascospore in 10% Indian ink. Scale bars: **a** = 500  $\mu\text{m}$ , **b** = 200  $\mu\text{m}$ , **c** = 100  $\mu\text{m}$ , **d–i** = 50  $\mu\text{m}$ , **j–n** = 20  $\mu\text{m}$

conidia, pycnidia with coleophoma-like characters and phialidic conidiogenesis cells. Fourteen genera are listed under Dictyosporiaceae (Iturrieta-González et al. 2018; Wijayawardene et al. 2018; Crous et al. 2019). We provide an updated phylogenetic tree of Dictyosporiaceae and propose a new species and a new host record of *Aquadictyospora*, *Dictyocheirospora* and *Pseudocoleophoma* on *Clematis* (Fig. 7).

#### *Aquadictyospora* Luo, Hyde & H.Y. Su

*Aquadictyospora* was introduced to accommodate a dictyosporous taxon on submersed decaying wood, and typified with *A. lignicola* Z.L. Luo et al. The genus is characterized by sporodochia, superficial, circular or subglobose conidiomata, micronematous conidiophores with monoblastic conidiogenesis cells, and uniformly medium brown dictyosporous conidia with a subglobose, hyaline cell at the basal end (Li et al. 2016). We introduce a second species of *Aquadictyospora* based on morphology (Fig. 8) and phylogenetic analyses (Fig. 7).

#### *Aquadictyospora clematidis* Phukhams., Bhat & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557125; *Facesoffungi number*: FoF 07250, Fig. 8.

*Etymology*: Name refers to the host plant, *Clematis*.

*Holotype*: MFLU 17–1488.

*Saprobic* on dead stems of *Clematis sikkimensis*. **Sexual morph**: Undetermined. **Asexual morph**: Hyphomycetous. *Colonies* 53–97  $\times$  121–213  $\mu\text{m}$  ( $\bar{x}$  = 72  $\times$  153  $\mu\text{m}$ ,  $n$  = 10), on natural substrate forming sporodochial conidiomata, superficial, compact, scattered, subglobose to oval, dark brown to reddish brown, velvety. *Mycelium* 2–3  $\mu\text{m}$  wide, immersed, consisting of branched septate hyphae. *Conidiophores* 6–11  $\times$  2–4  $\mu\text{m}$  ( $\bar{x}$  = 8  $\times$  3  $\mu\text{m}$ ,  $n$  = 10), micronematous, hyaline to pale brown, smooth. *Conidiogenous cells* 5–8  $\times$  3–5  $\mu\text{m}$ , holoblastic, monoblastic, solitary, discrete, determinate. *Conidia* 17–38  $\times$  15–24  $\mu\text{m}$  ( $\bar{x}$  = 32  $\times$  20  $\mu\text{m}$ ,  $n$  = 50), dictyosporous, compactly depressed, obovoid, appearing broadly rounded in upper half, heavily pigmented at the upper half, smooth, entirely reddish brown, with hyaline mammiform basal cell, smooth basal cell, 5–11  $\times$  4–11  $\mu\text{m}$  ( $\bar{x}$  = 8  $\times$  8  $\mu\text{m}$ ,  $n$  = 40), not complanate, secession involves splitting of the basal, without appendages.

*Culture characters*: Colonies on MEA reaching 50 mm diam. after 4 weeks at 25 °C. Cultures from above brownish beige at the centre, grey radiating outwardly, dense, raised with concave edge, circular, entire edge, umbonate, papillate

with fairly fluffy, wrinkled folded, covered with white aerial mycelium; reverse dark brown at the centre, faintly zonate, white mycelium at the edge.

*Material examined*: Thailand, Nan Province, on dead stem of *Clematis sikkimensis* (Hook. f. & Thomson) Drumm. ex Burkill, 2 May 2017, C. Phukhamsakda, CMTH26 (MFLU 17–1488, **holotype**); ex-type living culture, MFLUCC 17–2080.

*Host*: *Clematis sikkimensis*—(This study).

*Distribution*: Thailand—(This study).

*GenBank accession numbers*: LSU: MT214545; SSU: MT226664; ITS: MT310592; *tef1*: MT394727, *rpb2*: MT394679.

*Notes*: The species is assigned to *Aquadictyospora* based on its compatible morphological features such as superficial sporodochia with subglobose to oval conidiomata, micronematous conidiophores with monoblastic conidiogenous cell, and dictyosporous conidia with hyaline, mammiform basal cells (Li et al. 2016). *Aquadictyospora clematidis* has smaller non-cheiroid conidia (32  $\times$  20  $\mu\text{m}$ ) than *A. lignicola* which has larger cheiroid conidia (50  $\times$  24  $\mu\text{m}$ ). *Aquadictyospora clematidis* was found in a terrestrial habitat while *A. lignicola* was from submerged substrates (Fig. 8). In a BLASTn search of GenBank, the ITS sequence had 95% similarity while the *tef1* sequence had 95% similarity to the type species, *A. lignicola*. The new strain is introduced as a new species of *Aquadictyospora* based on polyphasic evidence.

#### *Dictyocheirospora* D'souza, Boonmee & K.D. Hyde

Boonmee et al. (2016) introduced *Dictyocheirospora* (typified by *D. rotunda*) for an aero-aquatic sporodochial fungus with cheiroid dictyosporous. Nineteen species are listed in Index Fungorum (2020) and interestingly, no sexual morph has been described for this genus (Wang et al. 2016; Tibpromma et al. 2018; Hyde et al. 2017, 2019a). We introduce *Dictyocheirospora clematidis* as a novel species and *D. xishuangbannaensis* as a new host record from *Clematis* based on compatible morphological and phylogenetic analysis (Fig. 9).

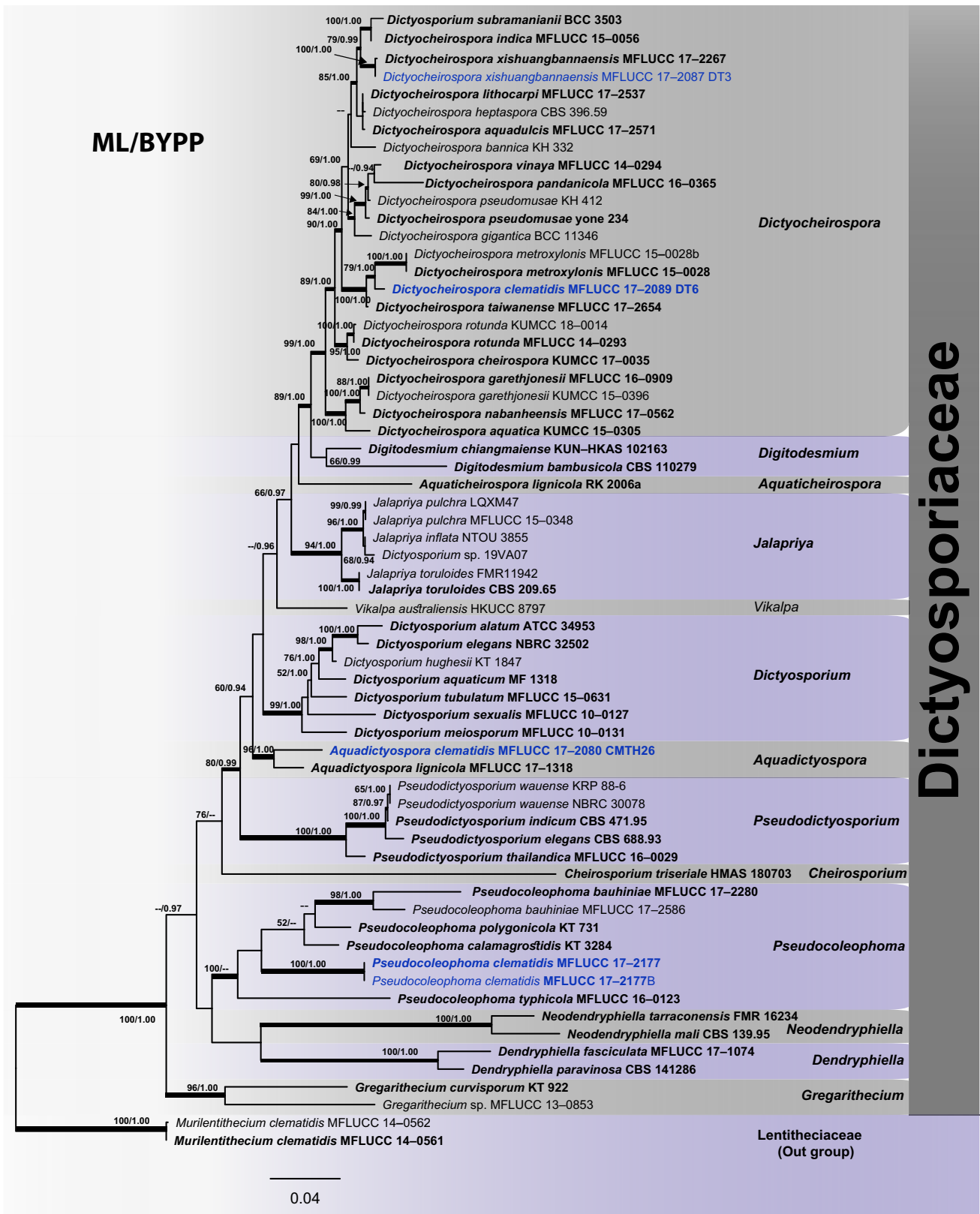
#### *Dictyocheirospora clematidis* Phukhams., D.J. Bhat & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557126; *Facesoffungi number*: FoF 07251, Fig. 9.

*Etymology*: Name refers to the host plant, *Clematis*.

*Holotype*: MFLU 17–1497.

*Saprobic* on dead stem of *Clematis sikkimensis*. **Sexual morph**: Undetermined. **Asexual morph**: Hyphomycetous. *Colonies* 200–340  $\mu\text{m}$  wide ( $\bar{x}$  = 265  $\mu\text{m}$ ,  $n$  = 20), on natural substrate forming sporodochial conidiomata, superficial, gregarious, scattered, punctiform, blackish brown, velvety, glistening, orbicular, with abundant sporulation, conidia



**Fig. 7** The best scoring RAxML tree with a final likelihood value of  $-15336.133569$  based on LSU, ITS and *tefl* sequence data for Dictyosporiaceae species. The topology and clade stability of the combined DNA analyses was compared to the single gene analyses. The tree is rooted with sequences of *Murilentithecium clematidis* (MFLUCC 14–0561, MFLUCC 14–0562) in Lentitheciaceae. The tree from the maximum likelihood analysis had similar topology to the Bayesian analyses. The matrix had 1009 distinct alignment patterns, with 37.31% undetermined characters and gaps. Estimated base frequencies were as follows; A=0.234307, C=0.254705, G=0.271061, T=0.239927; substitution rates AC=1.414609, AG=2.411925, AT=2.055489, CG=0.612591, CT=6.466528, GT=1.000000; gamma distribution shape parameter  $\alpha=0.761212$ . The species determined in this study are indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95)

readily liberated when agitated. Mycelium immersed, composed of brown, smooth, thin-walled, septate hyphae. Conidiophores  $15 \times 3 \mu\text{m}$ , micronematous, pale brown, smooth, thin-walled. Conidiogenous cells  $5\text{--}6 \times 3\text{--}4 \mu\text{m}$ , holoblastic, monoblastic, integrated, terminal, determinate, hyaline, smooth-walled. Conidia  $42\text{--}60 \times 15\text{--}30 \mu\text{m}$  ( $\bar{x}=50 \times 23 \mu\text{m}$ ,  $n=40$ ), solitary, acrogenous, cheiroid, with a basal connecting cell, discharges after mounted in water, cognac brown, consisting of 6–7 rows of cells, individual rows discoid. Conidial arm  $34\text{--}60 \times 7\text{--}10 \mu\text{m}$  ( $\bar{x}=52 \times 9 \mu\text{m}$ ,  $n=30$ ), digitate, cylindrical, inwardly curved at the tip, arising from a basal cell, 10–12 distosepta, slightly constricted at the septa, with large guttule in each cell.

**Culture characters:** Colonies on MEA reaching 40 mm diam. after 4 weeks at 25 °C. Cultures from above, black, dense, circular, entire edge, umbonate, papillate with aerial mycelium, wrinkled and folded, narrow fringe of submerged mycelium, covered with grey aerial mycelium; reverse black, white mycelium present at the edge.

**Material examined:** Thailand, Chiang Rai Province, Doi Tung, on dead stem of *Clematis sikkimensis*, 2 May 2017, C. Phukhamsakda & M.V. de Bult, CMTHDT06 (MFLU 17–1497, **holotype**); ex-type living culture, MFLUCC 17–2089.

**Host:** *Clematis sikkimensis*—(This study).

**Distribution:** Thailand—(This study).

**GenBank accession numbers:** LSU: MT214546; SSU: MT226665; ITS: MT310593; *tefl*: MT394728, *rpb2*: MT394680.

**Notes:** *Dictyocheirosora* species are highly diverse especially in tropical regions (Boonmee et al. 2016; Tibpromma et al. 2018; Yang et al. 2018a; Hyde et al. 2019a). *Dictyocheirosora clematidis* is similar to *D. metroxylonis* based on characters (Table 1), but the ITS sequence shows 97% similarity (2.8% nucleotide differences) while the *tefl* sequence has 96% similarity (4.6% nucleotide

differences). *Dictyocheirosora clematidis* clustered in the same clade as *D. taiwanense* but it has distinct characters. *Dictyocheirosora taiwanense* usually has 5 rows of cells in the conidia and they are longer than those of *D. clematidis* ( $78 \times 18$  vs  $50 \times 23 \mu\text{m}$ ). In the phylogenetic analysis, *D. clematidis* (MFLUCC 17–2089) clustered with *D. metroxylonis* (MFLUCC 15–0282) and *D. taiwanense* (MFLUCC 17–2654) but formed a well-separated clade with good support of 79% in ML and 1.00 in BYPP (Fig. 7).

***Dictyocheirosora xishuangbannaensis*** Tibpromma & K.D. Hyde, Fungal Divers 93:14 (2018), **new host record**

**Index Fungorum number:** IF554476; **Facesoffungi number:** FoF 04485, Fig. 10

**Saprobic** on dead stem of *Clematis sikkimensis*. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. Colonies 235–423  $\mu\text{m}$  wide ( $\bar{x}=326 \mu\text{m}$ ,  $n=20$ ), on natural substrate forming sporodochial conidiomata, superficial, gregarious, scattered, punctiform, blackish brown, velvety, glistening, orbicular, with abundant sporulation, conidia readily liberated when agitated. Mycelium 2  $\mu\text{m}$  wide, immersed, composed of hyaline to pale brown, smooth, thin-walled, septate hyphae. Conidiophores  $10\text{--}20 \times 3\text{--}6 \mu\text{m}$  ( $\bar{x}=12 \times 4 \mu\text{m}$ ,  $n=10$ ), micronematous, hyaline to light brown, smooth, thin-walled. Conidiogenous cells  $3\text{--}7 \times 2\text{--}6 \mu\text{m}$  ( $\bar{x}=5 \times 4 \mu\text{m}$ ,  $n=10$ ), holoblastic, integrated, terminal, determinate, hyaline, smooth-walled. Conidia  $32\text{--}53 \times 16\text{--}27 \mu\text{m}$  ( $\bar{x}=46 \times 20 \mu\text{m}$ ,  $n=50$ ), solitary, acrogenous, cheiroid, oblong or subglobose, with a basal connecting cell, discharges after mounted in water, cognac brown, heavily pigmented upper part, consisting of 6–7 rows of cells, rows digitate. Conidial arm  $40\text{--}55 \times 6\text{--}9 \mu\text{m}$  ( $\bar{x}=46 \times 6 \mu\text{m}$ ,  $n=30$ ), cylindrical, curved at both ends, arising from a basal cell, reddish brown, heavily pigmented at the upper part, 7–11 distoseptate, slightly constricted at the septa, with large guttule in each cell.

**Culture characters:** Colonies on MEA reaching 40 mm diam. after 4 weeks at 25 °C. Cultures from above, dark brown, dense, circular, edge entire, umbonate, papillate with white aerial mycelium, wrinkled and folded, narrow fringe of submerged mycelium; reverse dark brown, erose.

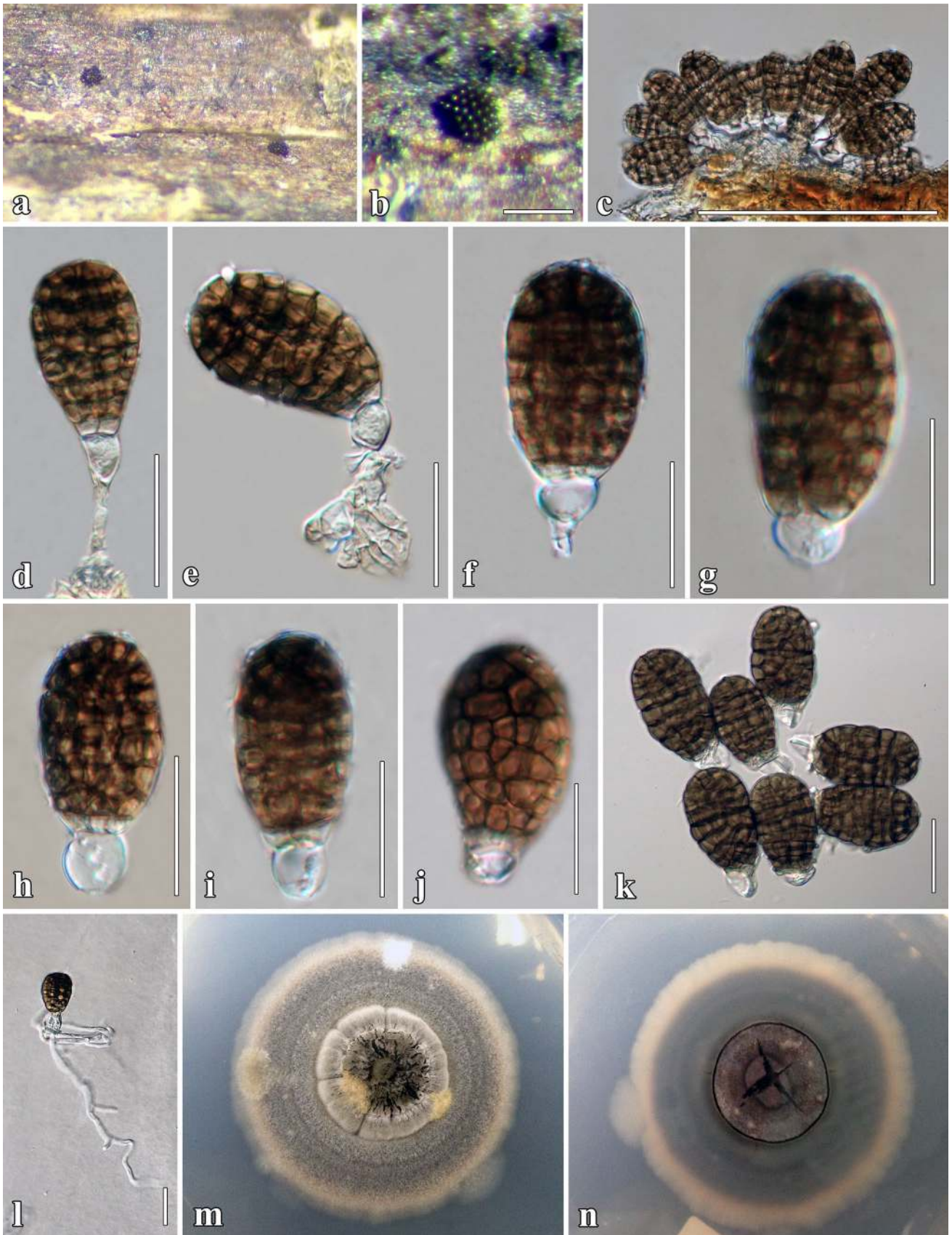
**Material examined:** Thailand, Chiang Rai Province, on dead stem of *Clematis sikkimensis*, 2 May 2017, C. Phukhamsakda & M.V. de Bult, CMTHDT03 (MFLU 17–1495); living culture, MFLUCC 17–2087.

**Hosts:** *Clematis sikkimensis*, *Pandanus* sp.—(Tibpromma et al. 2018; this study).

**Distribution:** China, Thailand—(Tibpromma et al. 2018; this study).

**GenBank accession numbers:** LSU: MT214547; SSU: MT226666; ITS: MT310594; *tefl*: MT394729.

**Notes:** *Dictyocheirosora xishuangbannaensis* was recorded from *Pandanus* by Tibpromma et al. (2018) and our



**Fig. 8** *Aquadictyospora clematidis* (MFLU 17–1488, **holotype**). **a**, **b** Sporodochia on natural substrate. **c** Vertical section through sporodochium. **d–e** Conidia attached to conidiophores. **f–k** Mature conidia. **l** Germinated conidia. **m**, **n** Culture characteristics on MEA. Scale bars: **b** = 100  $\mu$ m, **c** = 100  $\mu$ m, **d–l** = 20  $\mu$ m

collection resembles this species morphologically (Fig. 10). The first record of *D. xishuangbannaensis* was from southern China (Xishuangbanna, Yunnan Province), while our collection was found in the northern part of Thailand (Chiang Rai Province). Northern Thailand and Xishuangbanna both have a tropical climate and share similar weather in both the wet and dry seasons (Cao et al. 2006). It can be hypothesized that *D. xishuangbannaensis* generally occurs in tropical climates (Boonmee et al. 2016; Tibpromma et al. 2018; Yang et al. 2018a). This is the first record of *D. xishuangbannaensis* on *Clematis* species. We also provide sequence data and phylogenetic analyses in Fig. 7.

#### *Pseudocoleophoma* Tanaka & K. Hiray.

*Pseudocoleophoma* is characterized by its immersed to erumpent, ostiolate ascomata, cylindrical to clavate and short pedicellate asci and fusiform, 1-septate, sheathed ascospores. The genus produces coleophoma-like asexual morph with phialidic, doliform to lageniform conidiogenous cells and cylindrical, hyaline conidia. We introduce a new species in *Pseudocoleophoma* based on molecular and morphology (Figs. 7, 11).

#### *Pseudocoleophoma clematidis* Phukhams. & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557127; *Facesoffungi number*: FoF 07252, Fig. 11.

*Etymology*: Name refers to the host plant, *Clematis* from which the fungus was isolated.

*Holotype*: MFLU 16–0280.

*Saprobic* on dead stems of *Clematis vitalba*. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 130–150  $\times$  100–130  $\mu$ m ( $\bar{x}$  = 140  $\times$  110  $\mu$ m,  $n$  = 5), pycnidial, solitary, aggregated, uniloculate, immersed, with black shiny ostioles visible, globose to subglobose, coriaceous, subcoriaceous at the outer layers, thick-walled, black to dark brown, ostioles. *Ostioles* 30  $\times$  50  $\mu$ m, central, papillate, ovoid. *Conidiomatal wall* 20–30  $\mu$ m wide, of equal thickness, multilayered, outer layer composed of 8–10 layers of light brown to brown cells of *textura angularis*, lined with a thick hyaline layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 2–4  $\times$  1.5–4  $\mu$ m ( $\bar{x}$  = 2.5  $\times$  3  $\mu$ m,  $n$  = 30), holoblastic, phialidic, determinate, discrete, cylindrical to subcylindrical, smooth-walled, hyaline, arising from inner layers of conidioma. *Conidia* 5–8  $\times$  2–4  $\mu$ m ( $\bar{x}$  = 6  $\times$  4  $\mu$ m,  $n$  = 50), oval,

slightly curved towards the ends, aseptate, with 1(–2) guttules in each cell, hyaline when immature, yellowish brown at maturity, smooth-walled.

*Culture characters*: Colonies on MEA reaching 20 mm diam. after 2 weeks at 16 °C. Cultures from above, cream, dense, circular, umbonate, papillate with fluffy, covered with white aerial mycelium; reverse dark brown at the centre, cream radiating outwardly.

*Material examined*: Italy, Arezzo Province, Badia Tega—Ortignano Raggiolo, on dead aerial branch of *Clematis vitalba*, 9 March 2013, E. Camporesi, IT 1110 (MFLU 16–0280, **holotype**); ex-type living culture, MFLUCC 17–2177.

*Host*: *Clematis vitalba*—(This study).

*Distribution*: Italy—(This study).

*GenBank accession numbers*: LSU: MT214548, MT214549; SSU: MT226667; ITS: MT310595, MT310596; *tefl*: MT394730.

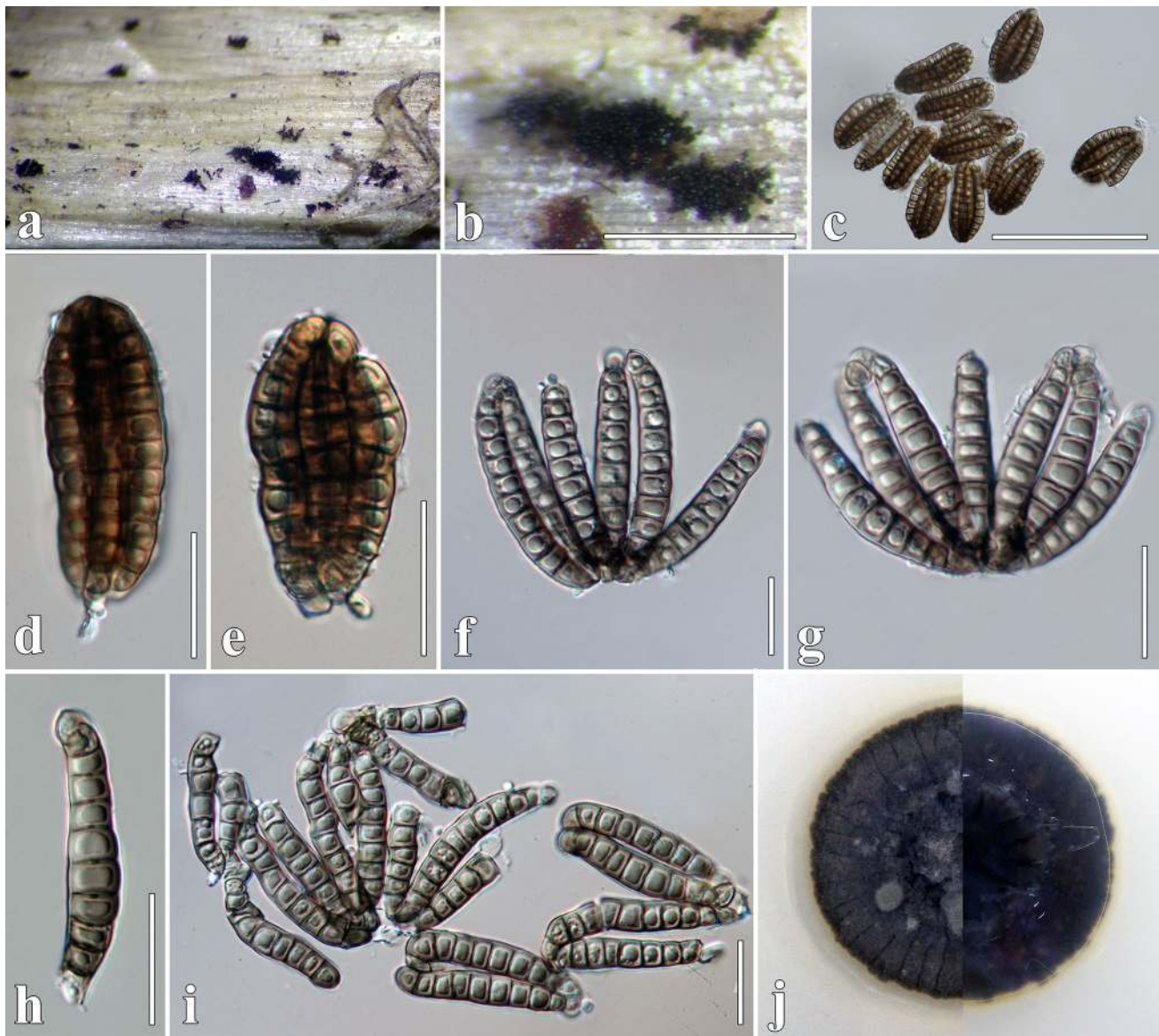
*Notes*: Based on the multi-gene phylogenetic analyses (Fig. 7), *Pseudocoleophoma clematidis* strain MFLUCC 17–2177 (Fig. 11) clusters between *P. calamagrostidis* (KT 3284) and *P. typhicola* (MFLUCC 16–0123). *Pseudocoleophoma clematidis* is different from other *Pseudocoleophoma* species by having pycnidial walls which are flat at the base and yellowish brown conidia (Tanaka et al. 2015; Hyde et al. 2016; Jayasiri et al. 2019, Fig. 11). This study confirmed its placement in *Pseudocoleophoma*. In a BLASTn search of GenBank, the closest match of the LSU sequence of MFLUCC 17–2177 is *P. calamagrostidis* (HHUF 30450) with 97% similarity, while the closest match of the ITS sequence is *P. polygonicola* (HHUF 27558) with 92% similarity.

#### **Didymellaceae** Gruyter, Aveskamp & Verkley

Valenzuela-Lopez et al. (2018) accepted 26 genera in Didymellaceae. Didymellaceous taxa frequently occur as plant pathogens, causing drooping and wilting of plant leaves or gummy stem blight leading to death of the plant (Sudisha et al. 2004; Vaghefi et al. 2012; Ahmadpour et al. 2017; Wijayawardene et al. 2017). The combined dataset of LSU, ITS and *rpb2* sequences for a multilocus analysis tree revealed distinct lineages in Didymellaceae (Fig. 12). In the present study, a cluster of fungi associated with *Clematis* species formed a distinct lineage, *Anthodidymella*, a novel genus in Didymellaceae, a novel species in *Xenodidymella*, and a new host record in *Neodidymelliopsis*.

#### *Anthodidymella* Phukhams., Camporesi & K.D. Hyde, **gen. nov.**

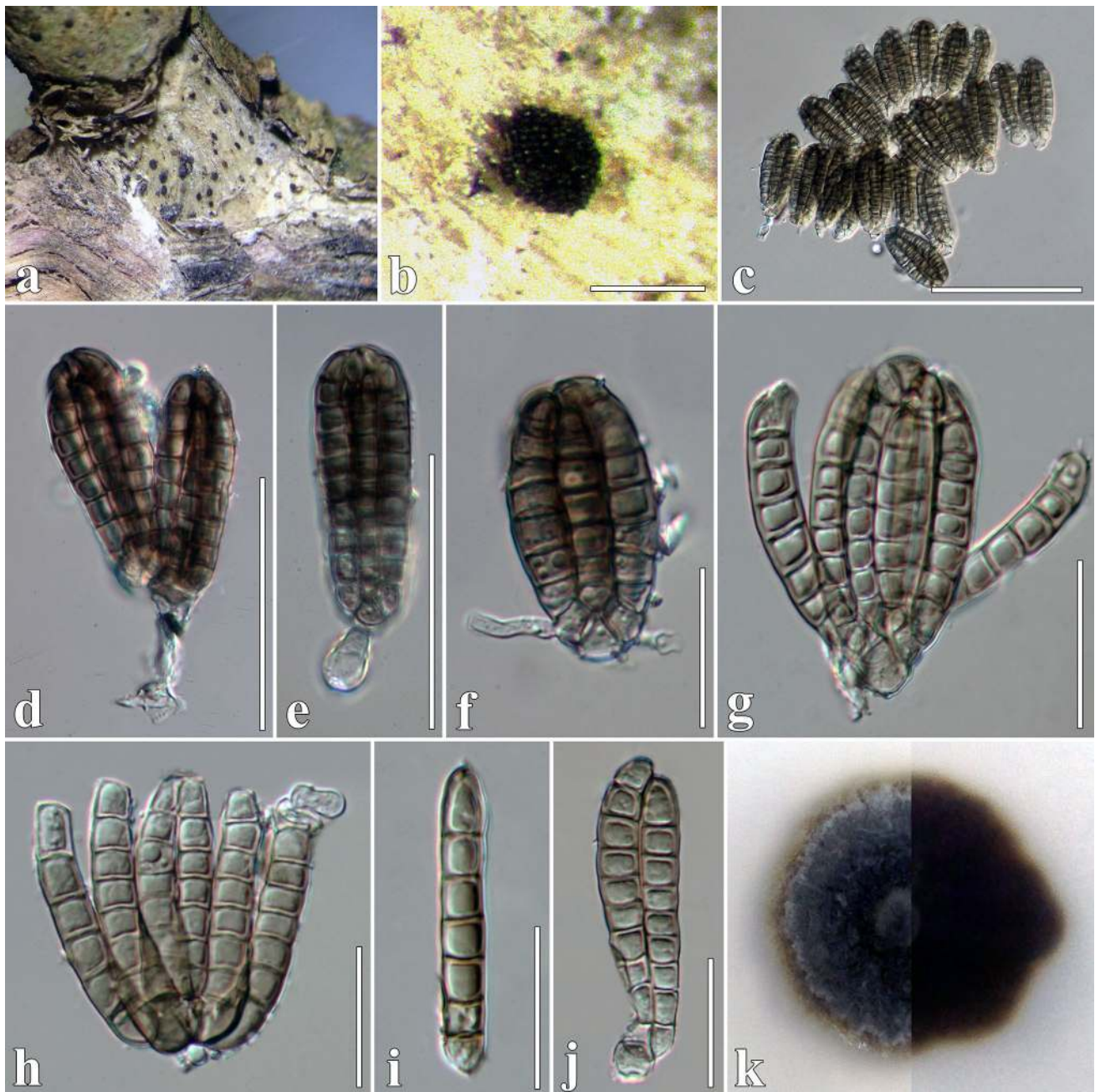
*Index Fungorum number*: IF557128; *Facesoffungi number*: FoF 07255, Fig. 13



**Fig. 9** *Dictyocheirospora clematidis* (MFLU 17-1497, holotype). **a, b** Sporodochia on natural substrate. **c** Sporodochium mounted in water. **d–i** Mature conidia. **j** Culture characteristics on MEA. Scale bars: **b** = 500  $\mu\text{m}$ , **c** = 100  $\mu\text{m}$ , **d–g** = 20  $\mu\text{m}$ , **h, i** = 10  $\mu\text{m}$

**Table 1** Synopsis of related *Dictyocheirospora* species and the new species from this study

Species	Conidia		References
	Average size ( $\mu\text{m}$ )	Shape	
<i>D. clematidis</i>	50 × 23	Cheiroid, acrogenous, cylindrical, cognac brown, consisting of 6–7 rows of cells	This study
<i>D. metroxylonis</i>	60 × 20	Cheiroid dictyospores, cylindrical, pale brown, consisting of 4–6 rows of cells	Phookamsak et al. (2019)
<i>D. taiwanense</i>	78 × 18	Cheiroid dictyospores, brown, ellipsoid to cylindrical, consisting of 5 rows of cells	Hyde et al. (2019a)

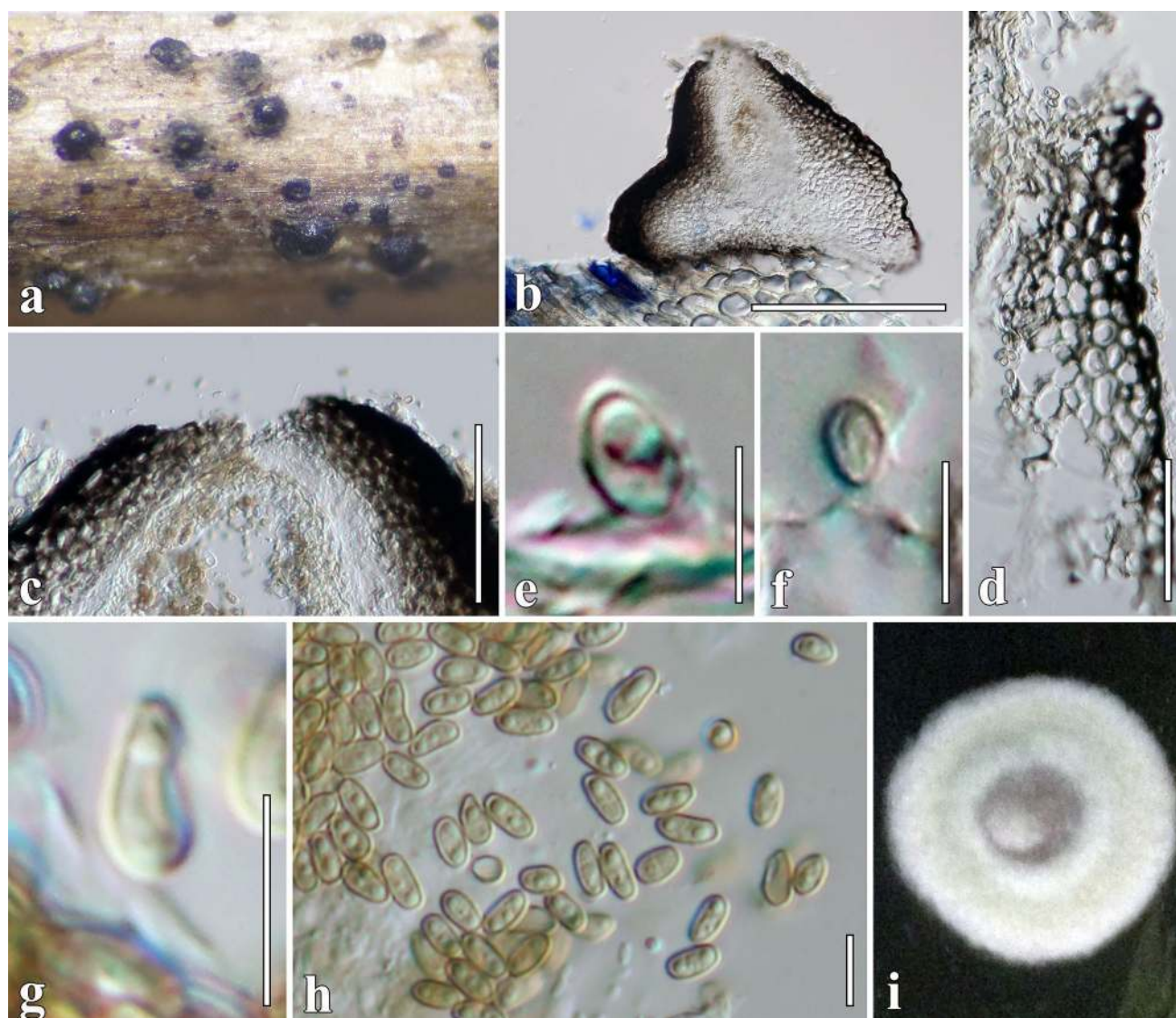


**Fig. 10** *Dictyocheirospora xishuangbannaensis* (MFLU 17–1495). **a, b** Sporodochia on natural substrate. **c** Sporodochium mounted in water. **d–h** Mature conidia. **i–j** Separated rows of conidium. **k** Cul-

ture characteristics on MEA. Scale bars: **b**=200  $\mu\text{m}$ , **c**=100  $\mu\text{m}$ , **d–e**=50  $\mu\text{m}$ , **f–g**=20  $\mu\text{m}$ . **h–j**=10  $\mu\text{m}$

**Etymology:** Anthos-meaning flower, *Anthodidymella* refer to species of *Didymella* that frequently occur on flowering plants.

**Saprobic** or necrotic on leaf and dead stems of herbaceous plants **Sexual morph:** *Ascomata* superficial, solitary or clustered, globose or subglobose to pyriform, with elongated ostioles. *Perithecial wall* consisting of *textura globulosa*.



**Fig. 11** *Pseudocoleophoma clematidis* (MFLU 16–0280, **holotype**). **a** Appearance of conidiomata on *Clematis vitalba*. **b** Vertical section through conidioma. **c** Ostiolar canal. **d** Section of conidioma wall.

**e–g** Conidiogenous cells and conidia. **h** Conidia. **i** Culture characteristics on MEA. Scale bars: **b**=200  $\mu$ m, **c**=50  $\mu$ m, **d**=20  $\mu$ m, **e–h**=5  $\mu$ m

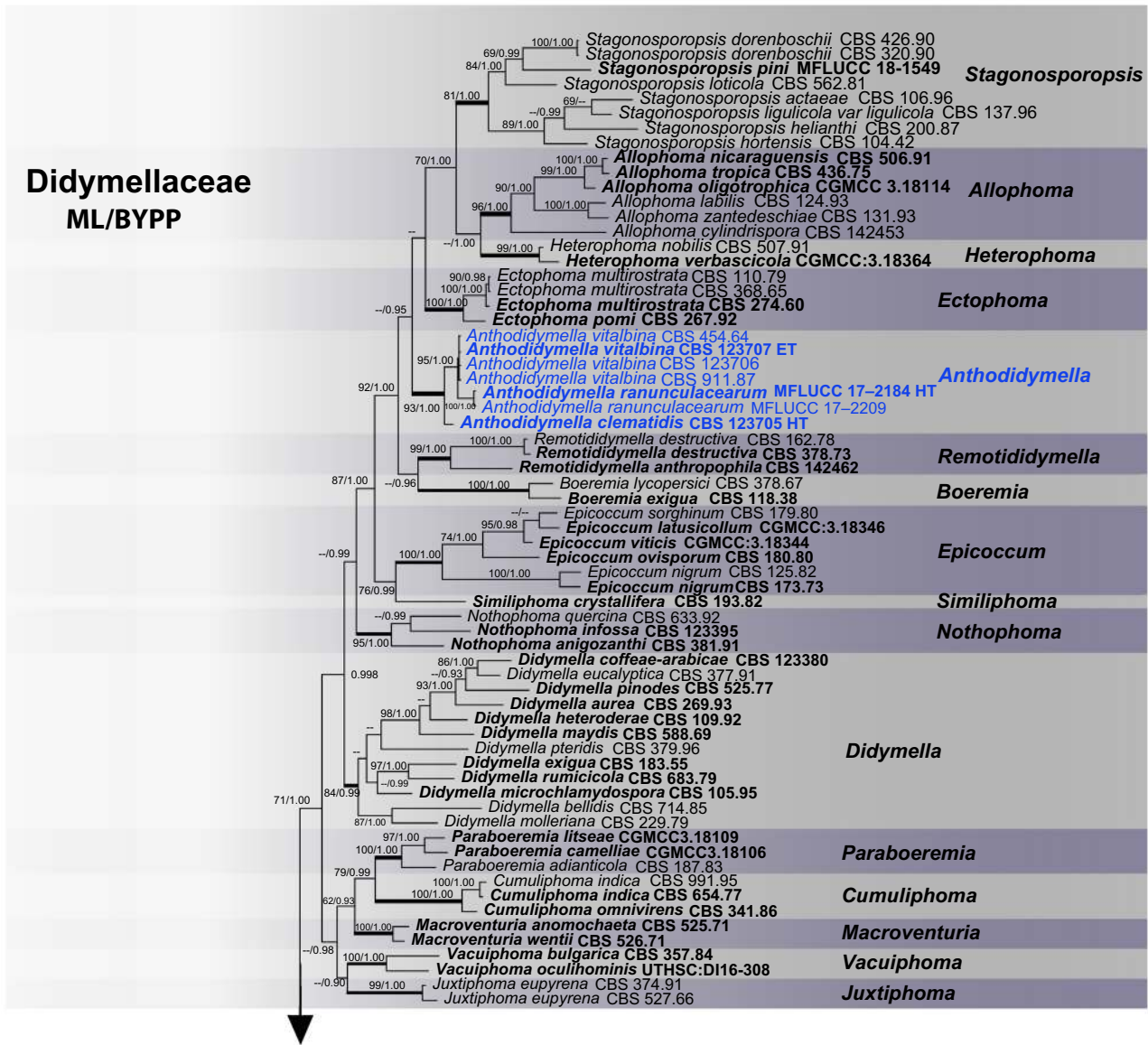
*Hamathecium* composed of numerous, dense, pseudoparaphyses. *Asci* 8-spored, bitunicate, cylindrical club-shaped pedicel. *Ascospores* uniseriate or partially overlapping, ovate to obpyriform, 1-septate, hyaline (Woudenberg et al. 2009). **Asexual morph:** *Conidiomata* pycnidial, uniloculate, immersed under host epidermis, subglobose to depressed, coriaceous, thin-walled, dark brown to brown, with papillate ostioles. *Conidiomatal wall* thin layers, pseudoparenchymatous, with brown cells of *textura globulosa*, lined with a hyaline cell-layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* phialidic, determinate, discrete, ampulliform, cylindrical to subcylindrical, hyaline. *Conidia* oblong or oval, rounded ends,

hyaline, aseptate or septate, smooth-walled. *Chlamydospores* absent.

*Type species: Anthodidymella ranunculacearum* Phukhams., Camporesi & K.D. Hyde

*Notes:* *Anthodidymella* is introduced for a strongly supported clade (93% ML/1.00 BYPP, Fig. 12) of *Didymella* species unit that is associated with *Clematis* (Aveskamp et al. 2010). *Anthodidymella clematidis* was described as *Phoma clematidina* as it clustered with other *Phoma clematidina* isolates (Woudenberg et al. 2009; Golzar et al. 2011). *Phoma clematidina* not only causes symptoms on *Clematis* species, but also is a saprobe on other hosts. It has been used as a control agent of *Clematis vitalba* in New Zealand (Gourlay et al. 2000). An updated study classified *Phoma*





**Fig. 12** Phylogram generated from maximum likelihood analysis based on combined LSU, ITS, and *rpb2* sequence data representing Didymellaceae species. Related sequences were taken from Chen et al. (2017) and Valenzuela-Lopez et al. (2018). One hundred and nine strains were included in the combined DNA analyses which comprised 2094 characters (964 characters for LSU, 531 characters for ITS, 599 characters for RPB2, including gap regions). *Leptosphaeria conoidea* (CBS 616.75) and *L. doliolum* (CBS 505.75) in Leptosphaeriaceae (Pleosporales) were used as out-group taxa. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree from the maximum likelihood analysis had similar topology to the Bayesian analyses. The best sorting RaxML tree with a final likelihood value of  $-18910.278845$  is

presented. The matrix had 602 distinct alignment patterns with 6.40% undetermined characters or gaps proportions. Estimated base frequencies were as follows: A=0.247270, C=0.227052, G=0.279182, T=0.246495; substitution rates AC=2.142238, AG=8.227445, AT=2.335028, CG=1.021789, CT=16.735281, GT=1.000000; gamma distribution shape parameter  $\alpha=0.490873$ . The GTR+I+G model was used for each partition in Bayesian analysis. The species determined in this study are indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95)

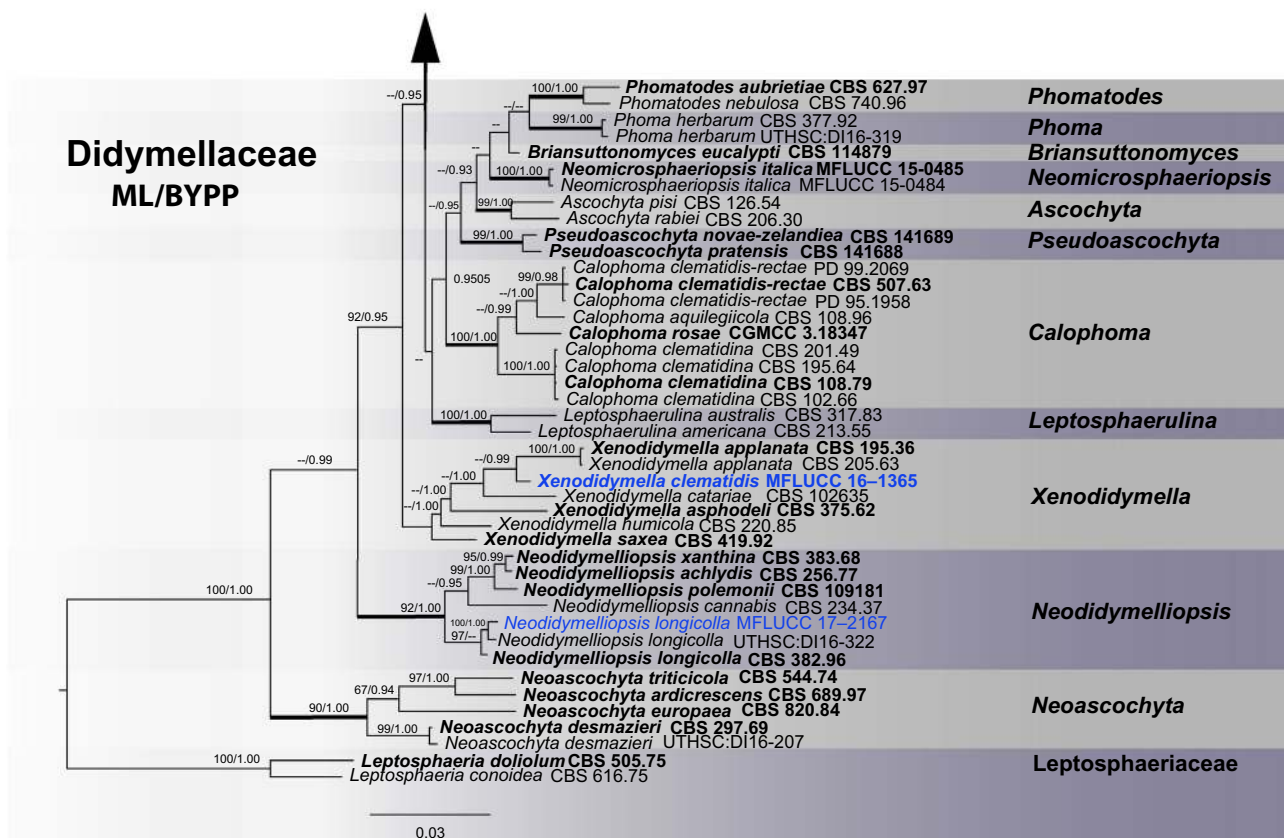


Fig. 12 (continued)

collections isolated from necrotic leaf tissues of *Clematis* species into *Anthodidymella*, *Calophoma*, and *Phoma* (Woudenberg et al. 2009; Valenzuela-Lopez et al. 2018, this study, Fig. 12).

*Anthodidymella* has similar morphology to *Didymella* in its solitary or clustered, globose ascomata, thin-walled cells of *textura globulosa*, with ovate to obpyriform, 1 septum, hyaline ascospores without a mucilaginous sheath. The asexual morph is pycnidial with phialidic, determinate, and discrete conidiogenous cells (Chen et al. 2017). However, *Anthodidymella* have broad-cylindrical asci, obpyriform ascospores while *Didymella* has oblong asci and broad-fusiform ascospores (Woudenberg et al. 2009; Aveskamp et al. 2010). The asexual morph of *Anthodidymella* has globose or flask-shaped, phialidic conidiogenous cells with oblong or elongated-oval conidia. The combined dataset

of the LSU, ITS, and *rpb2* sequences for Didymellaceae revealed a lineage including *Anthodidymella clematidis*, *A. ranunculacearum* (type species) and *A. vitalbina* (Fig. 12).

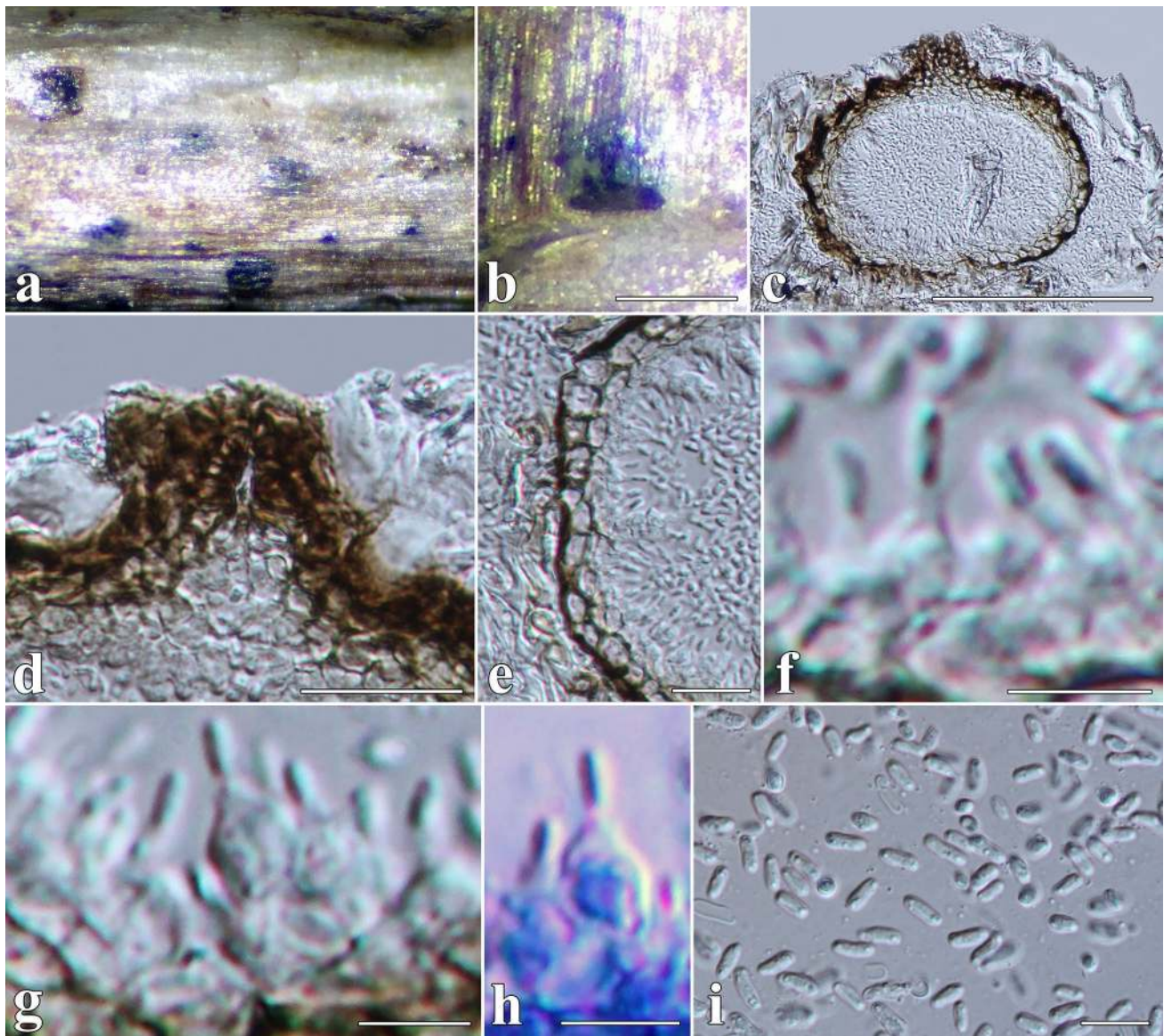
*Anthodidymella clematidis* (Woudenb., Spiers & Gruyter) Phukhams. & K.D. Hyde, **comb. nov.**

*Index Fungorum number*: IF557129; *Facesoffungi number*: FoF 07256

*Basionym*: *Didymella clematidis* Woudenb., Spiers & Gruyter in Woudenberg et al., Persoonia 22:60 (2009)

*Synonym*: *Phoma clematidina* (Thüm.) Boerema, Versl. Medsd. Plziektenk. Dienst Wageningen 153:17 (1979)

*Notes*: Since the fungus has been introduced before one fungus = one name (Taylor 2011), the isolate CBS 123705 bears two names for its pleomorphic life-cycles. The strain was originally described as *Phoma clematidina* from a



**Fig. 13** *Anthodidymella ranunculacearum* (MFLU 17–1468, **holotype**). **a** Appearance of conidiomata on *Clematis vitalba*. **b** Close up of conidioma on host substrate. **c** Vertical section through conidioma. **d** Ostiolar canal. **e** Section of partial conidioma wall. **f–h** Conidia. **h** conidiogenous cells in cotton blue. **i** Conidia. Scale bars: **b**=200  $\mu\text{m}$ , **c**=100  $\mu\text{m}$ , **d**=20  $\mu\text{m}$ , **e**=10  $\mu\text{m}$ , **f–i**=5  $\mu\text{m}$

necrotic leaf spot of *Clematis ligusticifolia* and developed both sexual morph and asexual morph characters in pure culture (Woudenberg et al. 2009). *Anthodidymella clematidis* was initially described as *Didymella clematidis* for its sexual morph epithet (= *Phoma clematidina* as asexual name) as it clustered with other *Phoma clematidina* isolates (Woudenberg et al. 2009; Golzar et al. 2011). The new combination, *Anthodidymella clematidis* is proposed for *Didymella clematidis* (CBS 123705).

*Host*: *Clematis ligusticifolia*—(Woudenberg et al. 2009).

*Distribution*: USA—(Woudenberg et al. 2009).

*Anthodidymella ranunculacearum* Phukhams., Camporesi & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557130; *Facesoffungi number*: FoF 07257, Fig. 13.

*Etymology*: The specific epithet reflects the host family, Ranunculaceae.

*Holotype*: MFLU 17–1468.

*Saprobic* on dead stems of *Clematis vitalba*. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 99–214  $\times$  130–246  $\mu\text{m}$  ( $\bar{x}$ =142  $\times$  169  $\mu\text{m}$ ,  $n$ =5), pycnidial, solitary, sometimes aggregated, uniloculate, immersed under epidermal layer, subglobose to depressed, coriaceous,

*Holotype*: MFLU 17–1468.

*Saprobic* on dead stems of *Clematis vitalba*. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 99–214  $\times$  130–246  $\mu\text{m}$  ( $\bar{x}$ =142  $\times$  169  $\mu\text{m}$ ,  $n$ =5), pycnidial, solitary, sometimes aggregated, uniloculate, immersed under epidermal layer, subglobose to depressed, coriaceous,

thin-walled, brown to dark brown, with ostiolate. *Ostioles* 25 × 42 µm, central, papillate, with pore. *Conidiomatal wall* 10–28(–36) µm wide, of 2–5 layers, each cell-layer 10 µm wide, light brown to brown cells of *textura globulosa*, heavily pigmented in the outer layers, lined with a hyaline innermost layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 2.5–5 × 1.5–3.5 µm ( $\bar{x}$  = 3.5 × 2 µm, n = 30), phialidic, determinate, discrete, ampulliform, cylindrical to sub-cylindrical, smooth-walled, hyaline, arising from the inner layer of conidioma. *Conidia* 6–10 × 2–5 µm ( $\bar{x}$  = 6 × 4 µm, n = 50), oblong or oval, slightly curved toward the ends, rounded ends, with 1(–2) guttules in each cell, hyaline, aseptate, smooth-walled.

**Culture characters:** Colonies on MEA reaching 20 mm diam. after 2 weeks at 25 °C. Cultures; above: greyish brown or dark green, dense, circular, umbonate, papillate, fluffy, covered with aerial mycelium, reverse dark brown.

**Material examined:** Italy, Forlì-Cesena Province, Valdinoco—Meldola, on dead aerial branch of *Clematis vitalba*, 3 February 2015, E. Camporesi, IT 2364 (MFLU 17–1536, **holotype**); ex-type living culture, MFLUCC 17–2184 = MFLUCC 17–2209.

**Host:** *Clematis vitalba*—(This study).

**Distribution:** Italy—(This study).

**GenBank accession numbers:** MFLUCC 17–2184; LSU: MT214550; SSU: MT226668; ITS: MT310597; *tefl*: MT394731; *rpb2*: MT394681; *act*: MT394620. MFLUCC 17–2209; LSU: MT214551; SSU: MT226669; ITS: MT310598; *tefl*: MT394732; *act*: MT394621.

**Notes:** *Anthodidymella ranunculacearum* (MFLUCC 17–2184) is similar to *A. vitalbina* (CBS 123707, ex-epitype), a strain recorded from the same host (Woudenberg et al. 2009). However, *A. ranunculacearum* differs from *A. vitalbina* in its thicker conidiomatal wall (10–36 vs 5.5–9.5 µm, Fig. 13). In a BLASTn search of GenBank, the ITS sequence had 99.5% similarity (2.25% nucleotide differences), while the *act* sequence had 91% similarity (77 nucleotide differences in 297 nucleotides). Thus, the new strain is introduced as a new species of *Anthodidymella* based on guidelines of Jeewon and Hyde (2016). Additionally, *A. ranunculacearum* is designated as the type species of *Anthodidymella* based on available material and an ex-type culture.

***Anthodidymella vitalbina*** (Petr.) Phukhams. & K.D. Hyde, **comb. nov.**

**Index Fungorum number:** IF557131; **Facesoffungi number:** FoF 07258.

**Basionym:** *Ascochyta vitalbae* Briard & Har. apud Briard, Rev. Mycol. (Toulouse) 13: 17. (1891).

≡ *Diplodina vitalbae* (Briard & Har.) Allesch., Rabenh. Krypt.-Fl., ed. 2. Pilze 6 (Lief. 69): 683. 1900 (1901).

**Synonym:** *Diplodina clematidina* Fautrey & Roum. apud Roum., Rev. Mycol. (Toulouse) 14: 105 (1892).

= *Didymella vitalbina* Petr, Anns mycol. 38(2/4): 348 (1940).

= *Phoma clematidina* (Thüm.) Boerema, Versl. Medsd. Plziektenk. Dienst Wageningen, 1978 153: 17 (1979).

**Notes:** *Anthodidymella vitalbina* was introduced for *Didymella vitalbina* (= *Phoma clematidina* as asexual name) which was reported from a necrotic leaf spot of *Clematis* species (Woudenberg et al. 2009). *Phoma clematidina* (strain CBS 123707) was isolated from *Clematis vitalba* and developed sexual and asexual morphs in culture. The sexual morph is named as *Didymella vitalbina* Petr. and was chosen as an epitype of *D. vitalbina* by Woudenberg et al. (2009). In the analyses of combined LSU, ITS, and *rpb2* sequence data of Didymellaceae, the ex-epitype strain (CBS 123707) and the related strains clustered with *Anthodidymella clematidis*. Therefore, we transfer *Didymella vitalbina* (= *Phoma clematidina*) to *Anthodidymella vitalbina* based on phylogenetic relationship of compatible morphology of both morphs. The nomenclature changes are also based on one fungus = one name protocol.

**Host:** *Clematis vitalba*—(Woudenberg et al. 2009).

**Distribution:** Austria, France, Switzerland—(Woudenberg et al. 2009).

***Neodidymelliopsis*** Qian & L. Cai

*Neodidymelliopsis* was introduced for one section of phoma-like species that reside within Didymellaceae and is typified by *N. cannabis* (Chen et al. 2017). The genus is characterized by immersed or erumpent subglobose to pyriform, ostiolate ascomata, cylindrical to clavate asci and subovoid to ellipsoidal, hyaline, septate ascospores. The asexual morph is phoma-like with pycnidial conidiomata, a 2–7-layered pseudoparenchymatous pycnidial wall, phialidic conidiogenous cells, and aseptate or occasionally 1-septate conidia (Chen et al. 2015, 2017). We introduce a new host record of *N. longicolla* from *Clematis vitalba* in Italy (Fig. 14).

***Neodidymelliopsis longicolla*** Hou, Crous & L. Cai, Stud. Mycol. 87: 153 (2017), **new host record**

**Index Fungorum number:** IF820006; **Facesoffungi number:** FoF 07259, Fig. 14.

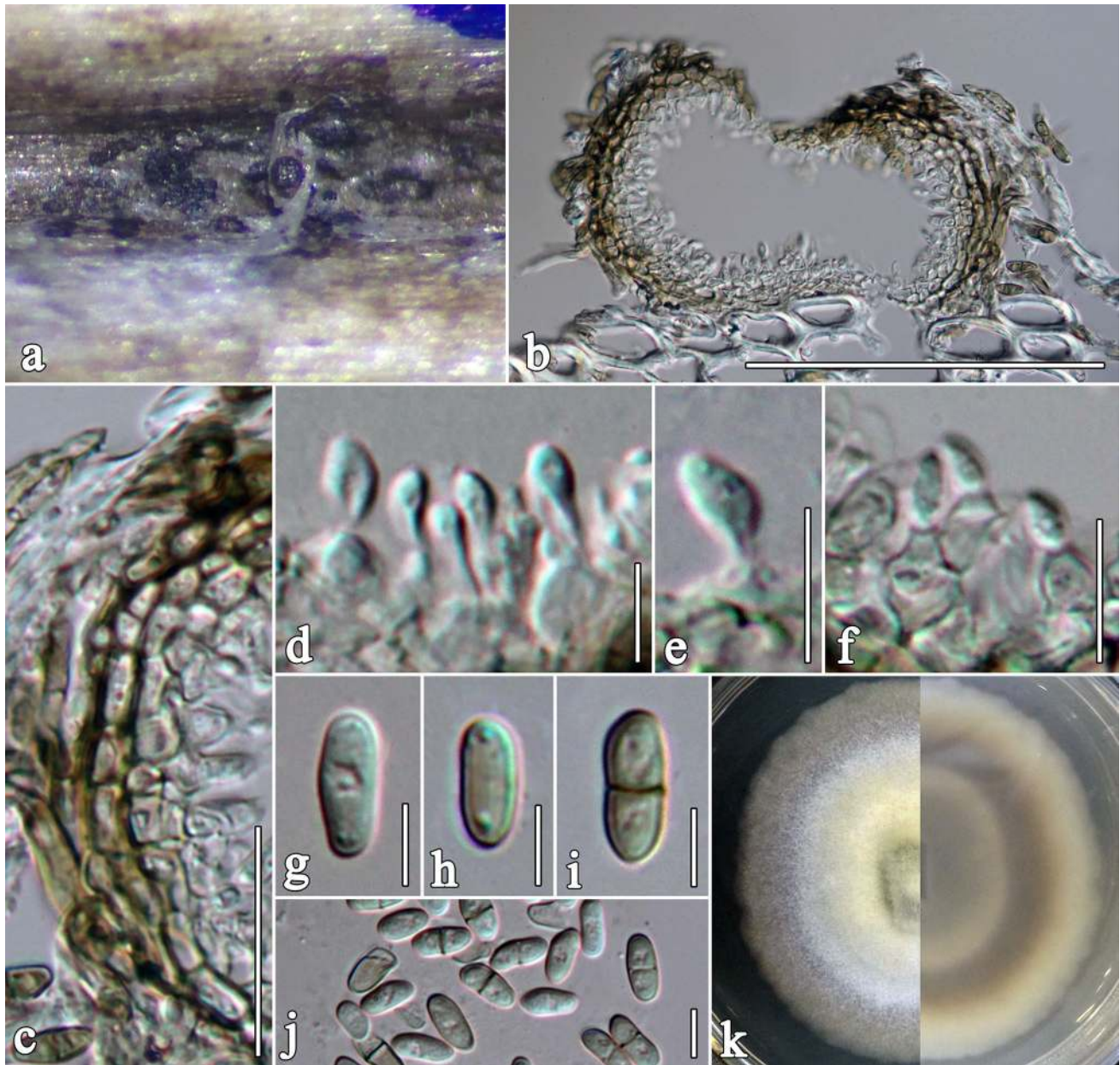
**Saprobic** on dead stems of *Clematis vitalba*. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* 70–95 × 124–134 µm ( $\bar{x}$  = 84 × 130 µm, n = 5), pycnidial, aggregated, uniloculate, superficial or covered by host epidermal layer, subglobose to depressed, cupulate when dried coriaceous, thick-walled, light brown to brown, with papillate ostioles. *Ostioles* 80 × 38 µm, central, papillate, opening by a pore. *Conidiomatal wall* 10–17(–27) µm wide, composed of 5–7 layers of light brown to brown cells of

*textura angularis*, heavily pigmented at the outer layers, lined with a hyaline layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells*  $2.5\text{--}7 \times 2\text{--}4.5 \mu\text{m}$  ( $\bar{x} = 4.5 \times 3.5 \mu\text{m}$ ,  $n = 30$ ), phialidic, annellidic, determinate, discrete, ampulliform, cylindrical to sub-cylindrical, smooth-walled, hyaline, arising from the inner layers of conidiomata. *Conidia*  $6.5\text{--}10 \times 2\text{--}4.5 \mu\text{m}$  ( $\bar{x} = 8 \times 4 \mu\text{m}$ ,  $n = 50$ ), oblong-elliptical, oval, slightly curved towards the ends, rounded ends, with 1(–2) guttules in each cell, initially aseptate and hyaline, becoming pale brown

and 1-septate at maturity, constricted at the septum, wall verrucose.

**Culture characters:** Colonies on MEA reaching 20 mm diam. after 2 weeks at 25 °C. Cultures from above, cream with white at the centre, medium dense, circular, umbonate, papillate, fluffy, covered with white aerial mycelium; reverse brown white cream at the edge.

**Material examined:** Italy, Forlì-Cesena Province, Castrocaro Terme, on dead aerial branch of *Clematis vitalba*, 19



**Fig. 14** *Neodidymelliopsis longicolla* (MFLU 20–0421). **a** Appearance of conidiomata on *Clematis vitalba*. **b** Vertical section through conidioma. **c** Section of conidioma wall. **d–f** Conidiogenous cells and

conidia. **g–j** Conidia. **k** Culture characteristics on MEA. Scale bars: **b** = 100  $\mu\text{m}$ , **c** = 20  $\mu\text{m}$ , **d–f** = 10  $\mu\text{m}$ , **g–j** = 10  $\mu\text{m}$

September 2012, E. Camporesi, IT739 (MFLU 20–0421); living culture, MFLUCC 17–2167.

**Hosts:** Soil in desert, *Clematis vitalba*—(Chen et al. 2017; this study).

**Distribution:** Israel, Italy—(Chen et al. 2017; this study).

**GenBank accession numbers:** LSU: MT214552; SSU: MT226670; ITS: MT310599; *tef1*: MT394733; *rpb2*: MT394682.

**Notes:** A new isolate of *Neodidymelliopsis longicolla* (MFLUCC 17–2167) was collected from *Clematis vitalba* in Italy. The new isolate formed a close relationship with the type (100% ML/1.00 BYPP). *Neodidymelliopsis longicolla* (CBS 382.96) was originally reported from a soil sample in Israel. The characters of the type strain were obtained from a culture on OA medium. The new collection differs slightly from the type material in having a shorter ostioles (Fig. 14).

#### *Xenodidymella* Chen & L. Cai

*Xenodidymella* typified by *X. applanata* ( $\equiv$  *Didymosphaeria applanata*) has *Phoma argillacea* as the asexual morph (Corlett 1981; Gruyter et al. 2013; Chen et al. 2015). *Xenodidymella* is distinct from other genera in Didymellaceae in having a thick peridium and ellipsoidal, allantoid or sub-cylindrical, unicellular conidia. *Xenodidymella* is reported from Europe and USA and consists of five species (Farr and Rossman 2020; Index Fungorum 2020). We introduce a novel species of *Xenodidymella clematidis* from *Clematis vitalba* in Italy and provide phylogenetic and morphological comparisons (Figs. 12, 15).

#### *Xenodidymella clematidis* Phukhams., Camporesi & K.D. Hyde, **sp. nov.**

**Index Fungorum number:** IF557132; **Facesoffungi number:** FoF 07260, Fig. 15.

**Etymology:** Refers to the host genus *Clematis*.

**Holotype:** MFLU 16–2288.

**Saprobic** on dead stems of *Clematis vitalba*. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* 220–375  $\times$  180–290  $\mu\text{m}$  ( $\bar{x}$  = 280  $\times$  230  $\mu\text{m}$ ,  $n$  = 5), pycnidial, aggregated, uniloculate, superficial or semi-immersed, sub-globose to depressed globose, cupulate, when dried coriaceous, thick-walled, brown to light brown, with ostioles. *Ostioles* central, papillate, opened-like pore. *Conidiomatal wall* 10–15(–25  $\mu\text{m}$  at apex) wide, composed of 7–9 layers of light brown to brown cells of *textura angularis*, heavily pigmented at the outer layers, lined with a hyaline layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 2–6(–12)  $\times$  2.3–3.3  $\mu\text{m}$  ( $\bar{x}$  = 5  $\times$  3  $\mu\text{m}$ ,  $n$  = 20), enteroblastic, phialidic, determinate, discrete, ampulliform, smooth-walled, hyaline, arising from the inner layers of conidiomata. *Conidia* 4–8  $\times$  2–5  $\mu\text{m}$  ( $\bar{x}$  = 6  $\times$  3.5  $\mu\text{m}$ ,  $n$  = 50), oblong-elliptical, oval, slightly

curved towards the ends, rounded ends, hyaline, with 1(–2) guttules in each cell, aseptate, verrucose.

**Culture characters:** Colonies on MEA reaching 20 mm diam. after 2 weeks at 16 °C. Cultures from above, grey with white aerial mycelium, dense, circular, umbonate, papillate, fluffy, reverse brownish white, cream at the edge.

**Material examined:** Italy, Forlì-Cesena Province, Monte Fumaiolo, dead aerial branch of *Clematis vitalba*, 6 August 2016, E. Camporesi, IT3054 (MFLU 16–2288, **holotype**); ex-type living culture, MFLUCC 16–1365.

**Host:** *Clematis vitalba*—(This study).

**Distribution:** Italy—(This study).

**GenBank accession numbers:** LSU: MT214553; ITS: MT310600; *act*: MT394622.

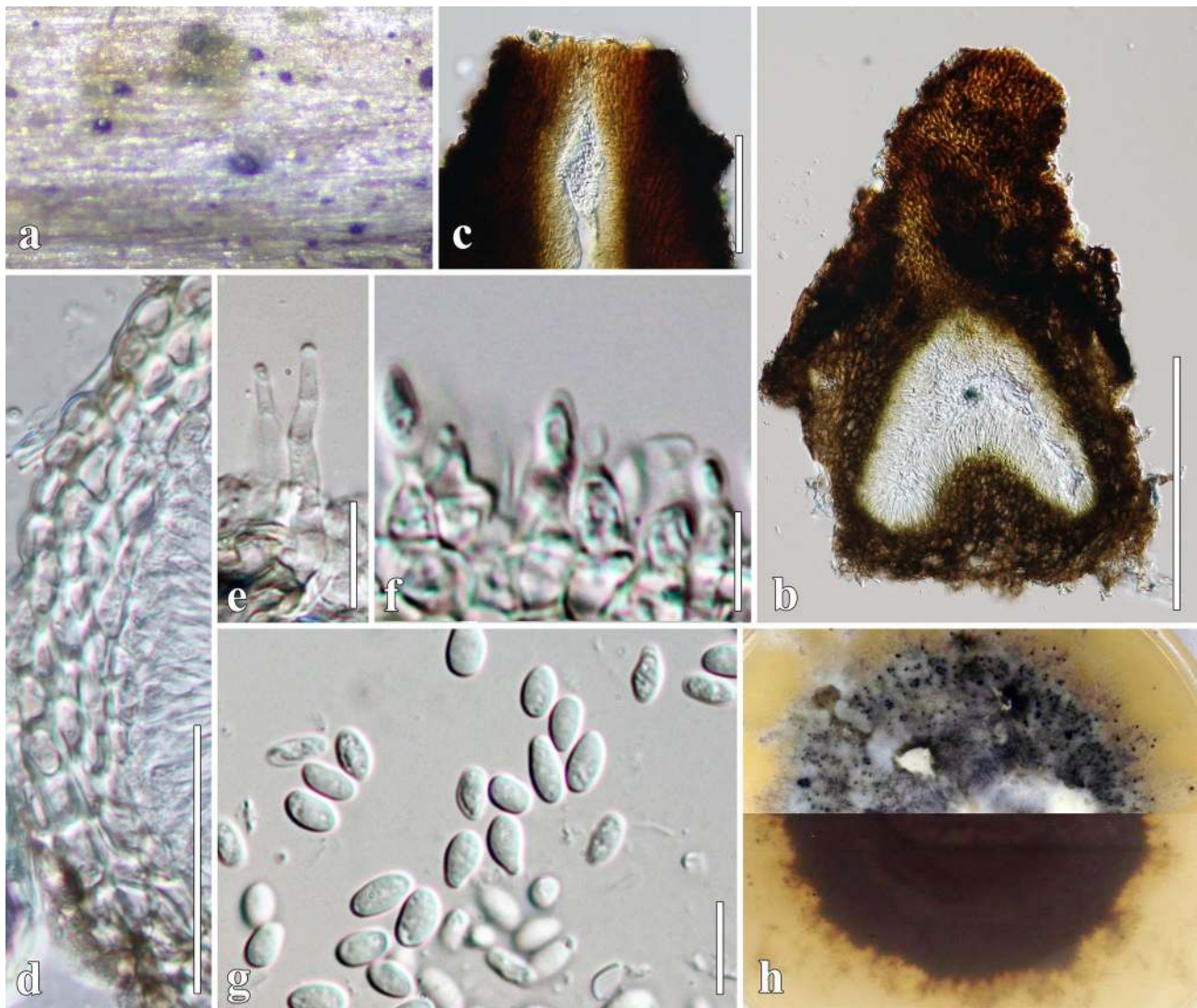
**Notes:** In the phylogenetic analysis, *Xenodidymella clematidis* clustered with *X. applanata* with strong support (1.00 in BYPP). *Xenodidymella clematidis* has a long ostiole, with oblong-elliptical or oval conidia, while *X. applanata* has a short ostiole and ellipsoidal conidia (Gruyter et al. 2002; Chen et al. 2015). *Xenodidymella applanata* is commonly a pathogen of raspberry (*Rubus* sp.). *Xenodidymella clematidis* was a saprobe on *Clematis vitalba* (Fig. 15). In a BLASTn search of GenBank, the ITS sequence had 98% similarity (11 nucleotides differences out of 488 nucleotides). The new strain is introduced as a new species of *Xenodidymella* herein.

#### Didymosphaeriaceae Munk

The latest treatment of Didymosphaeriaceae was by Ariyawansa et al. (2014a). The family is typified with *Didymosphaeria*. Several genera have been introduced to the family based on morphological and phylogenetic evidence (Tibpromma et al. 2018; Wanasinghe et al. 2018; Wijayawardene et al. 2018). Mapook et al. (2020) introduced *Chromolaenicola* Mapook & K.D. Hyde into Didymosphaeriaceae. Phylogeny and morphological comparisons revealed a novel species of *Chromolaenicola* and a new host record of *Didymosphaeria rubi-ulmifolii* from *Clematis* (Fig. 16).

#### *Chromolaenicola* Mapook & K.D. Hyde

Mapook et al. (2020) introduced *Chromolaenicola* (typified with *C. nanensis*) for a monophyletic clade of fungi described on *Chromolaena odorata*. *Chromolaenicola* is characterized by immersed to semi-immersed and coriaceous ascomata, cylindrical asci, and uniseriate, ellipsoid, muriform ascospores (Mapook et al. 2020). The asexual morph is pycnidial, with enteroblastic, phialidic conidiogenous cells, and oblong or oval to ellipsoid, globose to sub-globose conidia (Jayasiri et al. 2019; Mapook et al. 2020). We introduce a new species *Chromolaenicola clematidis* based on morphological comparison (Fig. 17) and phylogenetic analyses (Fig. 16).



**Fig. 15** *Xenodidymella clematidis* (MFLU 16–2288, holotype). **a** Appearance of conidiomata on *Clematis vitalba*. **b** Vertical section through conidioma. **c** Ostiolar canal. **d** Section of conidioma wall. **e**,

**f** Conidiogenous cells and conidia. **g** Conidia. **h** Culture characters on MEA. Scale bars: **b** = 200  $\mu$ m, **c**, **d** = 50  $\mu$ m, **e–g** = 10  $\mu$ m

***Chromolaenicola clematidis*** Phukhams. & K.D. Hyde, **sp. nov.**

*Index Fungorum* number: IF557133; *Facesoffungi* number: FoF 07253, Fig. 17.

*Etymology*: Named after the host genus, *Clematis*.

*Holotype*: MFLU 17–1483.

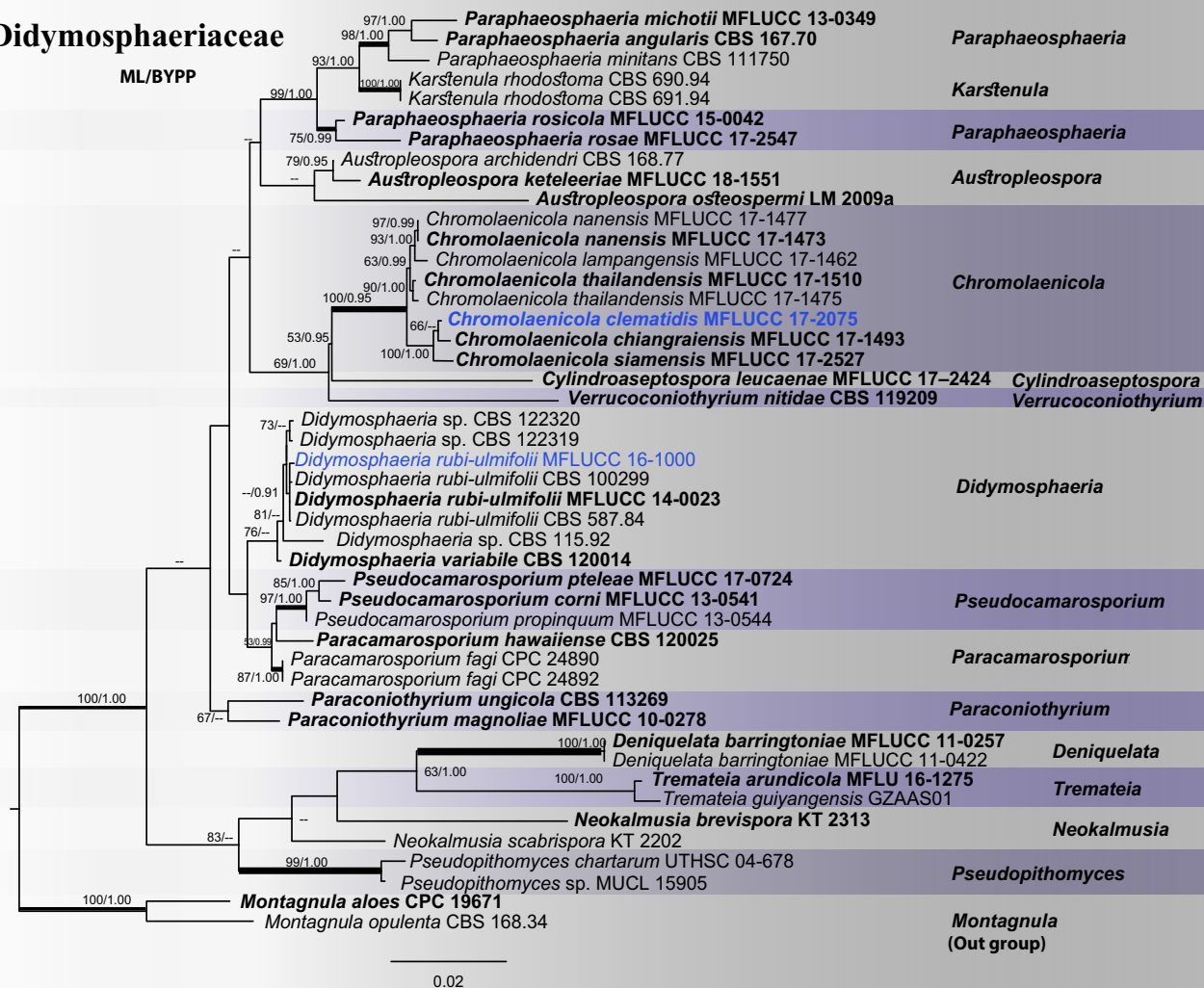
*Saprobic* on dead stem of *Clematis subumbellata*. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 76–145  $\times$  107–128  $\mu$ m ( $\bar{x}$  = 121  $\times$  117  $\mu$ m,  $n$  = 10), pycnidial, solitary, uniloculate, immersed, globose, coriaceous, thin, brown to light brown, ostiolate. *Conidiomatal wall* 5–10  $\mu$ m wide, uniform, wider at apex, composed of 3–5 layers of pale brown to bronze cells of *textura angularis*, lined with a thin, hyaline layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells*

2.6–4.5  $\times$  4–7  $\mu$ m ( $\bar{x}$  = 3.5  $\times$  5  $\mu$ m,  $n$  = 20), enteroblastic, phialidic, determinate, discrete, truncate, hyaline, smooth, arising from the inner layer of pycnidial wall. *Conidia* 7–10  $\times$  4.5–7  $\mu$ m ( $\bar{x}$  = 8.5  $\times$  6  $\mu$ m,  $n$  = 50), broad oblong or oval, rounded ends, hyaline when immature, reddish brown at maturity, 1-septate, with guttule in each cell, rough-walled, verrucose.

*Cultural characters*: Colonies on MEA reaching 30 mm diam. after 4 weeks at 25  $^{\circ}$ C. Cultures from above, dark brown, radiating outwardly, dense, umbonate, undulate edge, umbonate, papillate, fluffy, covered with white aerial mycelium; reverse dark brown, undulate.

*Material examined*: Thailand, Chiang Rai Province, Mae Sai District, dead stems of *Clematis subumbellata*, 20 March

## Didymosphaeriaceae



**Fig. 16** The best scoring RAxML tree with a final likelihood value of  $-12878.030199$  based on combined LSU, ITS, SSU, *rpb2* and *tub* sequence data of Didymosphaeriaceae. The tree is rooted with sequences of *Montagnula* species. Forty-six strains were included in the combined DNA sequence analyses which comprised 3681 characters (860 characters for LSU, 516 characters for ITS, 913 characters for SSU, 935 characters for *rpb2*, 457 characters for *tub*, including gap regions). The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree from the maximum likelihood analysis had similar topology to the Bayesian analyses. The matrix had 764 distinct alignment patterns, with 9.02% undetermined characters or gaps proportions. Estimated base frequen-

cies were as follows: A=0.239562, C=0.247759, G=0.271470, T=0.241209; substitution rates AC=1.719408, AG=2.576378, AT=1.444379, CG=0.958169, CT=9.083623, GT=1.000000; gamma distribution shape parameter  $\alpha=0.621443$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study are indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses at the genus level (BS  $\geq$  70%/BYPP  $\geq$  0.95)

2017, C. Phukhamsakda, CMTH19 (MFLU 17-1483, **holotype**); ex-type living culture, MFLUCC 17-2075.

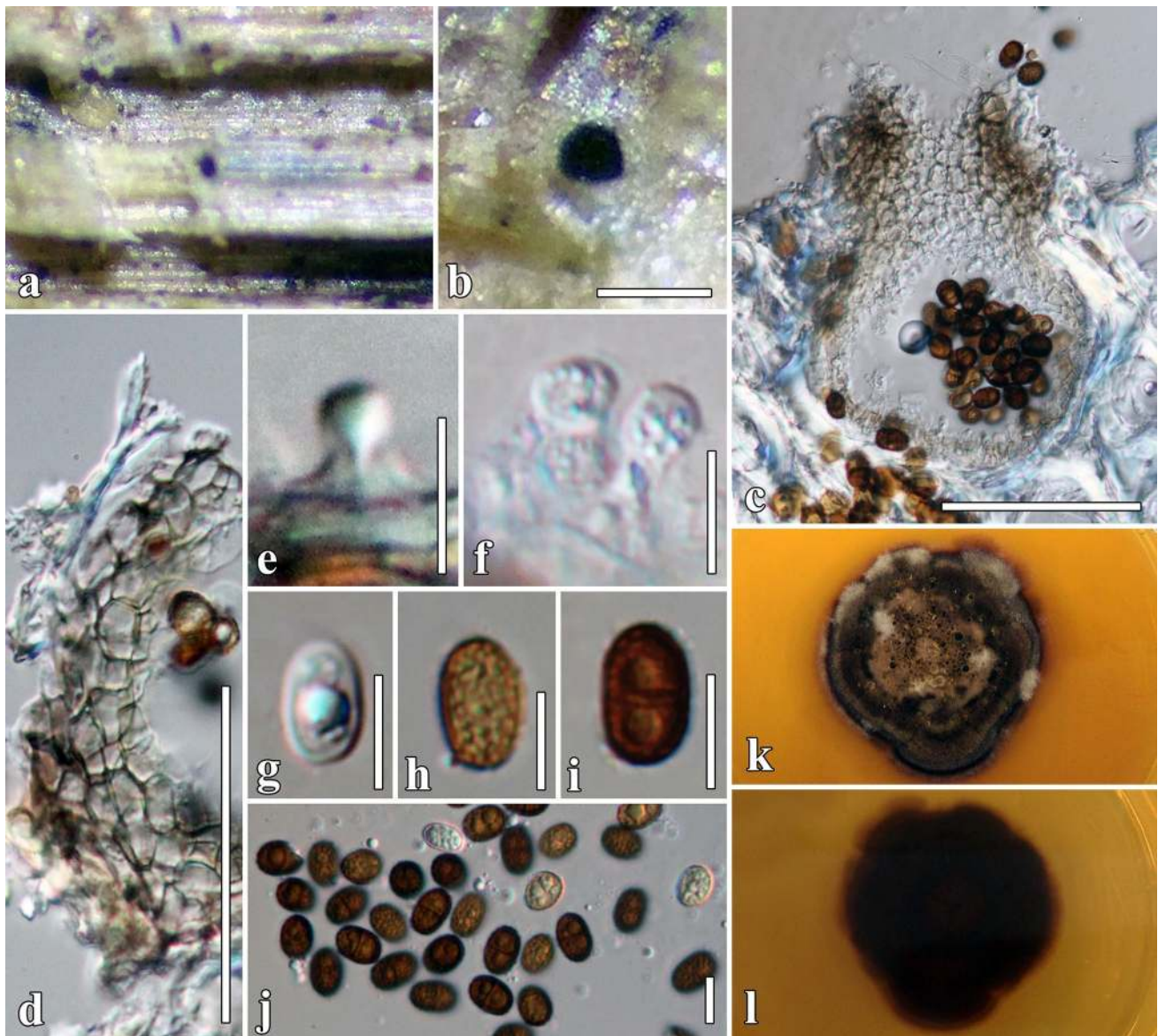
*Host*: *Clematis subumbellata*—(This study).

*Distribution*: Thailand—(This study).

*GenBank accession numbers*: LSU: MT214554; SSU: MT226671; ITS: MT310601; *rpb2*: MT394683.

*Notes*: In our phylogenetic analysis (Fig. 16), *Chromolaenicola clematidis* (MFLUCC 17-2075) clustered with *C. chiangraiensis* Mapook & K.D. Hyde with moderate statistical support (66% ML/0.82 BYPP). A comparison of the ITS sequences showed three nucleotide differences in 516 nucleotides, while the *rpb2* showed five nucleotide differences in 935 nucleotides. *Chromolaenicola clematidis* has smaller





**Fig. 17** *Chromolaenicola clematidis* (MFLU 17–1483, holotype). **a, b** Appearance of conidiomata on *Clematis subumbellata*. **c** Vertical section through conidioma. **d** Section of conidioma wall. **e–f** Con-

idiogenous cells and conidia. **g–j** Conidia. **k–l** Culture characters on MEA. Scale bars: **b** = 200  $\mu$ m, **c, d** = 50  $\mu$ m, **e–j** = 5  $\mu$ m

conidia than *C. chiangraiensis* (mean  $8.5 \times 6$  vs  $11 \times 7.5$   $\mu$ m) with guttules in each cell (Fig. 17, Table 2). The new strain is introduced as a new species of *Chromolaenicola* based on the guidelines proposed by Jeewon and Hyde (2016).

#### *Didymosphaeria* Fuckel

*Didymosphaeria* is characterized by trabeculate pseudoparaphyses (sensu Liew et al. 2000), which anastomose above the cylindrical asci, and uniseriate, 1-septate ascospores (Aptroot 1995; Ariyawansa et al. 2014b). The asexual morph of *Didymosphaeria* has been suggested to be *Ascochyta*, fusicladiella-like, *Periconia*, and phoma-like

species but a holomorphic connection has not been proven (Sivanesan 1984; Kirk et al. 2008; Ariyawansa et al. 2014b). *Didymosphaeria* is typified by *D. futilis*, however, fresh collections are needed to confirm its phylogenetic placement (Ariyawansa et al. 2014a; Wijayawardene et al. 2018). More than 500 epithets are listed under *Didymosphaeria* (Index Fungorum 2020), but only seven species were accepted by Aptroot (1995). Only three species of *Didymosphaeria* have phylogenetic evidence (*D. rubi-ulmifolii*, *D. variabile*, and *Didymosphaeria* sp. (as *Paraconiothyrium brasiliense* CBS 115.92, CBS 587.84, CBS 122319 and CBS 122320)) (Verkley et al. 2004). In this study, phylogenetic analyses based

**Table 2** A comparison of *Chromolaenicola* species discussed in this study

Species	Conidiomata ( $\mu\text{m}$ )	Conidiogenous cells ( $\mu\text{m}$ )	Conidia ( $\mu\text{m}$ )	Host
<i>Chromolaenicola clematidis</i>	76–145 $\times$ 107–128	2.6–4.5 $\times$ 4–7	7–10 $\times$ 4–7	<i>Clematis subumbellata</i>
<i>Ch. chiangraiensis</i> (MFLUCC 17–1493)	–	3.5–6.5 $\times$ 1–2	9–14 $\times$ 6–9	<i>Chromolaena odorata</i> (Asteraceae)
<i>Ch. siamensis</i> (MFLUCC 17–2527)	110–165 $\times$ 140–190	6.5–7.4 $\times$ 3.2–4.7	7–9 $\times$ 5–6	<i>Leucaena</i> sp. (Fabaceae)

on a combined dataset of the LSU, ITS, SSU, *rpb2*, and *tub* sequences revealed a new host record for *D. rubi-ulmifolii* on *Clematis heracleifolia* from China (Fig. 18).

***Didymosphaeria rubi-ulmifolii*** Ariyaw., Camporesi & K.D. Hyde, Phytotaxa 176:111 (2014), **new host record**

*Index Fungorum number*: IF808165; *Facesoffungi number*: FoF 07254, Fig. 18.

*Saprobic* on dead stem of *Clematis heracleifolia*. **Sexual morph**: Ariyawansa et al. (2014a). **Asexual morph**: *Conidiomata* 78–160  $\times$  75–244  $\mu\text{m}$  ( $\bar{x}$  = 110  $\times$  120  $\mu\text{m}$ ,  $n$  = 5), pycnidial, solitary, unilocular or multilocular, scattered, immersed or erumpent, under host epidermis, globose to compressed, brown to dark brown, without ostioles. *Pycnidial wall* 12–20(–30  $\mu\text{m}$  at apex) wide, composed of 4–5 brown cell layers of *textura angularis*, inner layer subhyaline, lining bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 1.8–5  $\times$  2–4  $\mu\text{m}$  ( $\bar{x}$  = 3  $\times$  3  $\mu\text{m}$ ,  $n$  = 20), enteroblastic, phialidic, determinate, smooth-walled, hyaline. *Conidia* 6–11  $\times$  2.5–4.5  $\mu\text{m}$  ( $\bar{x}$  = 9  $\times$  4  $\mu\text{m}$ ,  $n$  = 50), ellipsoid, 1 septum, constricted at septum, rounded ends, initially hyaline, pale brown at maturity, with 1–2 guttules, smooth-walled.

*Cultural characters*: Colonies on MEA reaching 30 mm diam. after 4 weeks at 16 °C. Cultures from above, olive brown at the centre, radiating outwardly, medium dense, circular, entire edge, umbonate, papillate, fairly fluffy, covered with white aerial mycelium; reverse dark brown at the centre, faintly zonate slightly present, white mycelium at the edge.

*Material examined*: China, Dali, on dead terrestrial stem of *Clematis heracleifolia* DC., 8 May 2016, C. Phukhamsakda, CMCN03 (MFLU 17–1460); living culture, MFLUCC 16–1000.

*Hosts*: *Coffea arabica*, *Rubus ulmifolius*, *Clematis heracleifolia*—(Verkley et al. 2004; Ariyawansa et al. 2014a; this study).

*Distribution*: Brazil, China, Italy—(Verkley et al. 2004; Ariyawansa et al. 2014a; this study).

*GenBank accession numbers*: LSU: MT214555; SSU: MT226672; ITS: MT310602; *tefl*: MT394734.

*Notes*: Ariyawansa et al. (2014a) introduced *Didymosphaeria rubi-ulmifolii* from a collection of *Rubus ulmifolius* in Italy. The ex-type strain (MFLUCC 14–0023) formed

a distinct clade with the type strain of *Paraconiothyrium brasiliense* (CBS 100299). Based on the multilocus phylogenetic analyses, our strain (MFLUCC 16–1000) formed a close relationship with the other *D. rubi-ulmifolii* strains with moderate support (Fig. 16), with no significant pairwise differences. Morphological comparison of *D. rubi-ulmifolii* (MFLUCC 16–1000) with the asexual morph report in *D. rubi-ulmifolii* (strain CBS 100299) show that they are different in the conidial characters (Fig. 18). Strain MFLUCC 16–1000 is saprobic and has 2-celled conidia while, CBS 100299 has single celled conidia in culture (Verkley et al. 2004). This study is the first record of *D. rubi-ulmifolii* on *Clematis* species.

**Hermatomycetaceae** Locq. ex A. Hashim. & Kaz. Tanaka

Hermatomycetaceae is typified by *Hermatomyces* and currently only known from asexual morph characters (Tibpromma et al. 2016, 2018; Koukol et al. 2018; Hyde et al. 2019a). We introduce a novel *Hermatomyces* species based on its distinct morphology with phylogenetic support (Figs. 19, 20).

***Hermatomyces*** Speg.

*Hermatomyces tucumanensis* is the type species. The genus has sporodochial conidiomata, and muriform, lenticular, hyaline or dematiaceous conidia of one or two types (Spegazzini 1911; Chang 1995; Tibpromma et al. 2016; Hashimoto et al. 2017; Hyde et al. 2019a). Examination of a *Clematis* collection revealed a novel species *Hermatomyces clematidis* based on its distinct morphological and phylogenetic relationship from other *Hermatomyces*. This is the first record of a *Hermatomyces* species on *Clematis* (Fig. 21).

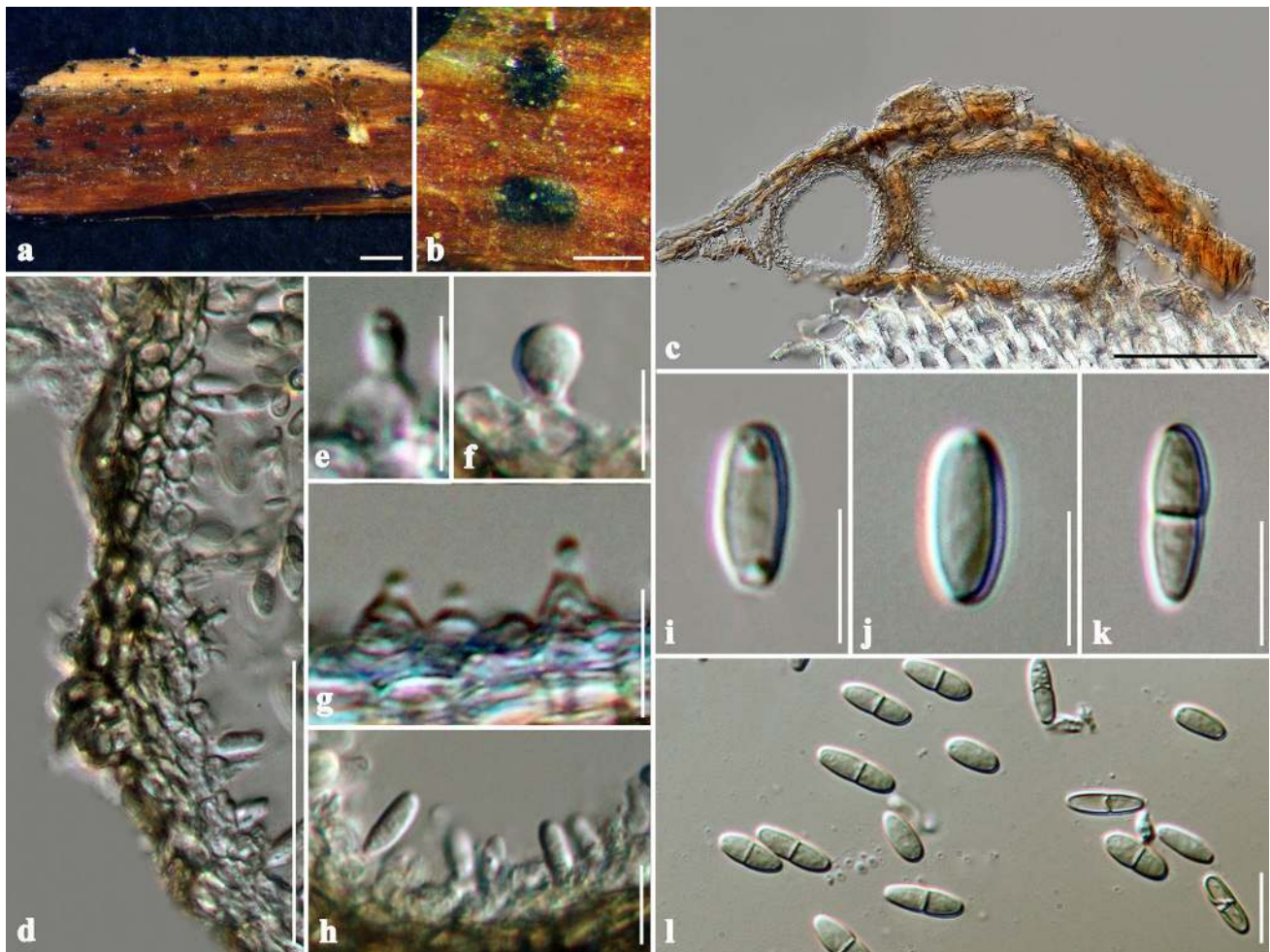
***Hermatomyces clematidis*** Phukhams., D.J. Bhat & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557134; *Facesoffungi number*: FoF 07244, Fig. 21.

*Etymology*: Name refers to the host plant, *Clematis*.

*Holotype*: MFLU 17–1493.

*Saprobic* on dead stem of *Clematis sikkimensis*. **Sexual morph**: Undetermined. **Asexual morph**: Hyphomycetous. *Colonies* on natural substrate forming sporodochial conidiomata, subiculate, superficial, scattered, circular or oval, blackish brown, velvety, glistening, orbicular, with



**Fig. 18** *Didymosphaeria rubi-ulmifolii* (MFLU 17–1535). **a, b** Appearance of conidiomata on *Clematis heracleifolia*. **c** Vertical section through conidioma. **d** Section of conidioma wall. **e–h** Con-

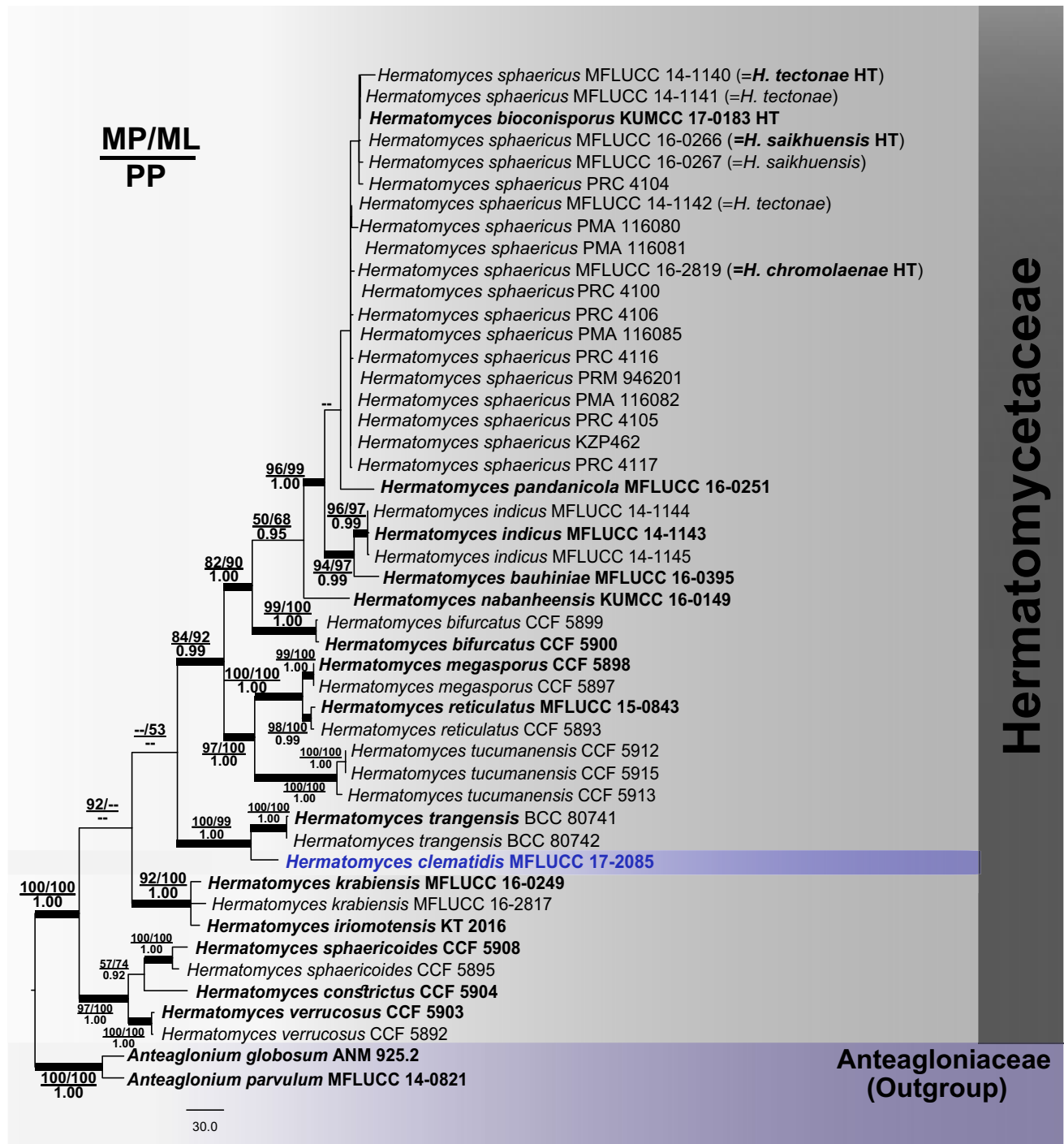
idiogenous cells and conidia. **i–l** Conidia. Scale bars: **a**=500  $\mu\text{m}$ , **b**=200  $\mu\text{m}$ , **c**=100  $\mu\text{m}$ , **d**=20  $\mu\text{m}$ , **e–k**=5  $\mu\text{m}$ , **l**=10  $\mu\text{m}$

abundant sporulation, conidia readily liberated when agitated, 410–565  $\mu\text{m}$  wide ( $\bar{x}$ =490  $\mu\text{m}$ ,  $n$ =20). *Mycelium* 2–4  $\mu\text{m}$  wide, mostly superficial, composed of a loose or compact network of repent, branched, septate, rough-walled, thick-walled, reddish brown to brown hyphae; subicular hyphae short, densely packed. *Conidiophores* 22–38  $\times$  2–5  $\mu\text{m}$ , micronematous or semi-macronematous, mononematous, cylindrical, erect, verruculose, aseptate, branched, often corresponding to conidiogenous cells, reddish brown to brown. *Conidiogenous cells* 7–13  $\times$  4–7  $\mu\text{m}$ , holoblastic, monoblastic, solitary, integrated, terminal, determinate, cylindrical or slightly subulate, subsphaerical or ampulliform, reddish brown to brown, sometimes hyaline. *Conidia* dimorphic solitary, smooth-walled; *lenticular conidia*: 30–45  $\times$  24–31  $\mu\text{m}$  ( $\bar{x}$ =40  $\times$  28  $\mu\text{m}$ ,  $n$ =30), muriform, smooth, disc-shaped, circular to oval in front view, central cells brown to reddish brown, peripheral cells hyaline to subhyaline, forming a wide and distinct ring, slightly

constricted at the septa, inside view composed of one column of 5–6 cells, end cells subhyaline to pale brown, often carrying remnant of conidiogenous cell at base; *cylindrical conidia*: 29–35  $\times$  12–14  $\mu\text{m}$  ( $\bar{x}$ =32  $\times$  13  $\mu\text{m}$ ,  $n$ =20), straight or flexuous, septate, constricted at the septa, consisting of one or two columns, usually separate at apex, each column with 4–5 transverse septa, obclavate, apical cell acute, basal cells rounded, smooth, hyaline.

*Culture characters*: Colonies on MEA reaching 50 mm diam. after 4 weeks at 25  $^{\circ}\text{C}$ . Cultures from above, white, dense, circular, margin erose, umbonate, papillate with fairly fluffy, wrinkled, folded, pale orange, covered with cream aerial mycelium; reverse dark brown at the centre, faintly zonate at the edge, greyish orange radiating outwardly.

*Material examined*: Thailand, Chiang Rai Province, on dead stem of *Clematis sikkimensis*, 2 May 2017, C. Phukhamsakda & M.V. de Bult, CMTHDT01 (MFLU



**Fig. 19** Phylogram generated from maximum parsimony analysis based on combined LSU, ITS, *tef1* and *rpb2* sequence data. Related sequences are taken from Nuankaew et al. (2019) and retrieved from GenBank. Forty-nine strains were included in the analysis of the combined DNA loci and comprise 3309 characters (826 characters for LSU, 514 characters for ITS, 948 characters for *tef1*, 1021 characters for *rpb2*, including gaps). The tree is rooted with *Anteaglonium globosum* (ANM 925.2) and *A. parvulum* (MFLUCC 14-0821) in Anteagloniaceae. Maximum parsimony analysis of 471 parsimony informative characters resulted in a most parsimonious tree (CI=0.674, RI=0.879, RC=0.593, HI=0.326). The best scoring RAxML tree had a final likelihood value of -10433.015131. The matrix had 802 distinct alignment patterns with 28.12% undetermined characters and gaps. Estimated base frequencies were: A=0.244372, C=0.264291, G=0.261114, T=0.230223; substitution rates AC=0.858179, AG=3.949480, AT=1.122065, CG=0.761732, CT=11.028490, GT=1.000000; gamma distribution shape parameter  $\alpha=0.151089$ . In our analysis, GTR+I + G model was used for each partition in Bayesian posterior analysis. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95). The ex-type strains are in bold and black. The newly generated sequence is in bold and blue

17–1493, **holotype**); ex-type living culture, MFLUCC 17–2085.

*Host*: *Clematis sikkimensis*—(This study).

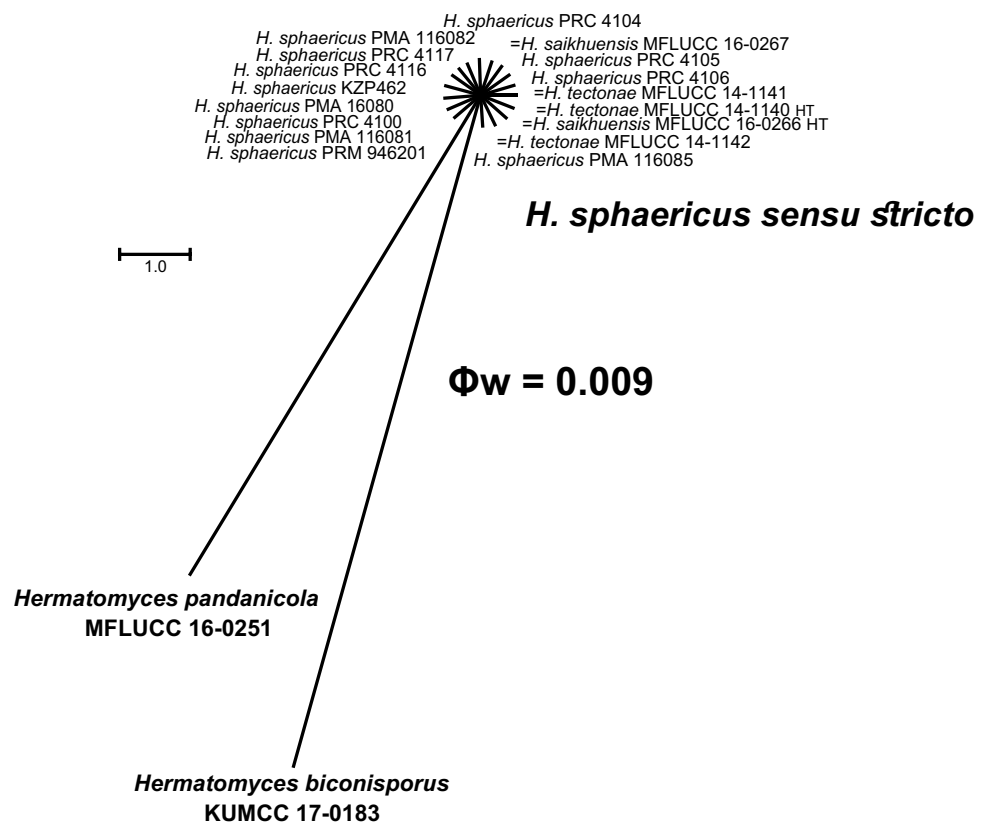
*Distribution*: Thailand—(This study).

*GenBank accession numbers*: LSU: MT214556; SSU: MT226673; ITS: MT310603; *tef1*: MT394735; *rpb2*: MT394684.

*Notes*: *Hermatomyces clematidis* is introduced as a new species based on its distinct morphology and phylogenetic results of a combined LSU, ITS, *tef1*, and *rpb2* dataset (Fig. 19). *Hermatomyces clematidis* matches the generic concept in having sporodochial conidiomata, with both lenticular and cylindrical conidia (Doilom et al. 2017; Hashimoto et al. 2017, Fig. 21). Morphological comparison with known *Hermatomyces* species shows it is similar to *H. tucumanensis*, however, the conidiophore of *H. clematidis* are straighter with larger conidia ((22–)27–35  $\times$  18–25 vs 30–45  $\times$  24–31  $\mu$ m) (Koukol et al. 2018). In the phylogenetic analysis, *H. clematidis* formed a close relationship with *H. trangensis* with strong support (100% MP/99% ML/1.00 BYPP). *Hermatomyces trangensis* is associated with sugar palm in southern Thailand (Nuankaew et al. 2019). *Hermatomyces trangensis* differs from *H. clematidis* because it lacks cylindrical conidia. In a BLASTn search of GenBank, the closest match of the LSU sequence of MFLUCC 17–2085 is *H. subiculosa* (MFLUCC 15–0843) with 97% similarity, while the closest match of the ITS sequence was *H. tectonae* (MFLUCC 14–1140) (NR\_154079) with 97% similarity.

Based on current evidence, phylogenetic results from the concatenated gene loci does not delineate *Hermatomyces*

**Fig. 20** The splits graph from the pairwise homoplasy index (PHI) test generated from the concatenated gene set of LSU, ITS, *tef1* and *rpb2* sequence data of closely related species using both LogDet transformation and splits decomposition. PHI test results ( $\Phi_w$ ) < 0.05 indicates significant recombination within the dataset



species, especially the *H. sphaericus* clade (Fig. 19). Therefore, we applied the GCPSR concept with the *H. sphaericus* clade. Seventeen isolates of *H. sphaericus* formed an isolated clade with two species (*H. biconisporus* and *H. pandanicola*) forming distant relationship lineage with low statistic support. However, the pairwise homoplasy index showed  $\Phi_w = 0.009$  when the degree of genealogical correlation model was applied between neighbouring strains of the clade (Fig. 20). These results are not congruent with the phylogenetic lineages shown in Fig. 19, which rather indicate that *H. biconisporus*, *H. pandanicola* and *H. sphaericus* should currently be treated as the same species.

### Leptosphaeriaceae Barr

Leptosphaeriaceae is typified by *Leptosphaeria*. Ariyawansa et al. (2015b) illustrated the members of Leptosphaeriaceae and included ten genera. Quaedvlieg et al. (2013) introduced an asexual morph genus, *Acicuseptoria* Quaedvlieg, Verkley & Crous to the family, however, a multilocus phylogenetic analysis (Fig. 22) reveals that *Acicuseptoria* clusters with *Paraleptosphaeria*. Crous and Groenewald (2017a) introduced *Querciphoma* isolated from stems and leaves of woody plants in a terrestrial environment. Members of this family usually have a single, papillate, immersed or erumpent, perithecial ascomata, with thick, scleroplectenchymatous or plectenchymatous cells and cylindrical asci with hyaline to brown, transversely septate ascospores (Hyde et al. 2013; Ariyawansa et al. 2015b). The asexual morphs in Leptosphaeriaceae can be coelomycetous or hyphomycetous (Gruyter et al. 2013; Hyde et al. 2013; Crous and Groenewald 2017). We introduce a novel *Alloleptosphaeria* species and describe a novel genus *Sclerenchymomyces* based on distinct morphology and phylogenetic support (Fig. 22).

#### *Alloleptosphaeria* Ariyaw., Wanas. & K.D. Hyde

*Alloleptosphaeria* was introduced as a monotypic genus to accommodate *A. italica* from *Clematis vitalba* in Italy (Ariyawansa et al. 2015b). It has immersed ascomata and a thin-walled peridium of reddish brown to dark brown pseudoparenchymatous cells (Ariyawansa et al. 2015b). In our phylogenetic analysis (Fig. 22), *Alloleptosphaeria* formed a distinct clade basal to *Leptosphaeria* sensu stricto. We introduce the second species of *Alloleptosphaeria* from *Clematis subumbellata* collected in Thailand (Fig. 23).

#### *Alloleptosphaeria clematidis* Phukhams. & K.D. Hyde, sp. nov.

*Index Fungorum* number: IF557109; *Facesoffungi* number: FoF 07286, Fig. 23.

*Etymology*: Epithet reflect the host genus *Clematis*.

*Holotype*: MFLU 17–1479.

*Saprobic* on dead stems of *Clematis subumbellata*.

**Sexual morph**: *Ascomata* 210–260 × 125–190 μm ( $\bar{x} = 237 \times 160$  μm, n = 5), on surface of the host, covered by a pseudoclypeus, visible as black spots, immersed, solitary, scattered, uniloculate, obpyriform, coriaceous, black to dark brown, rough-walled, with apical ostioles. *Ostioles* 70–85 × 50–70 μm ( $\bar{x} = 80 \times 60$  μm, n = 5), central, pale brown to dark brown, papillate, opening by a pore, ostioles with periphyses. *Peridium* 9–17 (–25 μm at apex) wide, thin, multilayered, pseudoparenchymatous cell type, comprising 4–5 layers of brown to dark brown cells of *textura angularis*, inner layers comprising thin, hyaline cells. *Hamathecium* composed of numerous, 5–3.5 μm wide ( $\bar{x} = 2.5$  μm, n = 50), dense, filiform branched, anastomosing above asci, reaching the ostioles, transversely septate, cellular pseudoparaphyses. *Asci* 75–125 × 8–15 μm ( $\bar{x} = 105 \times 10$  μm, n = 40), 8-spored, bitunicate, fissitunicate, cylindrical, apically rounded, short, bulbous pedicel, ocular chamber clearly visible when immature. *Ascospores* 15–25 × 6–9 μm ( $\bar{x} = 18 \times 7$  μm, n = 50), uniseriate, partially overlapping, broad fusiform, tapering towards the ends, round at both ends, with (1–)4–5 transverse septa, 1-longitudinal septum in each ascospore, initially hyaline, yellowish at maturity, slightly constricted at the septa, deeply constricted at the median septum, cell above median septum slightly wider than that below, verruculose, with guttule in each cell, with thin mucilaginous sheath. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA reaching 20 mm diam. after 4 weeks at 25 °C. Culture from above dark brown radiating outwardly, wrinkled folded in the middle, dense, circulate, flattened, umbonate, edge irregular, fairly fluffy; reverse black in the middle and dark brown at the edge, orange pigment slightly diffusing into the agar.

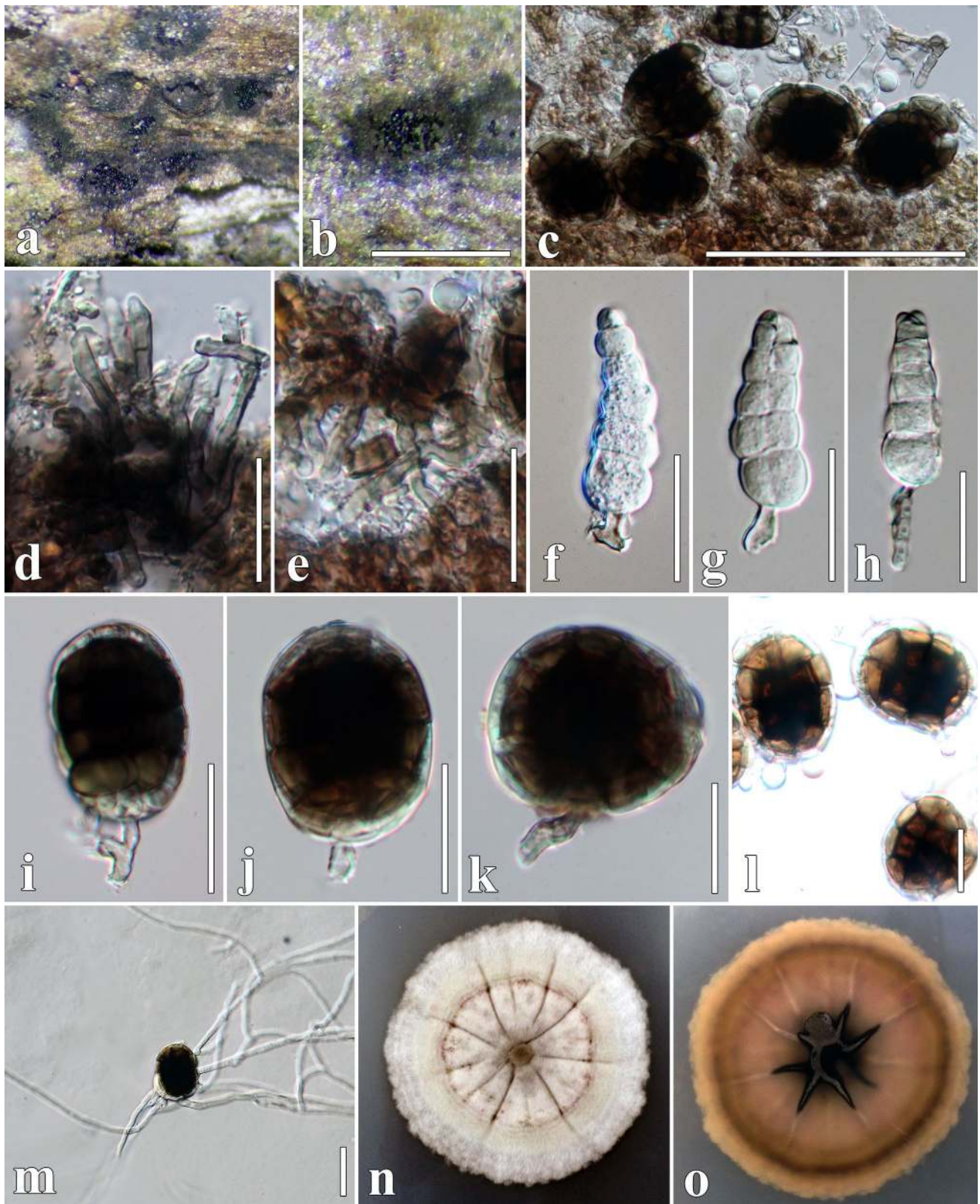
*Material examined*: Thailand, Chiang Rai Province, on dead stems of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH15 (MFLU 17–1479, **holotype**); ex-type living culture, MFLUCC 17–2071.

*Host*: *Clematis subumbellata*—(This study).

*Distribution*: Thailand—(This study).

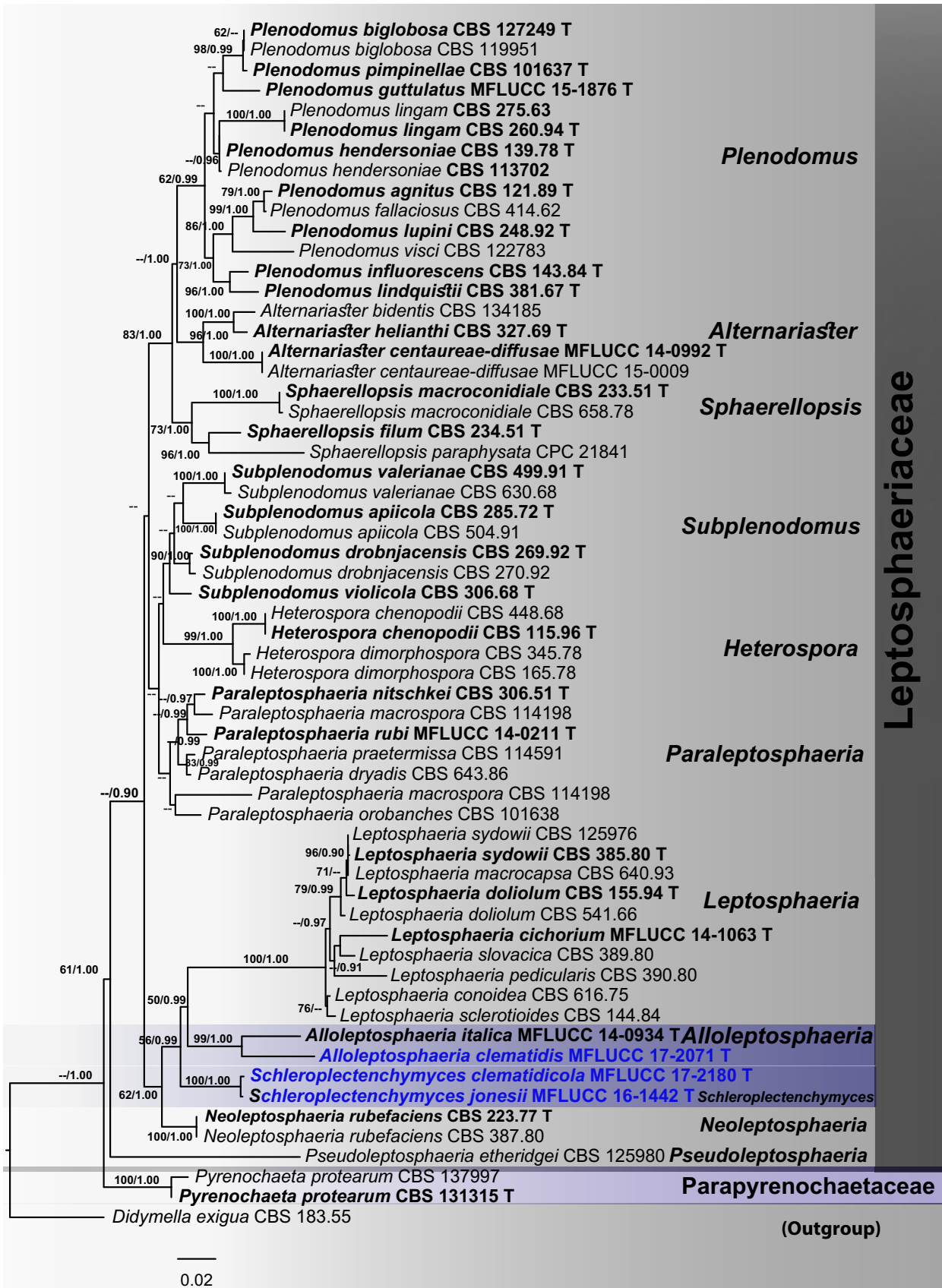
*GenBank accession numbers*: LSU: MT214557; SSU: MT226674; ITS: MT310604; *tefl*: MT394736; *rpb2*: MT394685.

*Notes*: *Alloleptosphaeria clematidis* is the first report of *Alloleptosphaeria* from Thailand and the second *Alloleptosphaeria* species known on *Clematis*. *Alloleptosphaeria clematidis* formed a robust clade with *A. italica* with strong support (97% ML/1.00 BYPP, Fig. 22). *Alloleptosphaeria clematidis* is compatible with the generic concept of *Alloleptosphaeria* in having immersed ascomata with a



**Fig. 21** *Hermatomyces clematidis* (MFLU 17–1493, holotype). **a, b** Sporodochia on natural substrate. **c** Vertical section of sporodochia. **d, e** Subicular hyphae. **f–h** Cylindrical conidia. **i–l** Mature lenticular

conidia. **m** Germinated conidium. **n, o** Culture characters on MEA. Scale bars: **b** = 500  $\mu$ m, **c** = 100  $\mu$ m, **d–m** = 20  $\mu$ m





**Fig. 22** The best scoring RAxML tree with a final likelihood value of  $-13918.604336$  based on combined LSU, SSU, ITS and *tefl* sequence data for Leptosphaeriaceae. The tree is rooted with a member of the Didymellaceae. Seventy strains were included in the combined gene sequence analyses, which comprise 3257 characters (831 characters for LSU, 951 characters for SSU, 566 characters for ITS, and 909 characters for *tefl*, including gap regions). The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The matrix had 649 distinct alignment patterns, with 45.29% of undetermined characters and gaps. Estimated base frequencies were as follows; A=0.244507, C=0.225295, G=0.271500, T=0.258698; substitution rates AC=1.503087, AG=2.814632, AT=2.230882, CG=0.467105, CT=7.217503, GT=1.000000; gamma distribution shape parameter  $\alpha=0.541381$ . In our analysis, GTR+I+G model was used for every partition in Bayesian analysis. The species determined in this study are indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (PP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. The supported values from all analyses are BS  $\geq$  70%/BYPP  $\geq$  0.95

thin-walled peridium of brown to dark brown or reddish brown, pseudoparenchymatous cells (Ariyawansa et al. 2015b). Based on morphology, *A. clematidis* is distinguished by its cylindrical asci and the presence of 1 longitudinal septum in its yellowish ascospore (Fig. 23). In a BLASTn search of GenBank, the closest match of the ITS region of MFLUCC 17–2071 is 92.5% similarity to *Subplenodomus iridicola* strain CBS 143395 (NR\_159068). Pairwise comparison of the ITS region of MFLUCC 17–2071 with *A. italica* (MFLUCC 14–0934) shows 97 nucleotide differences from 566 base pairs (17.13%).

***Sclerenchymomyces* Phukhams. & K.D. Hyde, gen. nov.**

*Index Fungorum number*: IF557110; *Facesoffungi number*: FoF 07287, Fig. 24

*Etymology*: Genus name reflects the characteristic of scleroplectenchyma tissue type.

*Saprobic* on dead or dead branch of herbaceous or woody plants in terrestrial habitats. **Sexual morph**: *Ascomata* covered by plant epidermis, located on the surface of host substrate, black, shiny, superficial to semi-immersed, solitary, globose, black, ostiolate. *Ostioles* central, short, filled with hyaline cells. *Peridium* composed of blackish to dark brown cells of *textura angularis*, thick, multilayered, scleroplectenchymatous cells, cells towards the inside lighter. *Hamathecium* composed of numerous, branched, septate, pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical, short-pedicellate. *Ascospores* uniseriate, partial overlapping, muriform, broad ellipsoidal, narrowed towards the ends, initially hyaline, becoming brown at maturity, constricted at median septum, guttulate, surrounded by a mucilaginous sheath (Wanasinghe et al. 2016). **Asexual morph**: *Conidiomata* pycnidial, solitary, sometimes aggregated, uniloculate or multiloculate, erumpent or superficial on host

substrate, with black shiny ostioles, globose to subglobose, coriaceous, dark brown to brown. *Ostioles* central, papillate, oblong. *Conidiomatal wall* thick-walled, multilayered, scleroplectenchymatous or pseudoparenchymatous cells, flat at base, composed of *textura angularis*, lined with a thick hyaline layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, phialidic, determinate, discrete, sub-cylindrical to truncate, smooth-walled, hyaline, arising from the inner layers of conidiomata. *Conidia* ellipsoid or cylindrical to oblong, rounded at both ends, slightly curved, hyaline when immature, yellowish at maturity, aseptate or septate, guttulate, smooth-walled.

*Type species*: ***Sclerenchymomyces clematidis* Phukhams. & K.D. Hyde**

*Notes*: *Sclerenchymomyces* is introduced for a lineage comprising *Sclerenchymomyces clematidis* and *S. jonesii* ( $\equiv$  *Neoleptosphaeria jonesii*) which received strong support (99% ML/1.00 BYPP, Fig. 22). Based on the multi-gene phylogenetic analyses, *Sclerenchymomyces* formed a separate lineage to *Alloleptosphaeria*, *Leptosphaeria* sensu stricto and *Neoleptosphaeria* (62% ML/1.00 BYPP). The members of Leptosphaeriaceae are remarkable by their characteristic scleroplectenchymatous or pseudoparenchymatous tissue types (Ariyawansa et al. 2015b). Two isolates of *Sclerenchymomyces* share similar characters in having black, shiny, superficial to semi-immersed ascomata with a multilayer of scleroplectenchymatous tissue type (Wanasinghe et al. 2016a, Fig. 24). *Sclerenchymomyces* shares similar pycnidial characters with *Leptosphaeria* sensu stricto and *Neoleptosphaeria* in the scleroplectenchymatous or plectenchymatous cell type in the peridium (Ariyawansa et al. 2015b). However, *Leptosphaeria* species only have transverse septa while *Sclerenchymomyces* has longitudinal septa in the ascospores (Fig. 24). *Sclerenchymomyces* has oblong, brown phragmoconidia in nature and phoma-like characters in culture (Gruyter et al. 2013; Wanasinghe et al. 2016). The combined dataset of the LSU, SSU, ITS and *tefl* sequences for Leptosphaeriaceae revealed a lineage of *Sclerenchymomyces* as a new genus from *Clematis* (Fig. 22).

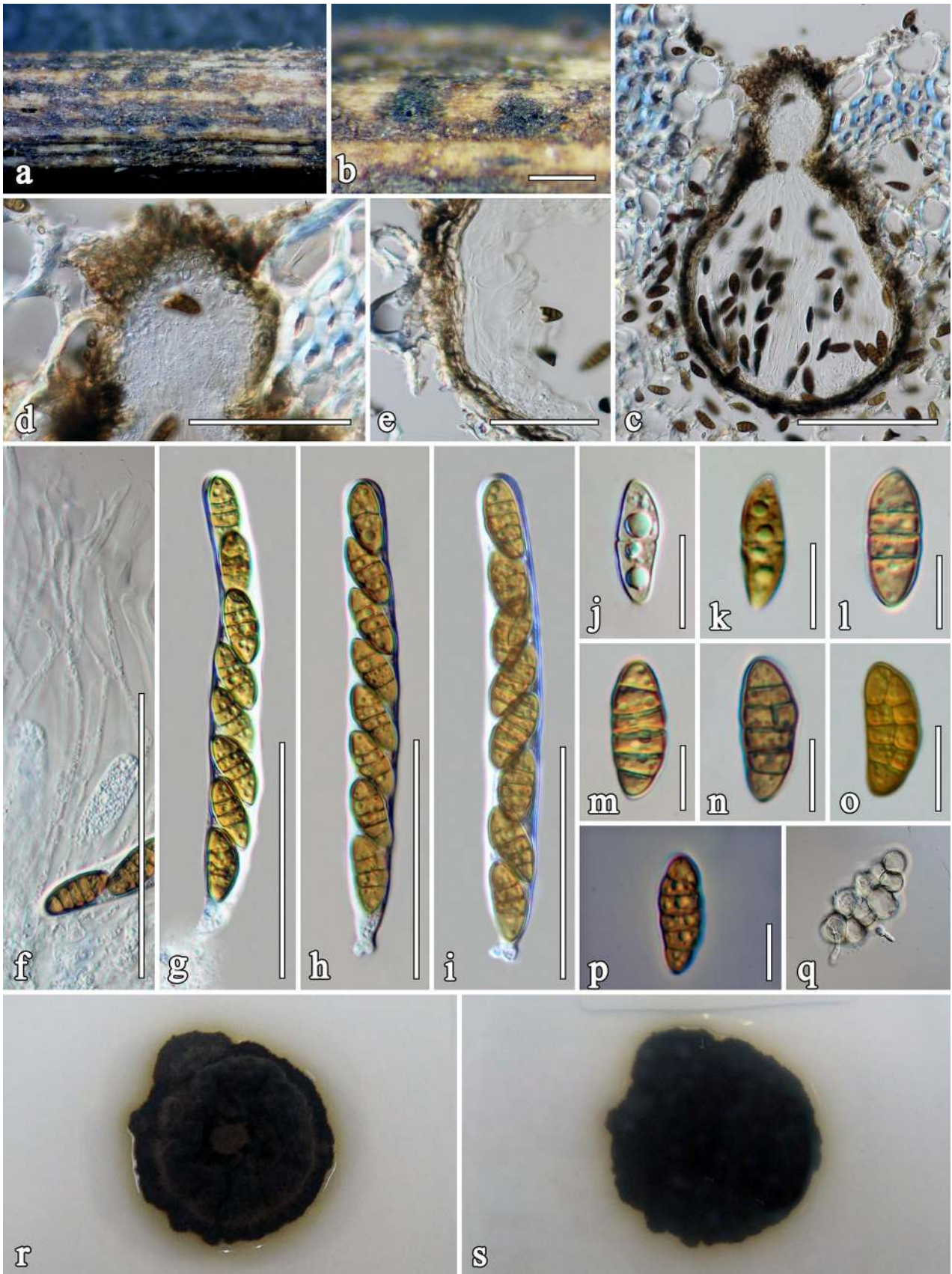
***Sclerenchymomyces clematidis* Phukhams. & K.D. Hyde, sp. nov.**

*Index Fungorum number*: IF557111; *Facesoffungi number*: FoF 07288, Fig. 24.

*Etymology*: Name refers to the host plant, *Clematis*.

*Holotype*: MFLU 16–2492.

*Saprobic* on dead stems of *Clematis vitalba*. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 187–225  $\times$  85–217  $\mu$ m ( $\bar{x}$ =210  $\times$  130  $\mu$ m, n=10), pycnidial, solitary, sometimes aggregated, uniloculate or multiloculate, erumpent or superficial on host substrate, with black shiny ostioles visible, globose to subglobose,



**Fig. 23** *Alloleptosphaeria clematidis* (MFLU 17–1479, holotype). **a** Appearance of ascomata on *Clematis subumbellata*. **b** Close up of ascoma on host substrate. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Pseudoparaphyses. **g–i** Asci. **j–p** Ascospores (**p** Ascospore in 10% Indian ink). **q** Germinated ascospore. **r, s** Culture characteristics on MEA. Scale bars: **b** = 200  $\mu$ m, **c** = 100  $\mu$ m, **d, f–i** = 50  $\mu$ m, **e** = 20  $\mu$ m, **j–p** = 10  $\mu$ m

coriaceous, dark brown to brown, ostiolate. *Ostioles* central, papillate, oblong. *Conidiomatal wall* 14–30(–35)  $\mu$ m wide, multilayered, scleroplectenchymatous cells, flat at base, outer layer composed of 5–9 layers of light brown to brown cells of *textura angularis*, lined with a thick hyaline layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 3–8  $\times$  1.5–4  $\mu$ m ( $\bar{x}$  = 5  $\times$  3  $\mu$ m,  $n$  = 30), enteroblastic, phialidic, determinate, discrete, sub-cylindrical to truncate, smooth-walled, hyaline, arising from the inner layers of conidiomata. *Conidia* 11–18  $\times$  2.5–5  $\mu$ m ( $\bar{x}$  = 14  $\times$  4  $\mu$ m,  $n$  = 50), broad cylindrical to oblong, rounded at both ends, hyaline when immature, yellowish at maturity, slightly curved, 3-septate, with 1(–2) guttules in each cell, smooth-walled.

**Culture characters:** Colonies on MEA reaching 40 mm diam. after 4 weeks at 25 °C. Cultures from above, cream-brown, sparse mycelia, circular, umbonate, papillate with fluffy, covered with white aerial mycelium; reverse dark brown at the centre, cream radiating outwardly.

**Material examined:** Italy, Forlì-Cesena Province, near Meldola, on dead aerial branch of *Clematis vitalba*, 15 November 2013, E. Camporesi, 1518C (MFLU 16–2492, **holotype**); ex-type living culture, MFLUCC 17–2180.

**Host:** *Clematis vitalba*—(This study).

**Distribution:** Italy—(This study).

**GenBank accession numbers:** LSU: MT214558; SSU: MT226675; ITS: MT310605; *tef1*: MT394737; *rpb2*: MT394686.

**Notes:** *Sclerenchymomyces clematidis* is distinct from *S. jonesii* in conidial characters (Fig. 24). *Sclerenchymomyces clematidis* has broad cylindrical to oblong, yellowish conidia with 3 septa and 1(–2) guttules, while *S. jonesii* has hyaline, aseptate conidia (Wanasinghe et al. 2016a). In a BLASTn search of GenBank, the LSU sequence of *S. clematidis* (strain MFLUCC 17–2180) was 98.7% similar to *S. jonesii* ( $\equiv$  *Neoleptosphaeria jonesii*), while the ITS sequence showed 97.28% similarity to NR\_152375. Pairwise comparison of the ITS sequence reveals nine bases pair differences (1.59%) between *S. clematidis* and *S. jonesii* (MFLUCC 16–1442). The *tef1* region shows seven bases pair difference between *S. clematidis* and *S. jonesii*.

***Sclerenchymomyces jonesii*** (Wanasinghe, Camporesi & K.D. Hyde) Phukhams. & K.D. Hyde, **comb. nov.**

**Basionym:** *Neoleptosphaeria jonesii* Wanasinghe, Camporesi & K.D. Hyde, in Wanasinghe, Camporesi & Hu, *Mycosphere* 7(9): 1373 (2016)

**Index Fungorum number:** IF552569; **Facesoffungi number:** FoF 02716

**Notes:** Wanasinghe et al. (2016a) introduced *Neoleptosphaeria jonesii* for a fungus whose morphology and phylogeny are related to *Neoleptosphaeria rubefaciens* (Gruyter et al. 2013; Wanasinghe et al. 2016a). The analyses of combined LSU, SSU, ITS and *tef1* sequence data for Leptosphaeriaceae showed that the ex-type strain of *Neoleptosphaeria jonesii* (MFLUCC 16–1442) clustered with *Sclerenchymomyces clematidis* (MFLUCC 17–2180), a fungal strain reported from the same host. Pairwise comparison of the ITS sequence data reveals 85 bases pair difference from 566 (15.9%, including gap region) between *N. jonesii* (MFLUCC 16–1442) and *N. rubefaciens* (CBS 223.77 and CBS 387.80). According to phylogeny (Fig. 22) coupled with morphology, we transfer *N. jonesii* to *Sclerenchymomyces*.

**Host:** *Clematis vitalba*—(Wanasinghe et al. 2016a).

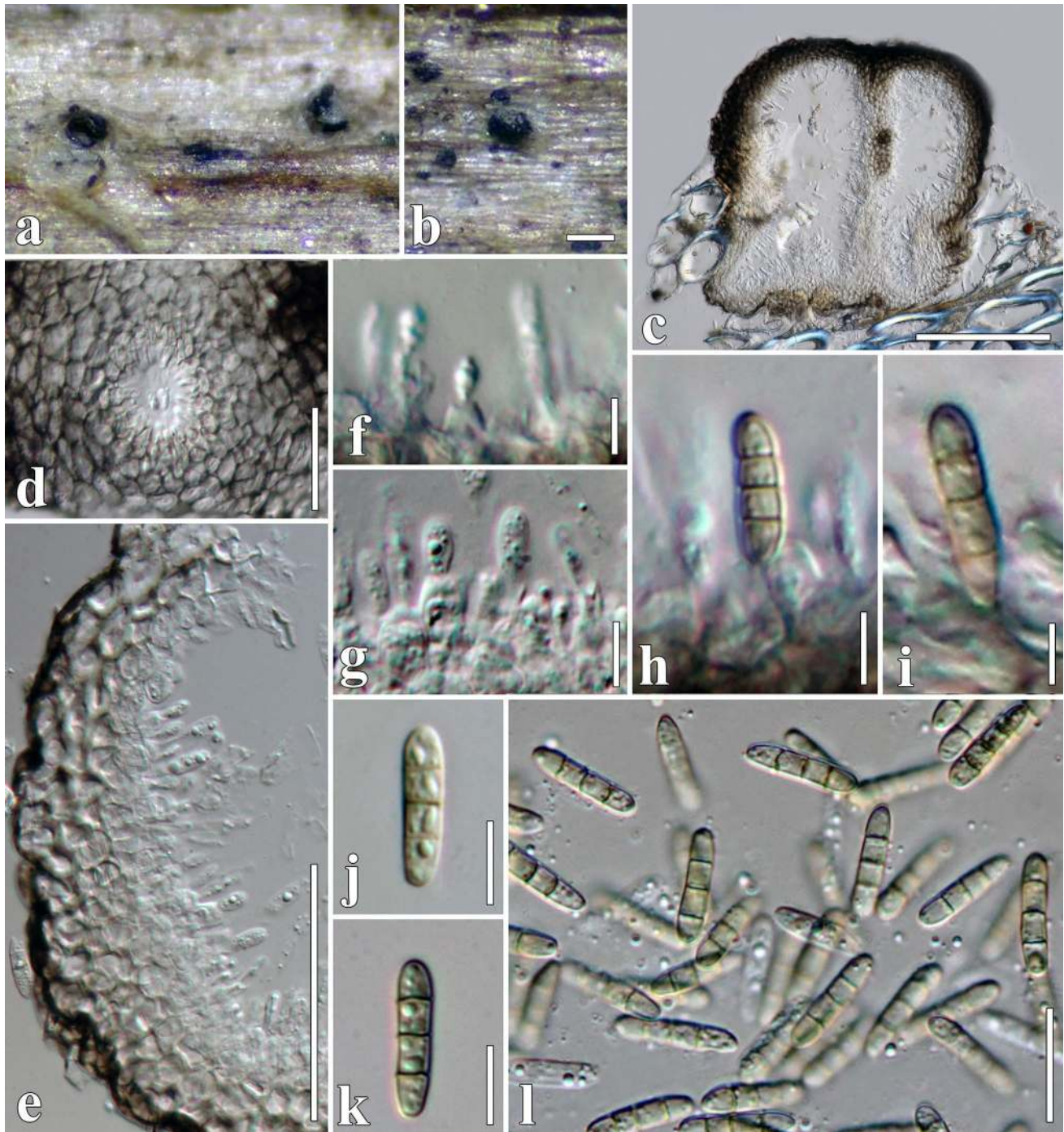
**Distribution:** Italy—(Wanasinghe et al. 2016a).

**Longiostiolaceae** Phukhams., Doilom & K.D. Hyde, **fam. nov.**

**Index Fungorum number:** IF557086; **Facesoffungi number:** FoF 07215, Fig. 25.

**Saprobic** on dead bark. **Sexual morph:** *Ascomata* immersed to semi-immersed, uniloculate, solitary, globose to subglobose, coriaceous, base flattened, ostiolate. *Ostioles* long, obtuse or dolabriform, central. *Peridium* thick, comprising several layers of scleroplectenchymatous or pseudoparenchymatous cell types, dark brown to black cells arranged in a *textura angularis* or *textura globosa*. *Hamathecium* composed of numerous, filiform, septate, branched, cellular pseudoparaphyses. *Asci* 4–8-spored, bitunicate, cylindrical to clavate, pedicellate, with ocular chamber. *Ascospores* biseriate, partial overlapping, broad fusiform, hyaline, brownish at the mature state, multi-septate, with or without mucilaginous sheath. **Asexual morph:** coelomycetous-like or hyphomycetous-like structures produced in culture. *Conidiomata* scattered, globose to subglobose, ostiolate. *Conidiomatal wall* thick, comprising of multilayered *textura angularis*, pale brown to brown cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* annellidic, doliiform to ampulliform. *Conidia* cylindrical, hyaline, bud scars disjunctors at base, multi-septate, smooth, without sheath (Matsumura et al. 2018). Colonies on MEA produce conidia structures on aerial mycelium, subglobose to ellipsoidal, initially hyaline, becoming black, aseptate, smooth-walled.

**Type genus:** ***Longiostiolum*** Doilom, Ariyaw. & K.D. Hyde, in Li et al., *Fungal Diversity* 78: 55 (2016)



**Fig. 24** *Sclerenchomyces clematidis* (MFLU 16–2492, **holotype**). **a** Appearance of conidiomata on *Clematis vitalba*. **b** Close up of conidiomata on host substrate. **c** Vertical section through conidiomata. **d**

Ostiole from above. **e** Section of conidioma wall. **f–i** Conidiogenous cells and conidia. **j–l** Conidia. Scale bars: **b** = 200  $\mu\text{m}$ , **c** = 100  $\mu\text{m}$ , **d** = 20  $\mu\text{m}$ , **e** = 50  $\mu\text{m}$ , **f–l** = 5  $\mu\text{m}$

**Notes:** Longiostioliaceae is introduced to accommodate two ascomycetous genera, *Crassiperidium* and *Longiostiolum*. *Longiostiolum* was introduced by Li et al. (2016) for a fungus associated with *Tectona grandis* from northern Thailand. Matsumura et al. (2018) reported *Crassiperidium* from twigs of *Fagus crenata* in Japan. *Crassiperidium* and *Longiostiolum* were associated with wood and share characters in having immersed to semi-immersed, uniloculate ascomata, thick-walled scleroplectenchymatous or pseudoparenchymatous cell, with hyaline ascospores turning brownish at maturity (Li et al. 2016; Matsumura et al. 2018). The asexual morphs of these two genera are different, with *Crassiperidium* forming pycnidial conidiomata with hyaline conidia, and *Longiostiolum* forming a hyphomycetous-like structure with globose, black conidia in culture (Fig. 25).

Matsumura et al. (2018) found that *Crassiperidium* was related to Cyclothyriellaceae (Pleosporales) based on phylogeny of an SSU, LSU and *rpb2* sequence dataset. *Longiostiolum* was assigned as an *incertae sedis* taxon in Pleosporales based on combined LSU, SSU, *rpb2* and *tef1* sequence data (Li et al. 2016). Phylogenetic analysis based on the LSU regions of *Crassiperidium* and *Longiostiolum* formed a clade together with moderate support (57% ML, data not shown) and sister to Cyclothyriellaceae. The concatenated dataset of LSU, SSU, ITS, *tef1* and *rpb2* showed *Crassiperidium* species (*C. octosporum* and *C. quadrisporum*) and *Longiostiolum tectonae* (MFLUCC 12–0562) forming a close relationship with strong support (98% ML/1.00 BYPP) and related to Cyclothyriellaceae (Fig. 2). Therefore, we introduce a new family to accommodate this distinct lineage.

**Type genus:** *Longiostiolum* Doilom, Ariyaw. & K.D. Hyde, in Li et al., Fungal Diversity 78: 55 (2016).

**Type species:** *Longiostiolum tectonae* Doilom, Bhat & K.D. Hyde, in Li et al., Fungal Diversity 78: 55 (2016).

**Genera included:** *Crassiperidium* Matsum. & Kaz. Tanaka (Matsumura et al. 2018); *Longiostiolum* Doilom, Ariyaw. & K.D. Hyde (Li et al. 2016).

**Hosts:** *Fagus crenata*, *Tectona grandis*—(Li et al. 2016; Matsumura et al. 2018).

**Distribution:** Japan, Thailand—(Li et al. 2016; Matsumura et al. 2018).

***Longiostiolum tectonae*** Doilom, Bhat & K.D. Hyde, in Li et al., Fungal Diversity 78: 55 (2016)

**Index Fungorum number:** IF 551900, **Facesoffungi number:** FoF 01882, Fig. 25.

**Notes:** Based on the phylogenetic analysis of the combined LSU, SSU, *rpb2* and *tef1* sequence data, the genus *Longiostiolum tectonae* (MFLUCC 12–0562) formed a distinct lineage in Pleosporales with no statistical support (Li et al. 2016). In a BLASTn search of GenBank, the closest match of the LSU sequence of MFLUCC 12–0562 is

*Crassiperidium octosporum* (strain MAFF 242971) with 97% similarity, while the ITS sequence showed 86.5% similarity.

**Host:** *Tectona grandis*—(Li et al. 2016).

**Distribution:** Thailand—(Li et al. 2016).

### **Lophiostomataceae** Sacc. [as ‘Lophiostomaceae’]

Lophiostomataceae was accepted by Mugambi and Huhndorf (2009). Members of this family are saprobes in terrestrial and aquatic habitats (Saccardo 1883; Tanaka and Harada 2003; Thambugala et al. 2015; Hashimoto et al. 2018). The family is characterized by carbonaceous ascomata with a slit-like ostiolar neck or opening (Zhang et al. 2009). A revision of Lophiostomataceae by Thambugala et al. (2015) based on multi-locus phylogeny along with the re-examination of holotype specimens revealed the boundaries of the family. Hashimoto et al. (2018) revised the classification based mainly on ascospore appendages and introduced several new genera. Mapook et al. (2020) introduced *Pseudocapulatispora* Mapook & K.D. Hyde and currently, there are 25 genera in the family (Wijayawardene et al. 2020). A phylogenetic analysis based on a combined LSU, ITS, SSU, *tef1* and *rpb2* sequence dataset (Figs. 26, 27) of lophiostomataceous taxa on *Clematis* from Europe and Asia revealed novel species of *Neovaginatispora*, *Pseudocapulatispora*, *Pseudolophiostoma* and *Sigarispora*, which are introduced with morphological support.

### ***Neovaginatispora*** Hashim., K. Hiray. & Kaz. Tanaka

Hashimoto et al. (2018) reanalysed Lophiostomataceae and segregated *Neovaginatispora fuckelii* (Sacc.) Hashim., K. Hiray. & Kaz. Tanaka from the type species of *Vaginatispora* based on phylogenetic analyses. *Neovaginatispora* is distinct in its thin, sub-carbonaceous peridium of uniform thickness (Thambugala et al. 2015). We introduce a second species of *Neovaginatispora* from *Clematis viticella* (Fig. 28).

***Neovaginatispora clematidis*** Phukhams., D. Ertz & C. Gerstmans & K.D. Hyde, **sp. nov.**

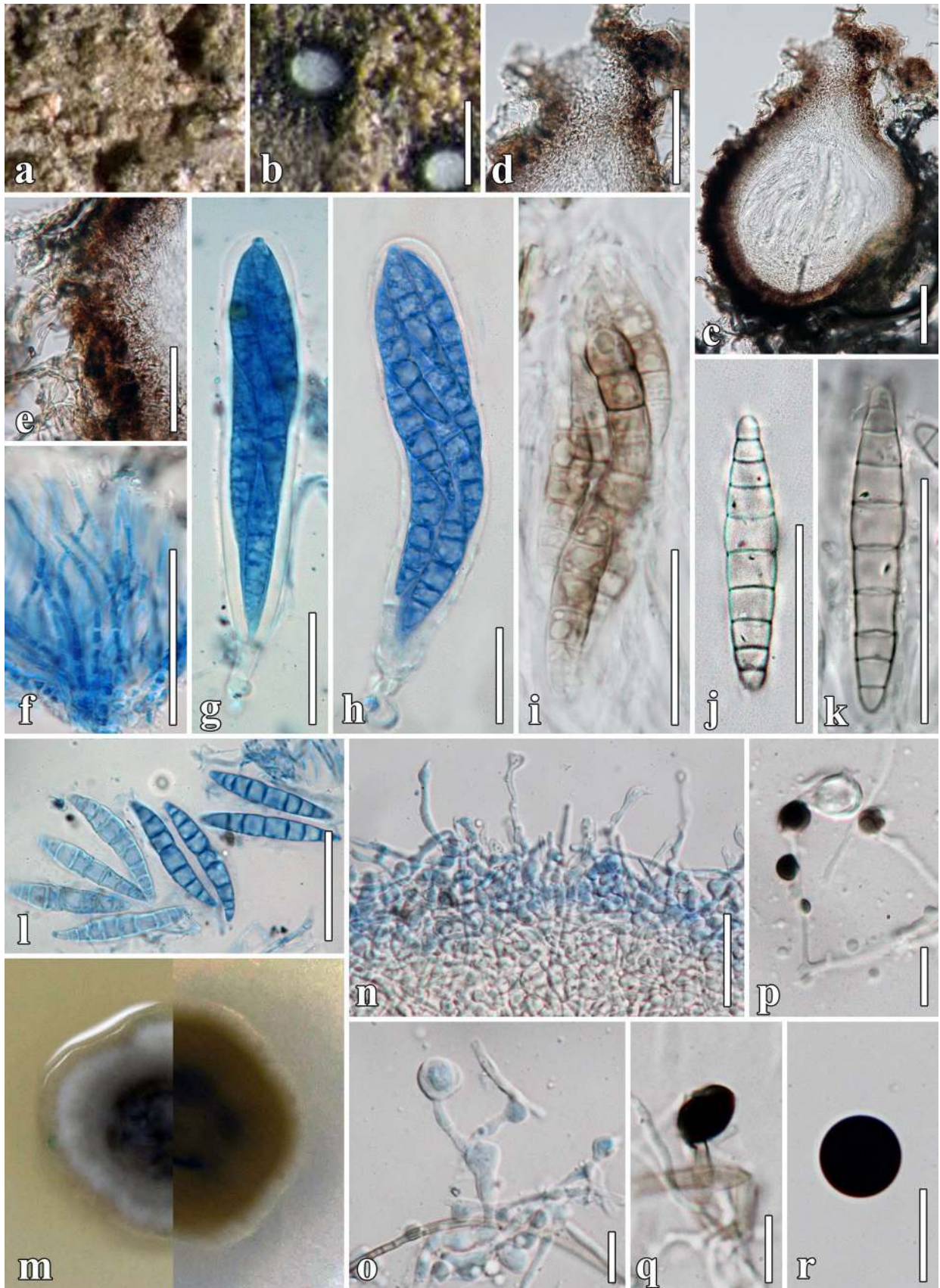
**Index Fungorum number:** IF557117; **Facesoffungi number:** FoF 07289, Fig. 28.

**Etymology:** Name refers to the host plant, *Clematis*.

**Holotype:** MFLU 17–1514.

**Saprobic** on dead stems of *Clematis viticella*.

**Sexual morph:** *Ascomata* 145–250 × 108–160 μm ( $\bar{x}$  = 172 × 134 μm, n = 10), solitary, gregarious, semi-immersed to erumpent, globose to compressed, coriaceous, dark brown to black, ostiolate. *Ostioles* 50–65 × 76–83 μm ( $\bar{x}$  = 58 × 80 μm, n = 5), with a crest-like apex, central, filled with hyaline periphyses. *Peridium* 13–28 μm wide ( $\bar{x}$  = 19 μm, n = 20), uniform, wider and heavily pigmented at the apex, composed of 5(–6) layers of somewhat



**Fig. 25** *Longiostiolum tectonae* (MFLU 15–3532, **holotype**). **a, b** Appearance of ascomata on host substrate. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Cellular pseudoparaphyses. **g–i** Asci. **j–l** Ascospores. **m** Culture characters on MEA. **n–q** Conidiogenous cell. **r** Conidia. Scale bars: **b** = 200  $\mu\text{m}$ , **c, d** = 100  $\mu\text{m}$ , **e–l** = 50  $\mu\text{m}$ , **n** = 10  $\mu\text{m}$ , **o–r** = 5  $\mu\text{m}$

flattened, thin-walled cells of *textura angularis*, cells towards the inside lighter, inner layer composed of thin hyaline gelatinous layer. *Hamathecium* composed of numerous, dense, 2–3  $\mu\text{m}$  wide, filamentous, branched, septate, pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 53–105  $\times$  9–12  $\mu\text{m}$  ( $\bar{x}$  = 78  $\times$  11  $\mu\text{m}$ ,  $n$  = 40), 8-spored, bitunicate, fissitunicate, cylindrical-clavate to clavate, with short, bulbous pedicel, apically rounded, with an ocular chamber. *Ascospores* 16–19  $\times$  5–7  $\mu\text{m}$  ( $\bar{x}$  = 16  $\times$  6  $\mu\text{m}$ ,  $n$  = 40), biseri-ate or partially overlapping, hyaline, broad fusiform with acute ends, tapering towards the ends, 1-euseptate, strongly constricted at the septum, upper cell broader than lower cell, smooth-walled, with two guttules in each cell, with 2  $\mu\text{m}$  wide globose appendages at both ends. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Cultures from above, dark brown, dense, circular, umbonate, rough surface, dull, undulate, radially furrowed, covered with grey aerial mycelium, oil droplets formed in the middle of cultures; reverse black radiating outwardly.

**Material examined:** Belgium, Flemish Brabant, Meise Botanic Garden, Bouchout Domain, dead stems of *Clematis viticella*, 13 June 2017, D. Ertz & C. Gerstmans, BRCV2 (MFLU 17–1514, **holotype**); ex-type living culture, MFLUCC 17–2156.

**Host:** *Clematis viticella*—(This study).

**Distribution:** Belgium—(This study).

**GenBank accession numbers:** LSU: MT214559; SSU: MT226676; ITS: MT310606; *tefl*: MT394738.

**Notes:** *Neovaginatispora clematidis* is compatible with the concept of *Neovaginatispora* in having thin, uniform peridium layers with short and globose appendages at both ends (Thambugala et al. 2015). *Neovaginatispora clematidis* was found on *Clematis viticella* in Belgium whereas *Neovaginatispora fuckelii* has mainly been isolated from herbaceous plant in Japan except the strain MFLUCC 17–2652 that was isolated from *Mangifera indica* in Taiwan. *Neovaginatispora clematidis* is somewhat similar to the type specimen of *N. fuckelii* (CBS 101952), but being distinguishable in its broad fusiform ascospores with a single eusepta (Fig. 28). *Neovaginatispora fuckelii* and *N. clematidis* are reported from different continents and closely related by morphology but distinct over the five gene loci phylogeny.

In the multigene phylogenetic analyses, the strain formed a strongly supported clade with *N. fuckelii* (98% ML/1.00

BYPP, Fig. 26). This clade contains four isolates of *N. fuckelii* and a single isolate of *N. clematidis* (Fig. 26). The ITS sequence shows six nucleotide differences while the *tefl* sequence has 10 nucleotide differences. In a BLASTn search of the GenBank, the ITS sequence has 98% similarity to *Vaginatispora aquatica* (MFLUCC 11–0083), while the *tefl* sequence has 93.49% similarity to *V. scabrispora* (LC312583). The GCPSR concept was applied to clade (a) for testing significant recombination between these isolates (Laurence et al. 2014). A pairwise homoplasmy index of  $\Phi_w = 1.0$  showed that there is no significant recombination of the gene flow in *N. clematidis* and *N. fuckelii* isolates (Fig. 27a). The new species of *Neovaginatispora* is therefore introduced based on morphological support and phylogenetic analysis.

### *Pseudocapulatispora* Mapook & K.D. Hyde

*Pseudocapulatispora* was introduced by Mapook et al. (2020) with *Pseudocapulatispora longiappendiculata* as the type species. The genus is characterized by slit-like ostioles, a peridium of *textura prismatica* and ascospores with relatively long appendages at the end of capped sheaths. We introduce a second species of *Pseudocapulatispora* from *Clematis subumbellata* based on morphology (Fig. 29) and phylogenetic analysis (Fig. 26).

### *Pseudocapulatispora clematidis* Phukhams. & K.D. Hyde, **sp. nov.**

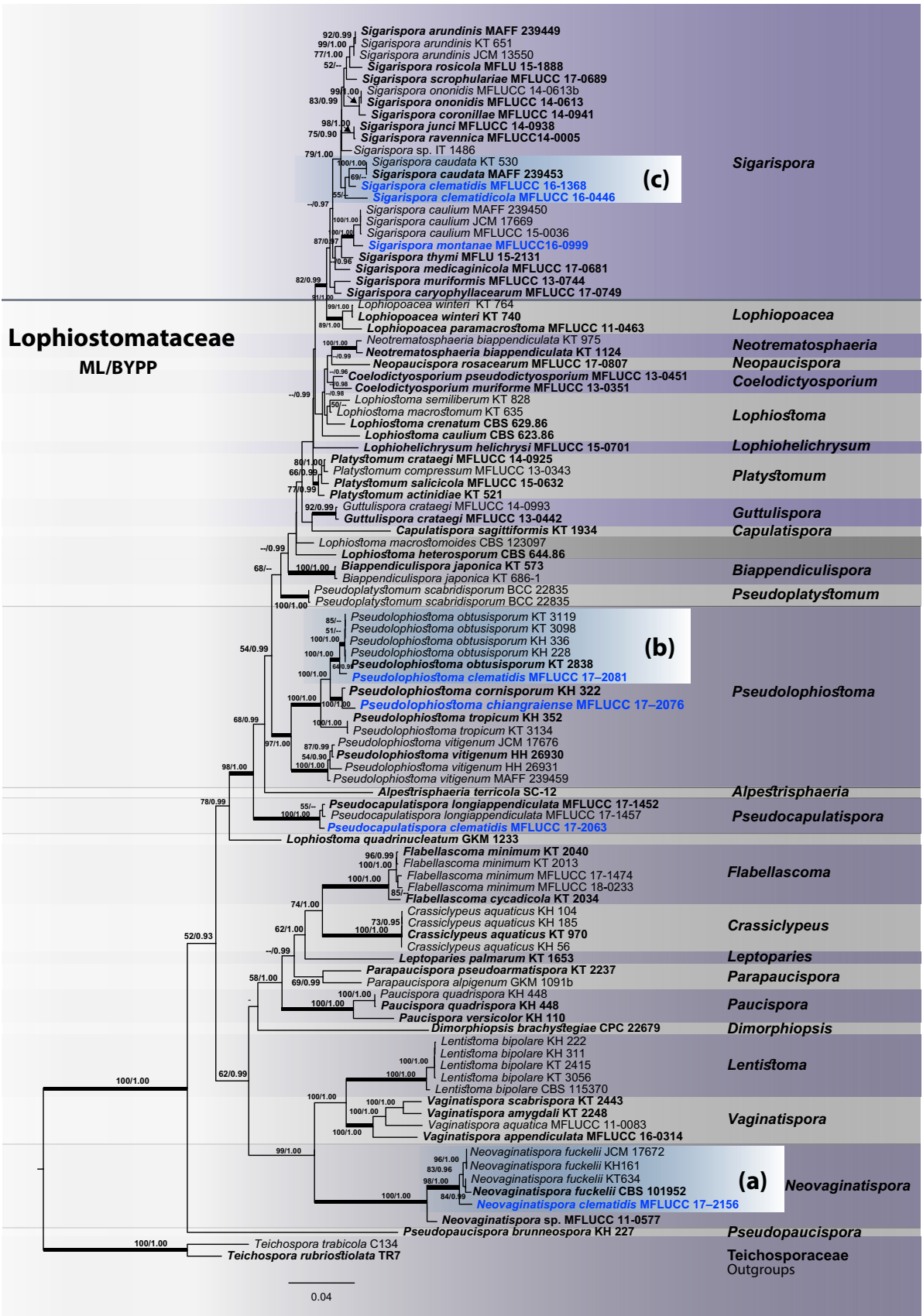
**Index Fungorum number:** IF557118; **Facesoffungi number:** FoF 07290, Fig. 29.

**Etymology:** The epithet reflects the host, *Clematis*.

**Holotype:** MFLU 17–1469.

**Saprobic** on dead stem of *Clematis subumbellata*.

**Sexual morph:** *Ascomata* 197–386  $\times$  160–252  $\mu\text{m}$  ( $\bar{x}$  = 300  $\times$  209  $\mu\text{m}$ ,  $n$  = 10), solitary, scattered, immersed, with only black shiny ostioles visible, subglobose to compressed, base flattened, coriaceous to carbonaceous at the apex, brown to black, with a developed pseudoclypeus, ostiolate. *Ostioles* 84–148  $\times$  56–86  $\mu\text{m}$  ( $\bar{x}$  = 107  $\times$  66  $\mu\text{m}$ ,  $n$  = 5), with a crest-like apex, central, long, elongated and laterally compressed, surrounded by a small blackened pseudoclypeus, with irregular wall, filled with hyaline periphyses. *Peridium* 10–23 (–38  $\mu\text{m}$  at apex) wide ( $\bar{x}$  = 23  $\mu\text{m}$ ,  $n$  = 20), uniform, wider at the apex, heavily pigmented at the apex, composed of 4 (–5) layers of *textura prismatica*, cells towards the inside lighter, inner layer composed of thin, hyaline gelatinous layer, at the apex fusing and indistinguishable from the host tissues. *Hamathecium* composed of numerous, dense, 1.5–3  $\mu\text{m}$  wide, filamentous, branched, septate, pseudoparaphyses, situated between and above the asci, embedded in a gelatinous matrix. *Asci* 68–117  $\times$  12–22  $\mu\text{m}$  ( $\bar{x}$  = 99  $\times$  18  $\mu\text{m}$ ,  $n$  = 40), 8-spored, bitunicate, fissitunicate, oblong to cylindrical-clavate, with short, furcate pedicel,





**Fig. 26** The best scoring RAxML tree with a final likelihood value of  $-33003.460353$  based on combined LSU, ITS, SSU, *tef1* and *rpb2* sequence data. The tree is rooted with *Teichospora rubriostiolata* (TR7) and *T. trubicola* (C134) in Teichosporaceae. One hundred and two strains were included in the combined sequence analyses which comprise 5255 characters (1300 characters for LSU, 921 characters for ITS, 1004 characters for SSU, 992 characters for *tef1* and 1038 characters for *rpb2*, including gaps). The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree from the maximum likelihood analysis had similar topology to the Bayesian analyses. The matrix had 1938 distinct alignment patterns with 36.36% undetermined characters or gaps proportions. Estimated base frequencies were as follows: A=0.248970, C=0.248285, G=0.267331, T=0.235414; substitution rates AC=1.347247, AG=3.679551, AT=1.191656, CG=1.272401, CT=7.723696, GT=1.000000; gamma distribution shape parameter  $\alpha=0.579807$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study are indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses at the genus level ( $BS \geq 70\%/BYPP \geq 0.95$ )

apically rounded, with an ocular chamber. *Ascospores*  $22\text{--}28 \times 7\text{--}12 \mu\text{m}$  ( $\bar{x} = 25 \times 9 \mu\text{m}$ ,  $n = 50$ ), biseriate or partially overlapping, ellipsoid, tapering towards the ends, acute ends, hyaline, 1-euseptate, strongly constricted at the septum, with guttule in each cell, slightly swollen near median septum, sheath drawn out from both ends to form polar appendages,  $14\text{--}25 \times 3\text{--}5 \mu\text{m}$  ( $\bar{x} = 19 \times 4 \mu\text{m}$ ,  $n = 50$ ), end caps visible at the ends of the appendages, with a lateral pad-like structure, up to  $4 \mu\text{m}$  wide at side. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at  $25 \text{ }^\circ\text{C}$ . Cultures from above, pale green to yellow brown in the middle, dense, circular, umbonate, surface rough, dull, fimbriate, radially furrowed, covered with yellow aerial mycelia, oil droplets formed in the middle of the culture; reverse cream radiating outwardly, yellow pigment diffusing in the agar.

**Material examined:** Thailand, Phayao Province, Phu Sang District, dead stems of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH05 (MFLU 17–1469, **holotype**); ex-type living culture, MFLUCC 17–2063.

**Hosts:** *Clematis subumbellata*—(This study).

**Distribution:** Thailand—(This study).

**GenBank accession numbers:** LSU; MT214560; SSU: MT226677; ITS: MT310607; *tef1*: MT394739; *rpb2*: MT394687.

**Notes:** In the phylogeny (Fig. 26), this species clustered with the type species, *Pseudocapulatispora longiappendiculata* (Mapook et al. 2020). Morphological comparison of *P. clematidis* and *P. longiappendiculata* revealed that *P. clematidis* (Fig. 29) has larger ascomata ( $300 \times 209$  vs  $240 \times 135 \mu\text{m}$ ), with shorter polar appendages ( $19 \times 4$  vs

$25 \times 4.5 \mu\text{m}$ ). The *tef1* sequence of *Pseudocapulatispora clematidis* (MFLU 17–1469) had 95% similarity to *P. longiappendiculata* (5% nucleotide differences in the *tef1* region). Therefore, the new strain is introduced as a new species based on morphology and phylogenetic evidence.

***Pseudolophiostoma*** Thambug., Kaz. Tanaka & K.D. Hyde

*Pseudolophiostoma vitigenum* is the type species. The genus was introduced for a lophiostomataceous taxon that formed a distinct clade from the type species of *Lophiostoma* (Hirayama and Tanaka 2011; Thambugala et al. 2015). Five species are listed in Index Fungorum (Index Fungorum 2020). We introduce two new species namely; *P. chiangraiensis* and *P. clematidis*, to accommodate species from Thailand which occurred on *Clematis* species (Figs. 30, 31).

***Pseudolophiostoma chiangraiense*** Phukhams. & K.D. Hyde, **sp. nov.**

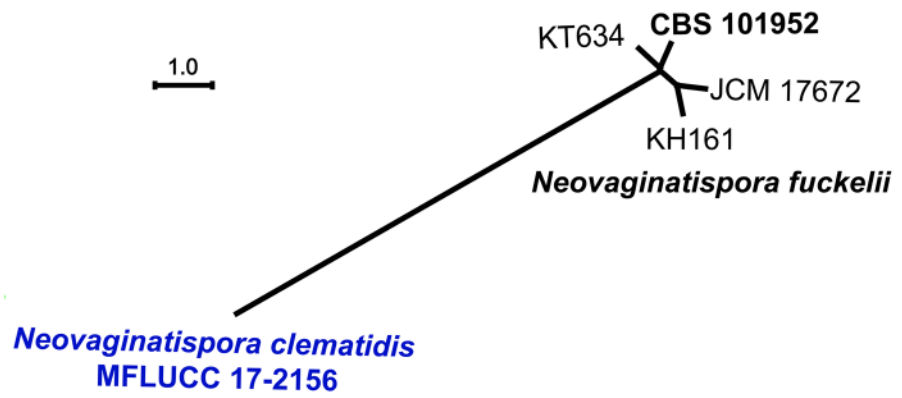
**Index Fungorum number:** IF557119; **Facesoffungi number:** FoF 07291, Fig. 30.

**Etymology:** The epithet reflects the location where the fungus was collected.

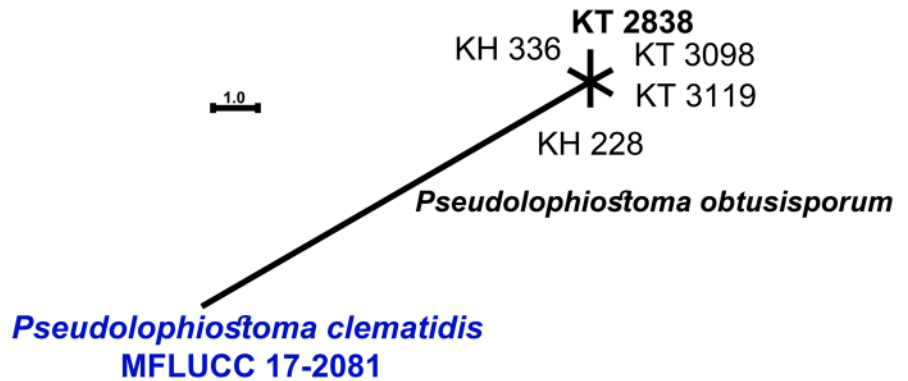
**Holotype:** MFLU 17–1484.

**Saprobic** on dead stem of *Clematis fulvicoma*. **Sexual morph:** *Ascomata*  $276\text{--}293 \times 94\text{--}294 \mu\text{m}$  ( $\bar{x} = 286 \times 239 \mu\text{m}$ ,  $n = 10$ ), solitary, scattered, sometimes gregarious, immersed, with only black shiny ostioles visible, subglobose or compressed, flattened base, coriaceous, carbonaceous at the apex, dark brown to black, with a well-developed clypeus, indistinguishable from substrate, ostiolate. *Ostioles*  $74\text{--}127 \times 88\text{--}129 \mu\text{m}$  ( $\bar{x} = 94 \times 103 \mu\text{m}$ ,  $n = 5$ ), with opening by a pore, central, elongated and laterally compressed, irregular wall, carbonaceous, black, filled with hyaline periphyses. *Peridium*  $14\text{--}32 \mu\text{m}$  wide ( $\bar{x} = 23 \mu\text{m}$ ,  $n = 20$ ), uniform, wider at the apex, heavily pigmented at the apex, composed of 3(–4) layers of *textura angularis* and *textura prismatica*, thin-walled cells, cells towards inside lighter than outside, somewhat flattened, inner layer composed of thin, hyaline gelatinous layer, at the apex fusing and indistinguishable from the host tissues. *Hamathecium* composed of numerous, dense, 2–3  $\mu\text{m}$  wide, filamentous, branched, septate, cellular pseudoparaphyses, embedded in a gelatinous matrix. *Asci*  $78\text{--}119 \times 11\text{--}16 \mu\text{m}$  ( $\bar{x} = 96 \times 13 \mu\text{m}$ ,  $n = 40$ ), 8-spored, bitunicate, fission-tunicate, broad cylindrical to cylindrical-clavate, with furcate pedicel, apically rounded, with an ocular chamber. *Ascospores*  $20\text{--}32 \times 4\text{--}7 \mu\text{m}$  ( $\bar{x} = 26 \times 6 \mu\text{m}$ ,  $n = 50$ ), uniseriate, overlapping, hyaline, becoming pale brown at senescence, broad-fusiform, tapering towards the ends, acute ends, 1-euseptate, strongly constricted at the septum, with 1(–2) guttules in each cell, slightly swollen near median septum, with 6–10  $\mu\text{m}$  wide sheath drawn-out to form polar appendages, sheath drawn out, with a pad-like structure, up to  $4 \mu\text{m}$  wide at side. **Asexual morph:** Undetermined.

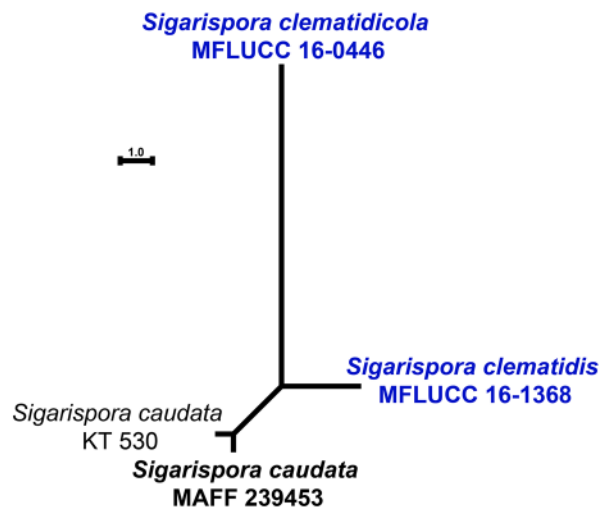
**Fig. 27** The splits graph from the pairwise homoplasy index (PHI) test generated from the concatenated gene set of LSU, ITS, SSU, *tef1* and *rpb2* sequence data of closely related species of *Neovaginatispora* (a), *Pseudolophiostoma* (b) and *Sigarispora* (c) using both LogDet transformation and splits decomposition. PHI test results ( $\Phi_w$ ) < 0.05 indicates significant recombination within the dataset. The strains determined in this study are in bold and blue



(a)  $\Phi_w = 1.0$



(b)  $\Phi_w = 1.0$



(c)  $\Phi_w = 1.0$

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Cultures from above, olive-brown to dark brown in the middle, dense, circular, convex with papillate surface, fluffy, rough surface, radially furrowed, covered with grey aerial mycelium, oil droplets formed in the middle of culture; reverse dark brown, radiating outwardly.

**Material examined:** Thailand, Chiang Rai Province, Mae Sai District, dead stems of *Clematis fulvicoma*, 20 March 2017, C. Phukhamsakda & M. van de Bult, CMTH21 (MFLU 17–1484, **holotype**); ex-type living culture, MFLUCC 17–2076.

**Host:** *Clematis fulvicoma*—(This study).

**Distribution:** Thailand—(This study).

**GenBank accession numbers:** LSU: MT214561; SSU: MT226678; ITS: MT310608; *tefl*: MT394740; *rpb2*: MT394688.

**Notes:** In the phylogenetic analysis, *Pseudolophiostoma chiangraiensis* (MFLUCC 17–2076) formed a close relationship with *P. cornisporum* KH 322 (100% ML/1.00 BYPP, Fig. 30). We compared *P. chiangraiensis* (MFLUCC 17–2076) with *P. cornisporum* (KH 322) and both have similar characters in being immersed, with only black shiny ostioles visible on the host, and broad-fusiform, 1-euseptate ascospores, with acute ends (Fig. 30). Ascomata of *P. chiangraiensis* are smaller than *P. cornisporum* (286 × 239 vs 650–700 × 580–650 µm). In a BLASTn search of GenBank, the ITS sequence had 99% similarity to *P. cornisporum* KH 322 (NR\_158930), while the *rpb2* gene was 98% similar to LC312602.

***Pseudolophiostoma clematidis* Phukhams. & K.D. Hyde, sp. nov.**

**Index Fungorum number:** IF557120; **Facesoffungi number:** FoF 07292, Fig. 31.

**Etymology:** The epithet reflects the host, *Clematis*.

**Holotype:** MFLU 17–1489.

**Saprobic** on dead stem of *Clematis fulvicoma*.

**Sexual morph:** Ascomata 352–370 × 139–243 µm ( $\bar{x}$  = 358 × 220 µm, n = 10), solitary, scattered, immersed, with only black shiny ostioles visible, with globose to compressed, flattened base, coriaceous to carbonaceous at the apex, dark brown to black, with a developed pseudoclypeus, ostiolate. *Ostiole* 140–159 × 60–80 µm ( $\bar{x}$  = 150 × 70 µm, n = 5), with a crest-like apex and with opening by a pore, central, elongated and laterally compressed, surrounded by a small blackened pseudoclypeus, irregular wall, filled with hyaline periphyses. *Peridium* 9–28 µm wide ( $\bar{x}$  = 14 µm, n = 20), uniform, wider at the apex, heavily pigmented at the apex, composed of 4(–5) layers of *textura prismatica*, cells towards the inside lighter, somewhat flattened, inner layer composed of thin, hyaline gelatinous layer, at the apex fusing and indistinguishable from the host tissues. *Hamathecium*

composed of numerous, dense, 2–3 µm wide, filamentous, branched, septate, pseudoparaphyses, situated between and above the asci, embedded in a gelatinous matrix. *Asci* 67–106 × 10–14 µm ( $\bar{x}$  = 88 × 12 µm, n = 40), 8-spored, bitunicate, fissitunicate, oblong to cylindrical-clavate, with short, furcate pedicel, apically rounded, with an ocular chamber. *Ascospores* 23–31 × 5–9 µm ( $\bar{x}$  = 26 × 7 µm, n = 50), biseriolate or partially overlapping, hyaline, broad-fusiform, tapering towards the ends, round at the end, 1-euseptate, strongly constricted at the septum, with 2(–3) guttules in each cell, slightly swollen near median septum, with 6–10 µm sheath drawn out to form polar appendages, with a lateral pad-like structure, up to 3 µm wide. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 50 mm diam. after 4 weeks at 25 °C. Cultures from above, olive gray to dark brown in the middle, dense, circular, umbonate, surface rough, dull, fimbriate, radially furrowed, covered with grey aerial mycelium, oil droplets formed in the middle of culture; reverse: dark brown radiating outwardly.

**Material examined:** Thailand, Chiang Rai Province, Mae Sai District, dead stems of *Clematis fulvicoma*, 20 March 2017, C. Phukhamsakda & M. van de Bult, CMTH27 (MFLU 17–1489, **holotype**); ex-type living culture, MFLUCC 17–2081.

**Host:** *Clematis fulvicoma*—(This study).

**Distribution:** Thailand—(This study).

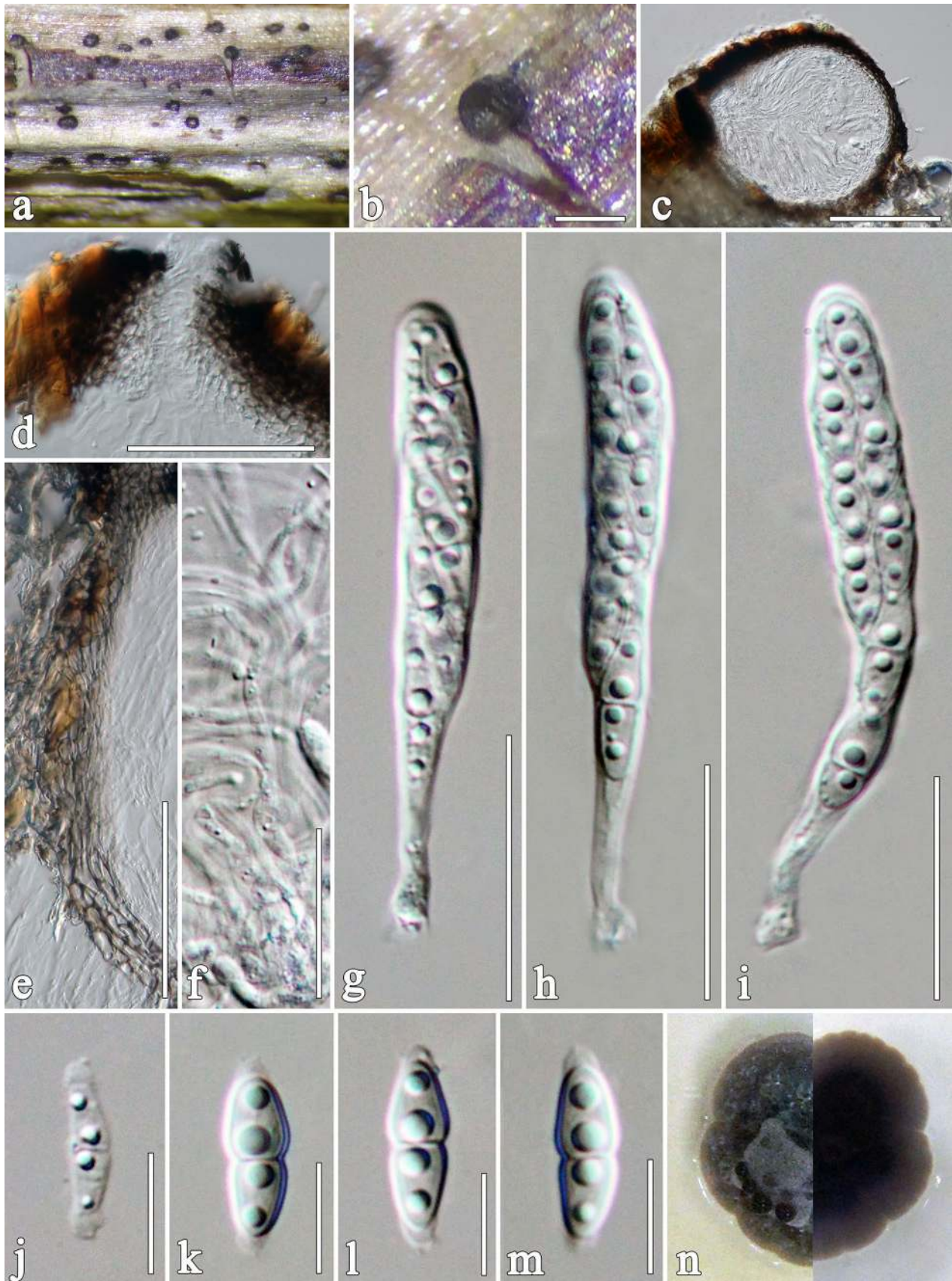
**GenBank accession numbers:** LSU: MT214562; SSU: MT226679; ITS: MN393004; *tefl*: MT394741; *rpb2*: MT394689.

**Notes:** *Pseudolophiostoma clematidis* (MFLUCC 17–2081) formed a sister clade to *P. obtusisporum* strains (100% ML/1.00 BYPP, Fig. 26). *Pseudolophiostoma obtusisporum* is commonly reported on herbaceous plants or palms in Japan (Hashimoto et al. 2018). The morphology of *P. clematidis* resembles *P. obtusisporum* except for the larger ascomata with thinner peridium (Fig. 31). A comparison of sequence data revealed that the two species differ in all studied loci (2 bp differences in ITS, 15 bp in *tefl* and 13 bp in *rpb2*). In a BLASTn search of GenBank, the ITS sequence had 99% similarity to the type specimen of *Pseudolophiostoma obtusisporum* (HHUF 30583), while *rpb2* sequence had 98% similarity to LC312605, derived from the same voucher specimen.

This strain was further evaluated for secondary metabolites and biological activities. *Pseudolophiostoma clematidis* (MFLUCC 17–2081) showed moderate growth of microbes and cytotoxicity in vitro (Macabeo et al. 2020).

***Sigarispora* Thambug. & K.D. Hyde**

*Sigarispora* was introduced by Thambugala et al. (2015) to accommodate *Lophiostoma ravennicum* and some lophiostomataceous taxa that formed a separate clade from the type species of *Lophiostoma*. *Sigarispora* is characterized



**Fig. 28** *Neovaginatispora clematidis* (MFLU 17–1514, holotype). **a** Appearance of ascomata on host surface. **b** Close up of ascoma on host substrate. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Pseudoparaphyses. **g–i** Asci. **j–m** Ascospores. **n** Culture characteristic on MEA. Scale bars: **b** = 200  $\mu$ m, **c** = 100  $\mu$ m, **d** = 50  $\mu$ m, **e–i** = 20  $\mu$ m, **j–n** = 10  $\mu$ m

by its immersed to semi-immersed ascomata, with a small crest-like ostioles, and brown cigar-shaped, multi-septate ascospores (Thambugala et al. 2015; Wanasinghe et al. 2018). Fourteen species are listed in Index Fungorum (Jayasiri et al. 2015; Index Fungorum 2020). We introduce three new species of *Sigarispora* from *Clematis* species, *S. clematidicola*, *S. clematidis* and *S. montana* (Figs. 32, 33, 34).

***Sigarispora clematidicola*** Phukhams., Camporesi & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557121; *Facesoffungi number*: FoF 07293, Fig. 32.

*Etymology*: The epithet reflects the host *Clematis*.

*Holotype*: MFLU 20–0419.

*Saprobic* on dead stems of *Clematis vitalba*. **Sexual morph**: *Ascomata* 255–288  $\times$  217–254  $\mu$ m ( $\bar{x}$  = 276  $\times$  238  $\mu$ m,  $n$  = 10), solitary, scattered immersed, with only black shiny ostioles present, dark brown to black, globose to compressed, coriaceous, rough-walled, sometimes with dark brown hyphae projecting from the peridium, pseudoclypeus, ostiolate. *Ostioles* 60–135  $\times$  67–156  $\mu$ m ( $\bar{x}$  = 97  $\times$  102  $\mu$ m,  $n$  = 5), with a crest-like apex, central, elongated and laterally compressed, irregular wall, filled with hyaline paraphyses. *Peridium* 13–48  $\mu$ m wide ( $\bar{x}$  = 29  $\mu$ m,  $n$  = 20), wider at the apex, thinner at the base, with 6–7 layers of lightly pigmented light brown to brown, thick-walled cells of *textura angularis*, lighter pigmented cells towards inside, somewhat flattened, inner layer composed of hyaline gelatinous layer, fusing and indistinguishable from the host tissues. *Hamathecium* numerous, dense, 2–3  $\mu$ m wide, filamentous, branched, septate, pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 101–125  $\times$  12–19  $\mu$ m ( $\bar{x}$  = 115  $\times$  16  $\mu$ m,  $n$  = 40), 8-spored, bitunicate, fissitunicate, broad cylindrical to clavate, with furcate pedicel, rounded at the apex, with an ocular chamber. *Ascospores* 22–29  $\times$  7–9  $\mu$ m ( $\bar{x}$  = 26  $\times$  8  $\mu$ m,  $n$  = 50), biserial or partially overlapping, initially hyaline, becoming yellowish brown at maturity, broad fusiform, tapering towards the end, mostly curved (3–)5-transversely euseptate, constricted at the septa, cells above central septum swollen, guttulate, indentations present, without or with 2–4  $\mu$ m sheath drawn out to form polar appendages. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA, slow-growing, reaching 20 mm diam. after 4 weeks at 25 °C. Culture centrally black dense, circular, flat, umbonate, surface rough; reverse: mycelium strongly radiating into agar, black.

*Material examined*: Italy, Forlì-Cesena Province, Viale Salinatore—Forlì, dead aerial branch of *Clematis vitalba*, 23 February 2015, E. Camporesi, IT2389-A (MFLU 20–0419, **holotype**); ex-type living culture, MFLUCC 16–0446.

*Host*: *Clematis vitalba*—(This study).

*Distribution*: Italy—(This study).

*GenBank accession numbers*: LSU: MT214563; SSU: MT226680; ITS: MT310609; *tefl*: MT394742.

*Notes*: Based on phylogenetic evidence, the isolates of *Sigarispora caudata* (KT 530, MAFF 239453), *S. clematidicola* (MFLUCC 16–0446) and *S. clematidis* (MFLUCC 16–1368) formed a moderately supported clade (Fig. 26). *Sigarispora clematidicola* (Fig. 32) and *S. clematidis* (Fig. 33) share morphological similarity such as brown ascospores with polar appendages, while *S. caudata* lacks polar appendages (Table 3). *Sigarispora clematidicola*, differs from *S. clematidis* in having smaller ascomata, asci and ascospores (Table 3). A pairwise comparison of *tefl* sequences of *S. clematidis* and *S. caudata* showed 98% similarity with 11 nucleotide differences. The *tefl* sequences of *S. clematidicola* and *S. clematidis* showed 30 nucleotide differences. A pairwise homoplasy index showed  $\Phi_w = 1.0$  when genealogical correlation model was applied between neighboring strains of clade (c) (Fig. 27c). The result is congruent with the phylogenetic lineages shown in Fig. 26. Thus, *S. caudata* (KT 530, MAFF 239453), *S. clematidicola* (MFLUCC 16–0446) and *S. clematidis* (MFLUCC 16–1368) are significantly different from each other based on molecular as well as morphological data.

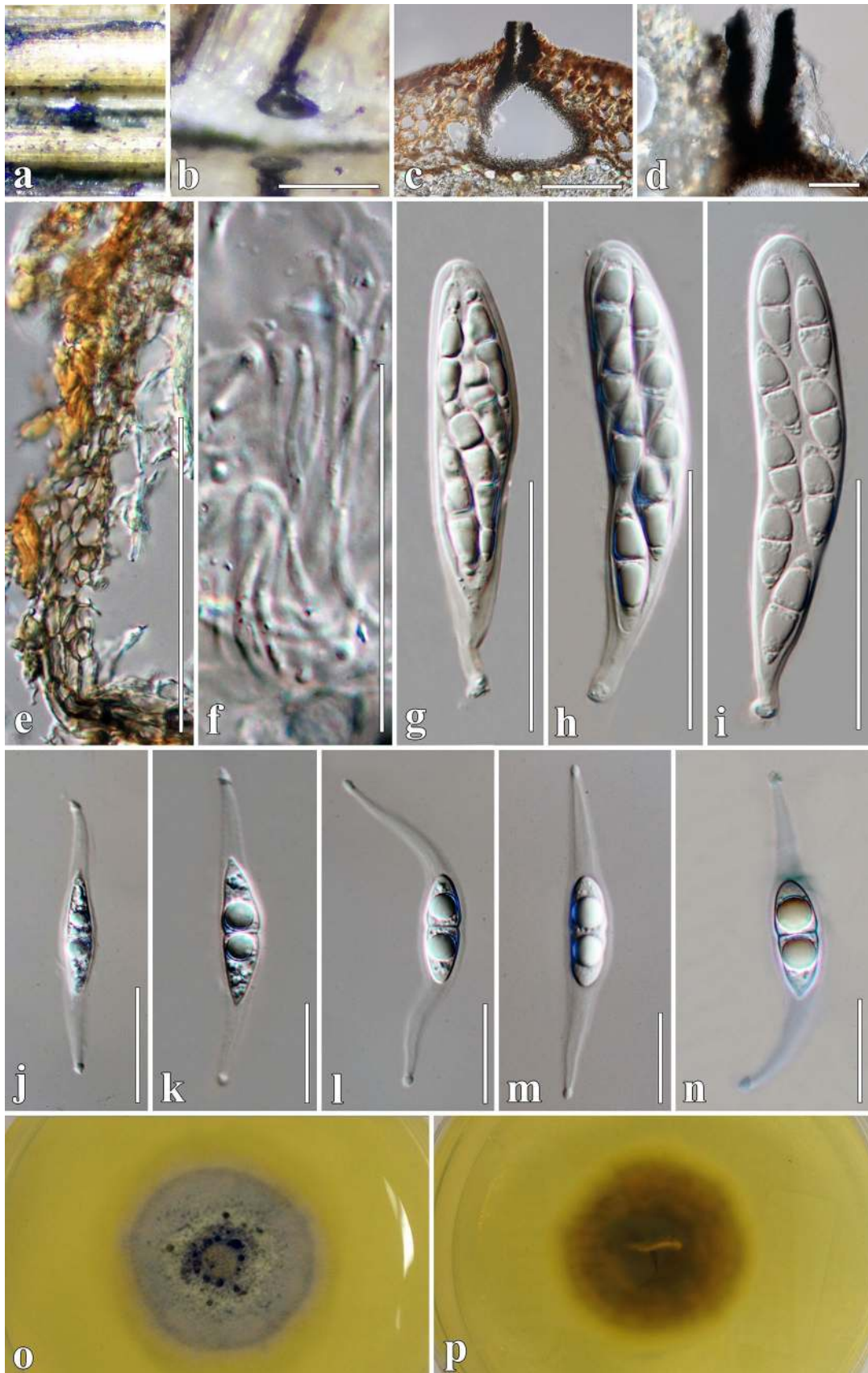
***Sigarispora clematidis*** Phukhams., & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557122; *Facesoffungi number*: FoF 07294, Fig. 33

*Etymology*: The epithet reflects the host *Clematis*.

*Holotype*: MFLU 20–0417

*Saprobic* on dead stem of *Clematis vitalba*. **Sexual morph**: *Ascomata* 416–493  $\times$  337–386  $\mu$ m ( $\bar{x}$  = 444  $\times$  369  $\mu$ m,  $n$  = 10), solitary, scattered, immersed, with only black shiny ostioles visible, globose to compressed, coriaceous, dark brown to black, rough-walled, ostiolate. *Ostioles* 151–187  $\times$  107–170  $\mu$ m ( $\bar{x}$  = 167  $\times$  129  $\mu$ m,  $n$  = 5), with a crest-like apex and with opening by a pore, central, elongated and laterally compressed, irregular wall, filled with hyaline paraphyses, pseudoparenchymatous cells. *Peridium* 6–46(–76  $\mu$ m at apex) wide ( $\bar{x}$  = 32  $\mu$ m,  $n$  = 20), wider at the apex, thinner at the base, with lightly pigmented, thick-walled cells of *textura angularis*, cell towards the inside lighter, somewhat flattened, inner layer composed of thick, hyaline gelatinous layer. *Hamathecium* composed of numerous, dense, 1.5–2.5  $\mu$ m wide, filamentous, branched, septate, pseudoparaphyses, situated between and above the asci embedded in a gelatinous matrix. *Asci* 96–145  $\times$  10–18  $\mu$ m ( $\bar{x}$  = 118  $\times$  14  $\mu$ m,  $n$  = 40), 8-spored,



**Fig. 29** *Pseudocapulatispora clematidis* (MFLU 17–1469, **holotype**). **a** Appearance of ascoma on host surface. **b** Close up of ascoma on host substrate. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Pseudoparaphyses. **g–i** Asci. **j–m** Ascospores. **n** Ascospore in cotton blue showing the end chambers. **o**, **p** Culture characteristic on MEA. Scale bars: **b** = 500  $\mu\text{m}$ , **c** = 200  $\mu\text{m}$ , **d–i** = 50  $\mu\text{m}$ , **j–n** = 20  $\mu\text{m}$

bitunicate, fissitunicate, clavate, with furcate pedicel, rounded at the apex, with an ocular chamber. *Ascospores* 22–30  $\times$  6–9  $\mu\text{m}$  ( $\bar{x}$  = 24  $\times$  7  $\mu\text{m}$ ,  $n$  = 60), biseriate or partially overlapping, broad fusiform, tapering towards the ends, initially hyaline, becoming yellowish brown at maturity, acute ends, mostly curved, 5–6 transversely eusepta, slightly constricted at the septa, cells above central septum swollen, guttulate, indentations present, with 5–10  $\mu\text{m}$  long sheath drawn out to form polar appendages. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 50 mm diam. after 4 weeks at 25 °C. Cultures from above, centrally black, dense, circular, flat, umbonate, surface rough, dull, fimbriate, radially furrowed, slightly covered with white aerial mycelium; reverse: mycelium strongly radiating into the agar, black with radiating brown outwardly.

**Material examined:** UK, Hampshire, Swanick Lake, dead stems of *Clematis vitalba*, 9 July 2016, E.B.G. Jones, GJ307 (MFLU 20–0417, **holotype**); ex-type living culture, MFLUCC 16–1368.

**Host:** *Clematis vitalba*—(This study).

**Distribution:** UK—(This study).

**GenBank accession numbers:** LSU: MT214564; SSU: MT226681; ITS: MT310610; *tefl*: MT394743.

**Notes:** See note under *Sigarispora clematidicola*.

***Sigarispora montanae*** Phukhams., Sue, K.D. Hyde, **sp. nov.**

**Index Fungorum number:** IF557124; **Facesoffungi number:** FoF 07295, Fig. 34.

**Etymology:** The epithet reflects the host species, *Clematis montana*.

**Holotype:** MFLU 20–0418.

**Saprobic** on dead stems of *Clematis montana*. **Sexual morph:** *Ascomata* 180–230  $\times$  140–200  $\mu\text{m}$  ( $\bar{x}$  = 200  $\times$  160  $\mu\text{m}$ ,  $n$  = 5), solitary, scattered, semi-immersed, with black shiny ostioles, globose, coriaceous, partial carbonaceous at the apex, dark brown to black, rough-walled, forming a clypeus like character, ostiolate. *Ostioles* (40–)70–100  $\times$  30–60  $\mu\text{m}$  ( $\bar{x}$  = 80  $\times$  50  $\mu\text{m}$ ,  $n$  = 5), with a crest-like apex, central, elongated and laterally compressed, irregular wall, filled with hyaline periphyses. *Peridium* 13–30  $\mu\text{m}$  wide ( $\bar{x}$  = 20  $\mu\text{m}$ ,  $n$  = 30), wider at the apex, thinner at the base, with 6–7 layers of lightly pigmented light brown to dark brown, thick-walled cells of *textura angularis*, cells towards the inside lighter, at the outside darker, inner layer composed of thick hyaline gelatinous layer, fusing

and indistinguishable from the host tissues. *Hamathecium* composed of numerous, dense, 1.2–1.5  $\mu\text{m}$  ( $\bar{x}$  = 1.3  $\mu\text{m}$ ,  $n$  = 30), filamentous, branched, septate, anastomosing, pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 105–130  $\times$  10–14  $\mu\text{m}$  ( $\bar{x}$  = 120  $\times$  15  $\mu\text{m}$ ,  $n$  = 20), 8-spored, bitunicate, fissitunicate, broad cylindrical to clavate, with a long, with furcate pedicel, rounded at the apex, with an ocular chamber. *Ascospores* 20–26  $\times$  5–7  $\mu\text{m}$  ( $\bar{x}$  = 22  $\times$  5  $\mu\text{m}$ ,  $n$  = 50), biseriate or partially overlapping, broad fusiform, tapering towards the ends, initially hyaline, becoming yellowish brown at maturity, mostly curved, 3(–5) transversely eusepta, constricted at the septa, cells above central septum swollen, indentations present, with 3–5  $\mu\text{m}$  sheath drawn out to form polar appendages. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA, reaching 25 mm diam. after 4 weeks at 16 °C. Cultures from above, centrally grey, bearing cream outwardly, dense, circular, flat, umbonate, surface rough; reverse cream with dark brown.

**Material examined:** China, Yunnan Province, Dali District, on dead stems of *Clematis montana*, 20 May 2016, C. Phukhamsakda, CMCR1 (MFLU 20–0418, **holotype**); ex-type living culture, MFLUCC 16–0999.

**Host:** *Clematis montana*—(This study).

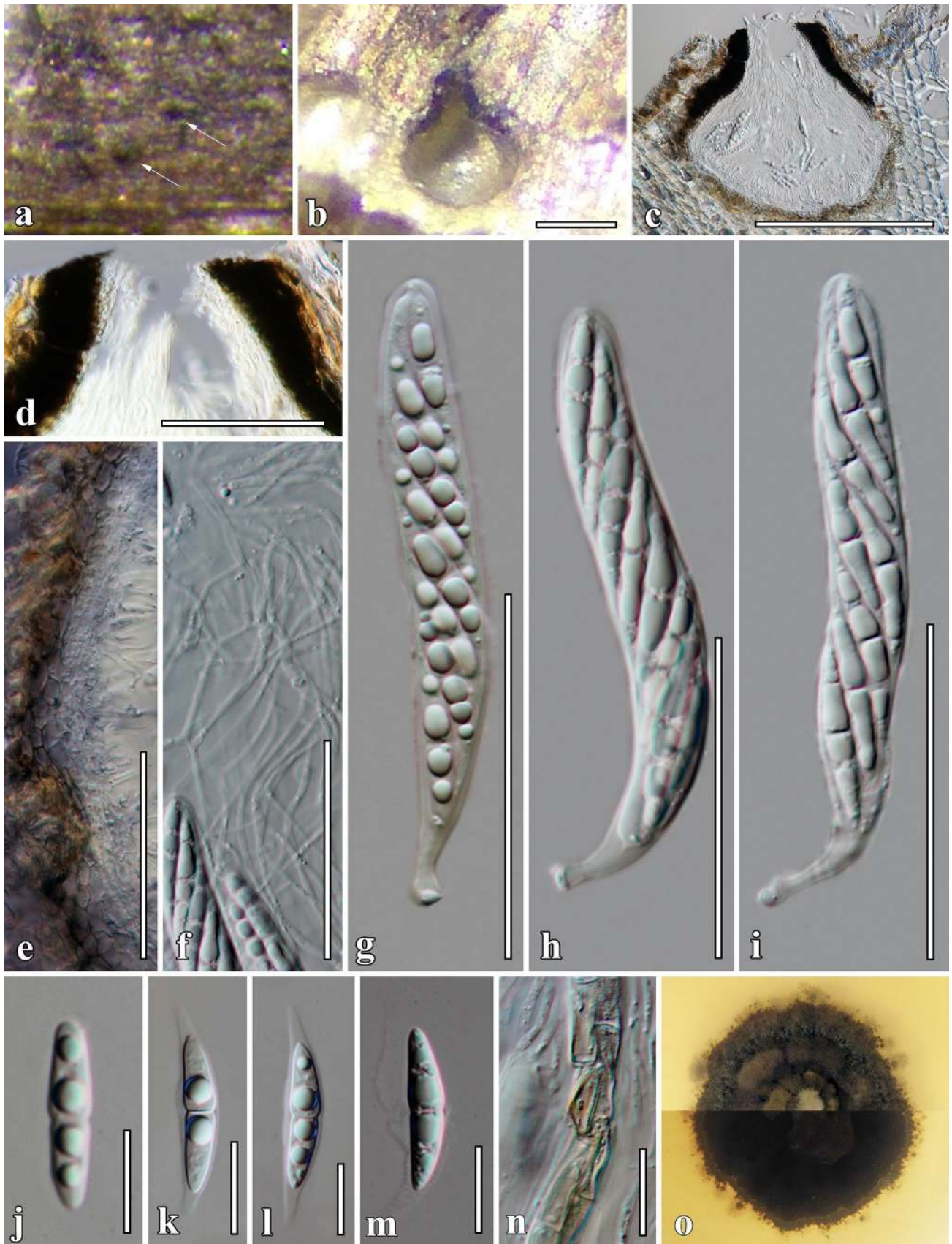
**Distribution:** China—(This study).

**GenBank accession numbers:** LSU: MT214565; SSU: MT226682; ITS: MT310611; *tefl*: MT394744.

**Notes:** In the phylogenetic analysis, *Sigarispora montanae* (Fig. 34) clustered basal to *Sigarispora caulium* with strong support (100% ML/1.00 BYPP, Fig. 26). *Sigarispora montanae* has relatively small ascomata and longer pedicels when compared with *S. caulium* (Thambugala et al. 2015, Table 3). In a BLASTn search of GenBank, the closest matches of *tefl* sequence of MFLUCC 16–0999 is 96.5% similar to *S. thymi* strain MFLU 15–2131 (MG829241). The *tefl* sequences of *S. montanae* had 98% similarity to *Sigarispora caulium* (MFLUCC 15–0036) with 15 nucleotide differences in the *tefl* region (Thambugala et al. 2015; Wanasinghe et al. 2018).

**Melanommataceae** Winter (= Pseudodidymellaceae)

*Melanomma* Nitschke ex Fuckel is the generic type (Winter 1885). Members of this family can occur on twigs or bark of various woody plants in terrestrial, marine or freshwater habitats. Melanommataceae is characterized by carbonaceous or coriaceous, gregarious, immersed to erumpent, globose to subglobose, papillate or epapillate, thick-walled, pseudoparenchymatous cells, trabeculate pseudoparaphyses, ascospores uniseriate or biseriate, fusoid to ellipsoidal, or muriform, hyaline or brown, 1 to multi-septate, with or without a mucilaginous sheath. Their asexual morphs can be hyphomycetes or coelomycetes (Sivanesan 1984; Huhndorf 1993; Liew et al. 2000; Tian et al. 2015; Wanasinghe et al. 2018). Wijayawardene et al. (2018) accepted 24 genera,





**Fig. 30** *Pseudolophiostoma chiangraiense* (MFLU 17–1484, holotype). **a** Appearance of ascoma on host surface. **b** Close up of ascoma on host substrate. **c** Vertical section of ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Pseudoparaphyses. **g–i** Asci. **j–m** Ascospores. **n** Senescent spores. **o** Culture characteristics on MEA. Scale bars: **b**, **c** = 200  $\mu\text{m}$ , **d–i** = 50  $\mu\text{m}$ , **j–n** = 10  $\mu\text{m}$

thereafter Wanasinghe et al. (2018) introduced five more genera to Melanommataceae. An analysis of fungal collections on *Clematis vitalba* revealed a novel genus, *Neobysso-sphaeria* based on a multi-gene phylogeny of LSU, SSU and ITS sequence data for Melanommataceae (Fig. 35).

***Neobysso-sphaeria*** Wanas., E.B.G. Jones & K.D. Hyde, **gen. nov.**

*Index Fungorum number*: IF557189; *Facesoffungi number*: FoF 07281, Fig. 36.

*Etymology*: Name refers to the similarity of its morphology to *Byssosphaeria*.

*Saprobic* on decaying wood or herbaceous plants in terrestrial habitats. **Sexual morph**: *Ascomata* immersed, ostioles orange, solitary or gregarious, globose to depressed-globose, coriaceous, ostiolate. *Ostioles* central, papillate, opening by a pore, filled with periphyses with orange pigment around the pore. *Peridium* thick, multilayered, outer layer composed of reddish brown cells of *textura angularis*, inner layer composed of thin and hyaline cells of *textura angularis*. *Hamathecium* composed of numerous, dense, filiform, branched, anastomosing, transversely septate, trabeculate pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical-clavate, pedicellate with an ocular chamber. *Ascospores* biserial, broadly fusiform, hyaline, constricted at the septa, guttulate in each cell, with or without a mucilaginous sheath. **Asexual morph**: Undetermined.

*Type species*: ***Neobysso-sphaeria clematidis*** Wanas., Phukhams., E.B.G. Jones & K.D. Hyde

*Notes*: *Neobysso-sphaeria* is established as a monotypic genus with *N. clematidis* as the type species. Based on multi-gene analyses, isolate MFLUCC 17–0794 formed a basal lineage to *Byssosphaeria*, but this placement is not supported by the statistical analyses. *Byssosphaeria* is characterized by superficial ascomata with bright yellow, orange or red colouration around the ostioles, hairy hypha protruding from the outside of peridium, long pedicellate asci, and hyaline or pale brown ascospores (Barr 1990; Tian et al. 2015). *Neobysso-sphaeria* is similar to *Byssosphaeria* in its orange apex (Zhang et al. 2012; Hyde et al. 2013). However, *Neobysso-sphaeria* is distinguished by its immersed ascomata with central papilla filled with periphyses, cellular pseudoparaphyses and broad fusiform and hyaline ascospores (Fig. 36). Our taxon failed to produce an asexual morph in culture and therefore it is not possible to compare the

asexual characteristics with species of *Byssosphaeria*. In the BLASTn search of GenBank, the closest match of the LSU region of MFLUCC 17–0794 is *Uzbekistanica yakut-khanika* (strain MFLUCC 17–0842) with 94.48% similarity (accession number MG829090). *Uzbekistanica* is however phylogenetically not closely related to our new collection (Fig. 35). Therefore, we believe it is taxonomically prudent to name our collection in a new genus until further studies are carried out with further taxonomic sampling and DNA based sequence analyses.

***Neobysso-sphaeria clematidis*** Wanas., Phukhams., E.B.G. Jones & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557190; *Facesoffungi number*: FoF 07282, Fig. 36.

*Etymology*: Named after the host genus, *Clematis*.

*Holotype*: MFLU 17–0614.

*Saprobic* on dead stems of *Clematis vitalba*. **Sexual morph**: *Ascomata* 525–550  $\times$  500–520  $\mu\text{m}$  ( $\bar{x}$  = 535  $\times$  510  $\mu\text{m}$ ,  $n$  = 5), immersed, ostiole part orange, solitary or gregarious, globose to depressed-globose, coriaceous, indistinguishable from host tissue, brown to pale brown, rough-walled, ostiolate. *Ostioles* 235–270  $\times$  190–230  $\mu\text{m}$  ( $\bar{x}$  = 250  $\times$  200  $\mu\text{m}$ ,  $n$  = 5), central, papillate, opening by a pore, filled with periphyses, with orange pigment around pore. *Peridium* 30–50 (–70  $\mu\text{m}$  at apex) wide, thick, multilayered, outer layer composed of heavily pigmented, reddish brown cells of *textura angularis*, inner layer composed of thin and hyaline cells of *textura angularis*. *Hamathecium* composed of numerous, 1.6–3  $\mu\text{m}$  ( $\bar{x}$  = 2.3  $\mu\text{m}$ ,  $n$  = 50), dense, filiform, branched, anastomosing, septate, cellular pseudoparaphyses. *Asci* 160–210  $\times$  20–30  $\mu\text{m}$  ( $\bar{x}$  = 185  $\times$  25  $\mu\text{m}$ ,  $n$  = 20), 8-spored, bitunicate, fissitunicate, cylindrical, long pedicellate with a furcated base, clavate, apically rounded, with ocular chamber. *Ascospores* 55–75  $\times$  8–14  $\mu\text{m}$  ( $\bar{x}$  = 60  $\times$  11  $\mu\text{m}$ ,  $n$  = 30), biserial, partially overlapping, broad fusiform, sometimes inequilateral, with acute ends, hyaline, 7-euseptate, constricted at the septa, cell above median septum enlarged, with guttules in each cell, rough-walled, without mucilaginous sheath. **Asexual morph**: Undetermined.

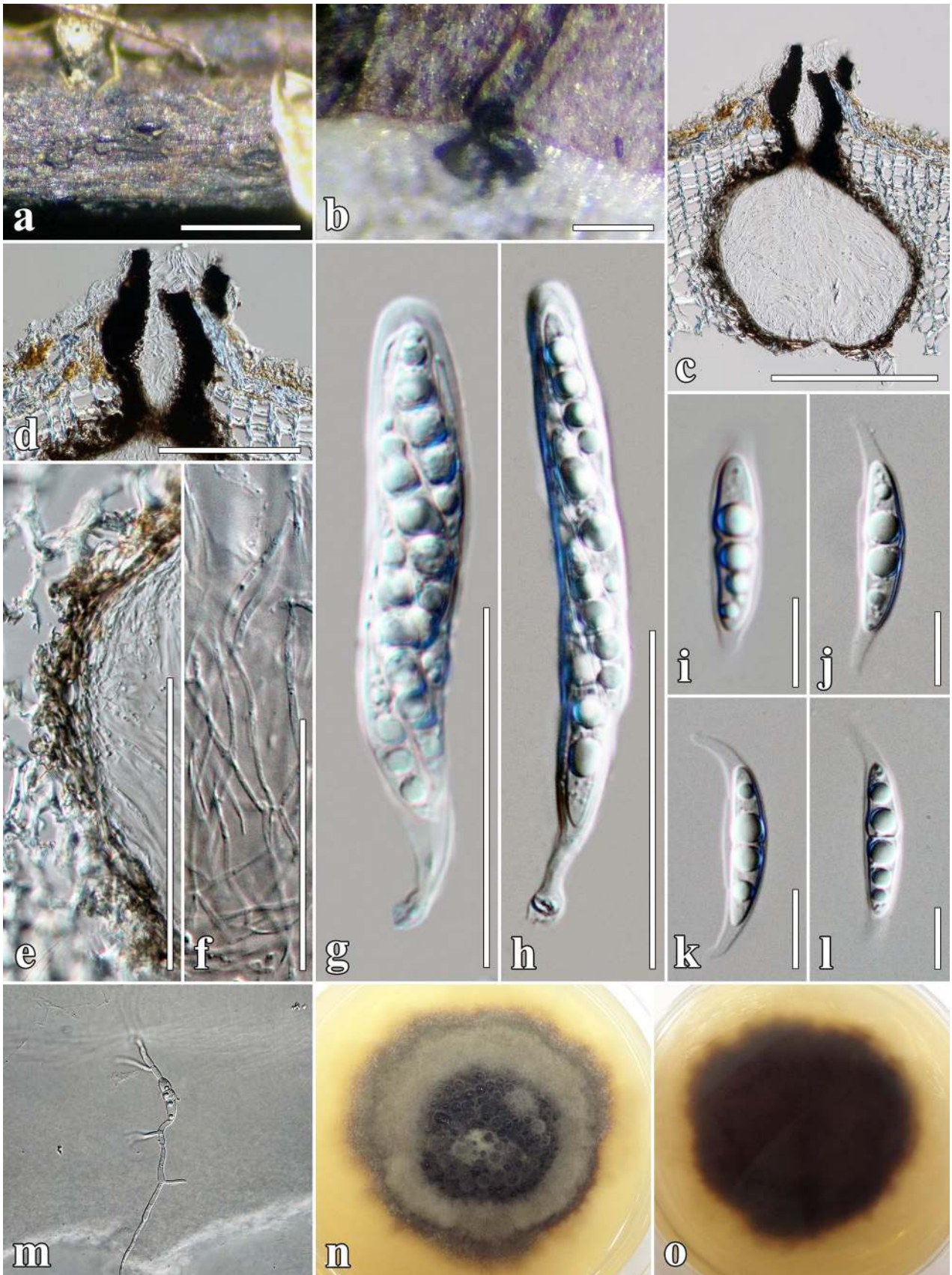
*Culture characters*: Colonies on MEA reaching 20 mm diam. after 4 weeks at 16 °C. Above cream with orange in the middle, with white edge, medium dense, flattened, umbonate, floccose; reverse: cream, thin, flat, circular.

*Material examined*: UK, Hampshire, Botley wood, on dead stems of *Clematis vitalba*, 25 May 2016, E.B.G. Jones, GJ 298 (MFLU 17–0614, **holotype**); ex-type living culture, MFLUCC 17–0794.

*Host*: *Clematis vitalba*—(This study).

*Distribution*: UK—(This study).

*GenBank accession numbers*: LSU: MT214566; SSU: MT408594.



**Fig. 31** *Pseudolophiostoma clematidis* (MFLU 17–1489, holotype). **a** Appearance of ascoma on host surface. **b** Close-up of ascoma on host substrate. **c** Vertical section of ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Pseudoparaphyses. **g–h** Asci. **i–l** Ascospores. **m** Germinated ascospore **n, o** Culture characteristics on MEA. Scale bars: **a**=500  $\mu\text{m}$ , **b, c**=200  $\mu\text{m}$ , **d, e**=100  $\mu\text{m}$ , **f–h**=50  $\mu\text{m}$ , **i–l**=10  $\mu\text{m}$

**Notes:** The new fungus morphologically resembles many genera in *Pleosporales* (e.g. *Angustimassarina*, *Aquastroma*, *Aquilomyces*, *Carinispora*, *Falciformispora*, *Keissleriella*, *Lophiopoacea*, *Lophiostoma*, *Parabambusicola*, *Quintaria*) in its cylindrical-clavate asci and hyaline, fusiform ascospores with large guttule in each cell (Zhang et al. 2012; Tanaka et al. 2015; Thambugala et al. 2015). However, these taxa are phylogenetically not closely related to *Neobysso-sphaeria clematidis* (see Notes *Neobysso-sphaeria* for further details). *Neobysso-sphaeria clematidis* differs from *Byssosphaeria* species by its immersed ascomata, lacking hairy hypha protruding from the outside of the peridium (Tian et al. 2015).

#### Neomassarinaceae Mapook & K.D. Hyde

The type genus of Neomassarinaceae is *Neomassarina*. The family is phylogenetically related to Sporormiaceae but the characters of the sexual morphs are different. Neomassarinaceae can be characterized by its crest-like ostiolar necks, with a carbonaceous texture, cylindrical to cylindrical-clavate asci and broad fusiform ascospores surrounded by a mucilaginous sheath (Thambugala et al. 2015; Tibpromma et al. 2017; Mapook et al. 2020). In this study, a dataset of LSU, SSU, ITS, *tef1* and *rpb2* were used for phylogenetic analyses (Fig. 2). A collection associated with *Clematis* formed a clade related to Neomassarinaceae. Thus, we introduce a new genus *Pseudohelminthosporium* and a first report of the asexual morph in Neomassarinaceae (Fig. 37).

#### *Pseudohelminthosporium* Phukhams. & K.D. Hyde, gen. nov.

**Index Fungorum number:** IF557191; **Facesoffungi number:** FoF 07283, Fig. 37.

**Etymology:** Referring to its similarity to *Helminthosporium*.

**Saprobic** on decaying wood or herbaceous plant material in terrestrial habitats. **Sexual morph:** Undetermined. **Asexual morph:** **Colonies** on host substrates, effuse, black, hairy, scattered, dark brown. **Mycelium** immersed from the substrate forming dark brown stroma-like aggregations. **Conidiophores** macronematous, simple, solitary, branched at the apex, stripes straight or flexuous, cylindrical, dark brown to reddish brown, multi-septate, with well-defined small pores at the apex, smooth or verruculose. **Conidiogenous cells** monotretic or polytretic, integrated, terminal on conidiophores, doliiform to oblong, pale brown. **Conidia**

phragmosporous, acrogenous, broad fusiform or obclavate, dark brown to reddish brown, distoseptate when young, becoming euseptate at maturity, verrucose, dark brown bud scars disjunctions present at the basal position, hyaline, elongate cells at the upper end of conidia, with guttules in each cell.

**Type species:** *Pseudohelminthosporium clematidis* Phukhams. & K.D. Hyde

**Notes:** *Pseudohelminthosporium* is established as a monotypic genus. Based on the multi-gene analysis (Fig. 2), the isolate MFLUCC 17–2086 formed a basal lineage with *Neomassarina* species with moderate support (0.93 BYPP). *Pseudohelminthosporium* is morphologically similar to *Helminthosporium* (Massariaceae) in having brown to dark brown phragmosporous conidia (Voglmayr and Jaklitsch 2017). *Helminthosporium* species have obclavate to rostrate, pale golden brown to brown conidia, with distoseptate and angular lumina. *Pseudohelminthosporium* is distinguishable by its solitary stipes with monotretic or polytretic conidiogenous cells, phragmosporous, broad fusiform or obclavate conidia, distoseptate when young, becoming euseptate at maturity, with hyaline, elongate cells at the upper end of the conidia and with large guttule in each cell (Fig. 37).

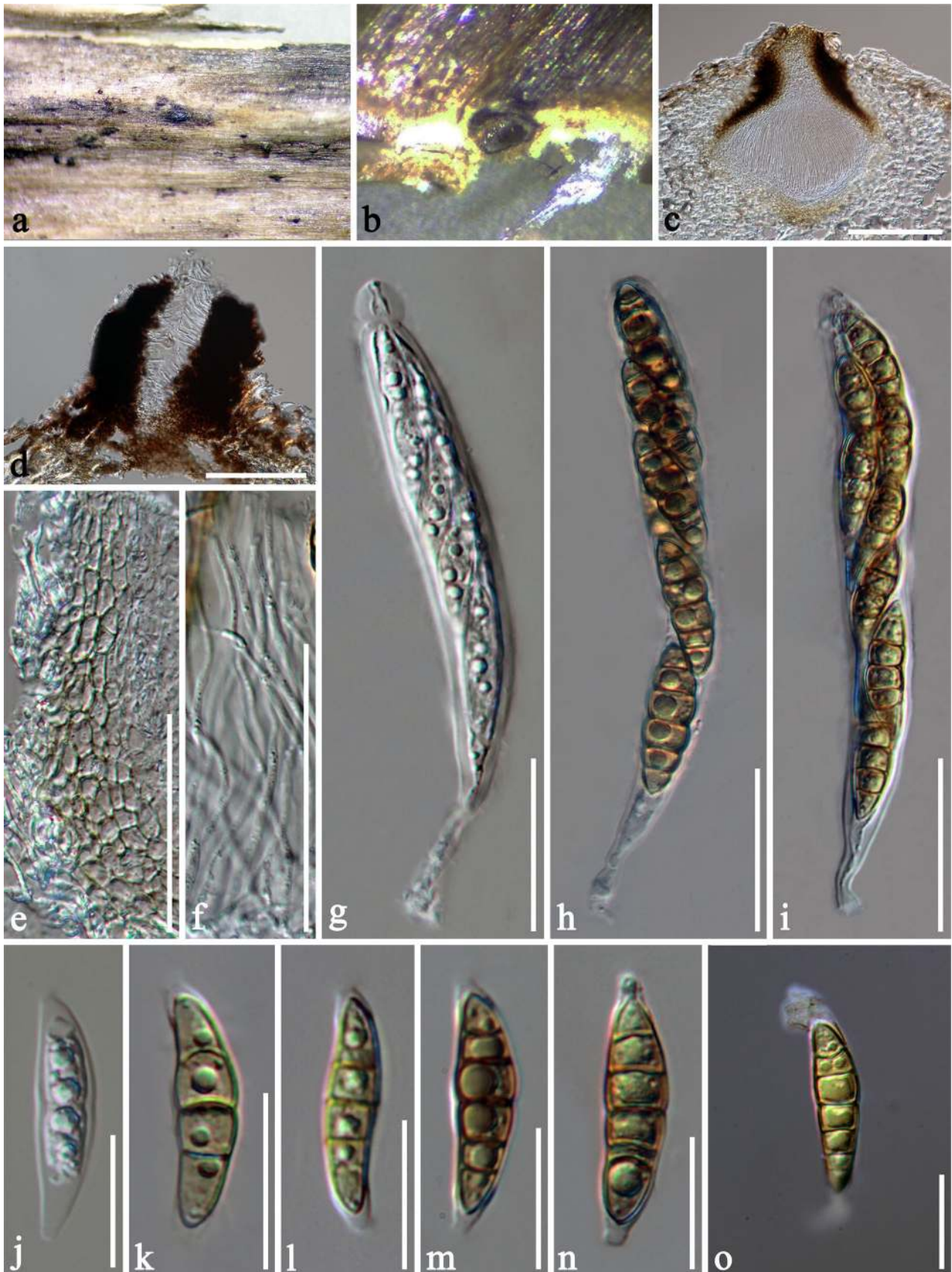
#### *Pseudohelminthosporium clematidis* Phukhams. & K.D. Hyde, sp. nov.

**Index Fungorum number:** FoF 07284; **Facesoffungi number:** FoF 07284, Fig. 37.

**Etymology:** The epithet “*clematidis*” refers to the host substrate.

**Holotype:** MFLU 17–1494.

**Saprobic** on decaying wood or herbaceous plant material in terrestrial habitats. **Sexual morph:** Undetermined. **Asexual morph:** **Colonies** on *Clematis sikkimensis* effuse, black, hairy, scattered, dark brown. **Mycelium** immersed, on the substrate surface forming stroma-like aggregations of dark brown sheet. **Conidiophores** 125–435  $\times$  9–20  $\mu\text{m}$  ( $\bar{x}$  = 230  $\times$  12  $\mu\text{m}$ ,  $n$  = 20), macronematous, simple, solitary, branched at the apex, stipes straight or flexuous, cylindrical, erect, septate, smooth, dark brown to reddish brown, 8–14 septa, brown, well-defined small pores with dark scar at the apex, smooth or verruculose. **Conidiogenous cells** 17–60  $\times$  9–12  $\mu\text{m}$  ( $\bar{x}$  = 40  $\times$  12  $\mu\text{m}$ ,  $n$  = 10), monotretic or polytretic, integrated, terminal, becoming intercalary, doliiform to oblong, pale brown. **Conidia** 63–142  $\times$  16–26  $\mu\text{m}$  ( $\bar{x}$  = 87  $\times$  20  $\mu\text{m}$ ,  $n$  = 20), phragmosporous, acrogenous, broad fusiform or obclavate, distoseptate at the early state, 3–5-euseptate at maturity, slightly constricted at septa, dark brown to reddish brown, verrucose, gradually tapering to 13  $\mu\text{m}$  at the distal end, with a dark brown to black scar present at the base, subhyaline, elongate cells at the upper end of conidia, with (1–)2 guttules in each cell.



**Fig. 32** *Sigarispora clematidicola* (MFLU 20–0419, **holotype**). **a** Appearance of ascoma on host surface. **b** Close up of ascoma. **c** Vertical section of ascoma. **d** Ostiolar canal. **e** Peridium. **f** Pseudoparaphyses. **g–i** Asci. **j–o** Ascospores (**o** Ascospore in 10% Indian ink; note the boundary of polar appendages). Scale bars: **c, d** = 100  $\mu\text{m}$ , **e, f** = 50  $\mu\text{m}$ , **g–i** = 20  $\mu\text{m}$ , **j–o** = 10  $\mu\text{m}$

**Culture characters:** Colonies on MEA reaching 40 mm diam. after 4 weeks at 25 °C. Culture from above, brownish grey, dark green, white in the center, forming cream fluffy mycelium at the edge, dense, umbonate, raised with concave edge, rough, dull, lobate, radially furrowed, with brown pigment slightly diffusing into the agar; reverse dark brown.

**Material examined:** Thailand, Chiang Rai Province, Mae Sai District, dead stems of *Clematis sikkimensis*, 24 June 2017, C. Phukhamsakda & M. van de Bult, CMTHDT02 (MFLU 17–1494, **holotype**); ex-type living culture, MFLUCC 17–2086.

**Host:** *Clematis sikkimensis*—(This study).

**Distribution:** Thailand—(This study).

**GenBank accession numbers:** LSU: MT214567; SSU: MT226683; ITS: MT310612; *tef1*: MT394627; *rpb2*: MT394690.

**Notes:** In a BLASTn search of GenBank, the closest match for the LSU sequence of MFLUCC 17–2086 was *Preussia terricola* strain CBS 317.65 (GQ203725) with 96.77% similarity. The closest match for the ITS region was *Forliomyces uniseptata* strain MFLUCC 15–0765 (NR\_154006). Based on the multi-locus phylogenetic support (Fig. 2), *Pseudohelminthosporium* formed a separate lineage related to *Neomassarina* species but lacked backbone support. Therefore, *Pseudohelminthosporium* is treated as a distinct genus in Neomassarinaeae.

### Nigrogranaceae Jaklitsch & H. Voglmayr

Nigrogranaceae was erected to accommodate a well-supported clade of *Nigrograna* in *Pleosporales*, with *Nigrograna* as the generic type (Jaklitsch and Voglmayer 2016). Nigrogranaceae was isolated from the bark of wood and is characterized by its immersed to erumpent ascomata, surrounded by a subiculum with only papillate ostioles seen on the host substrate. The remarkable characteristics of Nigrogranaceae include broad-fusiform to narrowly ellipsoid ascospores with 1–3-euseptate, and pale to chocolate brown ascospores. The asexual morph has pseudoparenchymatous pycnidia and rod-like to ellipsoid, 1-celled, hyaline or subhyaline conidia (Gruyter et al. 2013). Only *Nigrograna* is accepted in Nigrogranaceae (Wijayawardene et al. 2017, 2018, Fig. 38).

### *Nigrograna* Gruyter, Verkley & P.W. Crous

Gruyter et al. (2013) introduced *Nigrograna* for isolates reported as infectious human pathogen (Serrano et al. 1998;

Ahmed et al. 2018). The genus is typified by *Nigrograna mackinnonii* (Gruyter et al. 2013), and includes 13 species (Index Fungorum 2020) based on phylogenetic analyses of a concatenated dataset of LSU, SSU, ITS and *tef1* data (Fig. 38) coupled with morphology. *Nigrograna chromolaenae* and *N. oblique*, on *Clematis* species, are new host records (Figs. 39, 40).

### *Nigrograna chromolaenae* Mapook & K.D. Hyde, Fungal Divers (2020), **new host record**

**Facesoffungi number:** FoF 07297, Fig. 39.

**Saprobic** on dead stems of *Clematis fulvicoma*. **Sexual morph:** *Ascomata* 135–251  $\times$  87–238  $\mu\text{m}$  ( $\bar{x}$  = 178  $\times$  141  $\mu\text{m}$ ,  $n$  = 10), on the surface of the host, solitary, gregarious, immersed, the surface of host slightly swollen, subglobose to depressed, coriaceous, dark brown to brown, smooth-walled, papillate. *Ostioles* 119  $\times$  95  $\mu\text{m}$ , central, dark brown, filled with paraphyses. *Peridium* 9–15  $\mu\text{m}$  wide, multilayered, composed of 5–7 layers of dark brown cells of *textura angularis*, heavily pigmented at the outer layer, the inner layer composed of hyaline and thin layer. *Hamathecium* composed of numerous, 0.8–1.5  $\mu\text{m}$  wide ( $\bar{x}$  = 0.8  $\mu\text{m}$ ,  $n$  = 50), dense, filiform, branched, septate, pseudoparaphyses, anastomosing above asci. *Asci* 45–60  $\times$  7–9  $\mu\text{m}$  ( $\bar{x}$  = 46  $\times$  7  $\mu\text{m}$ ,  $n$  = 30), 8-spored, bitunicate, fissitunicate, cylindrical-clavate to clavate, with furcate pedicel, apically rounded, ocular chamber visible when immature. *Ascospores* 11–13  $\times$  3–5  $\mu\text{m}$  ( $\bar{x}$  = 12  $\times$  4  $\mu\text{m}$ ,  $n$  = 50), biseriolate, ellipsoid to broad fusiform, sometimes inequilateral, with rounded ends, cognac brown, (1–)3-septate, constricted at septum, cell above median septum enlarged, with guttule in each cell, smooth-walled, without mucilaginous sheath. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Cultures from above, dark brown, covered with white mycelia in the center, dense, irregular, umbonate, lobate, velvety, with flat parchment-like stromatic sheets; reverse dark brown, lobate.

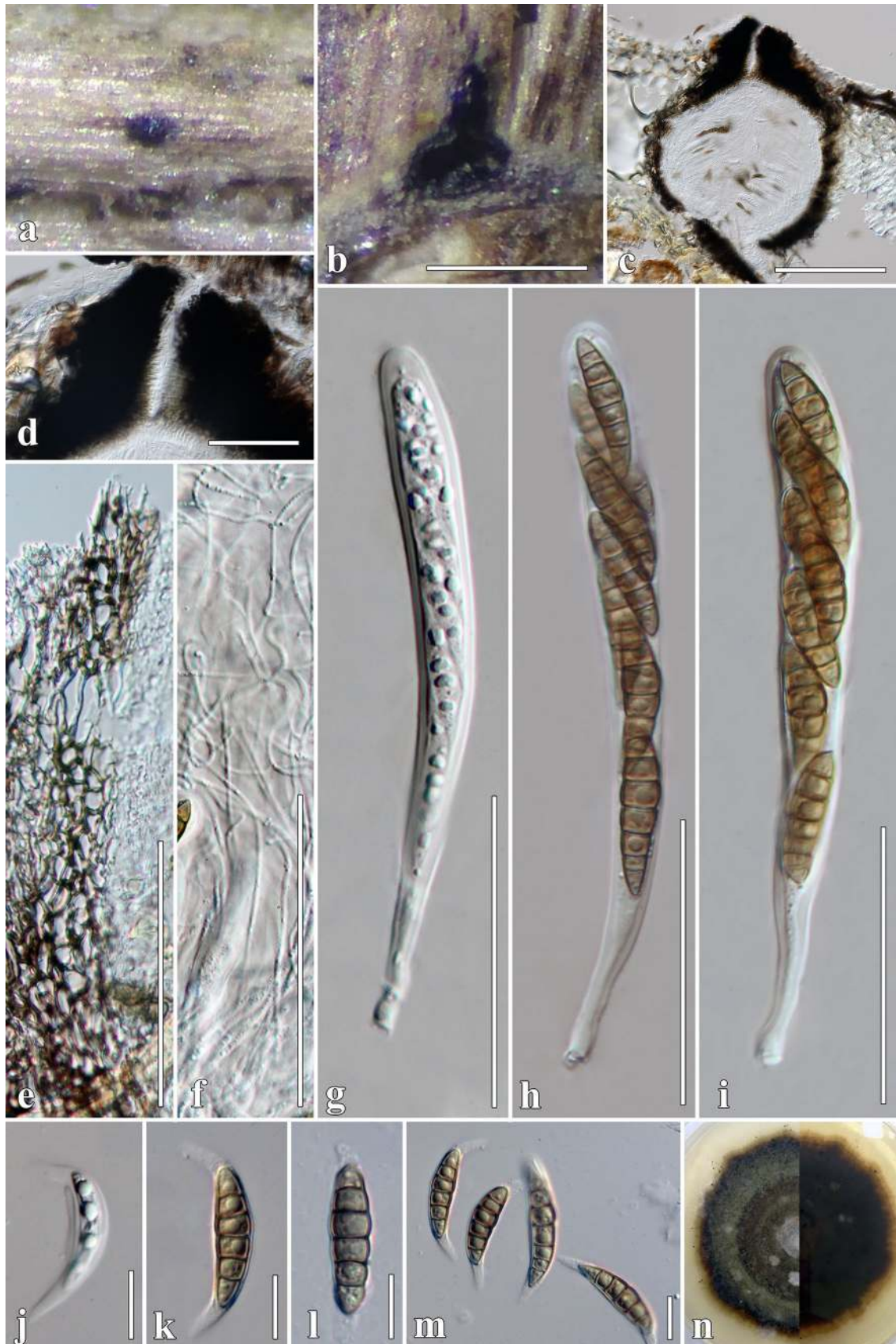
**Material examined:** Thailand, Chiang Rai Province, on dead branches of *Clematis fulvicoma*, 20 April 2017, C. Phukhamsakda, CMTH25 (MFLU 17–1487); living culture, MFLUCC 17–2079.

**Hosts:** *Chromolaena odorata*, *Clematis fulvicoma*—(Mapook et al. 2020; this study).

**Distribution:** Thailand—(Mapook et al. 2020; this study).

**GenBank accession numbers:** LSU: MT214568; SSU: MT226684; ITS: MT310613; *tef1*: MT394628; *rpb2*: MT394691.

**Notes:** Based on phylogenetic analyses (Fig. 38), MFLUCC 17–2079 clustered with *Nigrograna chromolaenae* (MFLUCC 17–1437). Mapook et al. (2020) introduced *N. chromolaenae* from a stem of *Chromolaena odorata* collected in Thailand. Characters of the ex-type strain such as ascomata, asci and ascospores were reported in Mapook



**Fig. 33** *Sigarispora clematidis* (MFLU 20–0417, holotype). **a** Appearance of ascoma on host surface. **b** Close up of ascoma. **c** Section of ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Pseudoparaphyses. **g–i** Asci. **j–m** Ascospores. **n** Culture characteristics on MEA. Scale bars: **b** = 500  $\mu\text{m}$ , **c** = 200  $\mu\text{m}$ , **d–i** = 50  $\mu\text{m}$ , **j–m** = 10  $\mu\text{m}$

et al. (2020) and were similar to our collection (Fig. 39). A comparison of the ITS and *tef1* sequence data revealed no significant difference between our new collection and the ex-type strain. Therefore, we introduce *Nigrograna chromolaenae* on *Clematis* as a new host record.

***Nigrograna obliqua*** Jaklitsch & H. Voglmayr, Studies in Mycology 85: 59 (2016), **new host record**

*Index Fungorum number*: IF817783; *Facesoffungi number*: FoF 07298, Fig. 40.

*Saprobic* on dead stems of *Clematis vitalba*. **Sexual morph**: *Ascomata* 193–450  $\times$  188–294  $\mu\text{m}$  ( $\bar{x}$  = 266  $\times$  258  $\mu\text{m}$ ,  $n$  = 5), on the surface of the host, solitary, sometimes gregarious, immersed, only black shiny ostioles visible, subglobose to depressed, coriaceous, brown to reddish brown, smooth-walled, papillate. *Ostioles* 80–165  $\times$  74–96  $\mu\text{m}$ , central, dark brown, filled with periphyses. *Peridium* 11–27  $\mu\text{m}$  wide, with brown hyphae projecting from the outer layer, composed of 8–10 layers of dark brown cells of *textura angularis*, heavily pigmented at outer layer, the inner layer hyaline and thin. *Hamathecium* composed of numerous, 1–1.3  $\mu\text{m}$  ( $\bar{x}$  = 1.2  $\mu\text{m}$ ,  $n$  = 50), dense, filiform, branched, transverse septate, pseudoparaphyses. *Asci* 60–101  $\times$  10–16  $\mu\text{m}$  ( $\bar{x}$  = 86  $\times$  12  $\mu\text{m}$ ,  $n$  = 20), 8-spored, bitunicate, fissitunicate, clavate to broad cylindrical, with furcate pedicel, apically rounded, ocular chamber visible when immature. *Ascospores* 16–20  $\times$  5–8  $\mu\text{m}$  ( $\bar{x}$  = 18  $\times$  6  $\mu\text{m}$ ,  $n$  = 50), biseriate, overlapping, ellipsoid to broad fusiform, sometimes inequilateral, with rounded ends, initially hyaline, pale brownish to brown at the maturity, (1–)3-eusepta, constricted at septa, cell above median septum enlarged, the second cell slightly wider than others, with guttule in each cell, smooth-walled, without mucilaginous sheath. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA reaching 30 mm diam. after 4 weeks at 16 °C. Cultures from above, dark brown, covered with greyish orange mycelia on the surface, dense, irregular, umbonate, lobate, velvety, flat parchment-like stromatic sheets developed, reverse dark brown, fimbriae.

*Material examined*: Italy, Forlì-Cesena Province, Corniolo—Santa Sofia, dead aerial branch of *Clematis vitalba*, 17 February 2014, E. Camporesi, IT 1726 (MFLU 16–0190); living culture, MFLUCC 14–0945.

*Hosts*: *Clematis vitalba*, *Ribes uva-crispa*, *Salix caprea*, *Sambucus nigra*, *S. racemosa*—(Jaklitsch et al. 2016; this study).

*Distribution*: Austria, France, Italy, UK—(Jaklitsch et al. 2016; this study).

*GenBank accession numbers*: LSU: MT214569; ITS: MT310614.

*Notes*: Our new collection is morphologically similar and phylogenetically related to the type species of *Nigrograna obliqua* (strain MF2). MFLUCC 14–0945 is not very different from that of the type species (Fig. 40). Jaklitsch and Voglmayr (2016) introduced four strains of *N. obliqua* from various shrubs and trees. In the phylogenetic analyses MFLUCC 14–0945 formed a close relationship with the ex-type strain reported from *Salix caprea* (84% ML/0.99 BYPP). Interestingly, three strains of *N. obliqua* (BW4, KE and MRP) formed a separate clade from the type strain. However, the morphological data of *N. obliqua* strains BW4, KE and MRP were not available for comparison. We report *N. obliqua* on *Clematis vitalba* as a new host record (Fig. 40).

### **Occultibambusaceae** Dai & K.D. Hyde

Occultibambusaceae is typified by *Occultibambusa* D.Q. Dai & K.D. Hyde. The species in this family are generally associated with monocotyledon such as *Occultibambusa*, *Seriascoma*, and *Versicolorisporium*. Doilom et al. (2017) reported *Neooccultibambusa*, another species from *Tectona grandis* and *Brunneofusispora* from dead wood in China. Occultibambusaceae is characterized by immersed, solitary to gregarious ascomata with ostioles, broadly cylindrical to clavate asci with broad-fusiform, hyaline to dark brown usually septate ascospores. Pycnidia are reported in the asexual morph of this family (Hatakeyama et al. 2008; Dai et al. 2017).

### ***Brunneofusispora*** Huang & K.D. Hyde

*Brunneofusispora sinensis* is the type species. The genus was recorded from dead wood near a river and is characterized by immersed, uniloculate ascomata, cylindrical to clavate and short pedicellate asci, and hyaline to brown, broadly fusiform ascospores (Phookamsak et al. 2019). *Brunneofusispora clematidis* is reported as a second species of *Brunneofusispora* on *Clematis* and is illustrated. An updated phylogenetic analysis of a concatenated dataset of LSU, *tef1*, ITS and SSU sequence data is presented in Fig. 41.

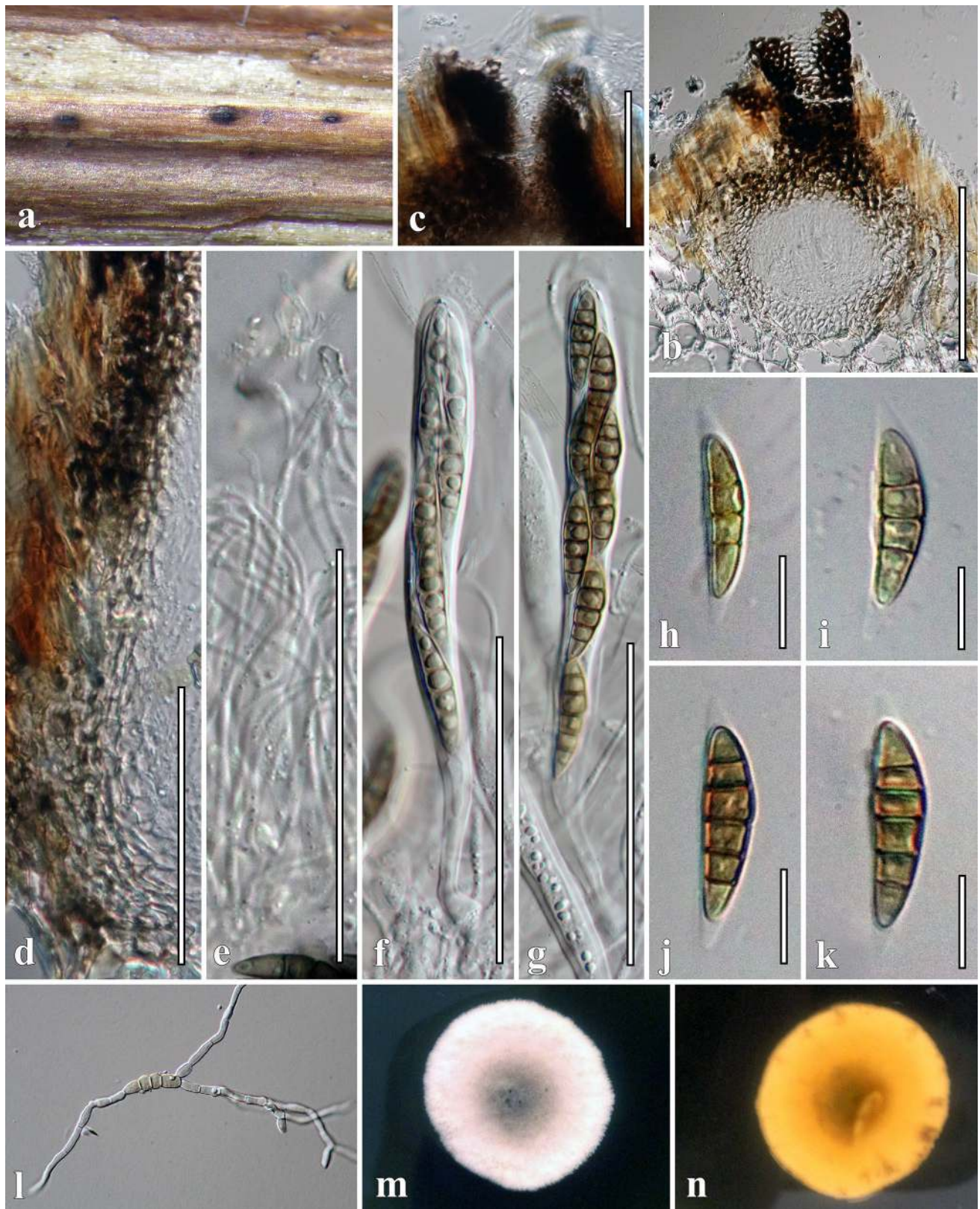
### ***Brunneofusispora clematidis*** Phukhams. & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557194; *Facesoffungi number*: FoF 07299, Fig. 42.

*Etymology*: Name refers to the host *Clematis*.

*Holotype*: MFLU 17–1478.

*Saprobic* on dead stems of *Clematis subumbellata*. **Sexual morph**: *Ascomata* 160–210  $\times$  165–186  $\mu\text{m}$  ( $\bar{x}$



**Fig. 34** *Sigarispora montanae* (MFLU 20–0418, holotype). **a** Appearance of ascomata on host surface. **b** Vertical section of ascoma. **c** Ostiolar canal. **d** Section of peridium. **e** Pseudoparaphy-

ses. **f, g** Asci. **h–k** Ascospores. **l** Germinated ascospore. **m, n** Culture characteristics on MEA. Scale bars: **b** = 100  $\mu$ m, **c–g** = 50  $\mu$ m, **h–k** = 10  $\mu$ m

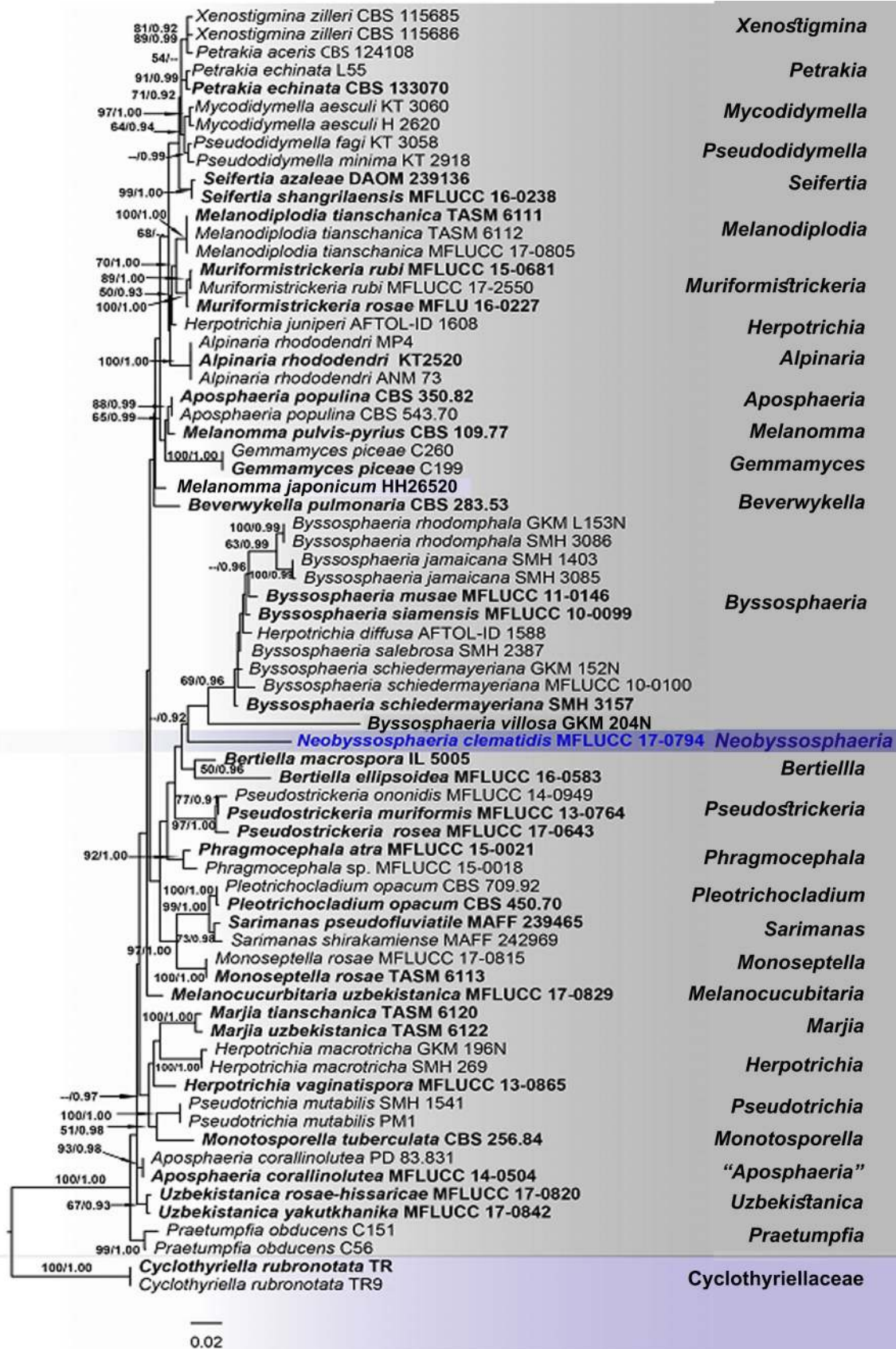


**Table 3** Morphological comparison of known *Sigarispora* species

Species	Ascomata ( $\mu\text{m}$ )	Asci ( $\mu\text{m}$ )	Ascospores			Host	References
			Size ( $\mu\text{m}$ )	Septa	Appendages ( $\mu\text{m}$ )		
<i>Sigarispora arundinis</i>	320–416 × 250–310	94–112.5 × 12.5–14.5	22–32.5 × 6.3–7.9	5	Both ends	<i>Phragmites australis</i>	Thambugala et al. (2015)
<i>S. caryophyllacearum</i>	420–500 × 400–500	80–120 × 10–12	30–40 × 7–8.5	5–9	Not present	Caryophyllaceae	Wanasinghe et al. (2018)
<i>S. caudata</i>	145–210 × 210–305	86–112.5 × 10.5–13	23.5–34.5 × 5.5–7	(4)–5–(6)	Not present	<i>Dactylis glomerata</i>	Thambugala et al. (2015)
<i>S. caulium</i>	180–340 × 200–280	75–100 × 12–14	18–25 × 5–8	5	Both ends	Herbaceous <i>Rosa canina</i>	Thambugala et al. (2015)
<i>S. clematidicola</i>	<b>255–288 × 217–254</b>	<b>101–125 × 12–19</b>	<b>22–29 × 7–9</b>	<b>(3)–5</b>	<b>2–4</b>	<b><i>Clematis vitalba</i></b>	<b>This study</b>
<i>S. clematidis</i>	<b>416–493 × 337–386</b>	<b>96–145 × 10–18</b>	<b>22–30 × 6–9</b>	<b>5–6</b>	<b>5–10</b>	<b><i>Clematis vitalba</i></b>	<b>This study</b>
<i>S. coronillae</i>	350–400 × 390–450	120–140 × 14–17	20–26 × 8–10	4–5 trans- versely eusepta, 2–4 vertical septa	Not present	<i>Coronilla emerus</i>	Thambugala et al. (2015)
<i>S. junci</i>	200–300 × 200–250	120–140 × 14–18	26–33 × 6.5–8	5–7	5–8 $\mu\text{m}$ of mucilaginous sheath	<i>Juncus</i> sp.	Wanasinghe et al. (2018)
<i>S. medicaginicola</i>	400–500 × 300–350	80–120 × 10–15	24–28 × 6.5–7	5–6	8–10	<i>Medicago falcata</i>	Wanasinghe et al. (2018)
<i>S. montanae</i>	<b>180–230 × 140–200</b>	<b>105–130 × 10–14</b>	<b>20–26 × 5–7</b>	<b>3(–5)</b>	<b>3–5</b>	<b><i>Clematis montana</i></b>	<b>This study</b>
<i>S. muriformis</i>	425–660 × 335–560	84–130 × 12–18	18–28 × 7–12	5–8 trans- versely eusepta, 2–3 longitudinal septa	Not present	<i>Helichrysum italicum</i>	Tibpromma et al. (2017)
<i>S. ononidis</i>	240 × 311	96–169 × 17–19	27–34 × 11–12	3–5	Not present	<i>Ononis spinosa</i>	Li et al. (2016)
<i>S. ravennica</i>	211–282 × 121–187	55–70 × 9–11	18–21 × 4–6	6	Not present, sometimes with terminal appendages	Poaceae	Thambugala et al. (2015)
<i>S. rosicola</i>	400–500 × 300–350	80–120 × 10–15	17–23 × 5–6	5	2–5	<i>Rosa</i> sp.	Wanasinghe et al. (2018)
<i>S. scrophulariae</i>	280–350 × 250–300	70–90 × 10–12	18–22 × 5–6	4–5	2–4	<i>Scrophularia donetzica</i>	Wanasinghe et al. (2018)
<i>S. thymi</i>	600–700 × 450–550	80–120 × 12–16	23–33 × 6–7	4–6	Not present	<i>Thymus marschallianus</i>	Wanasinghe et al. (2018)

= 185 × 176  $\mu\text{m}$ , n = 5), on the surface of the host, solitary, gregarious, uniloculate, semi-immersed, shiny, globose to depressed, coriaceous, dark brown to black, rough-walled, papillate, ostiolate. *Ostioles* 96–105 × 86–110  $\mu\text{m}$ , central, dark brown, filled with short periphyses. *Peridium* 8–17(–28)  $\mu\text{m}$  wide, multilayered, composed of 4–5 layers of dark brown cells of *textura prismatica*, heavily pigmented

at outer layer, the inner layer hyaline and thin. *Hamathecium* composed of medium dense, 1.7–4.5  $\mu\text{m}$  wide ( $\bar{x}$  = 2.7  $\mu\text{m}$ , n = 50), filiform, branched, septate, cellular pseudoparaphyses. *Asci* 45–117 × 12–24  $\mu\text{m}$  ( $\bar{x}$  = 73 × 17  $\mu\text{m}$ , n = 20), 8-spored, bitunicate, fissionate, clavate, with furcate pedicelate, apically rounded, ocular chamber visible when immature. *Ascospores* 17–35 × 5–10  $\mu\text{m}$  ( $\bar{x}$  = 27 × 9  $\mu\text{m}$ ,



Melanommataceae

0.02

**Fig. 35** The best scoring RAxML tree with a final likelihood value of  $-9510.121854$  based on combined LSU, SSU and ITS sequence data for Melanommataceae. The tree is rooted with members of the Cyclothyriellaceae. Seventy-one strains were included in the combined sequence analyses which comprised 2564 characters (921 characters for LSU, 1061 characters for SSU, 582 characters for ITS, including gap regions). The matrix had 602 distinct alignment patterns, with 31.43% of undetermined characters and gaps. Estimated base frequencies were as follows; A=0.254800, C=0.214111, G=0.278600, T=0.252489; substitution rates AC=2.132797, AG=2.875535, AT=1.394124, CG=0.797579, CT=9.867341, GT=1.000000; gamma distribution shape parameter  $\alpha=0.481484$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study, is indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. The supported values from all analyses are  $BS \geq 70\%$ /BYPP  $\geq 0.95$

$n=50$ ), biseriate, broad fusiform to ellipsoid, sometimes inequilateral, ends acute, initially hyaline, pale brown at maturity, 1-euseptate, constricted at septum, with guttule in each cell, smooth-walled with mucilaginous sheath. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Cultures from above, black, with greyish orange aerial mycelia on the surface, dense, irregular, umbonate, lobate, velvety, flat, parchment-like; reverse black, fimbriae.

**Material examined:** Thailand, Chiang Rai Province, on dead branches of *Clematis subumbellata*, 20 April 2017, C. Phukhamsakda, CMTH 14 (MFLU 17–1478, **holotype**); ex-type living culture, MFLUCC 17–2070.

**Host:** *Clematis subumbellata*—(This study).

**Distribution:** Thailand—(This study).

**GenBank accession numbers:** LSU: MT214570; SSU: MT226685; ITS: MT310615; *tef1*: MT394629; *rpb2*: MT394692.

**Notes:** In the phylogenetic analysis, *Brunneofusispora clematidis* clustered with the type species *B. sinensis* with strong support (100% ML/1.00 BYPP, Fig. 41). *Brunneofusispora clematidis* can be distinguished from *B. sinensis* by its thinner peridium layer (8–17(–28) vs 20–45  $\mu\text{m}$ ) and longer ascospores (Phookamsak et al. 2019). Comparison of 607 nucleotides across the ITS region reveals 74 bp (12.2%) differences between *B. clematidis* and *B. sinensis*. Therefore, *B. clematidis* is introduced as a new species (Fig. 42).

### Paradictyoarthriniaceae Doilom, Liu & K.D. Hyde

Paradictyoarthriniaceae was established to accommodate a hyphomycetes genus *Paradictyoarthrinium* (*P. diffractum*, type species) in aquatic and terrestrial habitats (Matsushima 1996; Liu et al. 2015, 2018). Multilocus phylogenetic analyses revealed a rock-inhabiting hyphomycete, *Coniosporium olivaceum* Link ( $\equiv$  *Sirodesmium olivaceum* CBS 395.59) that

is related to Paradictyoarthriniaceae (Ruibal et al. 2009). Wanasinghe et al. (2018) introduced the first sexual morph, *Xenomassariosphaeria* to Paradictyoarthriniaceae. We introduce an additional species of *Xenomassariosphaeria* on *Clematis vitalba* in Italy (Figs. 2, 43).

### *Xenomassariosphaeria* Jayasiri, Wanas. & K.D. Hyde

*Xenomassariosphaeria* was introduced for massariosphaeria-like species that formed a close relationship within Paradictyoarthriniaceae (Tanaka and Harada 2004; Wanasinghe et al. 2018). The genus is characterized by semi-immersed to erumpent, short papillate ascomata, unequal peridium of thick pseudoparenchymatous cells, cylindrical to cylindrical-clavate, subsessile to short pedicellate asci, with broad fusiform, hyaline to brown, asymmetric, multiseptate ascospores (Wanasinghe et al. 2018). Based on a multigene analysis of LSU, SSU, ITS, *tef1* and *rpb2* sequence data (Fig. 2), *Xenomassariosphaeria clematidis* formed a clade with the type species *X. rosae* with strong support (100% ML/1.00 BYPP, Fig. 2).

### *Xenomassariosphaeria clematidis* Wanas., Phukhams., Camporesi & K.D. Hyde, sp. nov.

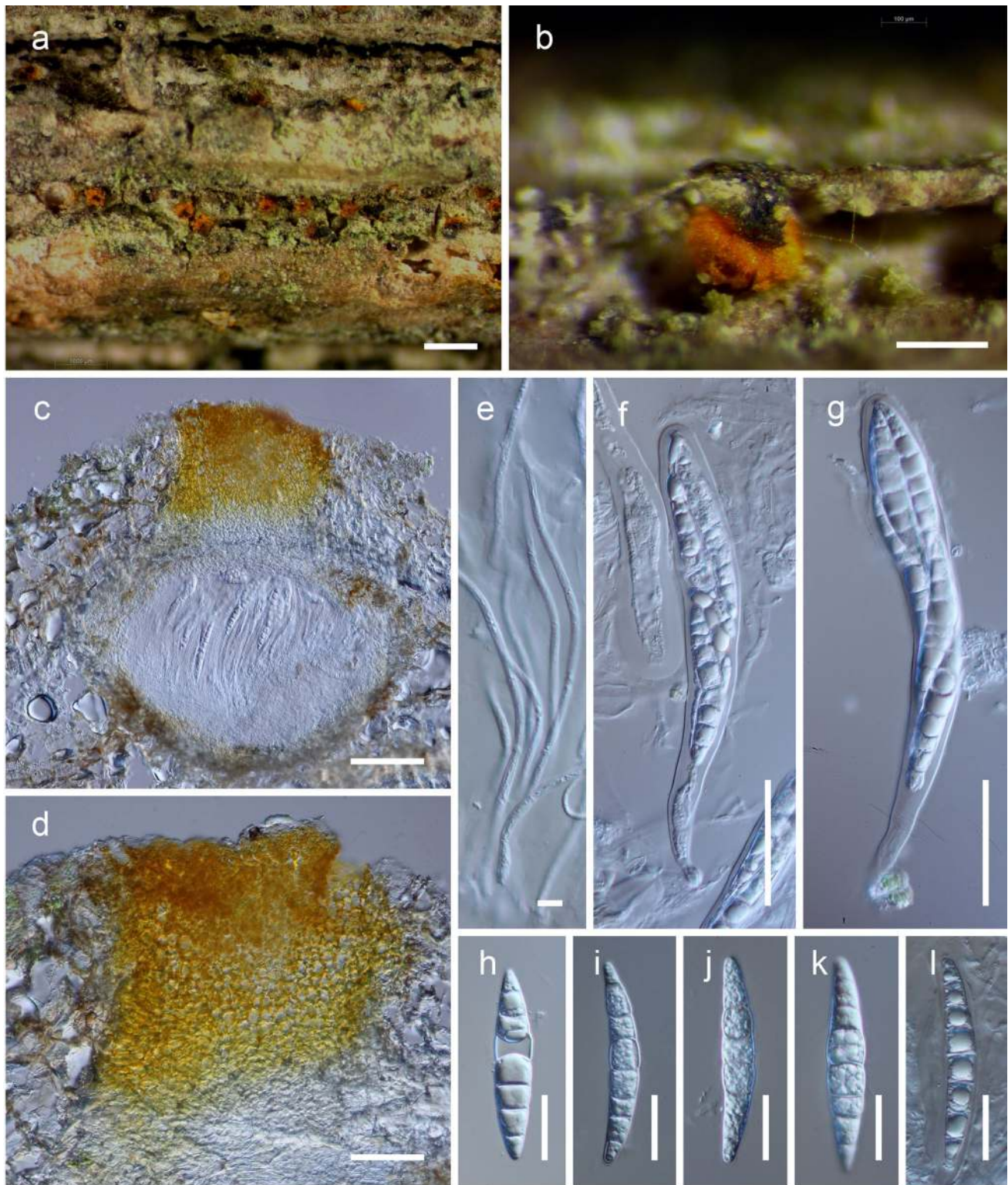
**Index Fungorum number:** IF557112; **Facesoffungi number:** FoF 07350, Fig. 43.

**Etymology:** Epithet reflects the host *Clematis*.

**Holotype:** MFLU 16–0119.

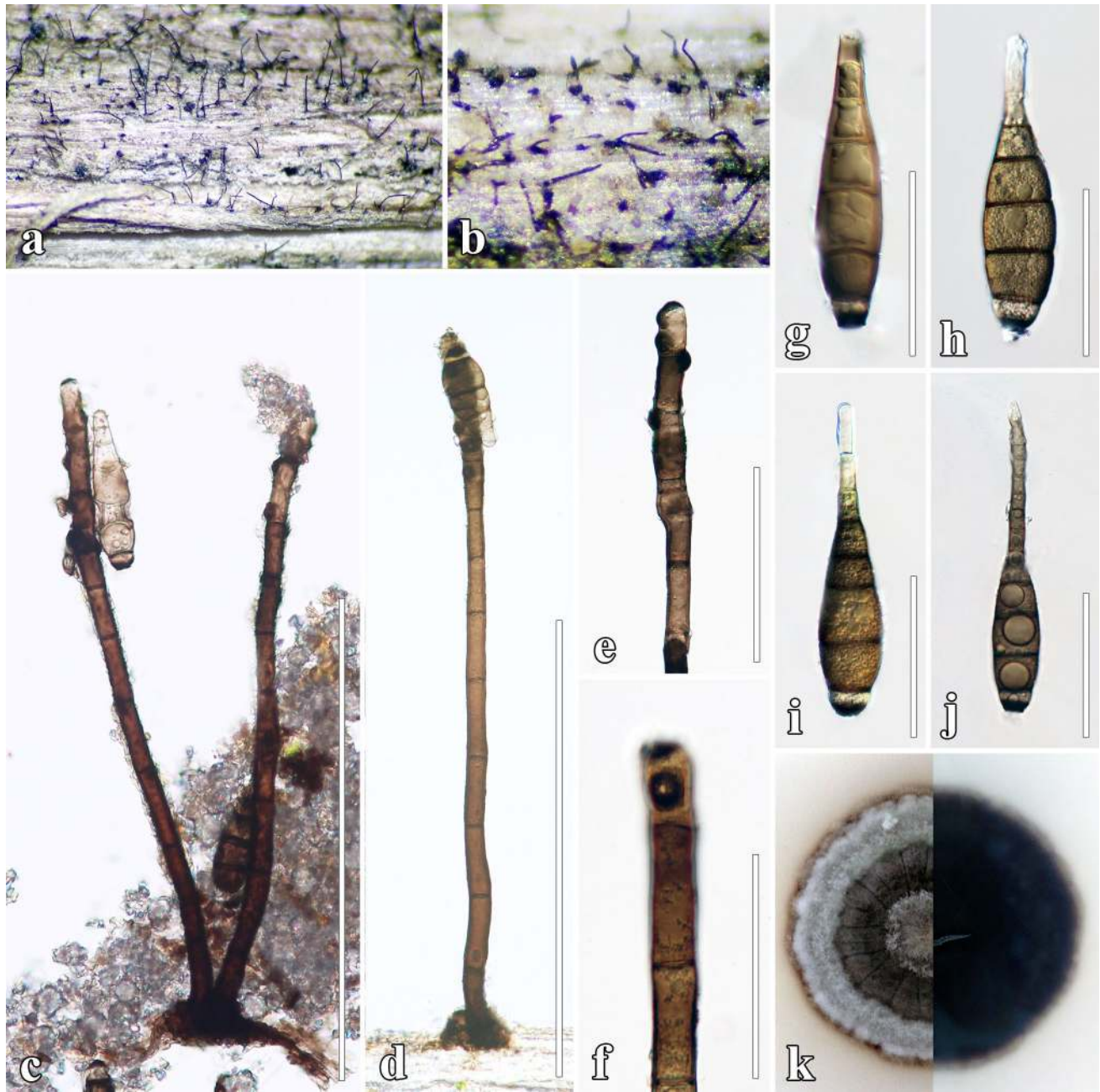
**Saprobic** on dead stems of *Clematis vitalba*. **Sexual morph:** *Ascomata* 273–283  $\times$  184–208  $\mu\text{m}$  ( $\bar{x}=275 \times 190 \mu\text{m}$ ,  $n=5$ ), solitary, scattered, immersed, with only black spot visible on the host surface, globose, coriaceous, dark brown to black, rough-walled, ostiolate. *Ostioles* centrally located, short papillate, with periphysoids. *Peridium* 12–37  $\mu\text{m}$  wide, composed of 5(–7 at apex) layers of dark brown outer layers of *textura angularis*, inner layer composed of hyaline gelatinous cells. *Hamathecium* composed of numerous, 2.3–3.5  $\mu\text{m}$  wide ( $\bar{x}=2.7 \mu\text{m}$ ,  $n=50$ ), dense, filiform, septate, branched, cellular pseudoparaphyses. *Asci* 91–120  $\times$  20–28  $\mu\text{m}$  ( $\bar{x}=107 \times 25 \mu\text{m}$ ,  $n=20$ ), 8-spored, bitunicate, fissitunicate, clavate to cylindrical-clavate, with furcate pedicel, with ocular chamber visible when immature. *Ascospores* 27–33  $\times$  7–11  $\mu\text{m}$  ( $\bar{x}=30 \times 9 \mu\text{m}$ ,  $n=50$ ), biseriate, naviculate, narrow towards the lower ends, initially hyaline, becoming yellowish to brown at maturity, 7–8 transversely eusepta, slightly constricted at the septa, third cells from apex usually enlarged, smooth-walled, guttulate and indentations present, without mucilaginous sheath. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 20 mm diam. after 4 weeks at 18 °C. Cultures from above, brown radiating yellowish towards the edge, dense, circular, flat, dull, fimbriate, radially furrowed, and slightly covered with



**Fig. 36** *Neobyssosphaeria clematidis* (MFLU 17–0614, holotype). **a, b** Appearance of ascomata on *Clematis vitalba*. **c** Vertical section through ascoma. **d** Close up of ostiolar canal. **e** Cellular pseudopara-

physes. **f, g** Asci. **h–l** Ascospores. Scale bars: **a** = 1 cm, **b** = 200  $\mu$ m, **c** = 100  $\mu$ m, **d, f–g** = 50  $\mu$ m, **e** = 5  $\mu$ m, **h–l** = 20  $\mu$ m



**Fig. 37** *Pseudohelminthosporium clematidis* (MFLU 17–1494, **holotype**). **a, b** Conidiophores on natural substrate (*Clematis sikkimensis*). **c, d** Mononematous conidiophores. **e, f** Conidiogenous cells and

conidia. **g–j** Conidia (Black basal conidia). **k** Culture characteristics on MEA. Scale bars: **c, d** = 500  $\mu$ m, **e** = 100  $\mu$ m, **f–j** = 50  $\mu$ m

white aerial mycelium; reverse black with radiating brown mycelium.

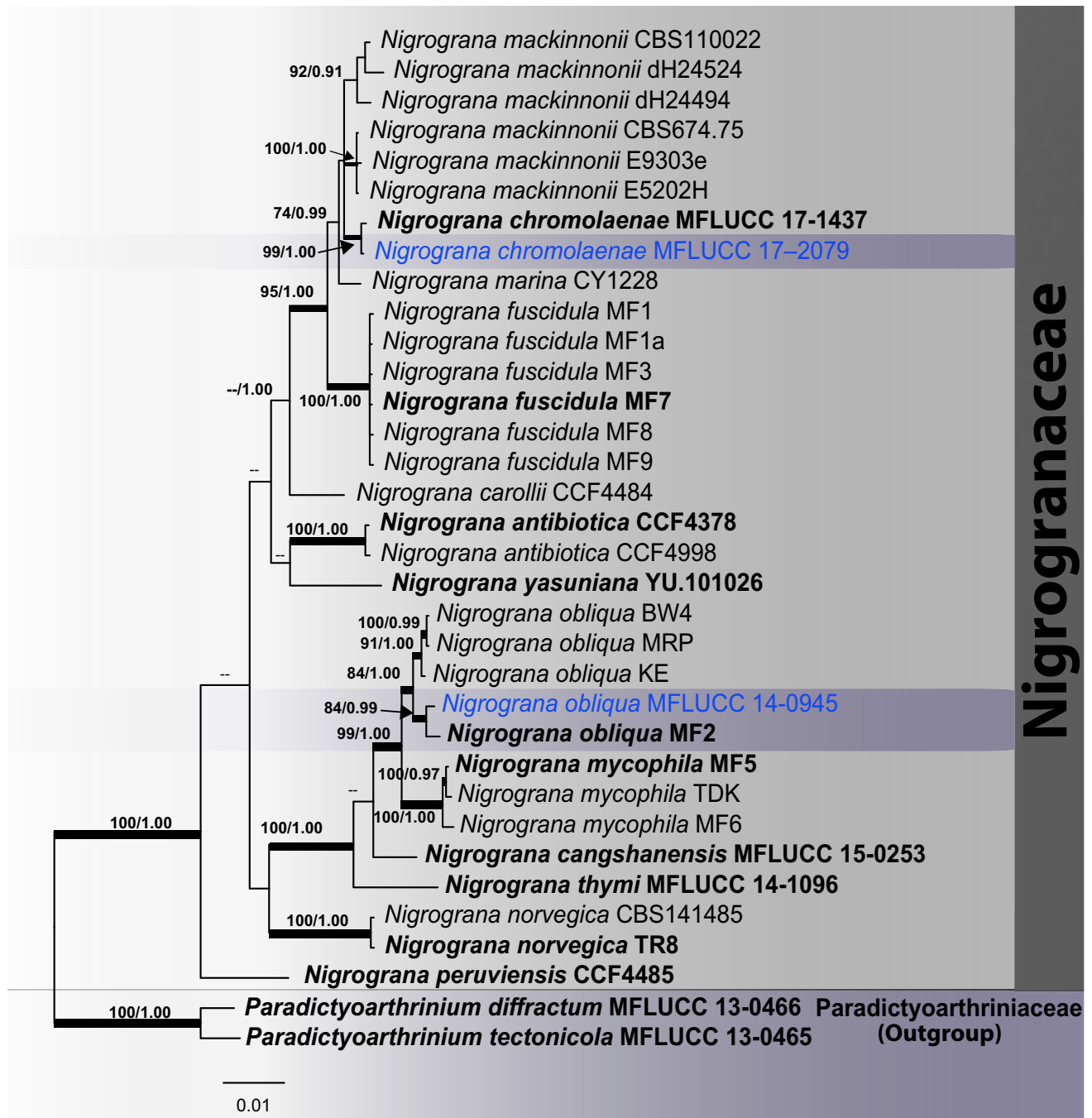
**Material examined:** Italy, Forlì-Cesena Province, Poggio alla Lastra—Bagno di Romagna, dead aerial stems of *Clematis vitalba*, 19 January 2013, E. Camporesi, IT1019 (MFLU 16–0119, **holotype**); ex-type living culture, MFLUCC 14–0923.

**Hosts:** *Clematis vitalba*—(This study).

**Distribution:** Italy—(This study).

**GenBank accession numbers:** LSU: MT214571; ITS: MT310616; *tef1*: MT394630.

**Notes:** In the phylogenetic analyses (Fig. 2), strain MFLUCC 14–0923 formed a close relationship with the type species *Xenomassariosphaeria rosae* (MFLUCC 15–0179) with strong support (100% ML/1.00 BYPP). *Xenomassariosphaeria clematidis* shares common features with *Xenomassariosphaeria* in having immersed ascomata with thick papilla, cylindrical or clavate asci, and brown, multiseptate



**Fig. 38** The Bayesian 50% majority-rule consensus phylogram based on combined LSU, ITS, SSU and *tef1* sequence data for Nigrogranaeae. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted with members of Paradictyoarthriniaaceae. Thirty-four strains were included in the combined gene sequence analyses which comprised 3202 characters (851 characters for LSU, 474 characters for ITS, 1027 characters for SSU, 850 characters for *tef1*, including gap regions). The tree from the maximum likelihood analysis had similar topology to the Bayesian analyses. The best scoring RAxML tree had a final likelihood value of  $-8012.999545$ . The matrix had 487 distinct alignment patterns with 20.65% of undetermined characters

and gaps. Estimated base frequencies were as follows; A=0.244997, C=0.242630, G=0.268950, T=0.243423; substitution rates AC=1.469986, AG=2.972149, AT=1.574187, CG=0.656303, CT=11.538665, GT=1.000000; gamma distribution shape parameter  $\alpha=0.62491$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study are indicated in blue. Bootstrap values (BS) greater than 70% BS (ML, left) and Bayesian posterior probabilities (BYPP) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 70% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95)

ascospores (Tanaka and Harada 2004; Wanasinghe et al. 2018). *Xenomassariosphaeria clematidis* is distinguished by its unique obovoid ascospores that are narrow towards the lower end, brown and multi-septate (Fig. 43).

In a BLASTn search of GenBank, the LSU sequence of *X. clematidis* (strain MFLUCC 14–0923) is 97.5% similar to *X. roumeguerei* (strain CBS 612.86, MH873692). The ITS region of *X. clematidis* (strain MFLUCC 14–0923) had 91.5% similarity with *X. roumeguerei* (strain CBS 612.86, MH862004). Thus, we introduce a novel species, *X. clematidis* based on morphological and phylogenetic evidence.

### Periconiaceae Nann.

Tanaka et al. (2015) verified Periconiaceae belonged in *Pleosporales* based on modern fungal systematics. Although sequence data is not available for the type species of *Periconia* (*P. lichenoides*), the morphological characters of extant *Periconia* species correspond to *P. lichenoides* (Mason and Ellis 1953; Tanaka et al. 2015). Periconiaceae is characterized by scattered, immersed to erumpent ascomata, pseudoparaphyses, oblong to cylindrical asci, and broadly fusiform, 1-septate, hyaline ascospores. Asexual characters include synnemata or noosia-like, macronematous, mononematous conidiomata, monoblastic to polyblastic, discrete and branched conidiogenous cells, and globose to ellipsoidal, aseptate, catenate, brown conidia (Tanaka et al. 2015).

### *Periconia* Tode

Over 190 species are listed under *Periconia* (Index Fungorum 2020). Based on phylogenetic analyses of a concatenated dataset of LSU, ITS, and *tef1* sequence data coupled with morphological characters, *P. verrucosa* is described as a new species from *Clematis viticella* (Figs. 44, 45).

### *Periconia verrucosa* Phukhams, Ertz, Gerstmans & K.D. Hyde, sp. nov.

*Index Fungorum number*: IF557143; *Facesoffungi number*: FoF 07296, Fig. 45.

*Etymology*: The epithet “*verrucosa*” refers to the surface of conidia being verrucose.

*Holotype*: MFLU 17–1516.

*Saprobic* on dead stems of *Clematis viticella*. **Sexual morph**: Undetermined. **Asexual morph**: Colonies effuse on the natural substrate, scattered, hairy, dark brown. *Mycelium* partly superficial, semi-immersed, branched, composed of pale brown, septate hyphae. *Conidiophores* 170–296 × 10–12 μm ( $\bar{x}$  = 225 × 11 μm, n = 20), macronematous, mononematous, solitary, gregarious, scattered, erect, stipes straight or slightly flexuous, with 3–4 short branches at the apex, cylindrical, smooth, dark brown, 2–4-septate, smooth or verruculose. *Conidiogenous cells* 11–26 × 6–14 μm ( $\bar{x}$  = 16 × 7 μm, n = 20), monoblastic or polyblastic, acropetally proliferating, integrated, terminal,

oblong, retrogressive, pale brown. *Conidia* 7–15 μm ( $\bar{x}$  = 12 μm, n = 50), in branched chains, acrogenous, globose, aseptate, thick-walled, hyaline when immature, dark brown to reddish brown at maturity, verrucose, bud scars or disjunctors present at the site of attachment, easily separating.

*Culture characters*: Colonies on MEA reaching 30 mm diam. after 2 weeks at 25 °C. Cultures from above, cream or white, mycelia medium dense, circular, umbonate, papillate, fluffy, slightly radiating outwardly; reverse: cream at the centre, radiating outwardly.

*Material examined*: Belgium, Flemish Brabant, Meise Botanic Garden, Bouchout Domain, on dead stems of *Clematis viticella*, 13 June 2017, D. Ertz & C. Gerstmans, BRCV4 (MFLU 17–1516, **holotype**); ex-type living culture, MFLUCC 17–2158.

*Hosts*: *Artemisia* sp., *Sasa kurilensis*, *Clematis viticella*—(Tanaka et al. 2015; this study).

*Distribution*: Belgium, Japan—(Tanaka et al. 2015; this study).

*GenBank accession numbers*: LSU: MT214572; SSU: MT226686; ITS: MT310617; *tef1*: MT394631.

*Notes*: Phylogenetic analysis included LSU, ITS and *tef1* sequence data with related sequences retrieved from GenBank (Fig. 44). *Periconia verrucosa* (MFLUCC 17–2158) formed a strongly supported clade (96% ML/0.99 BYPP) with three unnamed *Periconia* strains (KT 1820A, KT 1825 and S-900, Tanaka et al. 2015). These strains do not have morphological characters for comparison. A comparison of the ITS nucleotide bases shows that *P. verrucosa* (MFLUCC 17–2158) has one nucleotide difference with strain S-900 and one nucleotide difference with KT 1820A and KT 1825 in *tef1* region. This is regarded as not significant (Jeewon and Hyde 2016). We introduce *P. verrucosa* as a new species to accommodate this clade of *Periconia* (Fig. 45).

### Phaeoseptaceae Boonmee, Thambug. & K.D. Hyde

Phaeoseptaceae was established for lignicolous fungal lineages on wood. Five genera are included in the family: *Decaisnella*, *Lignosphaeria*, *Phaeoseptum* (generic type), *Pleopunctum* and putative strains of *Thyridaria macrostomoides* (Abdel-Wahab and Jones 2003; Zhang et al. 2012; Ariyawansa et al. 2015a; Thambugala et al. 2015; Hyde et al. 2018a; Liu et al. 2019; Phukhamsakda et al. 2019). Phaeoseptaceae members have ascomata immersed in host tissues, short papillate, anastomosed pseudoparaphyses, cylindrical-clavate, long pedicellate asci and broadly fusoid, single or multi-transverse septa, and hyaline to brown ascospores (Hyde et al. 2018a). Phaeoseptaceae had pycnidial or sporodochial characters as asexual morph (Liu et al. 2019). We describe a novel species of *Pleopunctum* recorded from *Clematis* in Thailand, based on a multi-gene analysis (Fig. 46) coupled with comparable morphology (Fig. 47).





**Fig. 39** *Nigrograna chromolaenae* (MFLU 17–1487). **a, b** Appearance of ascomata on *Clematis fulvicoma*. **c** Vertical section through ascoma. **d** Section of peridium. **e** Pseudoparaphyses. **f–h** Asci. **i–n** Ascospores. **o** Germinated ascospore. **p, q** Culture characteristics on MEA. Scale bars: **b** = 200  $\mu$ m, **c** = 100  $\mu$ m, **d–h** = 20  $\mu$ m, **i–o** = 10  $\mu$ m

### *Pleopunctum* Liu, K.D. Hyde & J.K. Liu

Liu et al. (2019) introduced the first asexual morph of *Pleopunctum* (typified by *P. ellipsoideum*) in Phaeosphaeraceae from decaying wood collected in China. The genus is characterized by sporodochial conidiomata, macronematous, mononematous conidiophores, monoblastic conidiogenous cells and muriform conidia that have a globose basal cell. The multilocus phylogeny (LSU, SSU, ITS, *tef1* and *rpb2*) revealed a new species of *Pleopunctum* from *Clematis* based on the morphology and well supported values from multi-gene phylogeny (Fig. 46).

### *Pleopunctum clematidis* Phukhams., Bhat & K.D. Hyde, sp. nov.

*Index Fungorum number*: IF557139; *Facesoffungi number*: FoF 07301, Fig. 47.

*Etymology*: The epithet “*clematidis*” refers to the host plant, *Clematis*.

*Holotype*: MFLU 17–1499.

*Saprobic* on dead branch of *Clematis sikkimensis* **Sexual morph**: Undetermined. **Asexual morph**: *Colonies* on natural substrate forming sporodochial conidiomata, 168–278  $\mu$ m wide, superficial, scattered, gregarious, oval, brown, velvety, glistening, orbicular, conidia readily liberated when agitated. *Mycelium* immersed, septate, smooth-walled, thin-walled, yellowish brown to brown hyphae, 3.5–4.5  $\mu$ m wide, subicular hyphae short, medium packed. *Conidiophores* 6.5–15.5  $\times$  2–5  $\mu$ m, micronematous, mononematous, cylindrical or truncate, erect, smooth or finely verruculose, aseptate, unbranched, often reduced to conidiogenous cells, initially hyaline, brown at maturity. *Conidiogenous cells* 3–8  $\times$  4–9  $\mu$ m, holoblastic, monoblastic, integrated, terminal, determinate, cylindrical or slightly truncate, subspherical or ampulliform, hyaline. *Conidia dimorphic*, solitary, smooth-walled; *lenticular conidia* 16–33  $\times$  15–23  $\mu$ m ( $\bar{x}$  = 25  $\times$  20  $\mu$ m, n = 50), muriform, smooth, broadly ellipsoidal to oval in front view, yellowish brown to brown, slightly constricted at the median septa, inner view composed of one column of 5–7 cells, end cells subhyaline to pale brown, often carrying remnant of conidiogenous cell at base; *cylindrical conidia* 15–35  $\times$  6–11  $\mu$ m ( $\bar{x}$  = 20  $\times$  8  $\mu$ m, n = 30), straight or flexuous, septate, constricted at the septa, consisting of one column, 2–3-septate, doliiform, broad clavate, narrow towards apex, apex rounded, basal cells globose or subglobose, smooth, hyaline.

*Culture characters*: Colonies on MEA at room temperature (25 °C) reaching 7 cm after 2 weeks. Cultures from

above, circular with lobate margin, olive mycelium, white at the margin, white aerial mycelium, smooth at the surface and raised; reverse beige, not sporulating.

*Material examined*: Thailand, Chiang Rai Province, Doi Tung, on dried stem of *Clematis sikkimensis*, 2 May 2017, C. Phukhamsakda & M.V. de Bult, CMTHDT08 (MFLU 17–1499, **holotype**); ex-type living culture, MFLUCC 17–2091.

*Host*: *Clematis sikkimensis*—(This study).

*Distribution*: Thailand—(This study).

*GenBank accession numbers*: LSU: MT214573; ITS: MT310618; *tef1*: MT394632; *rpb2*: MT394693.

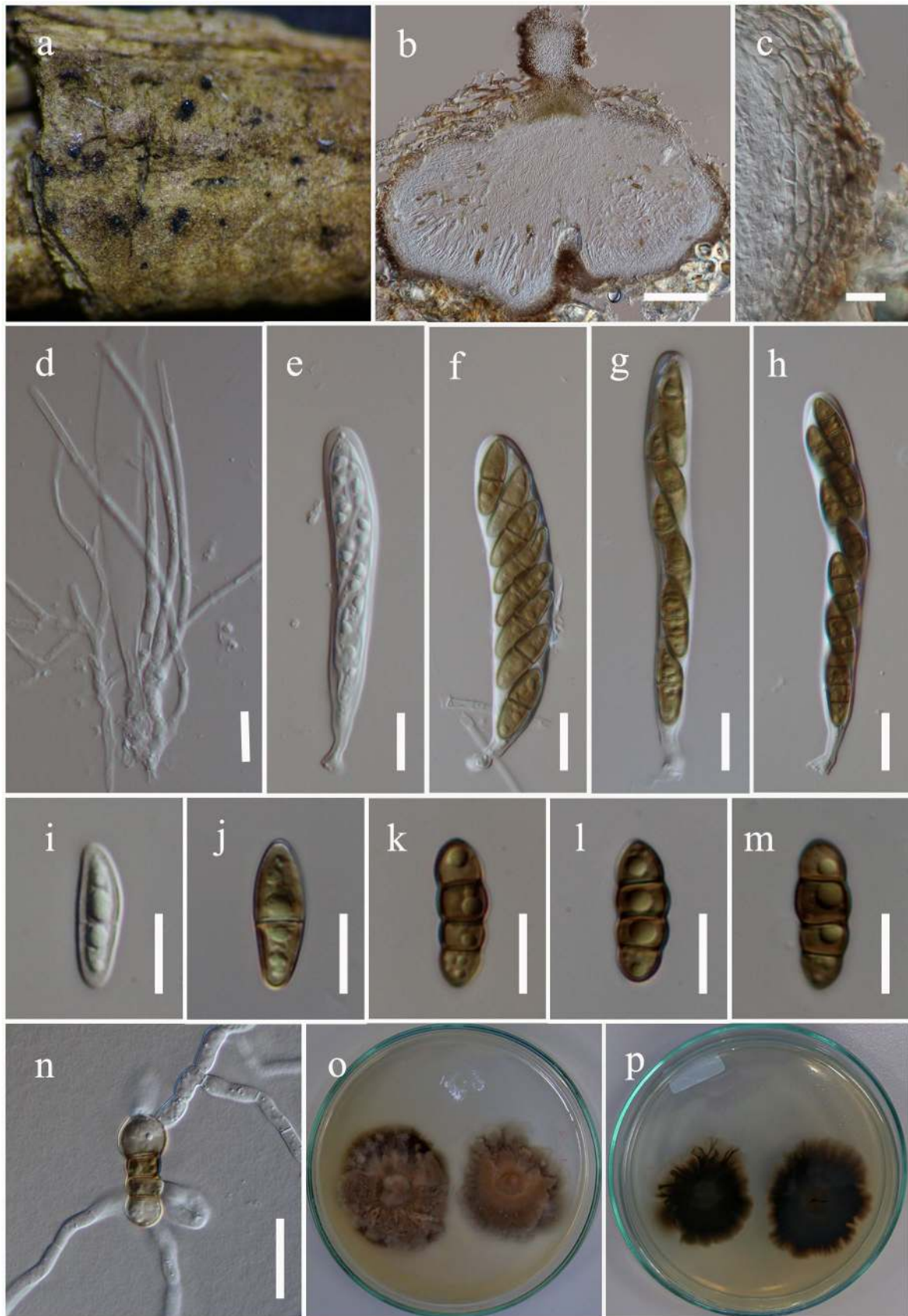
*Notes*: *Pleopunctum clematidis* (MFLUCC 17–2091) is similar to extant species of *Pleopunctum* in having sporodochial conidiomata, holoblastic, monoblastic conidiogenous cells and muriform lenticular conidia (Liu et al. 2019). The strain is distinguishable from other *Pleopunctum* species by its yellowish brown and smaller lenticular conidia. Additionally, *P. clematidis* has dimorphic conidia on the natural substrate. The dimorphic conidia type have been documented in Hermatomycetaceae, however, *P. clematidis* was without subicular hyphae (Tibpromma et al. 2018; Hyde et al. 2019a, Fig. 47). The multi-gene phylogeny of LSU, SSU, ITS, *tef1* and *rpb2* sequence data revealed that *P. clematidis* formed a sister lineage to *P. ellipsoideum* (MFLUCC 19–0390) and *P. pseudoellipsoideum* (MFLUCC 19–0391) with strong support (100% MP/100% ML/1.00 BYPP, Fig. 46). In a BLASTn search of GenBank, the closest match to MFLUCC 17–2091 is *Lignosphaeria fusispora* (strain MFLUCC 11–0377, KP888646) with 97.78% similarity in the LSU locus, while the closest match with the ITS sequence is 85.42% similar to KP899140.

### Phaeosphaeriaceae Barr

In this family, the taxa are mostly endophytes, pathogens or saprobes in various habitats (Quaedvlieg et al. 2013; Phookamsak et al. 2014). We follow the treatment of Hyde et al. (2020a) and report a novel genus and four new species based on molecular data coupled with morphological evidence.

### *Chaetosphaeronema* Moesz

*Chaetosphaeronema hispidulum* is the type species (Moesz 1915; Clements and Shear 1931). *Chaetosphaeronema* is characterized by immersed pycnidia with minute ostioles, enteroblastic, phialidic, determinate, discrete conidiogenous cells and cylindrical, hyaline, 1-septate conidia (Sutton 1980; Hyde et al. 2016). *Ophiobolus cirsii* (MFLUCC13–0218) is closely related to *Chaetosphaeronema* as suggested by Zhang et al. (2012). Five species are accepted under *Chaetosphaeronema* with two strains having sequence data (Phookamsak et al. 2019). Our phylogenetic analysis (Fig. 48) revealed two new species of



**Fig. 40** *Nigrograna obliqua* (MFLU 16–0190). **a** Appearance of ascomata on *Clematis vitalba*. **b** Vertical section through ascoma. **c** Section of peridium. **d** Pseudoparaphyses. **e–h** Asci. **i–m** Ascospores. **n** Germinated ascospore. **o, p** Culture characteristics on MEA. Scale bars: **b** = 100  $\mu\text{m}$ , **c–h** = 20  $\mu\text{m}$ , **i–n** = 10  $\mu\text{m}$

*Chaetosphaeronema* from *Clematis* species, with the sexual morph reported for the genus (Figs. 49, 50).

***Chaetosphaeronema clematidicola*** Phukhams., Ertz, Gerstmans & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557196; *Facesoffungi number*: FoF 07303, Fig. 49.

*Etymology*: The epithet reflects the host *Clematis*.

*Holotype*: MFLU 17–1508.

*Saprobic* on *Clematis patens*. **Sexual morph**: *Ascomata* 275–480  $\times$  260–405  $\mu\text{m}$  ( $\bar{x}$  = 394  $\times$  330  $\mu\text{m}$ ,  $n$  = 5), scattered or sometimes clustered, gregarious, semi-immersed, erumpent through host tissue, visible as raised, with only black shiny ostioles visible on the host surface, uniloculate, subglobose to compressed, dark brown to black, ostiolate, papillate. *Ostioles* 132–248  $\times$  97–120  $\mu\text{m}$  ( $\bar{x}$  = 174  $\times$  110  $\mu\text{m}$ ,  $n$  = 10), central, campanulate, composed of dark brown to black walled cells, rounded at the apex, with periphyses, ostioles filled with orange pigment at the pore. *Peridium* 13–35  $\mu\text{m}$  wide ( $\bar{x}$  = 23  $\mu\text{m}$ ,  $n$  = 20), thicker at apex, composed of several layers of pale brown to dark brown cells of a *textura angularis*, inner layer lined with subhyaline cells of *textura prismatica*. *Hamathecium* of numerous, 2–3.5  $\mu\text{m}$  wide ( $\bar{x}$  = 2.6  $\mu\text{m}$ ,  $n$  = 50), filamentous, cellular pseudoparaphyses, with distinct septa, embedded in mucilaginous matrix, anastomosing at the apex. *Asci* 139–208  $\times$  6–7  $\mu\text{m}$  ( $\bar{x}$  = 176  $\times$  6  $\mu\text{m}$ ,  $n$  = 20), 8-spored, bitunicate, broadly filiform to cylindrical, short, with furcate pedicel, apically rounded, ocular chamber visible when young. *Ascospores* 134–188  $\times$  1.5–7  $\mu\text{m}$  ( $\bar{x}$  = 162  $\times$  2.5  $\mu\text{m}$ ,  $n$  = 30), fasciculate, in parallel or spiral, scolecosporous, sometimes breaking at the septa, hyaline to yellowish brown, (18–)20(–23)-septate, not constricted at the septa, smooth-walled, with minute guttules in each cell. **Asexual morph**: Undetermined

*Culture characters*: Colonies growing on MEA reaching 40 mm after 4 weeks at 25 °C. Cultures from above, sparse, irregular, filamentous, flattened, smooth surface, with fimbriae edge, cream at the margin, white with pale yellowish at the centre; reverse colony cream at the margin and dark brown at the centre.

*Material examined*: Belgium, Flemish Brabant, Meise Botanic Garden, Bouchout Domain, dead stems of *Clematis patens* C. Morren & Decne., 13 June 2017, D. Ertz & C. Gerstmans, BRCP4 (MFLU 17–1508, **holotype**); ex-type living culture, MFLUCC 17–2151.

*Host*: *Clematis patens*—(This study).

*Distribution*: Belgium—(This study).

*GenBank accession numbers*: LSU: MT214574; SSU: MT226687; ITS: MT310619; *tefl*: MT394633; *rpb2*: MT394694.

*Notes*: *Chaetosphaeronema clematidicola* grouped with *Chaetosphaeronema* species (Fig. 48) with strong support (100% ML/1.00 BYPP). *Chaetosphaeronema clematidicola* (MFLUCC 17–2151) is similar to *Leptospora* (*L. rubella*) and *Pseudoophiobolus* based on their ascospore morphology, but MFLUCC 17–2151 differs in having orange colouration at the ostioles (Shoemaker 1976; Phookamsak et al. 2017). A morphological comparison of *Chaetosphaeronema clematidicola* with *Ophiobolus cirsii* (MFLUCC 13–0218) showed that it is similar in having fasciculate, cylindrical ascospores (Fig. 49). However, *Ophiobolus cirsii* has erumpent ascomata without pigment at the ostioles. Petrak (1944) mentioned that the ophiobolus-like characteristic is usually associated with *Chaetosphaeronema* species. Zhang et al. (2012) showed that *Chaetosphaeronema* is genetically related to Phaeosphaeriaceae. Phookamsak et al. (2014) subsequently confirmed the taxonomy placement and mentioned that ophiobolus-like species could be the sexual morph of *Chaetosphaeronema*. The asexual morph of *C. clematidicola* could not be obtained for morphological comparison.

In the phylogenetic analysis, *C. clematidicola* (MFLUCC 17–2151) grouped with *C. clematidis* (MFLUCC 17–2147), another species also on *Clematis*. A comparison of the ITS region (including of the 5.8S region) showed three nucleotide differences (588/599—98% with no gaps). A comparison of the *tefl* region revealed 12 base pair differences (842/874—96% with no gaps). Thus, we keep these isolates as distinct species.

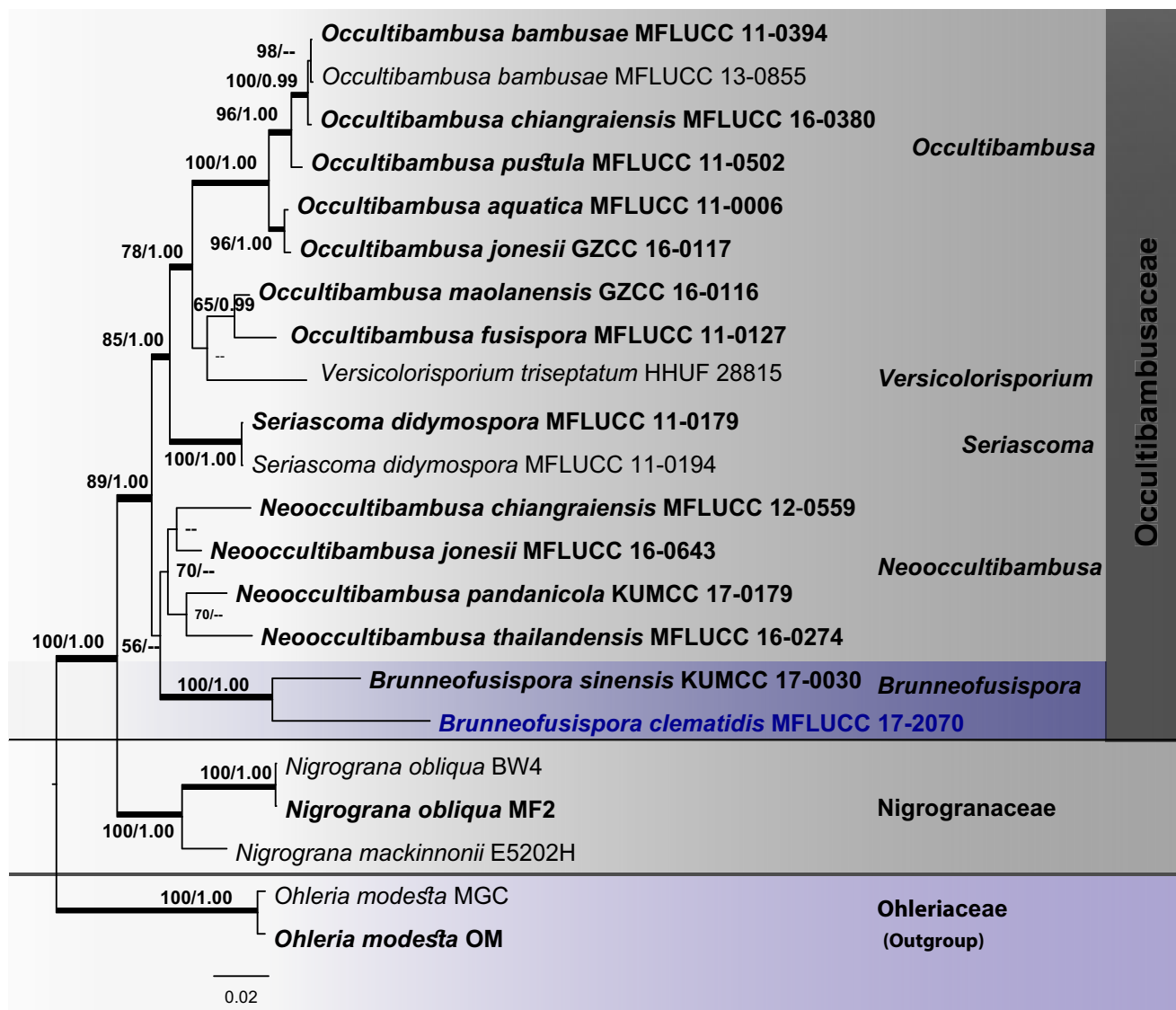
***Chaetosphaeronema clematidis*** Phukhams., Ertz, Gerstmans & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557195; *Facesoffungi number*: FoF 07302, Fig. 50

*Etymology*: The epithet name “*clematidis*” refers to the host substrate.

*Holotype*: MFLU 17–1504

*Saprobic* on dead branches of *Clematis orientalis*. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 175–348  $\times$  115–234  $\mu\text{m}$  ( $\bar{x}$  = 234  $\times$  159  $\mu\text{m}$ ,  $n$  = 5), pycnidial, solitary, sometimes aggregated, uniloculate, immersed, with only black shiny ostioles visible, globose to compressed, brown to dark brown, coriaceous, thick-walled, ostiolate, with minute papilla. *Ostioles* 41–139  $\times$  66–85  $\mu\text{m}$  ( $\bar{x}$  = 105  $\times$  73  $\mu\text{m}$ ,  $n$  = 5), central, oblong, lined with periphyses. *Conidiomatal wall* 12–32  $\mu\text{m}$  wide ( $\bar{x}$  = 20  $\mu\text{m}$ ,  $n$  = 20), comprises dark brown to light brown cells of *textura angularis*, lined with a hyaline layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 6–14  $\times$  1.5–3  $\mu\text{m}$  ( $\bar{x}$  = 10  $\times$  2.5  $\mu\text{m}$ ,  $n$  = 70), enteroblastic, phialidic, determinate, discrete, cylindrical to



**Fig. 41** Bayesian 50% majority-rule consensus phylogram based on combined LSU, *tef1*, ITS and SSU sequence data for Occultibambusaceae. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted with members of Ohleriaceae. Twenty-two strains were included in the combined sequence analyses which comprised 3213 characters (851 characters for LSU, 730 characters for *tef1*, 607 characters for ITS, 1025 characters for SSU, including gap regions). The tree from the maximum likelihood analysis had a similar topology to the Bayesian analyses. The best scoring RAxML tree had a final likelihood value of  $-8012.999545$ . The matrix had 763 distinct alignment patterns with 26.03% undetermined characters and gaps. Esti-

mated base frequencies were as follows; A=0.244085, C=0.246189, G=0.275394, T=0.234331; substitution rates AC=1.902831, AG=2.660713, AT=1.395112, CG=1.125830, CT=7.375307, GT=1.000000; gamma distribution shape parameter  $\alpha=0.601072$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study is indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95)

subcylindrical, hyaline, canal and collarette minute, smooth-walled, arising from the inner layers of conidioma. *Conidia* 10–15  $\times$  4–7  $\mu\text{m}$  ( $\bar{x}$  = 12  $\times$  5  $\mu\text{m}$ , n = 100), cylindrical, hyaline, slightly curved, with 1(–2) guttules in each cell, aseptate to 1 septum, smooth-walled.

**Culture characters:** Colonies on MEA reaching 20 mm diam. after 3 weeks at 25 °C. Cultures from above, cream

to pale yellow in the middle, with medium sparse mycelia, circular, umbonate, papillate fairly fluffy, covered with grey aerial mycelium, radially furrowed, dark brown pigment diffusing in the agar; reverse: dark brown at the centre, cream radiating outwardly.

**Material examined:** Belgium, Flemish Brabant, Meise Botanic Garden, Bouchout Domain, dead stems of

*Clematis orientalis* L., 13 June 2017, D. Ertz & C. Gerstmanns, BR01 (MFLU 17–1504, **holotype**); ex-type living culture, MFLUCC 17–2147.

*Host*: *Clematis orientalis*—(This study).

*Distribution*: Belgium—(This study).

*GenBank accession numbers*: LSU: MT214575; SSU: MT226688; ITS: MT310620; *tefl*: MT394634; *rpb2*: MT394695.

*Notes*: *Chaetosphaeronema clematidis* is similar to other *Chaetosphaeronema* species in having immersed pycnidia, unilocular, with enteroblastic, phialidic, cylindrical, a channel and collarete, minute conidiogenous cells and cylindrical, hyaline conidia (Sutton 1980; Hyde et al. 2016, Fig. 50). It is phylogenetically close to *C. achilleae* Huang & K.D. Hyde and *C. hispidulum* (Corda) Moesz, but differs by the lack of setae on top of the ostioles and by larger conidia. In the phylogenetic analysis, the strain formed a close relationship with *C. clematidicola* (see under *C. clematidicola* notes for more details).

***Dematiopleospora*** Wanas., Camporesi, E.B.G. Jones & K.D. Hyde

*Dematiopleospora* is typified by *D. mariae* Wanas., Camporesi, E.B.G. Jones & K.D. Hyde. The genus is characterized by brown setae filling the ostiolar canal, superficial ascomata and muriform ascospores with pale end cells. There are seven species in the genus (Wanasinghe et al. 2018, Fig. 51).

***Dermatiopleospora mariae*** Wanas., Camporesi, E.B.G. Jones & K.D. Hyde, in Wanasinghe, et al., Cryptog. Mycol. 35(2): 110 (2014), **new host record**

*Index Fungorum number*: IF550536; *Facesoffungi number*: FoF 07304, Fig. 52.

*Saprobic* on dead stems of *Clematis vitalba*. **Sexual morph**: *Ascomata* 131–210 × 156–300 μm ( $\bar{x}$  = 195 × 240 μm, n = 5), superficial, solitary, scattered, subglobose, flattened at base, dark brown to black, coriaceous, cupulate when dry, ostiolate. *Ostioles* 35–42 × 50–66 μm, papillate, brown, smooth, comprising short, light brown setae. *Peridium* 11–24 μm wide ( $\bar{x}$  = 19 μm, n = 20), thick, with 7–9 layers, outer layer heavily pigmented, comprising reddish to dark brown cells of *textura angularis*, inner layer composed of hyaline thin-walled cells of *textura angularis*. *Hamathecium* composed of numerous, 2–4 μm wide, filamentous, branched, septate, cellular pseudoparaphyses. *Asci* 100–125 × 13–19 μm ( $\bar{x}$  = 111 × 16 μm, n = 40), 8-spored, bitunicate, fissionate, cylindrical to cylindrical-clavate, pedicellate, thick-walled at the apex, with minute ocular chamber. *Ascospores* 20–26 × 7–10 μm ( $\bar{x}$  = 23 × 9 μm, n = 40), partially overlapping, 1–2-seriate, muriform, ellipsoidal to broad fusiform, slightly curved, ends acute, upper part wider than the lower part, 5–6-transversely septate, with 1–3 vertical septa, deeply constricted at the central septum, initially hyaline, becoming yellowish brown at maturity,

ends remaining lighter and without a mucilaginous sheath. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA reaching 20 mm diam. after 4 weeks at 18 °C. Culture from above, medium dense, circular, margin smooth, white, flat, surface rough; reverse cream radiating outwardly, dark brown in the middle.

*Material examined*: Italy, Forli-Cesena Province, Fiumana di Predappio dead and hanging branches of *Clematis vitalba*, 31 January 2013, E. Camporesi, IT 1037 (MFLU 16–0121).

*Hosts*: *Clematis vitalba*, *Ononis spinosa*—(Wanasinghe et al. 2014; this study).

*Distribution*: Italy—(Wanasinghe et al. 2014; this study).

*GenBank accession numbers*: LSU: MT214576; SSU: MT226689; ITS: MT310621; *tefl*: MT394635.

*Notes*: *Dermatiopleospora mariae* MFLU 16–0121 (Fig. 52) grouped with the type strain of *D. mariae* (MFLUCC 13–0612) with moderate statistical support of 60% ML (Fig. 51). *Dermatiopleospora mariae* (MFLUCC 13–0612) was originally described from *Ononis spinosa* in Italy. Morphological characters of our collection are similar to the type strain (Wanasinghe et al. 2014). The ITS sequence of our collection shows five base pair differences, however, the *tefl* data is identical to *D. mariae* (MFLUCC 13–0612).

***Leptospora*** Rabenh.

*Leptospora* is typified by *L. rubella* and clustered in Phaeosphaeriaceae (Hyde et al. 2016). Morphological characters of the holotype of *Leptospora* mentioned that the fungus stains host tissue red to purple and is red at the apical part of ostiolar canal (Rabenhorst 1857; Shoemaker 1976; Crous et al. 2006). Phylogenetic analyses of a combined LSU, SSU, ITS and *tefl* dataset revealed one new species and a new host record of *Leptospora* from *Clematis* species (Figs. 53, 54).

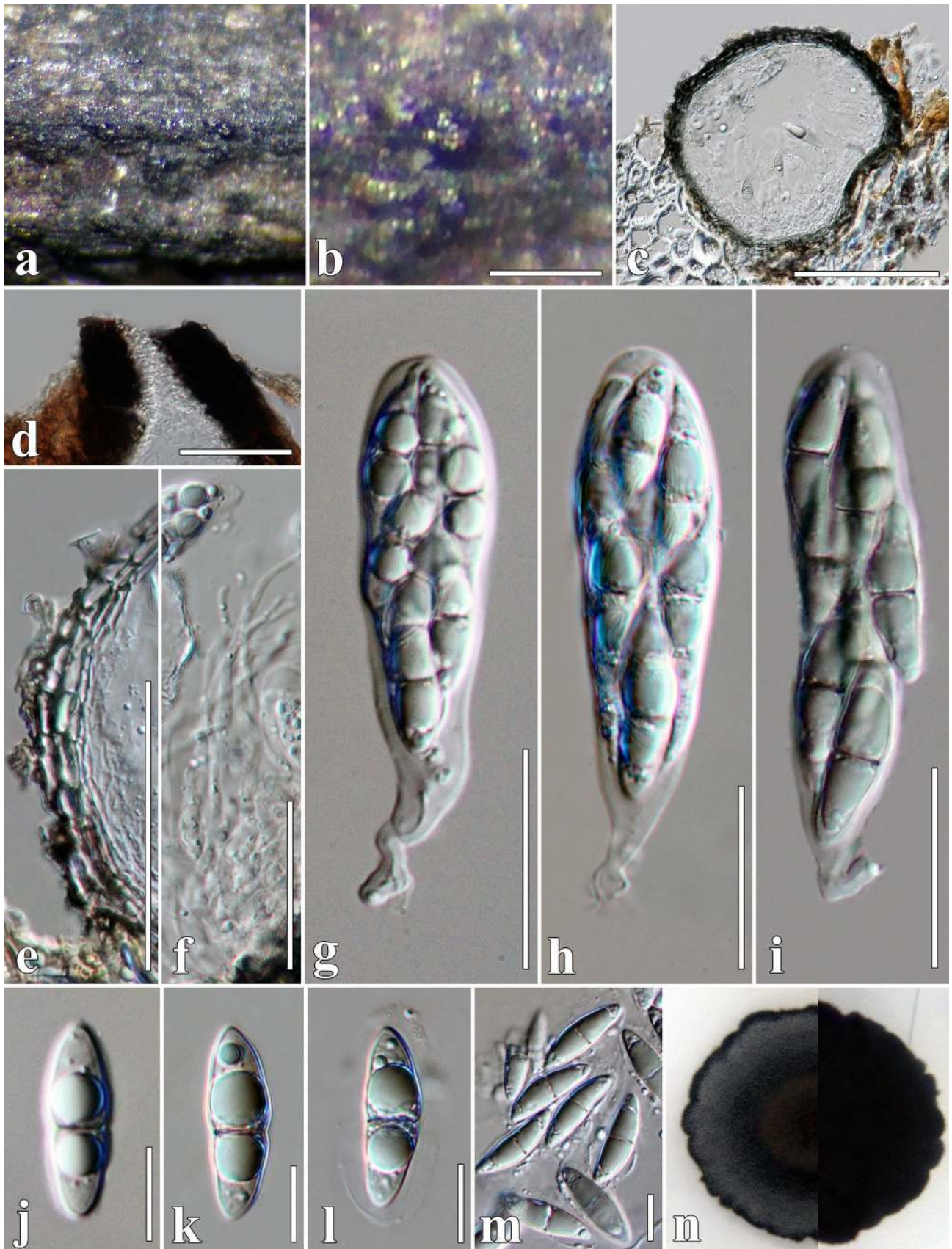
***Leptospora clematidis*** Phukhams., Ertz, Gerstmanns & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557197; *Facesoffungi number*: FoF 02441, Fig. 53.

*Etymology*: The specific name “*clematidis*” refers to the host.

*Holotype*: MFLU 17–1505.

*Saprobic* on *Clematis patens*. **Sexual morph**: *Ascomata* 95–245 × 127–247 μm ( $\bar{x}$  = 159 × 202 μm, n = 5), dark brown to black, scattered, sometimes semi-immersed, erupt through host epidermis, only black shiny dots are visible on the host surface, uniloculate, subglobose, compressed, dark brown to black, coriaceous, ostiolate. *Ostioles* 28–40 × 38–87 μm, central, pseudoclypeus, with paraphyses filling the ostiolar canal, pale brown to brown, with light orange pigment at the pore. *Peridium* 10–32 μm wide, uniform, composed of 4(–5) layers of cells arranged in *textura*



◀**Fig. 42** *Brunneofusispora clematidis* (MFLU 17–1478, **holotype**). **a, b** Appearance of ascomata on *Clematis subumbellata*. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Pseudoparaphyses. **g–i** Asci. **j–m** Ascospores. **n** Culture characteristics on MEA. Scale bars: **b**=200  $\mu$ m, **c**=100  $\mu$ m, **d, e**=50  $\mu$ m, **f, g–i**=20  $\mu$ m, **j–m**=10  $\mu$ m

*angularis*, brown to dark brown, inner layer lined with subhyaline cells of *textura angularis*. *Hamathecium* composed of numerous, 2–4  $\mu$ m wide, filamentous, cellular pseudoparaphyses, with distinct septa, embedded in mucilaginous matrix, anastomosing at the apex. *Asci* 67–96  $\times$  7–11  $\mu$ m ( $\bar{x}$  = 78  $\times$  10  $\mu$ m,  $n$  = 30), 8-spored, bitunicate, clavate, with short, furcate pedicel, apically rounded, ocular chamber present when young. *Ascospores* 17–33  $\times$  3–6  $\mu$ m ( $\bar{x}$  = 26  $\times$  5  $\mu$ m,  $n$  = 30), narrowly turbinate, rounded at apex, acute at the bottom, hyaline to yellowish brown, 3-septate, cell above median septa slightly enlarged, slightly constricted at the septa, smooth-walled, with minute guttule in each cell, polar appendages visible when immature. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C, from above: sparse, circulate, flattened, surface smooth, with fimbriate edge, cream at the margin, white with pale yellowish at the centre; colony below brown at the margin and centre.

**Material examined:** Belgium, Flemish Brabant, Meise Botanic Garden, Bouchout Domain, dead stems of *Clematis patens*, 13 June 2017, D. Ertz & C. Gerstmans, BRCPI (MFLU 17–1505, **holotype**); ex-type living culture, MFLUCC 17–2148, *ibid.* (MFLU 17–1509, paratypes); ex-paratype living culture, MFLUCC 17–2152.

**Host:** *Clematis patens*—(This study).

**Distribution:** Belgium—(This study).

**GenBank accession numbers:** MFLUCC 17–2148: LSU: MT214577; SSU: MT226690; ITS: MT310622; *tefl*: MT394636; *rpb2*: MT394696. MFLUCC 17–2152: LSU: MT214578; SSU: MT226691; ITS: MT310623; *tefl*: MT394637; *rpb2*: MT394697.

**Notes:** *Leptospora clematidis* shares common characters with *Leptospora* in having uniloculate ascomata, ostioles with light orange pigment, a peridium of thin-walled cells arranged in *textura angularis*, and light yellow ascospores (Hyde et al. 2016). *Leptospora clematidis* has similar morphology to *L. galii* (KUMCC 15–0521), the strain recorded from *Galium* sp. in Italy. However, our new species differs from *L. galii* in having larger ascomata that are subglobose with clavate, furcate asci (Hyde et al. 2016, Fig. 53).

Phylogeny (Fig. 48) reveals that *L. clematidis* forms a close relationship with *L. galii* (KUMCC 15–0521) with strong support (100% ML/1.00 BYPP, Fig. 48). A comparison of the ITS region (including 5.8S region) showed 10

nucleotide differences (585/594—98%, with a single gap). A comparison of the *tefl* region revealed 15 base pair differences (822/837—98%, with no gaps). Thus, we describe *L. clematidis* as a distinct species.

***Leptospora thailandica*** Phukhams. & K.D. Hyde, in Hyde et al. Fungal Diversity 80: 100 (2016), **new host record**

**Index Fungorum number:** IF552239; **Facesoffungi number:** FoF 02381, Fig. 54.

**Saprobic** on dead branches of *Clematis subumbellata*. **Sexual morph:** *Ascomata* 188–229  $\times$  159–179  $\mu$ m, immersed to erumpent through host tissue, only black shiny dots are visible on the host surface, solitary, scattered, globose to compressed, smooth, brown to dark brown. *Ostioles* 120–132  $\times$  91–95  $\mu$ m ( $\bar{x}$  = 125  $\times$  92  $\mu$ m,  $n$  = 5), papillate, oblong, dark brown to light brown, heavily pigmented outer layer, smooth, filled with paraphyses, reddish to orange pigment around pore. *Peridium* 12–18  $\mu$ m wide, up to 30  $\mu$ m at the apex, thin-walled, brown to dark brown, pseudoparenchymatous cells, composed of 5–7 layers of *textura angularis*, inner layers composed of hyaline gelatinous cells. *Hamathecium* composed of numerous, 2.8–5.5  $\mu$ m wide ( $n$  = 30), broad, branched, filamentous, septate, cellular pseudoparaphyses. *Asci* 63–107  $\times$  8–13  $\mu$ m ( $\bar{x}$  = 77  $\times$  10  $\mu$ m,  $n$  = 30), 8-spored, bitunicate, cylindrical to cylindrical-clavate, with short furcate pedicel, apically rounded, ocular chamber visible when immature. *Ascospores* 40–77  $\times$  2–5  $\mu$ m ( $\bar{x}$  = 58  $\times$  4  $\mu$ m,  $n$  = 40), fasciculate, scolecosporous, tapering towards the ends, hyaline when immature, pale brown at maturity, with minute guttule in each cell, with (7–)8–17-septate, slightly constricted at the septa, with 3–5  $\mu$ m sheath drawn out to form bipolar appendages, with a pad-like structure at the apices. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Culture from above brown, sparse, circular, faintly zonate, convex with moderate aerial mycelium, downy, with slightly irregular at margins; reverse brown at the edge, light brown at the centre, dense, margin rough, not pigmented.

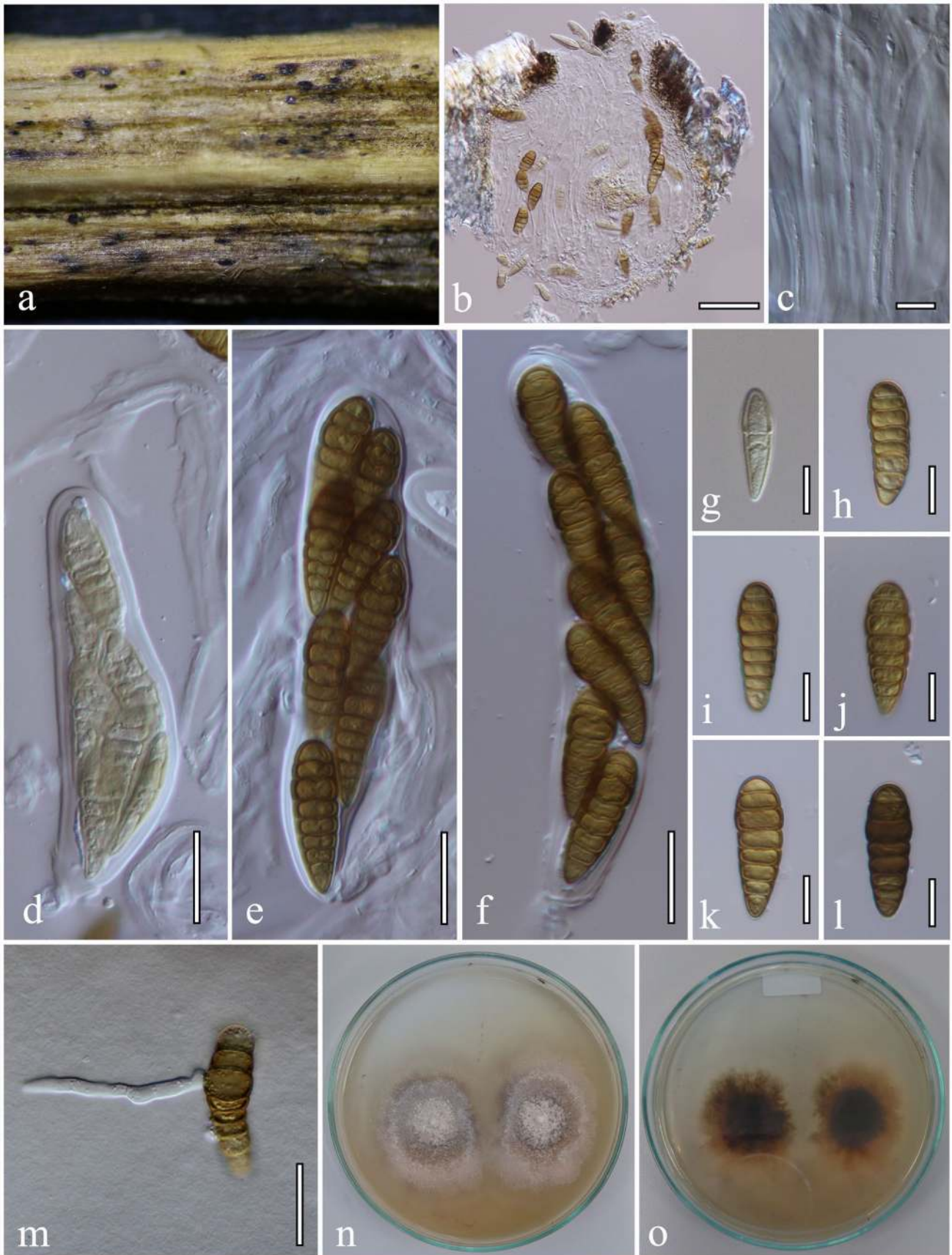
**Material examined:** Thailand, Phayao Province, on dead branches of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH10 (MFLU 17–1474); living culture, MFLUCC 17–2066.

**Hosts:** *Chromolaena odorata*, *Clematis patens*, *Duranta* sp.—(Hyde et al. 2016; Mapook et al. 2020; this study).

**Distribution:** Thailand—(Hyde et al. 2016; Mapook et al. 2020; this study).

**GenBank accession numbers:** LSU: MT214579; SSU: MT226692; ITS: MT310624; *tefl*: MT394638.

**Notes:** Our new collection of *Leptospora thailandica* (MFLUCC 17–2066) is morphologically similar to the type species (MFLUCC 16–0385) which was reported from





**Fig. 43** *Xenomassariosphaeria clematidis* (MFLU 16–0119, holotype). **a** Appearance of ascomata on host surface. **b** Vertical section of ascoma. **c** Pseudoparaphyses. **d–f** Asci. **g–l** Ascospores. **m** Germinated ascospore. **n, o** Cultures characteristics on MEA. Scale bars: **b** = 100  $\mu\text{m}$ , **c–f** = 20  $\mu\text{m}$ , **g–m** = 10  $\mu\text{m}$

*Duranta* sp. (Hyde et al. 2016). The collection MFLUCC 17–2066 stains the host substrate pinkish red (Fig. 54). Phylogenetic analysis of combined sequence data indicated that *L. thailandica* (MFLUCC 17–2066) clusters together with the type strain and the strain reported from *Chromolaena odorata* with strong support (100% ML/1.00 BYPP, Fig. 48). The ITS sequence of our collection shows two base pair differences from the type strain (from 577 characters, including gap regions), while the *tef1* region is 100% identical.

*Longispora* Phukhams. & K.D. Hyde, **gen. nov.**

*Index Fungorum number*: IF557198; *Facesoffungi number*: FoF 07305, Fig. 55.

*Etymology*: The generic epithet referring to the long ascospores.

*Saprobic* on herbaceous plant in terrestrial habitats. **Sexual morph**: *Ascomata* solitary, immersed to erumpent, subglobose to compressed, cupulate when dry, brown to dark brown, with brown hyphae projecting from the peridium, ostiolate. *Ostioles* papillate, oblong, brown to light brown, heavily pigmented at outer layer, smooth, filled with periphyses, with a reddish to orange pigment around the pore. *Peridium* thick-walled, wider at the apex, comprising brown-walled cells of *textura angularis*. *Hamathecium* composed of numerous, branched, filamentous, transversely septate, cellular pseudoparaphyses. *Asci* 8-spored, bitunicate, cylindrical-clavate, with short pedicel, with a visible ocular chamber. *Ascospores* fasciculate, scolecosporous, ends rounded, hyaline when immature, pale brown at maturity, multi-septate, deeply constricted at the swollen cell, slightly constricted at the other septa, not separating into part spores. **Asexual morph**: Undetermined.

*Type species*: *Longispora clematidis* Phukhams. & K.D. Hyde

*Notes*: *Longispora* is established as a monotypic genus with *L. clematidis* as the type species. The genus is typical of Phaeosphaeriaceae in having compressed globose, coriaceous, brown to dark brown ascomata, with a reddish to orange pigments around the ostiolar pore, cellular pseudoparaphyses and fasciculate, scolecosporous, pale brown and multi-septate ascospores (Rabenhorst 1857; Crous et al. 2006; Phookamsak et al. 2014). *Longispora* has morphological characters similar to *Leptospora* and the sexual morph character of *Chaetosphaeronema* and *Neosetophoma* (*N. camporesii*) in having pigmentation in the ostiolar canal (Hyde et al. 2016, 2020; this study). Moreover, the fasciculate, scolecosporous, ascospores are common in

Phaeosphaeriaceae such as in *Ophiobolus*, *Ophiosphaerella* or *Pseudoophiobolus* (Phookamsak et al. 2017). *Longispora* is distinguishable from other genera having scolecospores in Phaeosphaeriaceae in its cupulate ascomata with colouration around the ostiolar pore, and asci that are cylindrical-clavate, short with a bulbous pedicel, and ascospores that are deeply constricted at the swollen cell.

Based on the multi-gene phylogenetic analysis (Fig. 48), MFLU 20–0420 formed a basal lineage to *Leptospora* and *Populocrescentia* with strong support (96% ML/1.00 BYPP, Fig. 48). A BLAST result of the nucleotide sequences showed 98.84% similarity to *Phaeosphaeria oryzae* (CBS 110110) in the LSU region, while the ITS region showed 89.24% similarity to *Populocrescentia forlicesenensis* (MFLUCC 14–0651).

*Longispora clematidis* Phukhams. & K.D. Hyde, **sp. nov.**

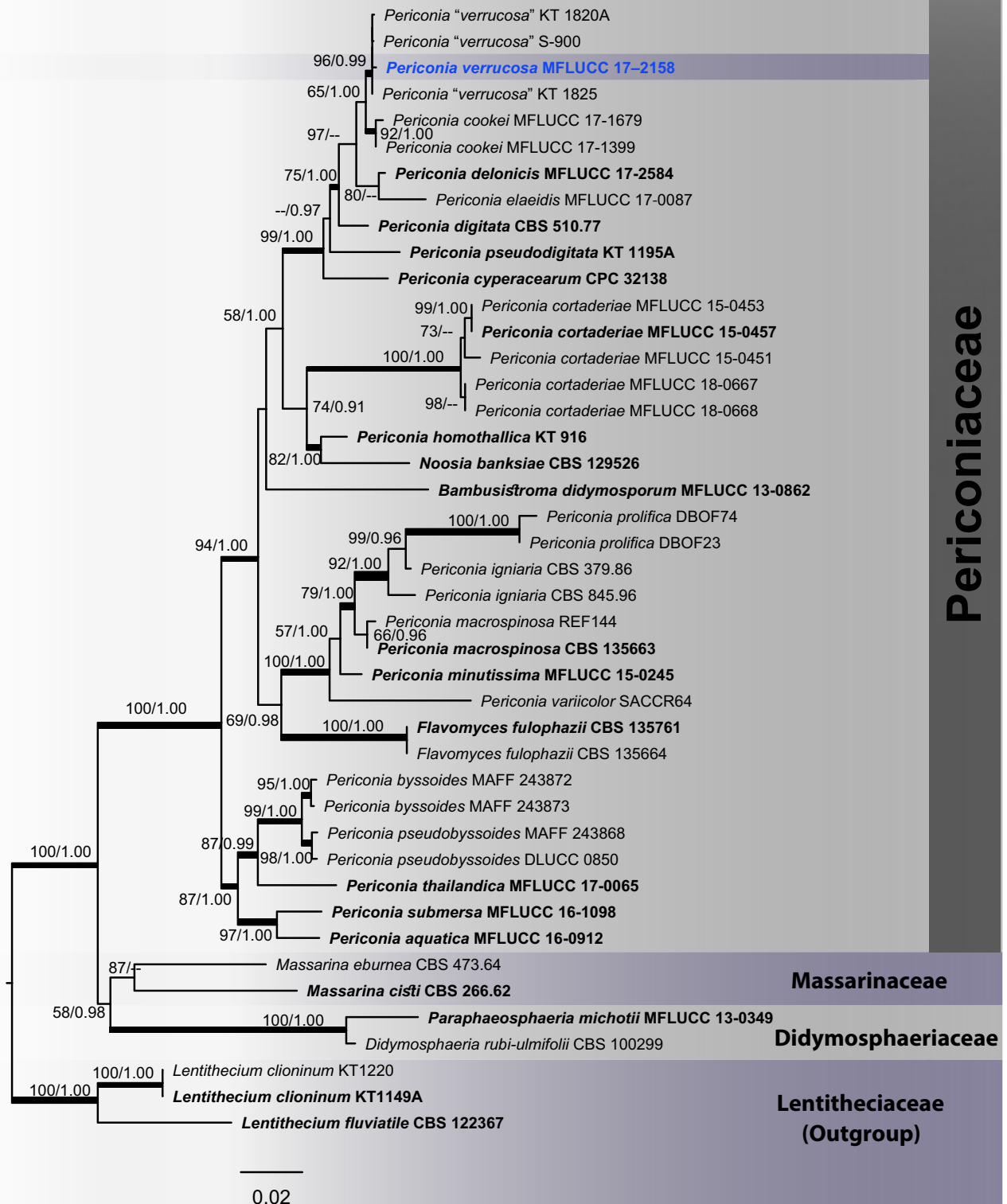
*Index Fungorum number*: IF557199; *Facesoffungi number*: FoF 07306, Fig. 55.

*Etymology*: The epithet reflects the host *Clematis*.

*Holotype*: MFLU 20–0420.

*Saprobic* on dead stems of *Clematis vitalba*. **Sexual morph**: *Ascomata* 324–340  $\times$  362–368  $\mu\text{m}$  ( $\bar{x}$  = 332  $\times$  365  $\mu\text{m}$ ,  $n$  = 5), solitary, scattered, immersed to erumpent through host tissue, only black shiny dots are visible on the host surface, subglobose to compressed, cupulate when dry, brown to dark brown, with brown hyphae projecting from the peridium, ostiolate. *Ostioles* 79–105  $\times$  75–85  $\mu\text{m}$  ( $\bar{x}$  = 94  $\times$  80  $\mu\text{m}$ ,  $n$  = 5), papillate, oblong, brown to light brown, heavily pigmented at outer layer, smooth, filled with periphyses, with a reddish to orange colouration around the pore. *Peridium* 15–30(–35)  $\mu\text{m}$  wide, up to 50  $\mu\text{m}$  at apex, wider at the apex, comprising 6(–8)-layers of brown-walled cells of *textura angularis*, outer layer heavily pigmented, with inner region comprising hyaline cells of *textura angularis*. *Hamathecium* composed of numerous, 2–4  $\mu\text{m}$  wide ( $n$  = 50), branched, filamentous, septate, cellular pseudoparaphyses. *Asci* 97–157  $\times$  9–15  $\mu\text{m}$  ( $\bar{x}$  = 128  $\times$  12  $\mu\text{m}$ ,  $n$  = 35), 8-spored, bitunicate, cylindrical-clavate, with short bulbous pedicel, apically rounded, with visible ocular chamber. *Ascospores* 93–124  $\times$  2–5  $\mu\text{m}$  ( $\bar{x}$  = 110  $\times$  4  $\mu\text{m}$ ,  $n$  = 40), fasciculate, scolecosporous, ends rounded, hyaline when immature, pale brown at maturity, with minute guttule in each cell, (17–)19–23-septate, swollen near the septa between the 11th or 13th or 14th cell, deeply constricted at the swollen cell, slightly constricted at the other septa, not separating into part spores, indentations present. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA reaching 20 mm diam. after 4 weeks at 16  $^{\circ}\text{C}$ . Culture slow growing, black, dense, rhizoid, raised with concave edge, rough, irregular at the margins; reverse: black, dense, not pigmented.



**Material examined:** Italy, Forlì-Cesena Province, Viale Salinatore—Forlì City, on dead aerial branch of *Clematis vitalba*, 23 February 2015, E. Camporesi, IT2389B (MFLU 20–0420, **holotype**).

**Hosts:** *Clematis vitalba*—(This study).

**Distribution:** Italy—(This study).

**GenBank accession numbers:** LSU: MT214580; SSU: MT226693; ITS: MT310625; *tef1*: MT394639.

**Fig. 44** The best scoring RAxML tree with a likelihood value of  $-10209.184183$  based on combined LSU, ITS and *tefl* sequence data for Periconiaceae. The tree is rooted with species of Lentitheciaceae. Forty-seven strains were included in the combined gene sequence analyses which comprised 2974 characters (1320 characters for LSU, 620 characters for ITS, 1034 characters for *tefl*, including gap regions). The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree from the maximum likelihood analysis had similar topology to Bayesian 50% majority-rule consensus phylogram. The matrix had 865 distinct alignment patterns with 33.43% undetermined characters and gaps. Estimated base frequencies were as follows; A=0.229606, C=0.269654, G=0.273255, T=0.227485; substitution rates AC=1.424251, AG=2.110026, AT=1.548277, CG=1.179391, CT=7.719698, GT=1.000000; gamma distribution shape parameter  $\alpha=0.673748$ . In our analysis, a GTR+I+G model was used for each partition in the Bayesian posterior analysis. The species determined in this study is indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% ML/0.90 BYPP. Thick branches represent significant support values from all analyses (ML  $\geq$  70%/BYPP  $\geq$  0.95)

*Notes:* *Longispora clematidis* is characterized by having sessile, cupulate ascomata, reddish orange ostioles, cellular pseudoparaphyses, cylindrical-clavate asci with a bulbous pedicel and filiform ascospores, which are multi-septate and swollen between the 11th–14th cells (Fig. 55). We introduce *L. clematidis* based on morphological and phylogenetic analyses.

***Pseudoophiobolus*** Phookamsak, Wanas., S.K. Huang, Camporesi & K.D. Hyde

*Pseudoophiobolus* is typified by *P. mathieui* (Westend.) Phookamsak, Wanas., S.K. Huang, Camporesi & K.D. Hyde. The genus was introduced to accommodate an ophiobolus-like taxon that is phylogenetically distant from *Ophiobolus* sensu stricto (Phookamsak et al. 2017). *Pseudoophiobolus* is distinguishable from other ophiobolus-like species in having semi-immersed to erumpent, papillate ascomata with pseudoparenchymatous cells, arranged in a *textura angularis* to *textura prismatica*, cellular pseudoparaphyses, and fasciculate, scolecosporous multi-septate ascospores with a swollen cell, that do not split into part spores. A new host record of *P. rosae* on *Clematis* is presented (Fig. 56).

***Pseudoophiobolus rosae*** Phookamsak, Wanas., Phukhams., Camporesi & K.D. Hyde in Phookamsak et al. Fungal Diversity 87: 330 (2017), **new host record**

*Index Fungorum number:* IF553928; *Facesoffungi number:* FoF 03805, Fig. 56.

*Saprobic* on dried stems of *Clematis vitalba*. **Sexual morph:** Ascomata 290–345  $\times$  240–305  $\mu\text{m}$  ( $\bar{x}=317 \times 274 \mu\text{m}$ ,  $n=5$ ), uniloculate, scattered, solitary, semi-immersed to superficial, ampulliform, globose, cupulate when dried, dark brown to black, with dark brown, septate mycelium at the base, ostiolate. *Ostioles* 117–135  $\times$  100–117  $\mu\text{m}$ , oblong,

apex rounded, short papillate, composed of several layers of dark pseudoparenchymatous cells, with opening by a pore, filled with hyaline periphyses. *Peridium* 12–30(–36)  $\mu\text{m}$  wide, slightly thickened, composed of 6(–9) layers of dark brown cells arranged in *textura angularis*, pseudoparenchymatous cells, inner layers comprising 2 layers of hyaline cells, arranged in *textura angularis*. *Hamathecium* composed of dense, 2–4  $\mu\text{m}$  wide ( $\bar{x}=2.5$ ,  $n=50$ ), wide, broad, branched, filamentous, septate, cellular pseudoparaphyses, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* 64–153  $\times$  9–14  $\mu\text{m}$  ( $\bar{x}=116 \times 12 \mu\text{m}$ ,  $n=30$ ), 8-spored, bitunicate, fissitunicate, cylindrical-clavate, with short bulbous pedicel, apically rounded, with well-developed ocular chamber. *Ascospores* (40–)75–110  $\times$  3–5  $\mu\text{m}$  ( $\bar{x}=88 \times 4 \mu\text{m}$ ,  $n=40$ ), fasciculate, scolecosporous, curved, pale yellowish to yellowish, with rounded ends, tapered towards the lower cells, swollen at the 8th or 10th cell, 17(–22)-septate, not constricted at the septa, smooth-walled. **Asexual morph:** Undetermined.

*Culture characters:* Colonies on MEA reaching 40 mm diam. after 4 weeks at 16 °C. Culture medium dense, circular, umbonate, surface smooth, edge erose, thinly hairy, green at the edge, yellowish to cream at the centre; reverse: cream at the margin, brown at the centre, not producing pigmentation in agar.

*Material examined:* Italy, Arezzo Province, Quota—Poppi City, dead aerial branch of *Clematis vitalba*, 5 June 2016, E. Camporesi, IT 2983A (MFLU 15–1014); living culture, MFLUCC 16–1364.

*Hosts:* *Clematis vitalba*, *Rosa canina*—(Phookamsak et al. 2017; This study).

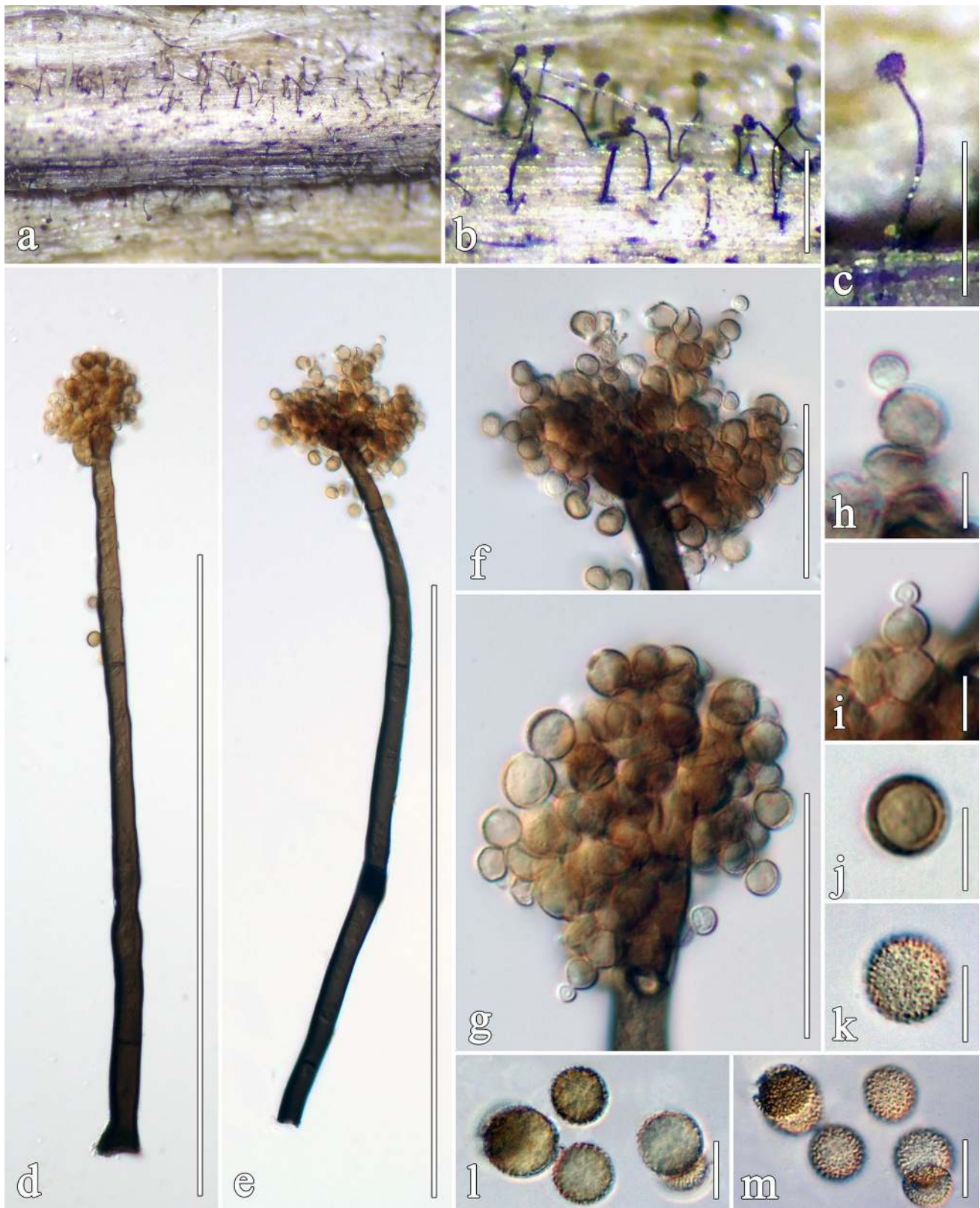
*Distribution:* Italy—(Phookamsak et al. 2017; This study).

*GenBank accession numbers:* LSU: MT214581; SSU: MT226694; ITS: MT310626; *tefl*: MT394640.

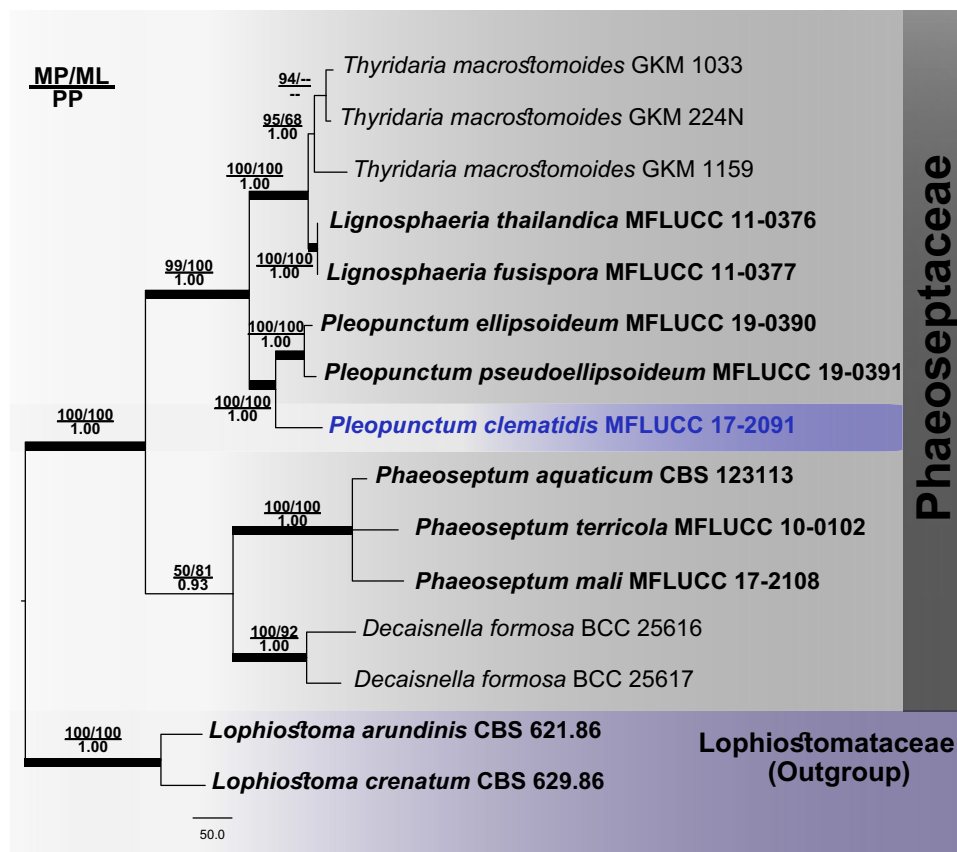
*Notes:* Our collection, MFLUCC 16–1364 formed a clade with the type strain of *Pseudoophiobolus rosae* (MFLUCC 17–1786) with strong support (100% ML/0.99 BYPP, Fig. 48). Our strain is morphologically similar to the type strain of *P. rosae* which was reported on *Rosa canina* (Phookamsak et al. 2017, Fig. 56). A nucleotide comparison of the ITS and *tefl* regions of MFLUCC 16–1364 and the type strain are 100% identical. Thus, we report a new host record of *Pseudoophiobolus rosae* on *Clematis*.

***Wojnowiciella*** Crous, Hern.-Restr. & M.J. Wingf.

*Wojnowiciella eucalypti* is the type species. The genus is characterized by a papillate, ostiolate conidiomata, ampulliform, phialidic conidiogenous cells, hyaline, aseptate microconidia and brown, multi-septate macroconidia (Wijayawardene et al. 2013; Crous et al. 2015a). The genus has morphological resemblance to *Wojnowicia* which was introduced by Saccardo (1892). However, Crous et al. (2015a)



**Fig. 45** *Periconia verrucosa* (MFLU 17–1516, holotype). **a, b** Sporodochia on natural substrate. **c** Close up of sporodochia. **d, e** Conidiophores. **f–i** Conidiogenous cells. **j–m** Conidia. Scale bars: **b** = 500  $\mu\text{m}$ , **c–e** = 250  $\mu\text{m}$ , **f, g** = 50  $\mu\text{m}$ , **h, i** = 10  $\mu\text{m}$ , **j–m** = 5  $\mu\text{m}$



**Fig. 46** Phylogram generated from maximum parsimony analysis of Phaeoseptaceae based on combined LSU, SSU, ITS, *tefl* and *rpb2* sequence data. Related sequences are taken from Liu et al. (2019) and retrieved from GenBank. Fifteen strains were included in the analysis of the combined loci and comprised 4077 characters (810 characters for LSU, 1017 characters for SSU, 480 characters for ITS, 895 characters for *tefl*, 875 characters for *rpb2*, including gaps). The tree is rooted with *Lophiostoma arundinis* (CBS 621.86) and *L. crenatum* (CBS 629.86) in Lophiostomataceae. Maximum parsimony analysis of 717 parsimony informative characters resulted in a most parsimonious tree (CI=0.797, RI=0.757, RC=0.603, HI=0.203). The best scoring RAxML tree received a final likelihood value of  $-12889.704989$ . The matrix had 769 distinct alignment pat-

terns, with 37.49% undetermined characters and gaps. Estimated base frequencies were: A=0.241361, C=0.260527, G=0.275500, T=0.222613; substitution rates AC=1.309734, AG=3.237095, AT=1.407451, CG=1.436805, CT=9.313126, GT=1.000000; gamma distribution shape parameter  $\alpha=0.742589$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. Bootstrap values (BS) from maximum parsimony (MP, left), maximum likelihood (ML, right) higher than 50% BS and Bayesian posterior probabilities (BYPP, below) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95). The ex-type strains are in bold and black. The newly generated sequence is in bold and blue

discussed that the type species of *Wojnowicia*, *W. hirta* is compatible with the generic concept of *Septoriella*. Thus, *Wojnowicia* was synonymised as a member of *Septoriella*. Our collection associated with *Clematis viticella* revealed a novel species *W. clematidis* from Belgium (Fig. 57).

***Wojnowiciella clematidis*** Phukhams., Ertz, Gerstmans & K.D. Hyde, **sp. nov.**

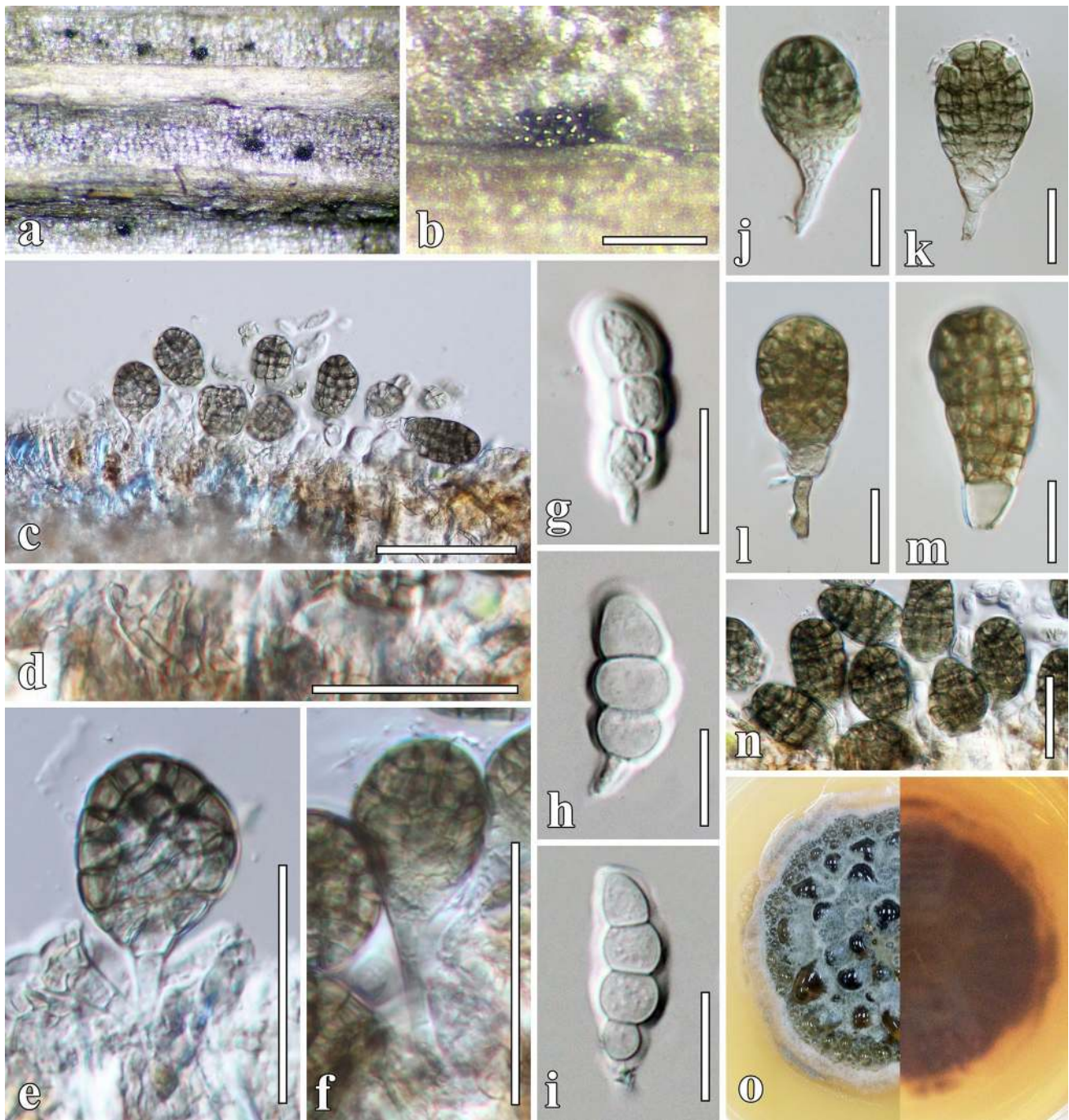
*Index Fungorum number*: IF557200; *Facesoffungi number*: FoF 07307, Fig. 58.

*Etymology*: The epithet refers to the host plant, *Clematis*.  
*Holotype*: MFLU 17-1517.

*Saprobic* on dried stems of *Clematis viticella*.

**Sexual morph**: *Ascomata* 228–240  $\times$  223–250  $\mu$ m,

( $\bar{x}$  = 236  $\times$  240  $\mu$ m, n = 5), solitary, gregarious, scattered, immersed to erumpent through host epidermis, black shiny dots are visible on the host surface, uniloculate, subglobose, compressed, dark brown to black, coriaceous, apapillate, ostiolate. *Ostioles* central, pseudoclypeate, dark brown. *Peridium* 15–45  $\mu$ m wide, composed of 7(–9) layers of cells arranged in *textura angularis*, brown to dark brown, inner layer lined with sub-hyaline cells of *textura angularis*. *Hamathecium* composed of numerous, 2–4  $\mu$ m wide, filamentous, cellular pseudoparaphyses, with distinct septa, embedded in a mucilaginous matrix, anastomosing at the apex. *Asci* 97–136  $\times$  12–17  $\mu$ m ( $\bar{x}$  = 116  $\times$  15  $\mu$ m, n = 30), 8-spored, bitunicate, broadly cylindrical to cylindrical-clavate, with short, furcate pedicel, apically rounded, with



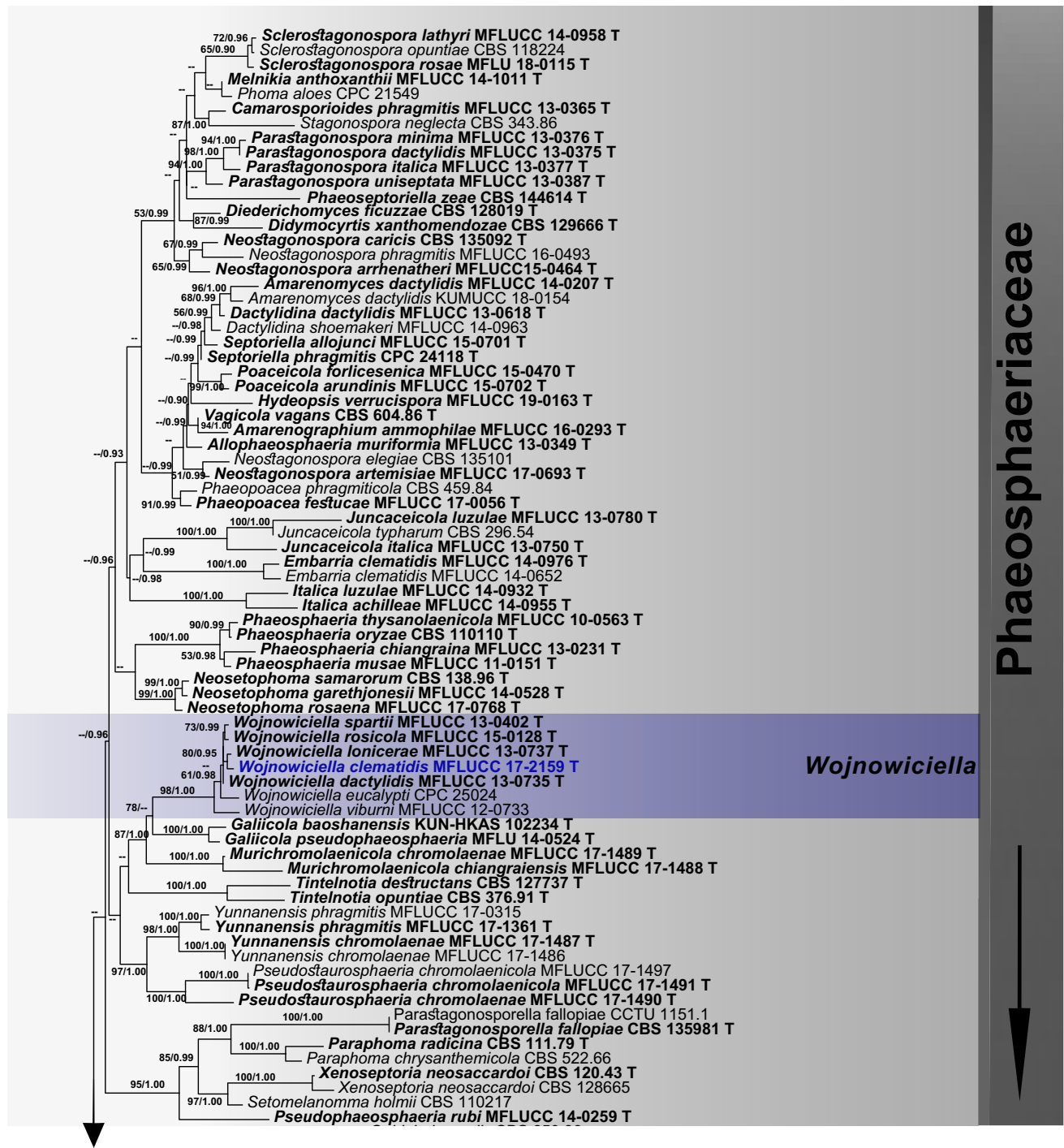
**Fig. 47** *Pleopunctum clematidis* (MFLU 17–1499, holotype). **a, b** Sporodochia on natural substrate. **c** Vertical section of sporodochia. **d** Subicular hyphae. **e–f** Cylindrical conidia and lenticular conidia

on host substrate. **g–i** Cylindrical conidia. **j–n** Mature lenticular conidia. **o** Culture characteristics on MEA. Scale bars = **b** = 500  $\mu$ m, **c** = 100  $\mu$ m, **d–n** = 20  $\mu$ m

well-developed ocular chamber. *Ascospores* 24–38  $\times$  4–9  $\mu$ m ( $\bar{x}$  = 29  $\times$  7  $\mu$ m,  $n$  = 30), biseriata, partially overlapping, obovoid to sub-fusiform, rounded at apex, acute at the ends, hyaline to yellowish brown, 4–6-septate, with an oblique or longitudinal septum in the central 2–3 cells, above median septum slightly enlarged, constricted at the cell above

median septum, smooth-walled, with minute guttule in each cell, without a mucilaginous sheath. **Asexual morph:** Undetermined.

*Culture characters:* Colonies on MEA reaching 30 mm diam. after 4 weeks at 25  $^{\circ}$ C. Culture from above sparse, circular, flattened, surface smooth, with fimbriae at the edge,



**Fig. 48** The best scoring RAxML tree with a final likelihood value of  $-34209.688899$  based on a combined LSU, SSU, ITS and *tefl* dataset for Phaeosphaeriaceae. One hundred and fifty-seven strains were included in the combined genes sequence analyses which comprised 3290 characters (822 characters for LSU, 603 characters for ITS, 991 characters for SSU and 874 characters for *tefl*, including gap regions). The tree is rooted with *Staurosphaeria*. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree from the maximum likelihood analysis had similar topology to the Bayesian 50% majority-rule consensus phylogram. The matrix had 1355 distinct alignment patterns, with 23.28% of undeter-

mined characters and gaps. Estimated base frequencies were as follows; A=0.241166, C=0.264679, G=0.235933, T=0.258221; substitution rates AC=1.039932, AG=3.142919, AT=2.044232, CG=0.762621, CT=4.795230, GT=1.000000; gamma distribution shape parameter  $\alpha=0.455752$ . In our analysis, GTR+I + G model was used for each partition in Bayesian posterior analysis. The species determined in this study are indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. The significant support values from all analyses are  $BS \geq 70\%/BYPP \geq 0.95$

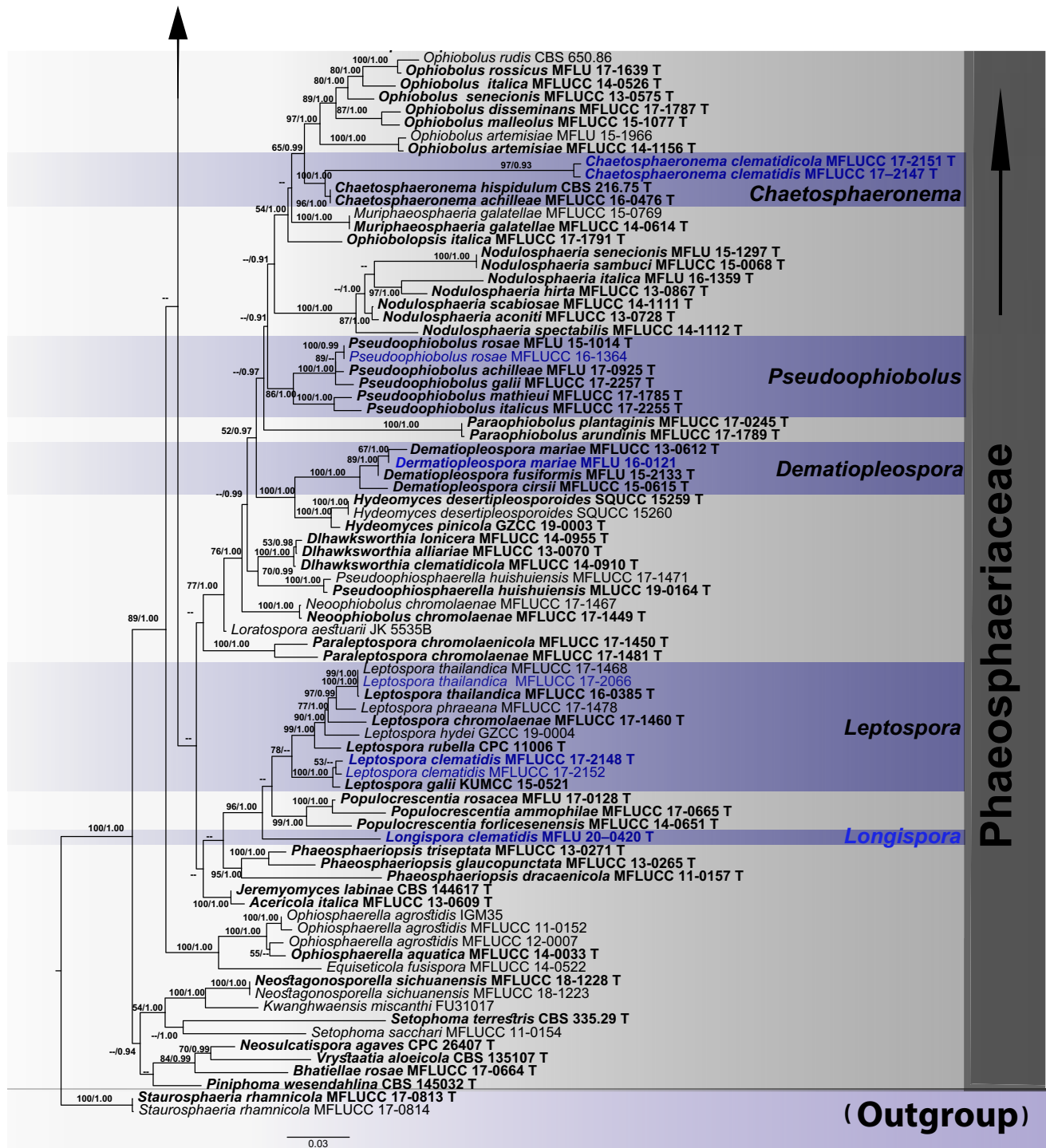


Fig. 48 (continued)

cream at the margin, white at the centre, colony from below brown.

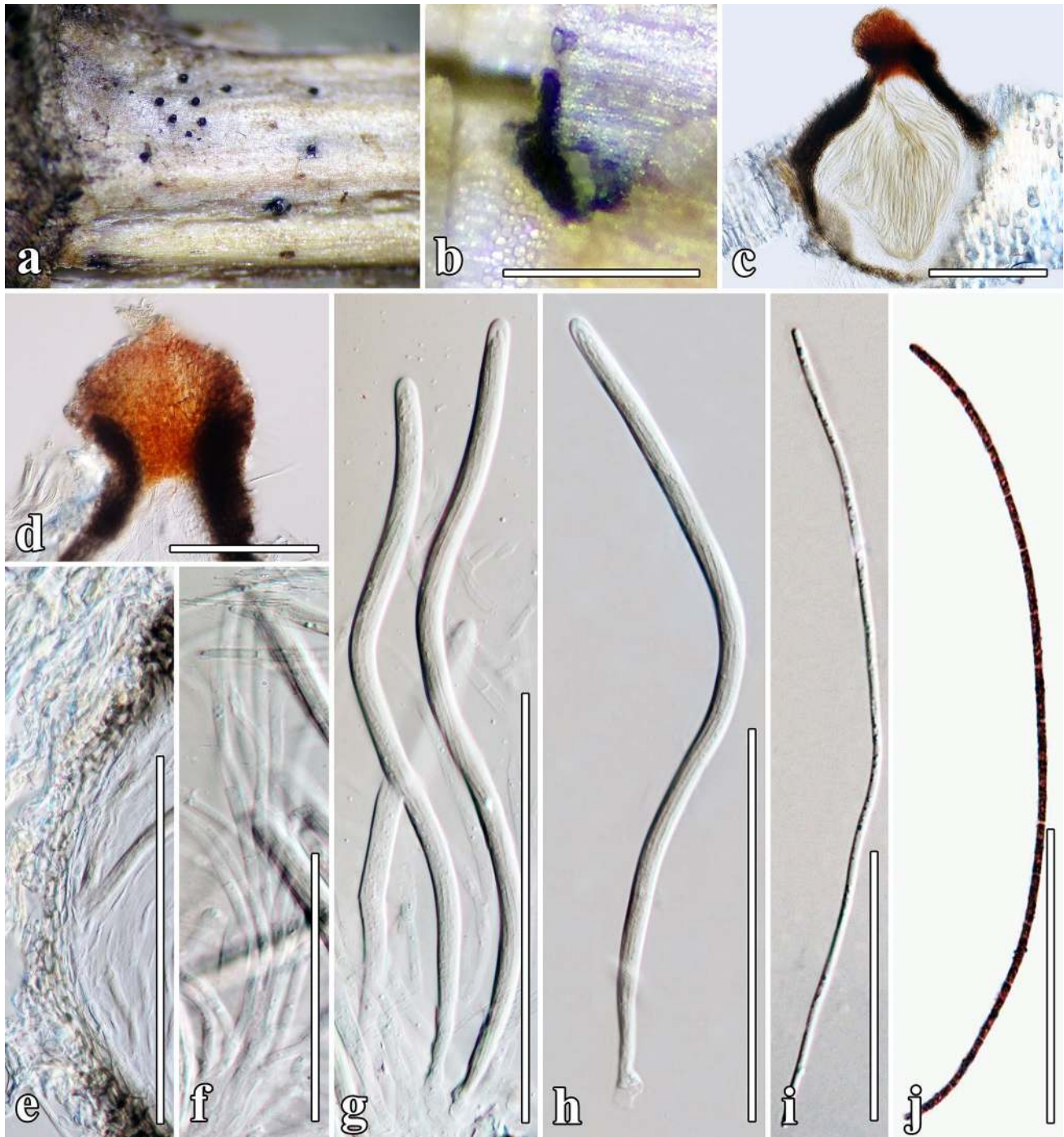
*Material examined:* Belgium, Flemish Brabant, Meise Botanic Garden, Bouchout Domain, dead stems of *Clematis viticella*, 13 June 2017, D. Ertz & C. Gerstmans,

BRCV5 (MFLU 17–1517, **holotype**); ex-type living culture, MFLUCC 17–2159.

*Host:* *Clematis viticella*—(This study).

*Distribution:* Belgium—(This study).





**Fig. 49** *Chaetosphaeronema clematidicola* (MFLU 17–1508, holotype). **a** Appearance of ascomata on *Clematis patens*. **b** Close up of ascoma on host substrate. **c** Vertical section through ascoma. **d** Osti-

olar canal. **e** Section of peridium. **f** Pseudoparaphyses. **g–h** Asci. **i–j** Ascospores (**j** Ascospore in 10% India ink). Scale bars: **b** = 500  $\mu$ m, **c** = 200  $\mu$ m, **d–h** = 100  $\mu$ m, **i–j** = 50  $\mu$ m

*GenBank accession numbers*: LSU: MT214582; SSU: MT226695; ITS: MT310627; *tef1*: MT394641; *rpb2*: MT394698.

*Notes*: *Wojnowiciella clematidis* (strain MFLUCC 17–2159) is introduced as a novel species based on its

distinctive morphology and phylogeny (Figs. 57, 58). The species is similar to other *Wojnowiciella* species in having immersed to erumpent, dark brown to black, coriaceous, ostiolate, apapillate ascomata, and hyaline to yellowish brown ascospores (Crous et al. 2015a). *Wojnowiciella*

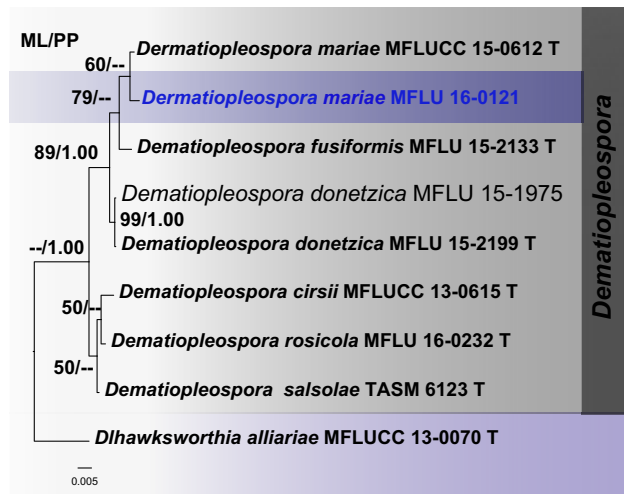


**Fig. 50** *Chaetosphaeronema clematidis* (MFLU 17–1504, **holotype**). **a** Appearance of conidiomata on *Clematis orientalis*. **b** Close up of conidioma on host substrate. **c** Vertical section through conidioma. **d** Ostiolar canal. **e** Section of conidiomatal wall. **f–i** Conidiog-

enous cells and conidia (**f, g** conidiogenous cells in cotton blue). **j–l** Conidia. Scale bars: **b**=500  $\mu\text{m}$ , **c**=200  $\mu\text{m}$ , **d**=50  $\mu\text{m}$ , **e**=20  $\mu\text{m}$ , **f–l**=10  $\mu\text{m}$

*clematidis* has similar characters to *W. italica* (MFLUCC 13–0447), but differs in having cylindrical to cylindrical-clavate asci and obovoid to sub-fusiform ascospore (Hyde et al. 2016). Phylogeny (Fig. 57) revealed that *W. clematidis* (MFLUCC 17–2159) formed a close relationship

with *W. dactylidis* (CBS 145077) with good support (77% MP/84% ML/1.00 BYPP). *Wojnowiciella dactylidis* strain CBS 145077 formed a separate clade from the type species (*W. dactylidis* MFLUCC 13–0735), however the strain CBS 145077, which was isolated from *Dypsisis* sp. only forms



**Fig. 51** The Bayesian 50% majority-rule consensus phylogram based on combined LSU, SSU, ITS and *tefl* sequence data for *Dematiopleospora* species. Nine strains were included in the combined analyses which comprised 3407 characters (884 characters for LSU, 1033 characters for SSU, 617 characters for ITS and 873 characters for *tefl*, including gap regions). The tree is rooted with *Dhawksworthia allariae* (MFLUCC 13-0070). Single gene analyses were also performed to compare the topology and clade stability with combined gene analyses. Tree topology generated under the maximum likelihood analysis was similar to Bayesian analyses. The best scoring RAXML tree was obtained with a final likelihood value of  $-5814.019143$ . The matrix had 162 distinct alignment patterns, with 24.34% of undetermined characters and gaps. Estimated base frequencies were as follows; A=0.252414, C=0.229288, G=0.264353, T=0.253945; substitution rates AC=0.248381, AG=2.072002, AT=1.746797, CG=1.084349, CT=5.244757, GT=1.000000; gamma distribution shape parameter  $\alpha=0.904425$ . In our analysis, GTR+I+G model was used for each partition in the Bayesian posterior analysis. The species determined in this study is indicated in blue. Bootstrap values (BS) from maximum likelihood (ML, left) of more than 50% BS and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. The significantly supported values from all analyses are  $BS \geq 70\%/BYPP \geq 0.95$

microconidia in culture (Crous et al. 2019). A comparison of nucleotides between *W. clematidis* (MFLUCC 17-2159) and *W. dactylidis* (CBS 145077) showed that the ITS region (including the 5.8S region) has a single nucleotide difference (550/561—98%, including gaps). The comparison of the *tefl* region revealed three base pair differences (869/440, no gaps). Based on current evidence and the lack of asexual morph character from this study, these species are considered as distinct.

### Pleosporaceae Nitschke

The family was revisited by Ariyawansa et al. (2015c), with additional taxa and resegmentation in Wanasinghe et al. (2017). We introduce a new species and new host records of *Stemphylium* from *Clematis vitalba*, based on a morphology

and concatenated phylogenetic analysis of LSU, ITS, SSU and *gadh* sequence data (Fig. 59).

### *Stemphylium* Wallr.

*Stemphylium* is a dematiaceous hyphomycete genus and the asexual morph has been recorded in *Pleospora* allies (Ariyawansa et al. 2015c; Woudenberg et al. 2017). Based on morphological studies, multi-gene phylogeny analyses, and ecological evidence, the use of *Stemphylium* over *Pleospora* has been recommended (Köhl et al. 2009; McNeill et al. 2012; Rossmann et al. 2015; Woudenberg et al. 2017; Wijayawardene et al. 2018). *Stemphylium* is typified with *S. botryosum*. Phylogenetic placement of *S. botryosum* is verified by Woudenberg et al. (2017). Based on the phylogenetic analysis of the combined LSU, ITS and *gadh* dataset (Fig. 59), we report a new host record for *S. vesicarium* and describe a new species *S. clematidis* from *Clematis vitalba* (Figs. 60, 61).

### *Stemphylium clematidis* Wanas., Camporesi & K.D. Hyde, sp. nov.

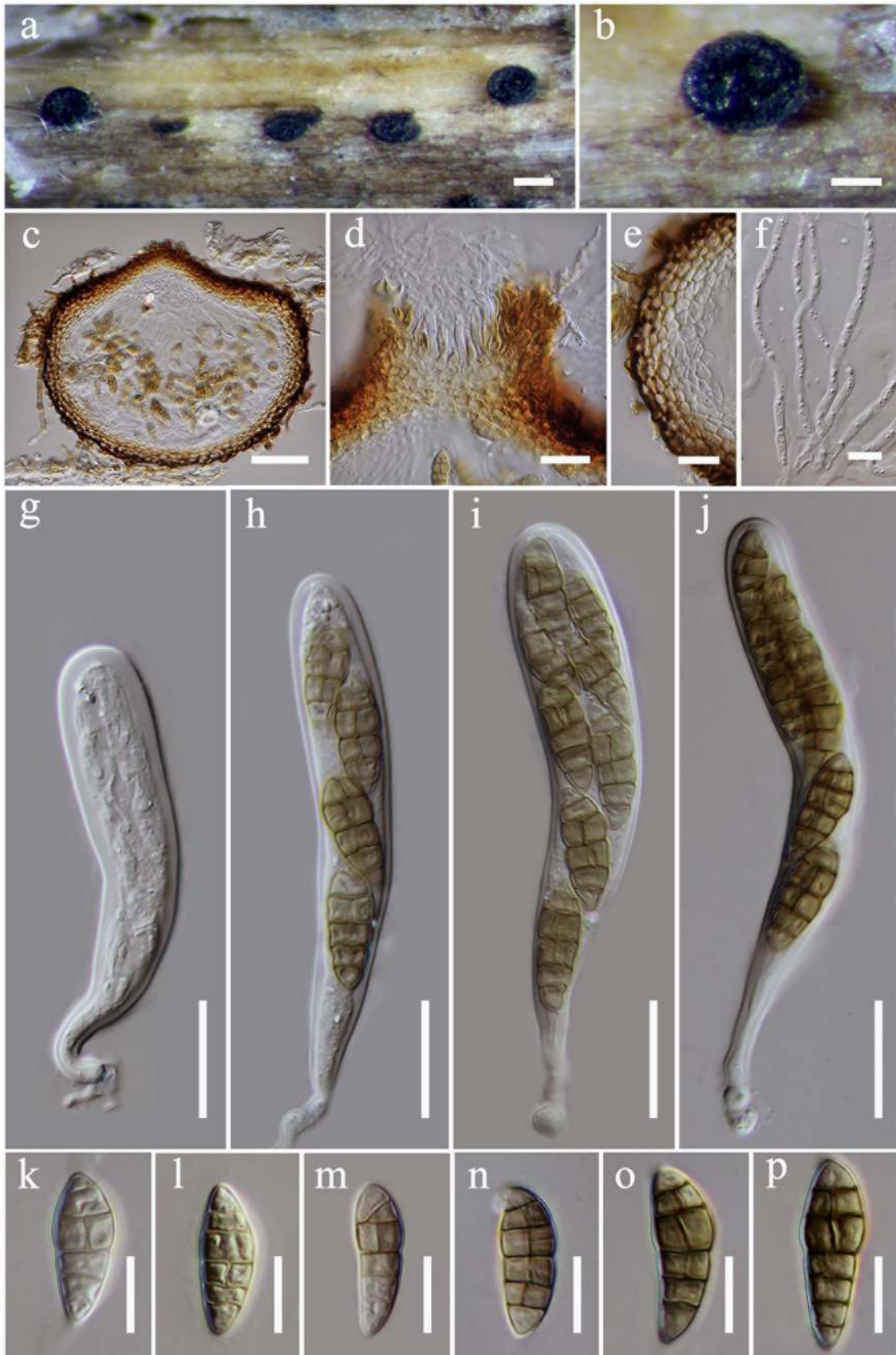
*Index Fungorum number*: IF557305; *Facesoffungi number*: FoF 07327, Fig. 60.

*Etymology*: Refers to the name of the host.

*Holotype*: MFLU 16-0176.

*Saprobic* on dead stem of *Clematis vitalba*. **Sexual morph**: *Ascomata* 190–230 × 290–320  $\mu\text{m}$  ( $\bar{x}=220 \times 303 \mu\text{m}$ ,  $n=20$ ), solitary, scattered, uniloculate, under epidermal layer of host to superficial, compressed globose to subglobose, coriaceous, black, ostiolate. *Ostioles* central, papillate, with variable walls, opening by a pore, filled with hyaline periphyses. *Peridium* 30–43  $\mu\text{m}$  wide ( $\bar{x}=36 \mu\text{m}$ ,  $n=20$ ), uniform, composed of 7 layers of *textura angularis*, black, heavy pigment at outer layer, cells towards the inside lighter, inner layer thin, hyaline gelatinous. *Hamathecium* composed of numerous, dense, 3–4  $\mu\text{m}$  wide, filamentous, branched, septate, cellular pseudoparaphyses, situated between and above the asci embedded in a gelatinous matrix. *Asci* 127–172 × 29–38  $\mu\text{m}$  ( $\bar{x}=150 \times 35 \mu\text{m}$ ,  $n=30$ ), 8-spored, bitunicate, fissitunicate, clavate to broad cylindrical-clavate, with short, with furcate pedicel, apically rounded, with an ocular chamber. *Ascospores* 24–37 × 12–18  $\mu\text{m}$  ( $\bar{x}=32 \times 13 \mu\text{m}$ ,  $n=50$ ), biseriate or partially overlapping, broad-fusiform, tapering towards the rounded ends, dictyosporous, 1(–2)-longitudinal euseptate, 7–9-transverse euseptate, constricted at the septa, strongly constricted at the median septum, cells above median septum wider than below, yellowish, with 8–10  $\mu\text{m}$  wide mucilaginous drawn out sheath. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA reaching 30 mm diam. after 4 weeks at 16 °C. Culture from above cream,



**Fig. 52** *Dermatiopleospora mariae* (MFLU 16–0121). **a** Appearance of ascomata on *Clematis vitalba*. **b** Close up of ascoma on host substrate. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Pseudoparaphyses. **g–j** Asci. **k–p** Ascospores. Scale bars: **a** = 500  $\mu\text{m}$ , **b** = 100  $\mu\text{m}$ , **c–j** = 20  $\mu\text{m}$ , **k–p** = 10  $\mu\text{m}$

orange aerial mycelium, dense, fluffy at the edge, umbonate, rough, lobate; reverse cream radiating white outwardly.

**Material examined:** Italy, Forlì-Cesena Province, Marsignano-Predappio, dead stems of *Clematis vitalba*, 31 March 2015, E. Camporesi, IT 1537 (MFLU 16–0176, **holotype**); ex-type living culture, MFLUCC 14–0937.

**Host:** *Clematis vitalba*—(This study).

**Distribution:** Italy—(This study).

**GenBank accession numbers:** LSU: MT214583; SSU: MT226696; ITS: MT310628; *gapdh*: MT394626.

**Notes:** *Stemphylium clematidis* has a pleospora-like sexual morph. The isolate formed a sister clade with *S. majusculum*, however the species is distinguishable by somewhat larger ascospores (Ramsey 1934; Simmons 1969, Fig. 60). The asexual morph was not obtained from culture. A pairwise comparison of the ITS sequence showed six nucleotide differences out of 555 nucleotides in the ITS regions (including gaps), while *gapdh* showed five nucleotide differences out of 595 nucleotides. The new strain is introduced as a new species of *Stemphylium* based on guidelines proposed by Jeewon and Hyde (2016).

***Stemphylium rosae*** (Wanas. et al.) Phukhams. & K.D. Hyde, **comb. nov.**

**Index Fungorum number:** IF557603; **Facesoffungi number:** FoF 0404

**Basionym:** *Pleospora rosae* Wanas., Camporesi, E.B.G. Jones & K.D. Hyde, in Wanasinghe et al., *Fungal Diversity* [153] (2018).

**Notes:** Based on analyses of combined LSU, ITS and *gapdh* sequence data for *Stemphylium* (Fig. 60), two isolates of *Pleospora rosae* and *P. rosae-caninae* clustered with *Stemphylium* sensu stricto. These two strains are compatible with the *Stemphylium* concept. Thus, we synonymize *Pleospora rosae* under *Stemphylium rosae* and *Pleospora rosae-caninae* under *Stemphylium rosae-caninae*. The nomenclature change is based on one fungus = one name protocol.

**Host:** *Rosa canina*—(Wanasinghe et al. 2018).

**Distribution:** Italy—(Wanasinghe et al. 2018).

***Stemphylium rosae-caninae*** (Wanas. et al.) Phukhams. & K.D. Hyde, **comb. nov.**

**Index Fungorum number:** IF557604; **Facesoffungi number:** FoF 04047

**Basionym:** *Pleospora rosae-caninae* Wanas., Camporesi, E.B.G. Jones & K.D. Hyde, in Wanasinghe et al., *Fungal Diversity* [157] (2018).

**Notes:** See notes under *Stemphylium rosae-caninae*.

**Host:** *Rosa canina*—(Wanasinghe et al. 2018).

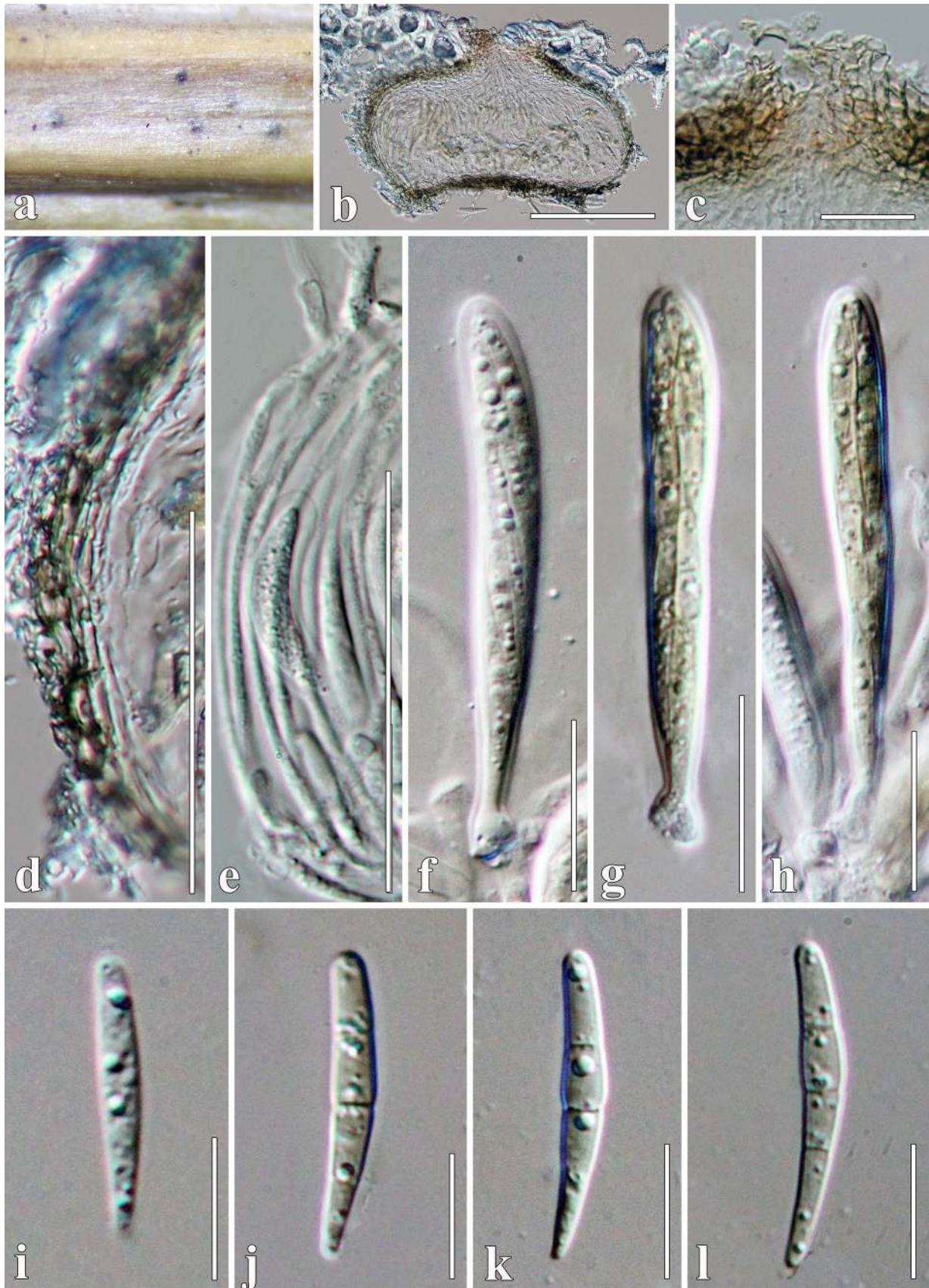
**Distribution:** Italy—(Wanasinghe et al. 2018).

***Stemphylium vesicarium*** (Wallr.) E.G. Simmons, *Mycologia* 61(1): 9 (1969), **new host record**

**Index Fungorum number:** IF339660; **Facesoffungi number:** FoF 04472, Figs. 61, 62.

**Saprobic** on dead stem of *Clematis vitalba*. **Sexual morph:** *Ascomata* 170–257  $\times$  148–250  $\mu\text{m}$  ( $\bar{x}$  = 220  $\times$  205  $\mu\text{m}$ ,  $n$  = 10), solitary, scattered, uniloculate, under epidermal layer of host, to superficial, globose to compressed, coriaceous, black, ostiolate. *Ostioles* central, papillate, with variable walls, opening by a pore, filled with hyaline periphyses. *Peridium* 25–49  $\mu\text{m}$  wide ( $\bar{x}$  = 40  $\mu\text{m}$ ,  $n$  = 20), thick, uniform, composed of 7–12 layers of *textura angularis*, black, heavy pigmented at the outer layer, cells toward the inside cells of lighter, inner layer composed of thin hyaline gelatinous layer. *Hamathecium* composed of numerous, dense, 1.6–2.25  $\mu\text{m}$  wide ( $\bar{x}$  = 1.8  $\mu\text{m}$ ,  $n$  = 20), filamentous, branched, septate, cellular pseudoparaphyses, situated between and above the asci embedded in a gelatinous matrix. *Asci* 103–138  $\times$  16–26  $\mu\text{m}$  ( $\bar{x}$  = 118  $\times$  23  $\mu\text{m}$ ,  $n$  = 30), 8-spored, bitunicate, fissitunicate, oblong to cylindrical-clavate with short, furcate pedicel, apically rounded, with an ocular chamber. *Ascospores* 20–30  $\times$  6–12  $\mu\text{m}$  ( $\bar{x}$  = 26  $\times$  10  $\mu\text{m}$ ,  $n$  = 50), biseriate or partially overlapping, muriform, tapering towards the ends, ends rounded, dictyosporous, 1(–2)-longitudinal euseptate, 4–10-transversely euseptate, constricted at the septa, strongly constricted at the median septum, yellowish, with 5–12  $\mu\text{m}$  wide drawn out mucilaginous sheath. **Asexual morph:** *Colonies* effuse on the surface of culture, scattered, hairy, and fluffy. *Mycelium* white, with 2–5  $\mu\text{m}$  wide, effuse, hyaline, septate. *Conidiophores* 10–57  $\times$  3–5  $\mu\text{m}$  ( $\bar{x}$  = 35  $\times$  4  $\mu\text{m}$ ,  $n$  = 20), macronematous, mononematous, simple, branched, stipes straight or flexuous, cylindrical, erect, septate, smooth, dark brown to brown, 3(–6)-septate, with 1–2 primary branches, irregularly branched at the upper parts, brown, smooth. *Conidiogenous cells* 5–12  $\times$  4–10  $\mu\text{m}$  ( $\bar{x}$  = 9  $\times$  7  $\mu\text{m}$ ,  $n$  = 20), monotretic, integrated or terminal, on conidiophores, dolii-form to oblong, pale brown. *Conidia* 14–31  $\times$  13–32  $\mu\text{m}$  ( $\bar{x}$  = 21  $\times$  21  $\mu\text{m}$ ,  $n$  = 30), in branched chains, acrogenous, folded, muriform, dictyosporous, 1(–2)-transversely euseptate, 1–3-horizontal euseptate, constricted at septa, dark brown to brown, bud scars or disjunctors present at the site of attachment, easily separated.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 16 °C. Cultures from above, dark green, fluffy, with dark green aerial mycelium, dense, fluffy at the edge, umbonate, rough, lobate, faintly zonate; reverse: dark green, radiating.



**Fig. 53** *Leptospora clematidis* (MFLU 17–1505, holotype). **a** Appearance of ascomata on *Clematis patens*. **b** Vertical section through ascoma. **c** Ostiolar canal. **d** Section of peridium. **e** Pseudoparaphyses. **f–h** Asci. **i–l** Ascospores. Scale bars: **b** = 100  $\mu$ m, **c**, **f–h** = 20  $\mu$ m, **d**, **e** = 50  $\mu$ m, **i–l** = 10  $\mu$ m

**Material examined:** Italy, Forli-Cesena Province, Viale Salinatore—Forli, dead stems of *Clematis vitalba*, 23 February 2015, E. Camporesi, IT 2983M (MFLU 16–1109); living culture, MFLUCC 16–0998.

**Hosts:** *Abies* sp., *Allium cepa*, *A. sativum*, *Asparagus officinalis*, *Brassica nigra*, *Brassica pekinensis*, *Cirsium* sp., *Citrus* sp., *Clematis vitalba*, *Cremanthodium discoideum*, *Cremanthodium discoideum*, *Dahlia pinnata*, *Dianthus caryophyllus*, *Lathyrus odoratus*, *Leucadendron* sp., *Linum usitatissimum*, *Lunaria annua*, *Lunaria rediviva*, *Malus domestica*, *Malus sieversii*, *Mangifera indica*, *Medicago sativa*, *Phaseolus vulgaris*, *Pisum sativum*, *Populus tomentosa*, *Pyrus sinkiangensis*, *Sedum spectabile*, *Solanum lycopersicum*, *Tamaric* sp., *Trigonella foenum-graecum*—(Simmons 1969; Simonyan 1981; Câmara et al. 2002; Köhl et al. 2009; Arzanlou et al. 2012; Ariyawansa et al. 2015c; Woudenberg et al. 2017; Farr and Rossman 2020; this study).

**Distribution:** Australia, Canada, China, Denmark, Germany, India, Italy, Netherlands, New Zealand, Portugal, South Africa, Tunisia, UK (England), USA (California)—(Simmons 1969; Simonyan 1981; Câmara et al. 2002; Köhl et al. 2009; Arzanlou et al. 2012; Ariyawansa et al. 2015c; Woudenberg et al. 2017; Farr and Rossman 2020; this study).

**GenBank accession numbers:** LSU: MT214584; SSU: MT226697; ITS: MT310629; *tefl*: MT394642.

**Notes:** *Stemphylium vesicarium* (= *Pleospora herbarum*) is a plant pathogen distributed on a range of wild and cultivated hosts (Köhl et al. 2009; Arzanlou et al. 2012; Woudenberg et al. 2017). Phylogenetic analysis of combined LSU, ITS and *gapdh* regions show that our isolate clusters with *S. vesicarium* (Fig. 59). The morphological comparison of our isolate (MFLUCC 16–0998) with *S. vesicarium* (CPC 29939) which was reported in Woudenberg et al. (2017) showed that our collection has 4–10-transverse eusepta ascospores (Figs. 61, 62). Our collection is similar to the type strain of *S. vesicarium* (STR) which was described by Simmons (1969). A pairwise comparison of the ITS regions show that our isolate is 100% identical to the type strain of *S. vesicarium* (CBS 192.86). This is the first report of *S. vesicarium* on *Clematis*.

### **Pseudoberkleasmiaceae** Phukhams. & K.D. Hyde

Pseudoberkleasmiaceae was introduced to accommodate a berkleasmiium-like species that has phylogenetic stability in Pleosporales (Hyde et al. 2019a). The family comprises *Pseudoberkleasmiium acacia*, *Pseudoberkleasmiium*

*chiangmaiense* and *P. pandanicola* (the generic type). We describe the first record of *P. chiangmaiense* on *Clematis* from Thailand (Figs. 2, 63).

### **Pseudoberkleasmiium** Tibpromma & K.D. Hyde

The monotypic genus *Pseudoberkleasmiium* was introduced with *P. pandanicola* Tibpromma & K.D. Hyde as the type species. *Pseudoberkleasmiium* is characterized by hyaline, subglobose conidiogenous cells and acrogenous, broadly ellipsoidal to obovoid, muriform, brown or olivaceous green, and guttulate conidia. There is no sexual morph report in this family. A collection on *Clematis sikkimensis* reveals an additional strain of *Pseudoberkleasmiium chiangmaiense* according to phylogenetic and morphological evidence (Figs. 2, 63).

### **Pseudoberkleasmiium chiangmaiense** Y.Z. Lu & K.D. Hyde, in Hyde et al., Fungal Diver (2019), **new host record**

**Index Fungorum number:** IF555595; **Facesoffungi number:** FoF 05310, Fig. 63.

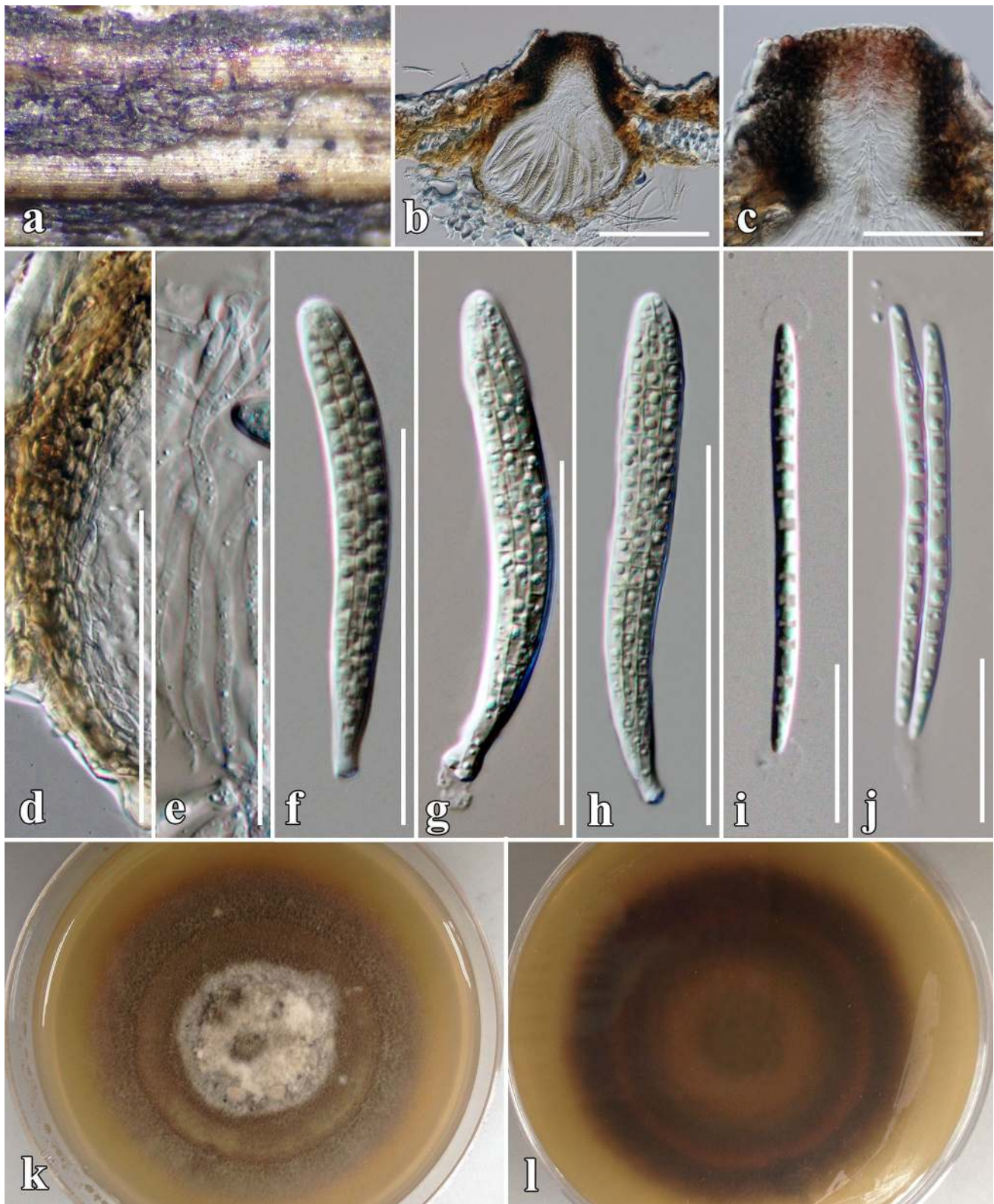
**Saprobic** on dead stems of *Clematis sikkimensis*. **Sexual morph:** Undetermined. **Asexual morph:** *Hyphomycetous*, colonies on natural substrate forming sporodochial conidiomata, 109–460  $\mu$ m wide, superficial, scattered, gregarious, oval, brown, velvety, glistening, orbicular, conidia readily liberated when agitated. *Mycelium* immersed in the substrate, composed of septate, branched, smooth, hyaline to pale brown, 2.5  $\mu$ m wide hyphae. *Conidiophores* 10–25  $\times$  2–5  $\mu$ m, micronematous, mononematous, cylindrical or truncate, erect, hyaline, smooth-walled. *Conidiogenous cells* 9–15  $\times$  6–15  $\mu$ m ( $\bar{x}$  = 12  $\times$  11  $\mu$ m, n = 20), holoblastic, monoblastic, integrated, terminal, determinate, subglobose, cylindrical or slightly truncate, guttulate, hyaline. *Conidia* 24–32  $\times$  13–18  $\mu$ m ( $\bar{x}$  = 27  $\times$  16  $\mu$ m, n = 50), acrogenous, solitary, broadly ellipsoidal to obovoid, flattened, dictyosporous, muriform, apex rounded, basal cells globose or subglobose, in side view composed of one column of 4–6 cells, guttulate, smooth-walled, brown, usually with conidiogenous cell attached, bud scars or disjunctors present at the site of attachment.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Cultures from above black, dense, circular, umbonate, papillate, fluffy, slightly radiating, wrinkled folded; reverse black, lifting media up in the centre, wrinkled.

**Material examined:** Thailand, Chiang Rai Province, Doi Tung, on dried stem of *Clematis sikkimensis*, 2 May 2017, C. Phukhamsakda & M.V. de Bult, CMTHDT05 (MFLU 17–1496); living culture, MFLUCC 17–2088.

**Hosts:** *Clematis fulvicoma*, Undetermined decaying wood—(Hyde et al. 2019a; this study).

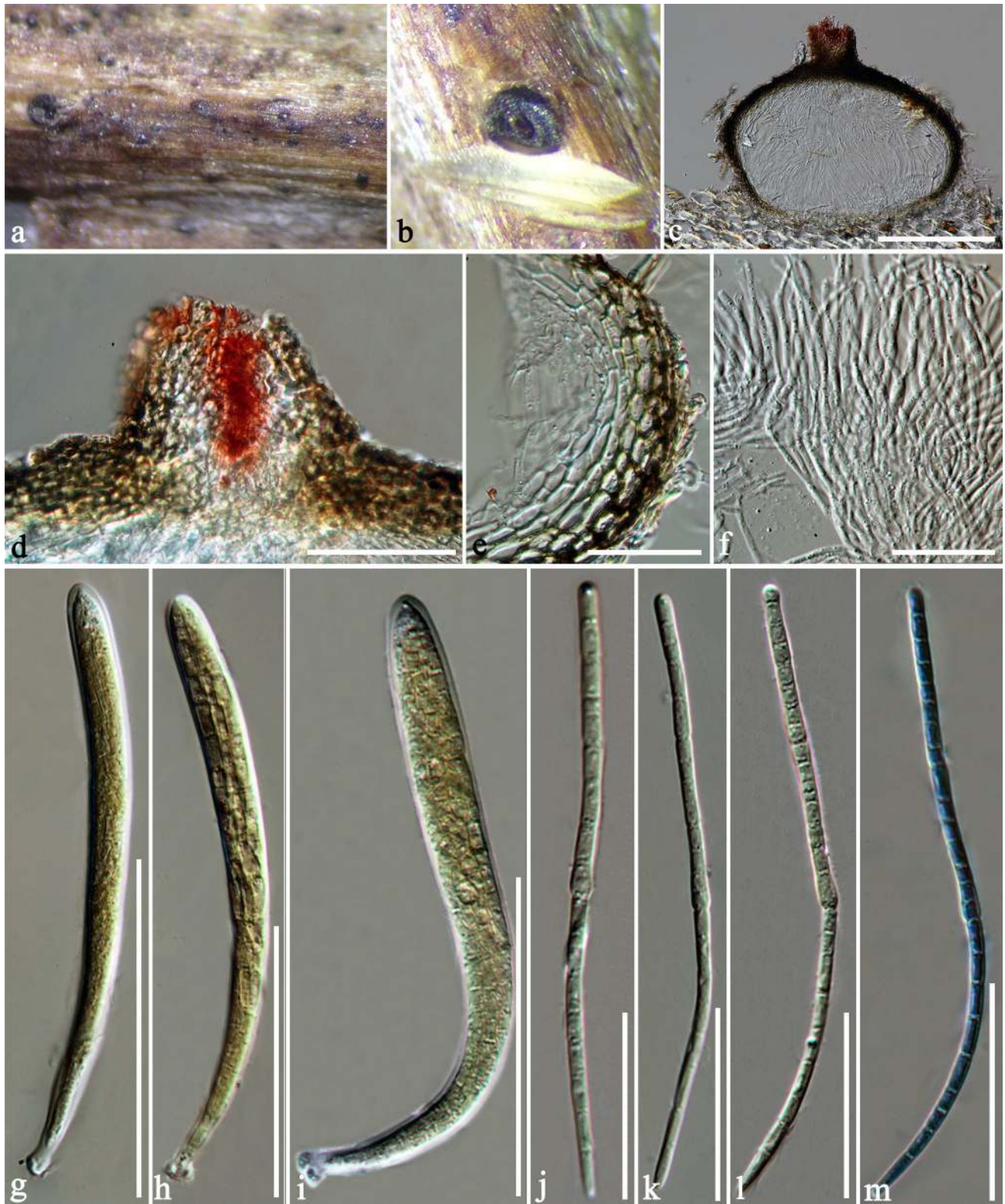
**Distribution:** Thailand—(Hyde et al. 2019a; this study).



**Fig. 54** *Leptospora thailandica* (MFLU 17–1474). **a** Appearance of ascomata on *Clematis subumbellata*. **b** Vertical section through ascoma. **c** Ostiolar canal. **d** Section of peridium. **e** Pseudoparaphyses.

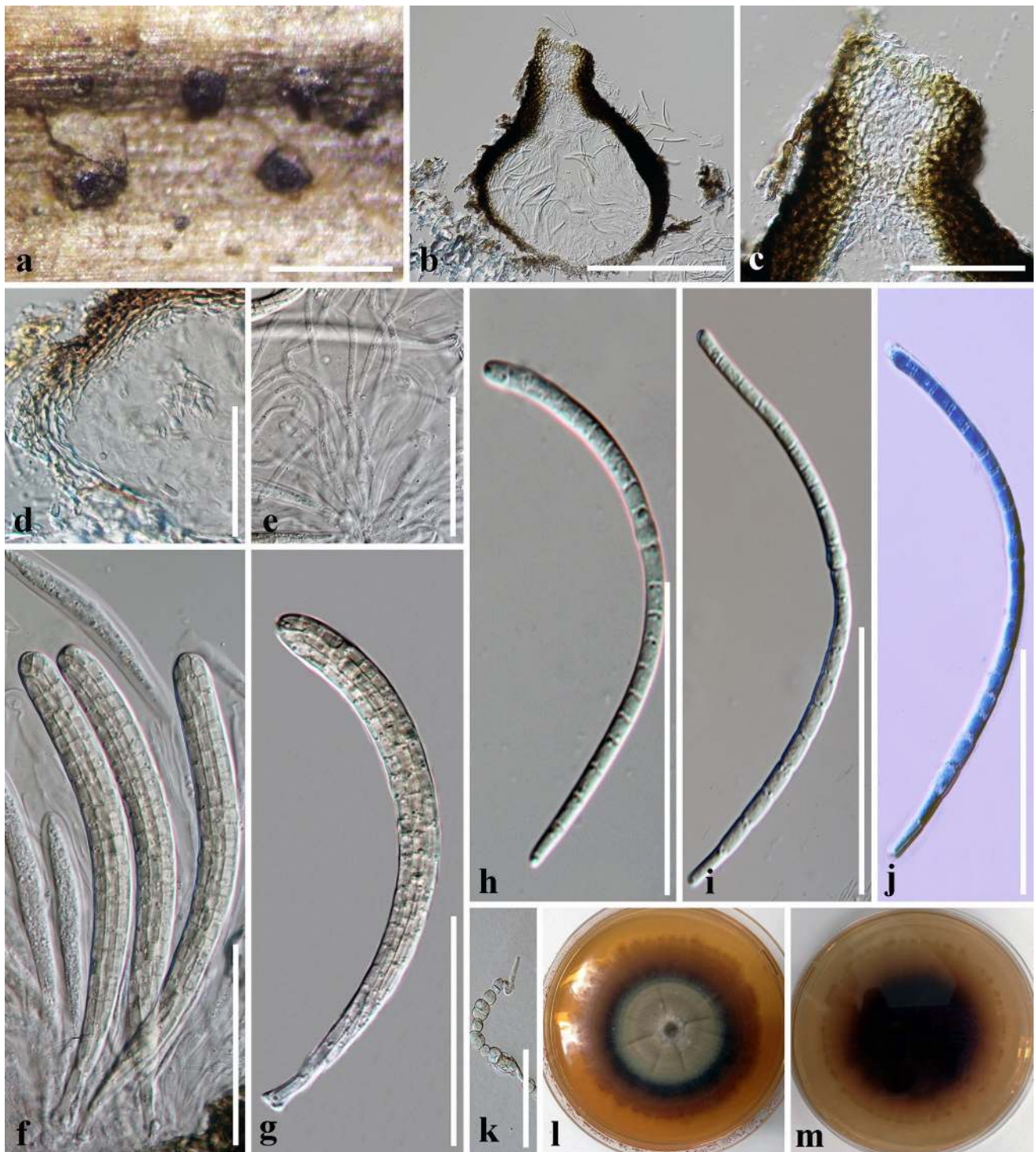
**f–h** Asci. **i, j** Ascospores. **k, l** Culture characteristics on MEA. Scale bars: **b** = 100  $\mu$ m, **c–h** = 50  $\mu$ m, **i, j** = 20  $\mu$ m





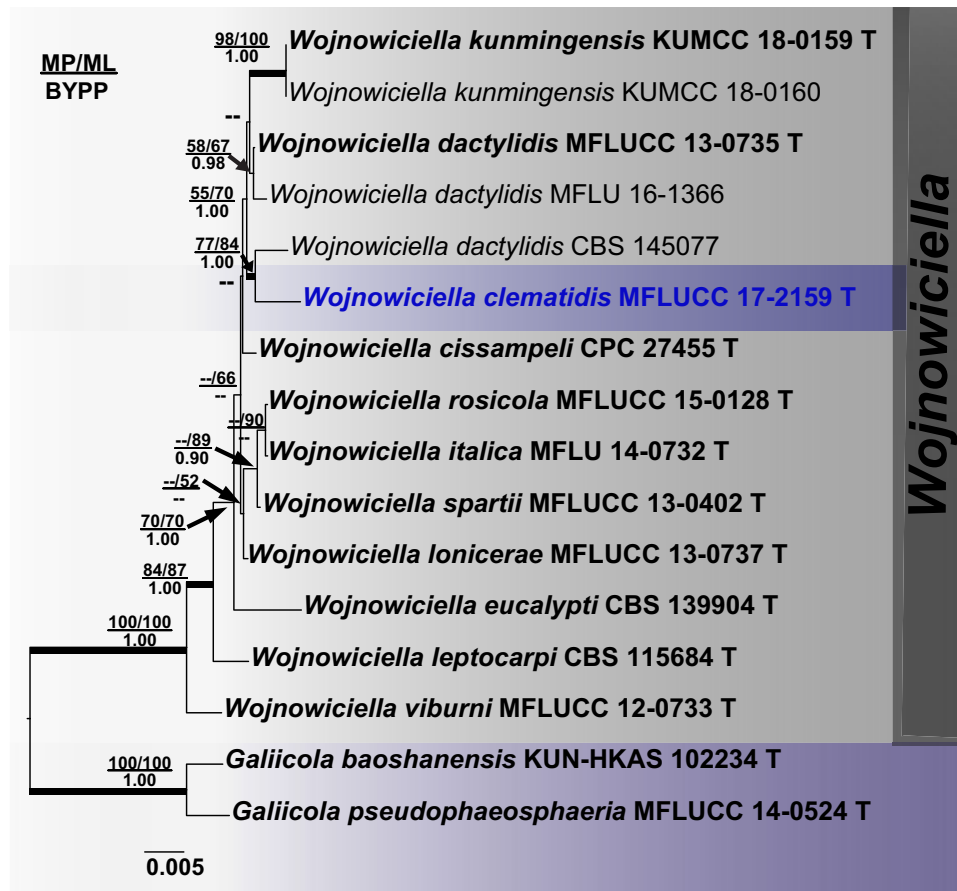
**Fig. 55** *Longispora clematidis* (MFLU 20–0420, holotype). **a, b** Appearance of ascomata on *Clematis vitalba*. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Pseudo-

paraphyses. **g–i** Asci. **j–m** Ascospores (**m** Ascospore in cotton blue). Scale bars: **c** = 100  $\mu$ m, **d–f, j–m** = 20  $\mu$ m, **g–i** = 50  $\mu$ m



**Fig. 56** *Pseudoophiobolus rosae* (MFLU 15–1014). **a** Appearance of ascomata on *Clematis vitalba*. **b** Vertical section through ascoma. **c** Ostiolar canal. **d** Section of peridium. **e** Pseudoparaphyses. **f–g** Asci.

**h–j** Ascospores (**j** in cotton blue). **k** Germinated ascospore. **l, m** Culture characteristics on MEA. Scale bars: **a**=500  $\mu$ m, **b**=200  $\mu$ m, **c–k**=50  $\mu$ m



**Fig. 57** Phylogram obtained from maximum likelihood based on combined LSU, SSU, ITS, *tef1* and *rpb2* sequence data. The tree is rooted with *Galiicola baoshanensis* (KUN-HKAS 102234) and *Galiicola pseudophaeosphaeria* (MFLUCC 14-0524). Related sequences are retrieved from GenBank with 16 strains included in the analysis of the combined loci and comprises 4177 characters (820 characters for LSU, 1012 characters for SSU, 561 characters for ITS, 905 characters for *tef1*, 879 characters for *rpb2*, including gaps). The best scoring RAxML tree had a final likelihood value of  $-7456.746869$ . The matrix had 277 distinct alignment patterns with 31.92% undetermined characters and gaps. Estimated base frequencies were: A=0.244763, C=0.241642, G=0.263061, T=0.250533; substitution rates AC=1.095229, AG=1.632728, AT=1.392298,

CG=0.793970, CT=6.972828, GT=1.000000; gamma distribution shape parameter  $\alpha=6.674062$ . Maximum parsimony analysis of 111 parsimony informative characters resulted in a most parsimonious tree (CI=0.916, RI=0.858, RC=0.786, HI=0.084). In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. Bootstrap values (BS) from maximum parsimony (MP, left), maximum likelihood (ML, right) higher than 50% BS and Bayesian posterior probabilities (BYPP, below) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% ML/0.90 BYPP. Thick branches represent significant support values from all analyses (ML $\geq$ 70%/BYPP $\geq$ 0.95). The ex-type strains are in bold and black. The newly generated sequence is in bold and blue

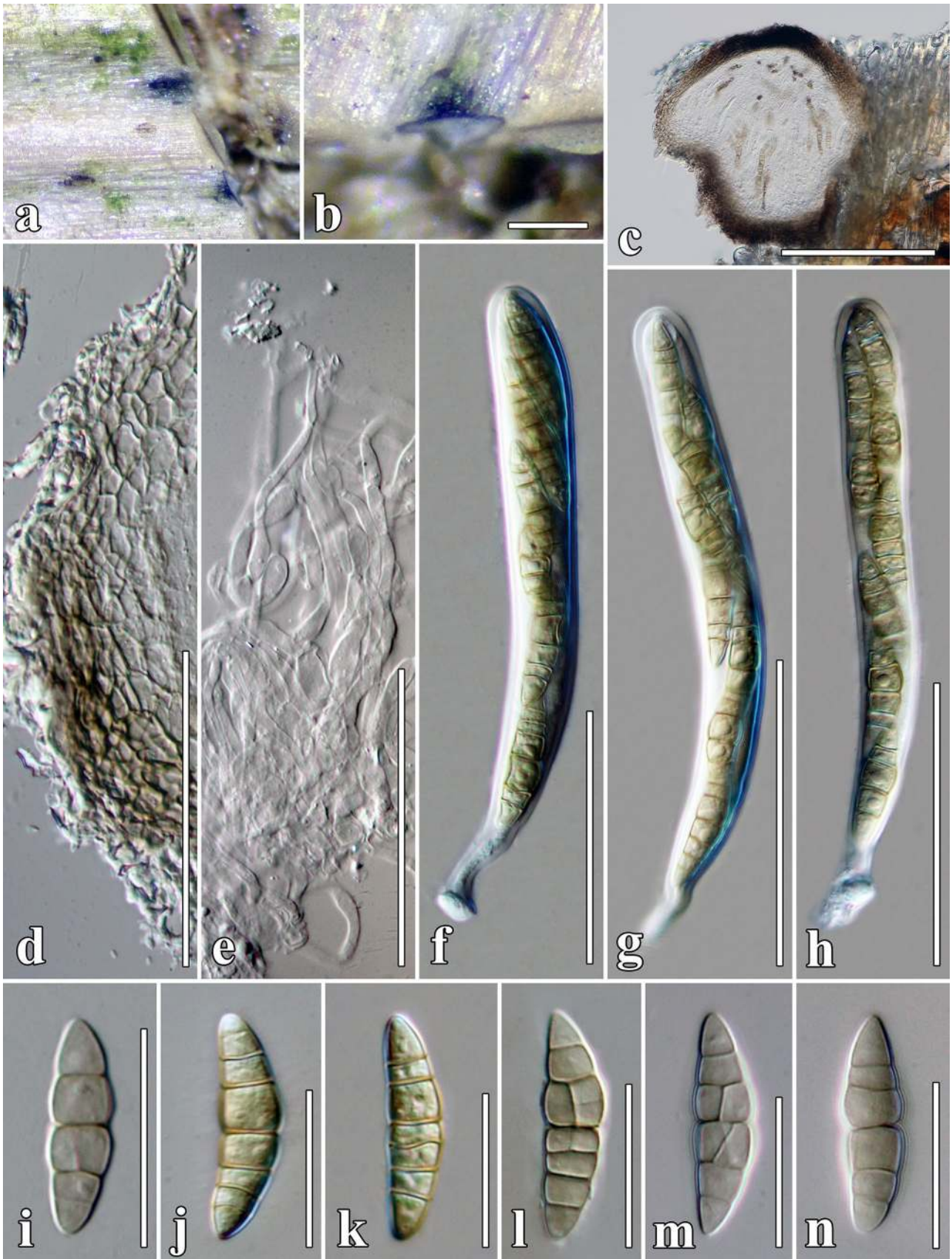
**GenBank accession numbers:** LSU: MT214585; SSU: MT226698; ITS: MT310630; *tef1*: MT394643; *rpb2*: MT394699.

**Notes:** *Pseudoberkleasium changmaiense* (MFLUCC 17-2088) is similar to the type except for culture characters (Hyde et al. 2019a, Fig. 63), however, our collection was grown on MEA while the type was cultured on PDA. Phylogenetically, *P. changmaiense* (strain MFLUCC 17-2088) formed a clade with the type strain (MFLUCC 17-1809) with strong support (100% ML/1.00 BYPP, Fig. 2). The type strain was isolated from decaying wood collected in Chiang Mai, therefore, we present a new host record of *P. changmaiense* on *Clematis sikkimensis* (Fig. 63).

**Pseudomassarinaeae** Phukhams. & K.D. Hyde, **fam. nov.**

**Index Fungorum number:** IF557104; **Facesoffungi number:** FoF 07212, Fig. 64.

**Saprobic** on dried herbaceous plants. **Sexual morph:** *Ascomata* immersed, only ostioles visible, uniloculate, obpyriform to subglobose, light brown to brown, coriaceous. *Ostioles* central, brown to dark brown, carbonaceous, papillate. *Peridium* thin, multilayered, comprising thin-walled, light brown to brown cells of *textura angularis*, inner layers comprising hyaline cells. *Hamathecium* of dense, filiform, branched, pseudoparaphyses. *Asci* 8-spored, bitunicate, fission-tunicate, thick-walled, oblong, apically rounded, with furcate pedicel. *Ascospores* biserial, overlapping, hyaline, broad



**Fig. 58** *Wojnowiciella clematidis* (MFLU 17–1517, holotype). **a, b** Appearance of ascomata on host surface. **c** Vertical section through ascoma. **d** Section of peridium. **e** Pseudoparaphyses. **f–h** Asci. **i–n** Ascospores. Scale bars: **b, c** = 200  $\mu\text{m}$ , **d–h** = 50  $\mu\text{m}$ , **i–n** = 20  $\mu\text{m}$

fusiform, tapering towards the ends, acute at both ends, with transverse septa, with mucilaginous sheath. **Asexual morph:** Undetermined.

*Type genus:* *Pseudomassarina* Phukhams. & K.D. Hyde

*Notes:* The new family Pseudomassarinaceae is introduced to accommodate a monotypic genus, *Pseudomassarina* (Fig. 64). Phylogenetic analysis which included related families in Pleosporales, showed that Pseudomassarinaceae formed a distinct lineage related to Amorosiaceae, Halotthiaceae, Lophiostomataceae, Neomassarinaceae, Phaeoseptaceae and Sporormiaceae (Fig. 2). The family is morphologically similar to Lophiostomataceae, Neomassarinaceae and Neomassarinaceae in having hyaline, ellipsoid to fusiform, septate ascospores (Thambugala et al. 2015; Ariyawansa et al. 2018; Mapook et al. 2020). Pseudomassarinaceae is distinguished by its immersed, coriaceous ascomata with crest-like, carbonaceous ostioles, oblong and short pedicellate asci and wide mucilaginous sheath surrounding the ascospores.

***Pseudomassarina* Phukhams. & K.D. Hyde, gen. nov.**

*Index Fungorum number:* IF557097; *Facesoffungi number:* FoF 07213, Fig. 64.

*Etymology:* The genus epithet reflects its morphological similarity to *Massarina* species.

*Saprobic* on decaying plants in terrestrial habitats. **Sexual morph:** *Ascomata* on surface of the host, visible as black spots, immersed, solitary, uniloculate, obpyriform, brown, rough-walled, coriaceous, with apical ostioles. *Ostioles* central, brown to dark brown, partially carbonaceous, papillate, filled with periphyses. *Peridium* multilayered, comprising thin-walled, light brown to brown cells of *textura angularis*, inner layers comprising hyaline cells. *Hamathecium* of dense, filiform, branched, transverse septa, pseudoparaphyses. *Asci* 8-spored, bitunicate, fissionate, thick-walled, oblong, apically rounded, short pedicellate, with an ocular chamber. *Ascospores* biserial, overlapping, hyaline, broad fusiform, tapering towards the ends, with transverse septum, guttulate, smooth-walled, with mucilaginous sheath. **Asexual morph:** Undetermined.

*Type species:* *Pseudomassarina clematidis* Phukhams, Camporesi & K.D. Hyde

*Notes:* *Pseudomassarina*, is typified by *P. clematidis* and formed a distinct lineage in Pleosporales (Fig. 2). The morphological comparison showed that our collection has unique characters among related taxa in Pleosporales (Zhang et al. 2012; Hyde et al. 2013; Thambugala et al. 2015; Ariyawansa et al. 2018; Mapook et al. 2020). In a BLASTn

search of GenBank, the closest match of the LSU sequence of MFLU 16–0493 is *Preussia terricola* (strain CBS 317.65) with 97.43% similarity. The closest match with the ITS sequence is *Preussia polymorpha* (strain CBS 117679) with 84.49% similarity.

***Pseudomassarina clematidis* Phukhams, Camporesi & K.D. Hyde sp. nov.**

*Index Fungorum number:* IF557098; *Facesoffungi number:* FoF 07214, Fig. 64.

*Etymology:* The epithet reflects *Clematis*.

*Holotype:* MFLU 16–0493.

*Saprobic* on dead stems of *Clematis vitalba*. **Sexual morph:** *Ascomata* 150–220  $\times$  80–130  $\mu\text{m}$  ( $\bar{x}$  = 190  $\times$  110  $\mu\text{m}$ ,  $n$  = 5), on surface of the host, visible as black spots, immersed, only ostioles visible, solitary, scattered, uniloculate, obpyriform to subglobose, light brown to brown, rough-walled, coriaceous, with apical ostioles. *Ostioles* central, 65–70  $\times$  50–55  $\mu\text{m}$ , brown to dark brown, papillate, opening by a pore, filled with periphyses. *Peridium* 10–20  $\mu\text{m}$  wide, multilayered, comprising of 4–5 layers of thin-walled light brown to brown cells of *textura angularis*, inner layers comprising hyaline cells. *Hamathecium* of dense, 1.3–1.5  $\mu\text{m}$  wide ( $\bar{x}$  = 1.3  $\mu\text{m}$ ,  $n$  = 50), filiform, branched, septate, pseudoparaphyses. *Asci* 55–80  $\times$  9–15  $\mu\text{m}$  ( $\bar{x}$  = 60  $\times$  10  $\mu\text{m}$ ,  $n$  = 30), 8-spored, bitunicate, fissionate, thick-walled, oblong, apically rounded, with short, furcate pedicel, ocular chamber clearly visible. *Ascospores* 16–25  $\times$  3–7  $\mu\text{m}$  ( $\bar{x}$  = 20  $\times$  5  $\mu\text{m}$ ,  $n$  = 50), biserial, overlapping, hyaline, broad fusiform, tapering towards the ends, acute at both ends, with 1 transverse septum, with large guttules in each cell, deeply constricted at the septum, cell above septum longer and wider than below cell, smooth-walled, with 4–6  $\mu\text{m}$  wide mucilaginous sheath. **Asexual morph:** Undetermined.

*Material examined:* Italy, Forlì-Cesena Province, Fiumicello-Premilcuore, on dead aerial branch of *Clematis vitalba*, 20 March 2016, E. Camporesi, IT 2335 (MFLU 16–0493, **holotype**).

*Host:* *Clematis vitalba*—(This study).

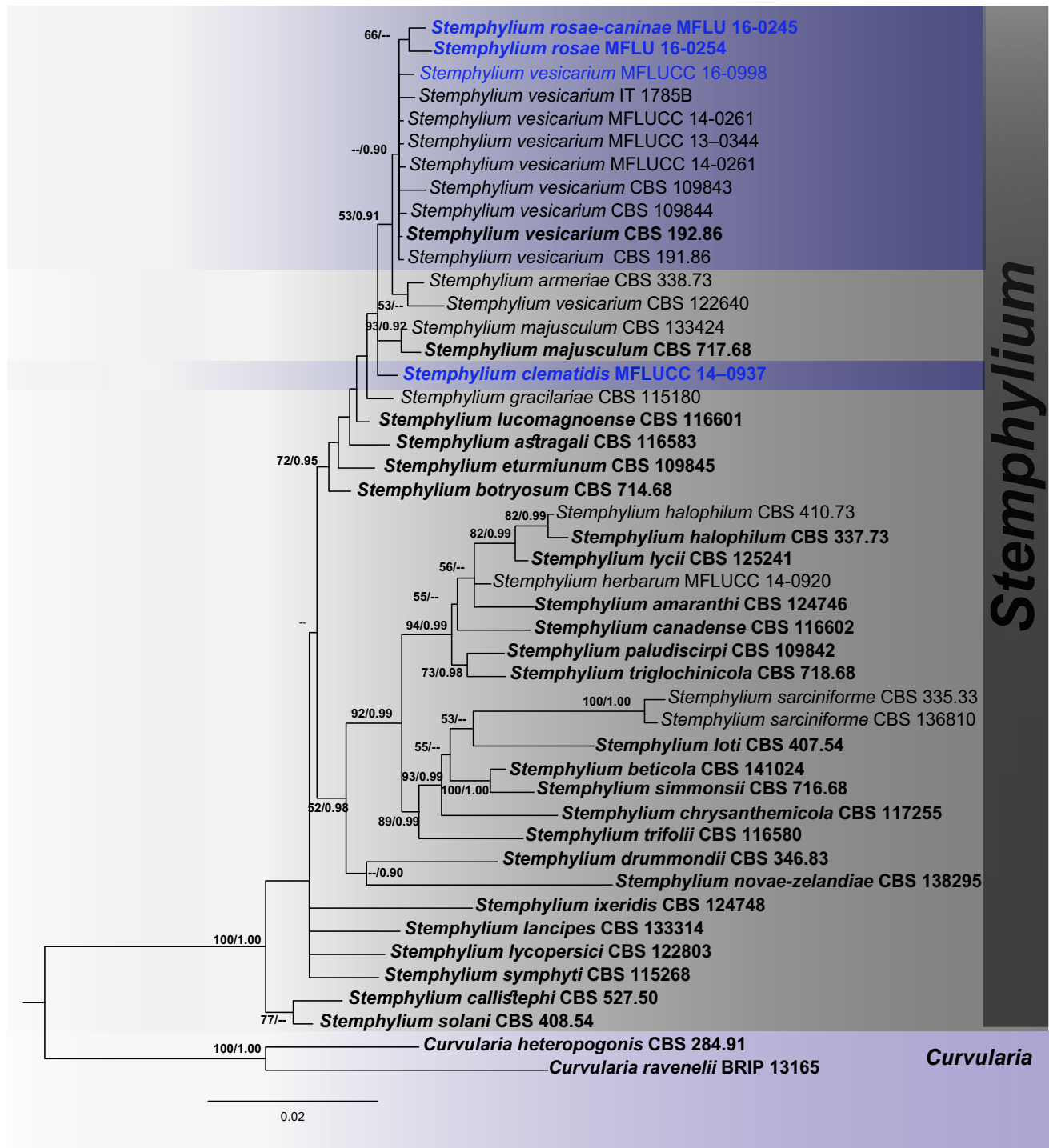
*Distribution:* Italy—(This study).

*GenBank accession numbers:* LSU: MT214586; SSU: MT226699, MT310631; ITS: MT415397; *tef1*: MT394644; *rpb2*: MT394700.

*Notes:* See note under *Pseudomassarina*.

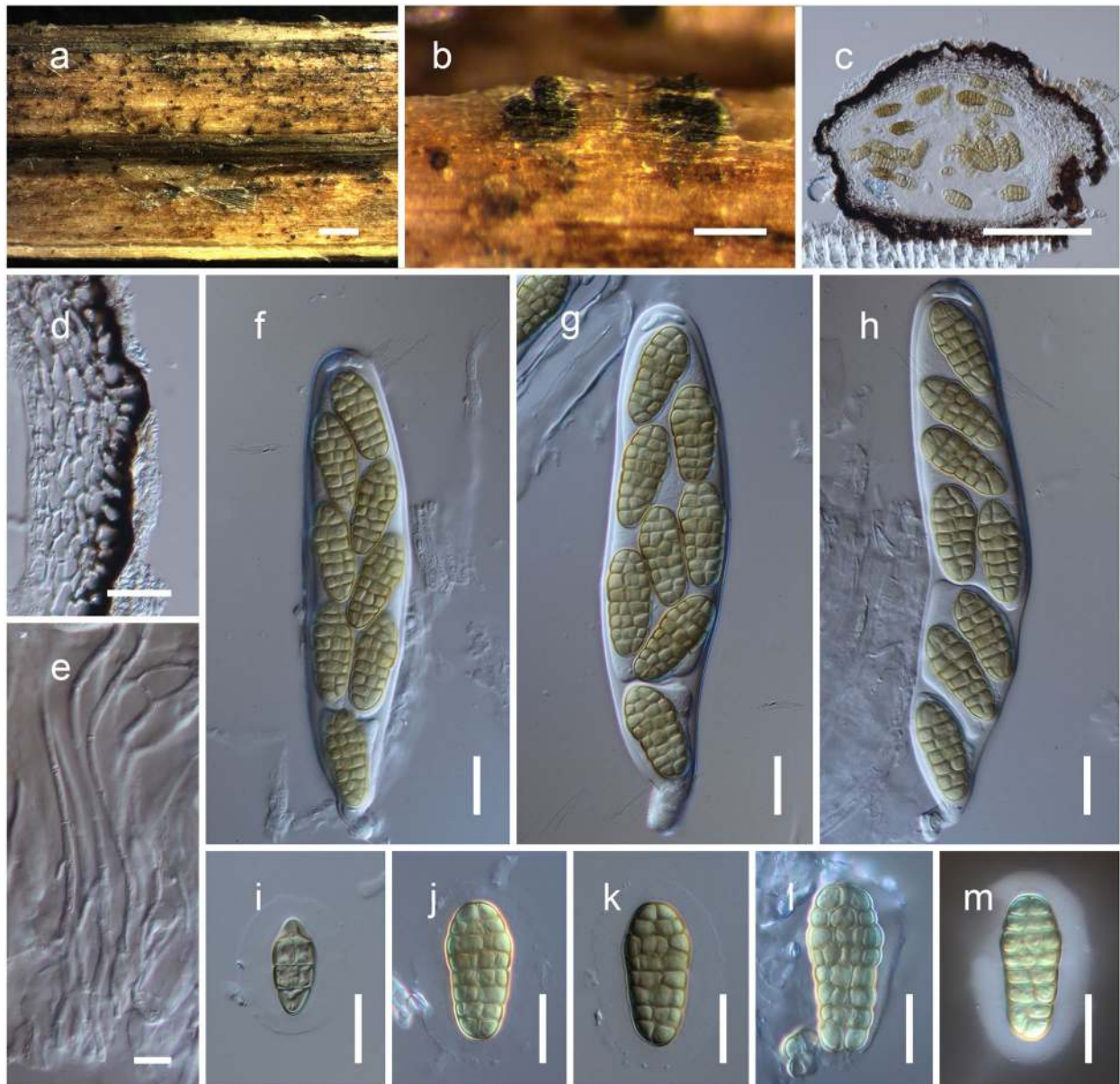
**Pseudolophiotremataceae** K.D. Hyde & S. Hongsanan

Pseudolophiotremataceae was introduced by Hongsanan et al. (2018) with *Pseudolophiotrema elymicola* as the generic type. The multi-locus analysis (Fig. 2) showed that *Clematidis italica* (MFLUCC 15–0084) clustered with *P. elymicola*. Thus, we place *Clematidis italica* (Fig. 65) in Pseudolophiotremataceae.



**Fig. 59** The Bayesian 50% majority-rule consensus phylogram based on combined LSU, ITS and *gadh* data for *Stemphylium*. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted with *Curvularia* species. Forty-six strains were included in the combined gene sequence analyses which comprised 2052 characters (902 characters for LSU, 555 characters for ITS, 595 characters for *gadh*, including gap regions). The best scoring RAxML tree had a likelihood value of  $-6557.926637$ . The matrix had 441 distinct alignment patterns with 29.84% undetermined characters and gaps. Estimated base frequencies were as follows; A=0.245289, C=0.253669, G=0.255798,

T=0.245244; substitution rates AC=1.484723, AG=3.274866, AT=0.996508, CG=1.050822, CT=5.664441, GT=1.000000; gamma distribution shape parameter  $\alpha=0.781417$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study are indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95)



**Fig. 60** *Stemphylium clematidis* (MFLU 16–0176, **holotype**). **a** Appearance of ascomata on host surface. **b** Close up of ascomata. **c** Vertical section through ascoma. **d** Peridium. **e** Pseudoparaphyses. **f–h**

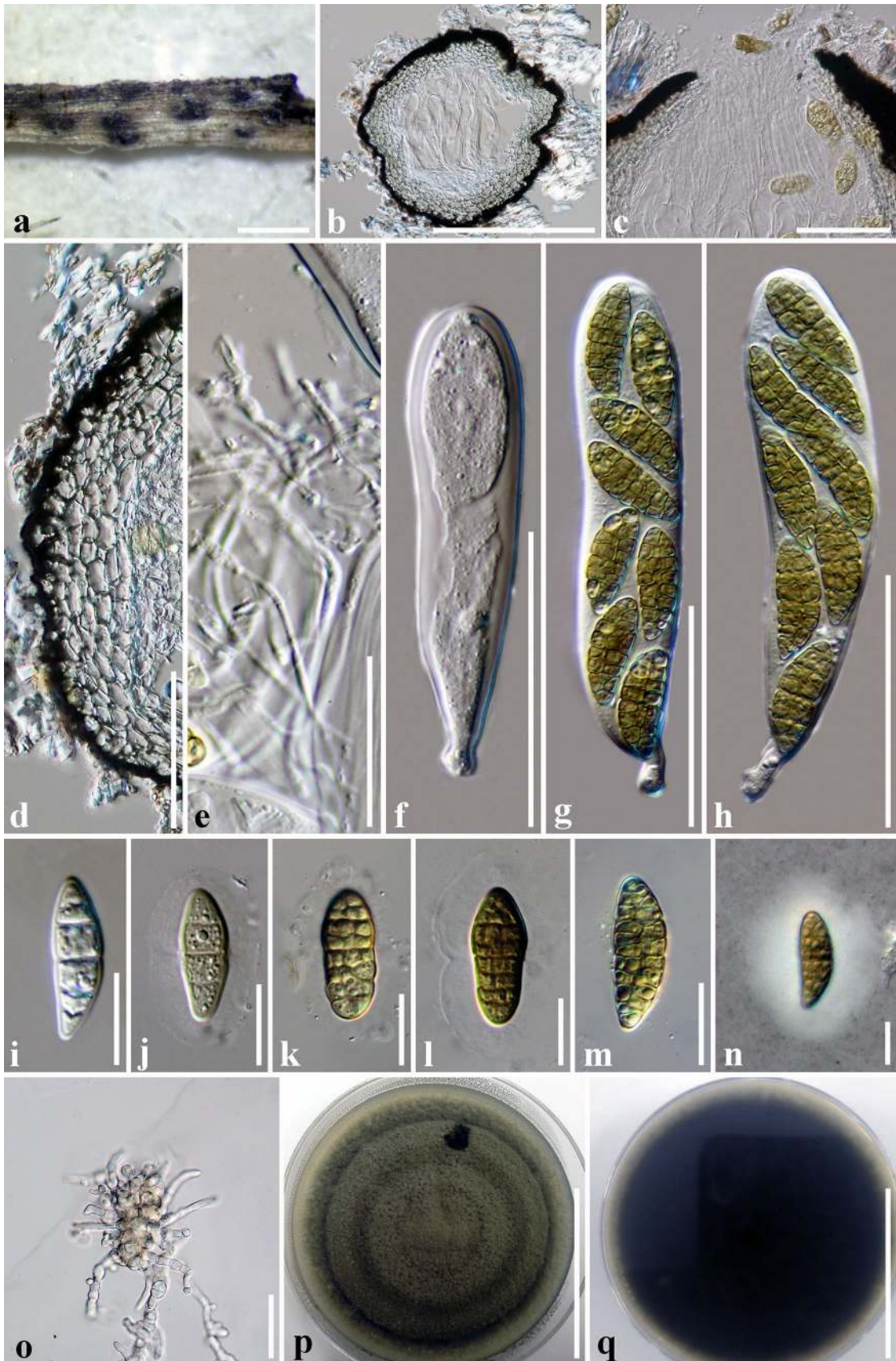
Asci. **i–m** Ascospores (**m** Ascospore stained in 10% India ink). Scale bars: **a** = 500  $\mu$ m, **b** = 200  $\mu$ m, **c** = 100  $\mu$ m, **d–h** = 20  $\mu$ m, **i–m** = 10  $\mu$ m

*Clematidis italica* Tibpromma, Camporesi & K.D. Hyde, in Li et al., Fungal Diversity 78: 60 (2016)

*Index Fungorum* number: IF 551867, *Facesoffungi* number: FoF 01813, Fig. 65.

*Notes*: Li et al. (2016) introduced *Clematidis italica* (strain MFLUCC 15–0084) based on analyses of combined LSU and SSU sequence data. Subsequently, Hashimoto et al. (2017) introduced *Pseudolophiotrema elymicola* (MAFF 239600) based on analyses of a SSU, ITS, LSU, *tef1* and

*rpb2* dataset (Jaklitsch et al. 2018). In our LSU phylogenetic tree for Pleosporales, *Clematidis* and *Pseudolophiotrema* formed a closely related clade with strong support (94% ML/0.98 BYPP, data not shown). Consequently, in a combined dataset of LSU, SSU, ITS, *tef1* and *rpb2*, *Clematidis italica* (MFLUCC 15–0084) and *Pseudolophiotrema elymicola* (JCM 13090) formed a well-supported clade (100% ML/1.00 BYPP) basal to Anteagloniaceae (Fig. 2). A similar topology is shown in Hashimoto et al. (2017) wherein *P.*





◀**Fig. 61** *Stemphylium vesicarium* (MFLU 16–1109). **a** Appearance of ascomata on host surface. **b** Vertical section of ascoma. **c** Ostiolar canal. **d** Section of peridium. **e** Pseudoparaphyses. **f–h** Asci. **i–n** Ascospores (**n** Ascospore in 10% Indian ink). **o** Germinated ascospore. **p, q** Culture characteristics on MEA. Scale bars: **a**=500  $\mu\text{m}$ , **b**=200  $\mu\text{m}$ , **c–h**=50  $\mu\text{m}$ , **i–n**=10  $\mu\text{m}$ , **o**=20  $\mu\text{m}$ , **p, q**=50 mm

*elymicola* formed a distinct clade from Lophiotremataceae. As the epithet “clematidis” is similar to the plant family Clematidaceae/Clemataceae, it is difficult to introduce a generic epithet. Therefore, *Pseudolophiotrema elymicola* is selected as a generic type for Pseudolophiotremataceae (Hongsanant et al. 2020).

### Roussoellaceae Liu, Phookamsak, Dai & K.D. Hyde

Roussoellaceae was introduced by Liu et al. (2014) and family members have steadily increased (Jaklitsch and Voglmayr 2016; Tibpromma et al. 2017, 2018; Wanasinghe et al. 2018; Jiang et al. 2019; Karunarathna et al. 2019; Phookamsak et al. 2019; Mapook et al. 2020). We provide an updated phylogenetic analysis for Roussoellaceae based on a concatenated LSU, ITS, *tef1*, *rpb2*, and SSU sequence dataset. New host records and new species occurring on *Clematis* species in Thailand are described based on morphological characteristics coupled with multigene phylogenetic analyses (Fig. 66).

#### *Neorousoella* Liu et al.

*Neorousoella* was introduced with the single species, *N. bambusae* Phookamsak, J.K. Liu & K.D. Hyde. Phylogenetic placement of the species in Roussoellaceae was demonstrated by Karunarathna et al. (2019) and Mapook et al. (2020). A multigene phylogenetic analysis (Fig. 66) reveals two novel species *N. clematidis* and *N. fulvicomae*, and the first record of *N. heveae* from a *Clematis* species.

#### *Neorousoella clematidis* Phukhams. & K.D. Hyde, sp. nov.

*Index Fungorum number*: IF557113; *Facesoffungi number*: FoF 07328, Fig. 67.

*Etymology*: Name refers to the host genus, *Clematis*.

*Holotype*: MFLU 17–1467

*Saprobic* on dried stems of *Clematis subumbellata*. **Sexual morph**: *Ascomata* 230–250  $\times$  155–160  $\mu\text{m}$  ( $\bar{x}$ =235  $\times$  158  $\mu\text{m}$ ,  $n$ =5), only ostioles present on the surface of host, solitary, gregarious, erumpent, semi-immersed, globose to subglobose, black to rust brown, coriaceous, rough-walled, ostiolate. *Ostioles* central, 84–90  $\times$  67–70  $\mu\text{m}$ , dark brown to black, papillate, ostiolar canal lined with paraphyses. *Peridium* 12–29  $\mu\text{m}$  wide, outer layer composed of 5–7 layers of brown to chestnut brown cells of *textura angularis*, with thin, hyaline inner layer. *Hamathecium* of dense, 0.8–1.5  $\mu\text{m}$  wide ( $\bar{x}$ =1.3  $\mu\text{m}$ ,  $n$ =40), filiform, branched, septate, trabeculate pseudoparaphyses. *Asci* 62–82  $\times$  4–7  $\mu\text{m}$

( $\bar{x}$ =71  $\times$  5  $\mu\text{m}$ ,  $n$ =30), (2–)8-spored, bitunicate, fissitunicate, cylindrical, apically rounded, with bulbous pedicel, ocular chamber visible when young. *Ascospores* 9–11  $\times$  3–5  $\mu\text{m}$  ( $\bar{x}$ =10  $\times$  4  $\mu\text{m}$ ,  $n$ =50), uniseriate, partially overlapping, ellipsoid with rounded ends, olive to yellowish brown, 1-septate, constricted at septum, guttulate in each cell, rough-walled, at maturity, slightly longitudinally ribbed, without a mucilaginous sheath. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA reaching 20 mm diam. after 4 weeks at 25 °C. Culture from above, brown, radiating outwards, dense, irregular in shape, umbonate, dull, edge erose, downy, covered with fairly white mycelium; reverse brown at the middle dark brown at the edge, with domes on the media.

*Material examined*: Thailand, Chiang Rai Province, on dead branches of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH03 (MFLU 17–1467, **holotype**); ex-type living culture, MFLUCC 17–2061.

*Host*: *Clematis subumbellata*—(This study).

*Distribution*: Thailand—(This study).

*GenBank accession numbers*: LSU: MT214587; SSU: MT226700; ITS: MT310632; *tef1*: MT394645; *rpb2*: MT394701.

*Notes*: *Neorousoella clematidis* shares common characters with *Neorousoella* in having uniloculate ascomata without a clypeus, cylindrical to broad filiform asci with bulbous pedicel and 1-septate ascospores (Liu et al. 2014). Our collection is distinguishable by having immersed and globose ascomata with papillate ostioles (Fig. 67). In the phylogenetic analysis, *N. clematidis* (MFLUCC 17–2061) formed a strongly supported clade (100% ML/1.00 BYPP, Fig. 66) with *N. fulvicoma* (MFLUCC 17–2073) which was an asexual morph (Fig. 68). A comparison of the ITS region (including 5.8S region) showed 7.7% base pair differences (with gaps) and 3.4% base pair differences (with gaps) in the *tef1* region, which is evidence for new species rank.

The isolate MFLUCC 17–2061 was evaluated for the potential of secondary metabolites against *Bacillus subtilis*, *Escherichia coli*, *Mucor plumbeus* and *Schizosaccharomyces pombe*. The strain demonstrated moderate inhibitory activities against *Bacillus subtilis* and against conidia development in *Mucor plumbeus*. This isolate is suitable for further evaluation of secondary metabolites.

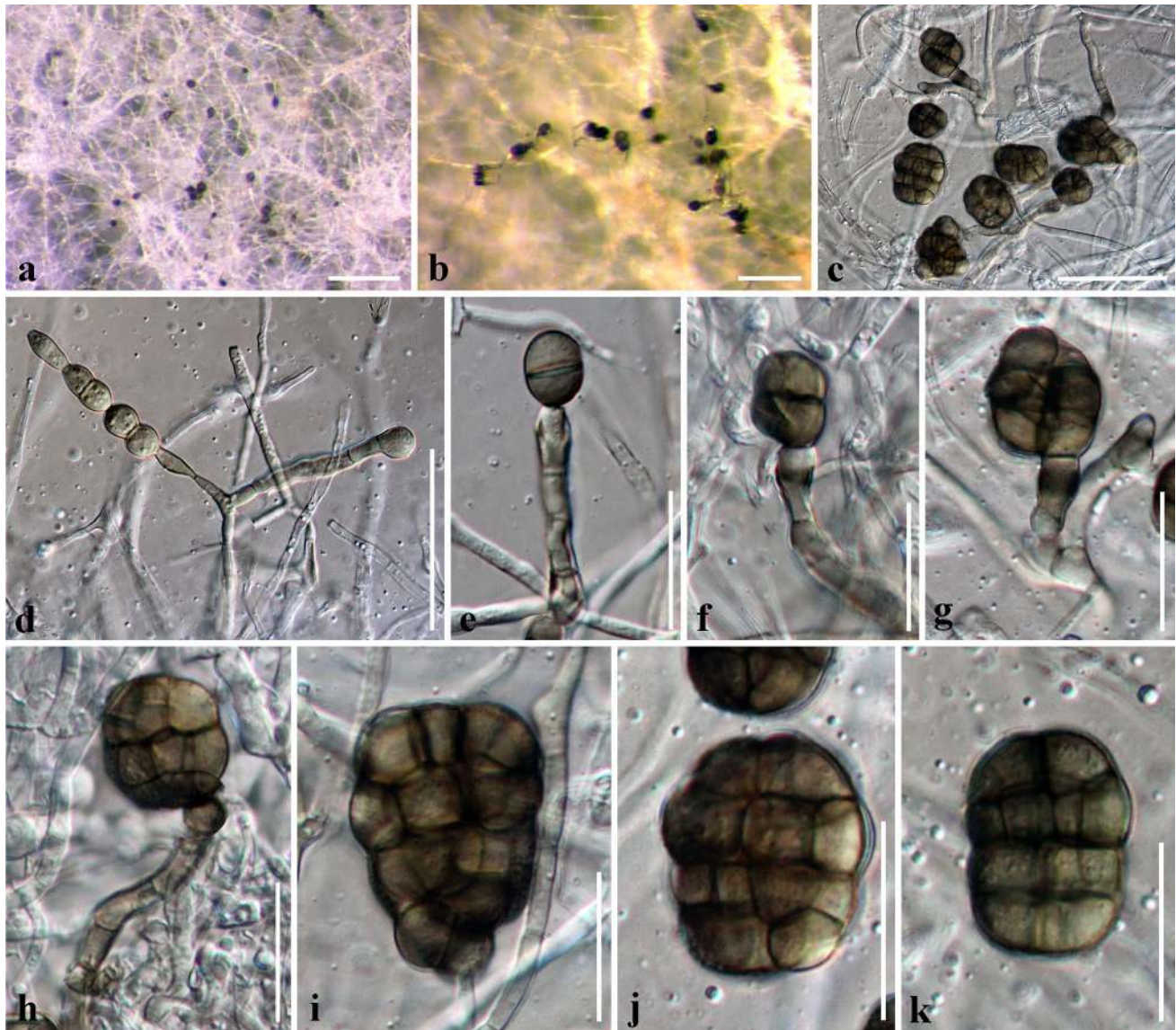
#### *Neorousoella fulvicomae* Phukhams. & K.D. Hyde, sp. nov.

*Index Fungorum number*: IF557114; *Facesoffungi number*: FoF 07329, Fig. 68.

*Etymology*: Name refers to *Clematis fulvicoma*.

*Holotype*: MFLU 17–1481.

*Saprobic* on dried stems of *Clematis fulvicoma*. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata*



**Fig. 62** *Stemphylium vesicarium* (MFLUCC 16–0998). **a** Appearance of asexual morph on the surface of MEA. **b, c** Close up of conidiophore. **d–k** Conidia. Scale bars: **a** = 200  $\mu$ m, **b** = 100  $\mu$ m, **c, d** = 50  $\mu$ m, **e–k** = 20  $\mu$ m

140–241  $\times$  137–155  $\mu$ m ( $\bar{x}$  = 207  $\times$  146  $\mu$ m,  $n$  = 5), pycnidial, solitary, sometimes aggregated, uniloculate, immersed, visible as minute, black, shiny ostioles, subglobose, coriaceous, thick-walled, dark brown to brown, with papilla, ostiolate. *Ostioles* 51–60  $\times$  49–55  $\mu$ m, central, oblong, papillate. *Conidiomatal wall* 12–18  $\mu$ m wide ( $\bar{x}$  = 15  $\mu$ m,  $n$  = 20), thick, outer layer composed of 5–7 layers of brown to light brown cells of *textura angularis*, lined with a hyaline layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 2.5–5  $\times$  1.5–2  $\mu$ m ( $\bar{x}$  = 4  $\times$  1.7  $\mu$ m,  $n$  = 20), enteroblastic, phialidic, annelidic, determinate, discrete, truncate, smooth-walled, hyaline, arising from the inner layers of conidiomata. *Conidia* 3–6  $\times$  2–3  $\mu$ m ( $\bar{x}$  = 4.5  $\times$  2.5  $\mu$ m,  $n$  = 100), oval, slightly

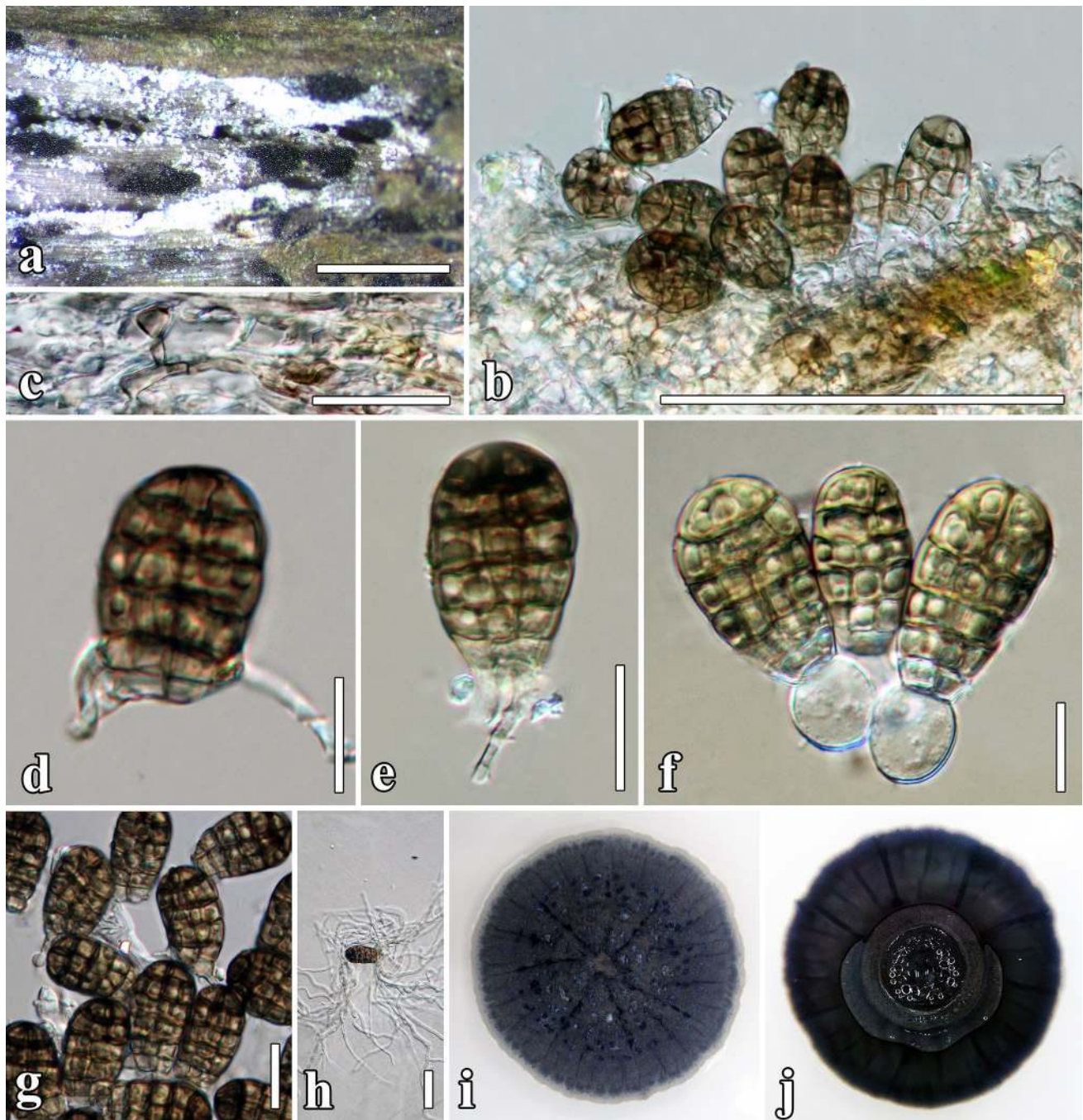
curved, hyaline when immature, brown at maturity, with 1(–2) guttules in each cell, aseptate, smooth-walled.

*Culture characters*: Colonies on MEA reaching 50 mm diam. after 4 weeks at 25  $^{\circ}$ C. Cultures from above, black, dense, circular, margin undulate, umbonate, fluffy, wrinkled, folded, covered with brown aerial mycelium; reverse black at the centre radiating outwardly.

*Material examined*: Thailand, Chiang Rai Province, on dead branches of *Clematis fulvicoma*, 20 March 2017, C. Phukhamsakda, CMTH17 (MFLU 17–1481, **holotype**); ex-type living culture, MFLUCC 17–2073.

*Host*: *Clematis fulvicoma*—(This study).

*Distribution*: Thailand—(This study).



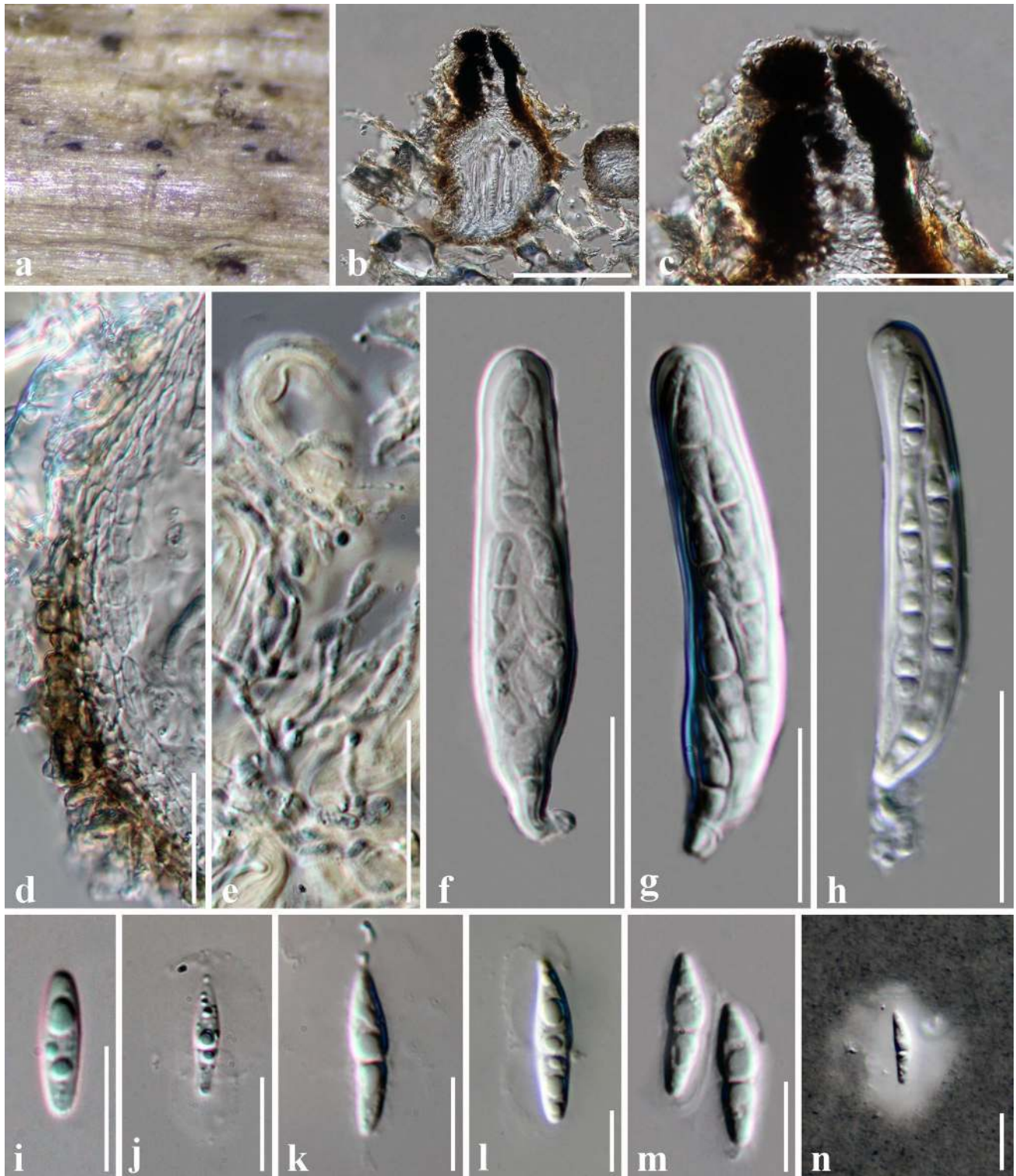
**Fig. 63** *Pseudoberkleasium chiangmaiense* (MFLU 17–1496). **a** Sporodochia on natural substrate. **b** Vertical section through sporodochia. **c** Hyphae. **d–g** Mature lenticular conidia. **h** Germinated

conidia. **i, j** Culture characteristics on MEA. Scale bars=**a**=1 cm, **b**=100  $\mu$ m, **c, g–h**=20  $\mu$ m, **d–f**=10  $\mu$ m

*GenBank accession numbers:* LSU: MT214588; SSU: MT226701; ITS: MT310633; *tef1*: MT394646; *rpb2*: MT394702.

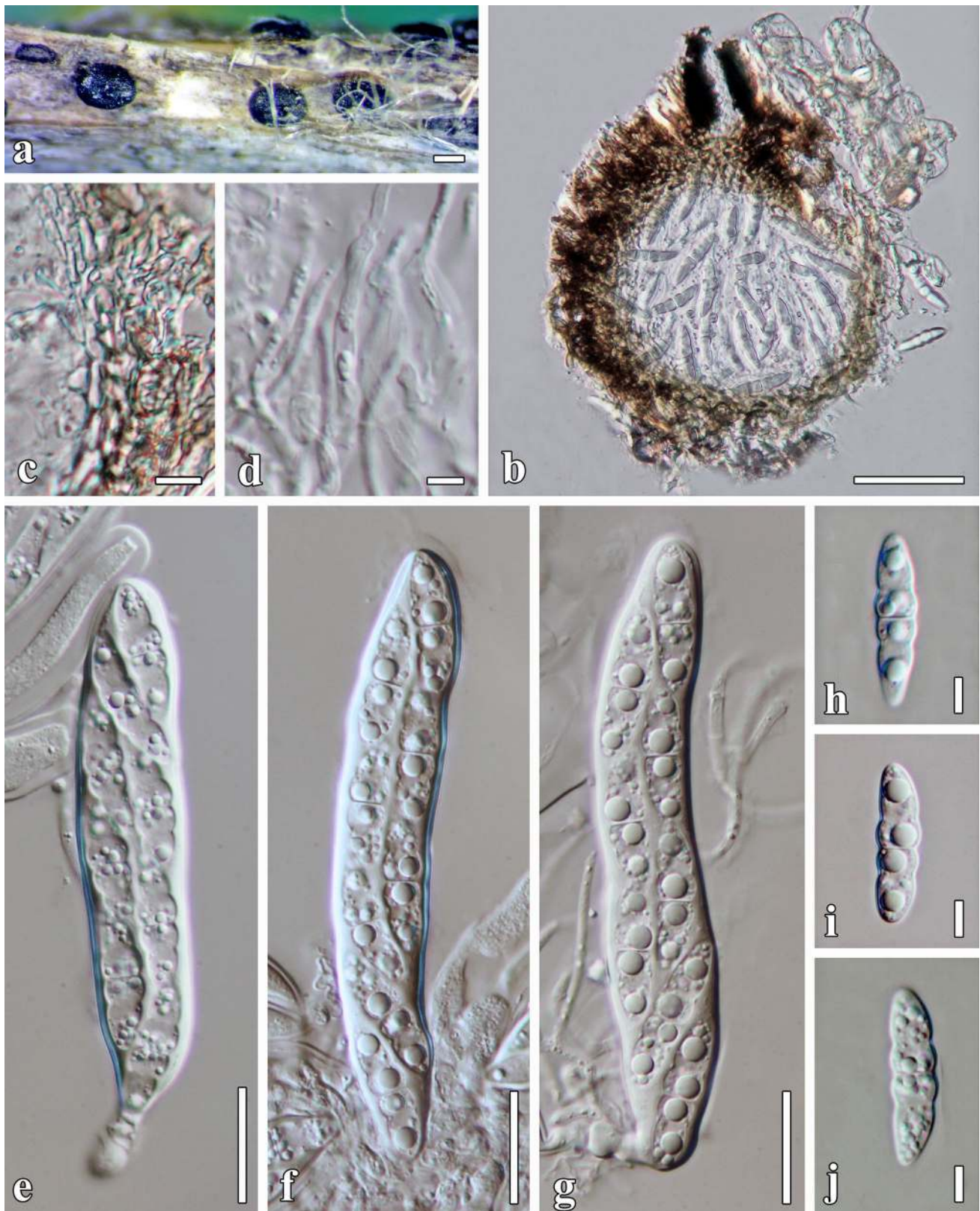
*Notes:* In the phylogenetic analysis of combined sequence data *Neorousoella fulvicomae* clustered in a separate clade with *N. clematidis* (100% ML/1.00 BYPP, Fig. 66). *Neorousoella fulvicomae* is similar to *Neorousoella* in having

immersed, uniloculate, globose to subglobose pycnidia, annellidic conidiogenesis cells, and aseptate, 2-guttulate conidia (Liu et al. 2014). *Neorousoella fulvicomae* is distinguishable by having papillate ostioles and short conidiogenesis cells and brown conidia with 1–2 guttules in each cell (Fig. 68). *Neorousoella fulvicomae* was evaluated for potential of secondary metabolites in the same manner as *N.*

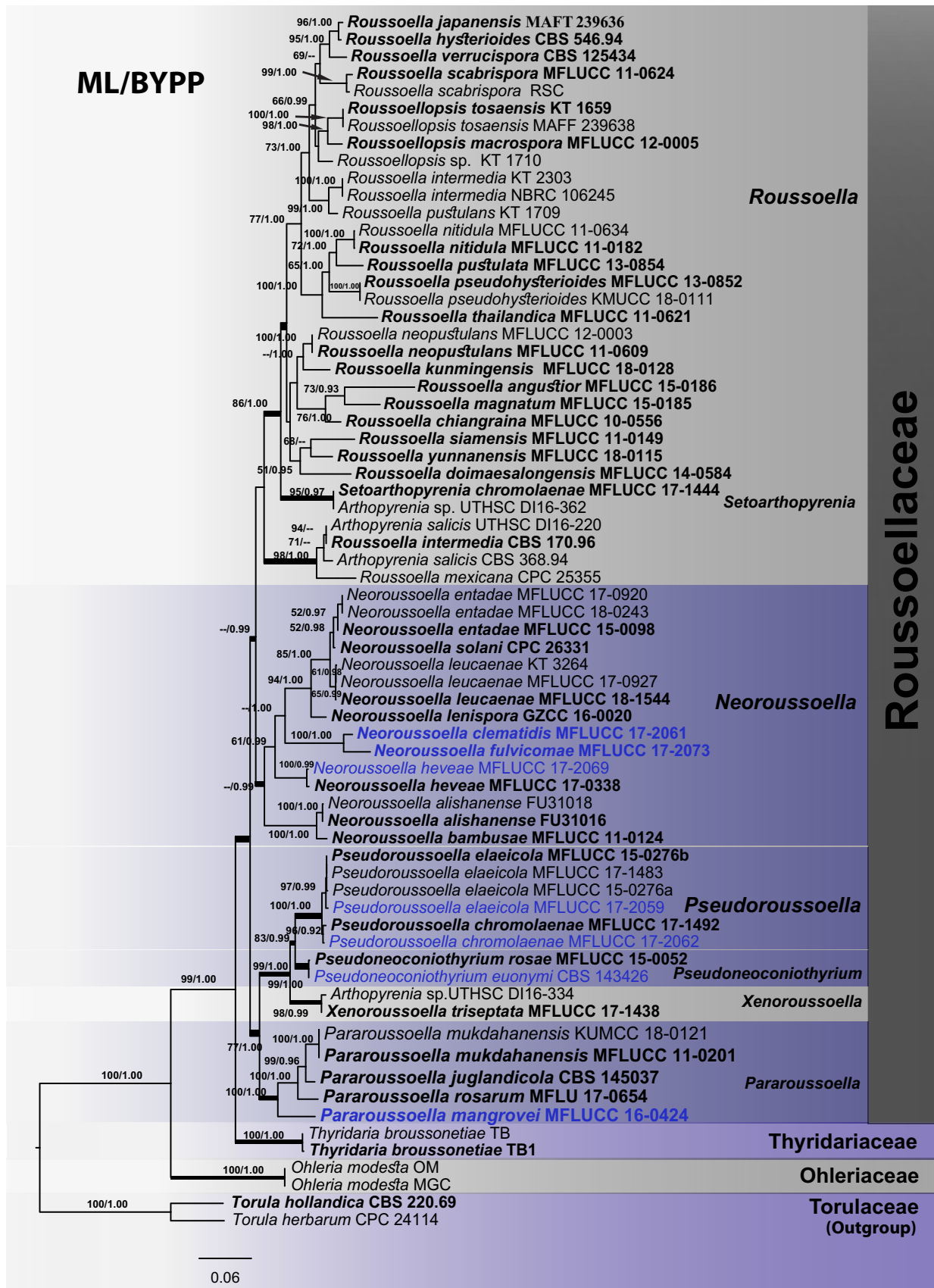


**Fig. 64** *Pseudomassarina clematidis* (MFLU 16–0493, holotype). **a** Appearance of ascomata on host surface. **b** Vertical section of ascoma. **c** Ostiolar canal. **d** Section of peridium. **e** Pseudoparaphy-

ses. **f–h** Asci. **i–m** Ascospores. **n** Ascospore in 10% Indian ink. Scale bars: **b** = 100  $\mu$ m, **c** = 50  $\mu$ m, **d–h** = 20  $\mu$ m, **i–n** = 10  $\mu$ m



**Fig. 65** *Clematidis italica* (MFLU 14–0669, holotype). **a** Appearance of ascomata on host substrate. **b** Vertical section of ascoma. **c** Section of peridium. **d** Pseudoparaphyses. **e–g** Asci. **h–j** Ascospores. Scale bars: **a** = 200  $\mu\text{m}$ , **b** = 50  $\mu\text{m}$ , **c** = 10  $\mu\text{m}$ , **d, h–j** = 5  $\mu\text{m}$ , **e–g** = 20  $\mu\text{m}$



**Fig. 66** Best scoring RAxML tree with a final likelihood value of  $-27212.762040$  based on combined LSU, ITS, *tef1*, *rpb2*, and SSU sequence data for Roussoellaceae. The tree is rooted with members of Torulaceae. Sixty-nine strains were included in the combined analyses which comprised 4382 characters (819 characters for LSU, 567 characters for ITS, 906 characters for *tef1*, 1052 characters for *rpb2*, 1038 characters for SSU, including gaps). The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree from the maximum likelihood analysis had similar topology to the Bayesian 50% majority-rule consensus phylogram. The matrix had 1501 distinct alignment patterns with 38.60% of undetermined characters and gaps. Estimated base frequencies were as follows: A=0.245133, C=0.255324, G=0.268618, T=0.230925; substitution rates AC=1.766964, AG=5.243182, AT=2.353818, CG=1.339749, CT=11.449776, GT=1.000000; gamma distribution shape parameter  $\alpha=0.473912$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The isolates of this study are in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses ( $BS \geq 70\%/BYPP \geq 0.95$ ) at the genus and family levels

*clematidis*. Interestingly, isolate MFLUCC 17–2073 does not produce growth inhibitory activity against *Bacillus subtilis* and *Mucor plumbeus*.

***Neorousoella heveae*** Senwana, Phookamsak & K.D. Hyde, in Phookamsak et al., Fungal Divers [66] (2019), **new host record**

*Index Fungorum number*: IF555287; *Facesoffungi number*: FoF 07330, Fig. 69.

*Saprobic* on dried stems of *Clematis subumbellata*. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata*  $128\text{--}263 \times 142\text{--}304 \mu\text{m}$  ( $\bar{x}=177 \times 200 \mu\text{m}$ ,  $n=5$ ), pycnidial, solitary, gregarious, uniloculate, immersed, visible only as minute ostioles, globose, coriaceous, thick-walled, dark brown to brown, ostiolate. *Ostioles*  $40 \times 77 \mu\text{m}$ , central, papillate, oblong, opening by a pore. *Conidiomatal wall*  $24\text{--}31 \mu\text{m}$  wide ( $\bar{x}=26 \mu\text{m}$ ,  $n=20$ ), thick, 7–8 layers, outer layer composed of brown cells of *textura angularis*, lined with a hyaline layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells*  $2\text{--}4 \times 1.5\text{--}2 \mu\text{m}$  ( $\bar{x}=3 \times 2 \mu\text{m}$ ,  $n=50$ ), enteroblastic, phialidic, determinate, discrete, truncate, smooth-walled, hyaline, arising from the inner layers of the conidiomata. *Conidia*  $3.5\text{--}4.5 \times 2.3\text{--}3 \mu\text{m}$  ( $\bar{x}=4 \times 2.5 \mu\text{m}$ ,  $n=100$ ), oval, hyaline when immature, olive brown at maturity, slightly curved at the ends, with 1(–2) guttules in each cell, aseptate, smooth-walled.

*Culture characters*: Colonies on MEA reaching 50 mm diam. after 4 weeks at 25 °C. Cultures from above, dark brown to brown, dense, irregular, margin lobate, umbonate, wrinkled folded, thinly hairy, covered with brown aerial mycelium; reverse black at the centre, radiating outwardly.

*Material examined*: Thailand, Chiang Rai Province, on dead stems of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH13 (MFLU 17–1477); living culture, MFLUCC 17–2069.

*Hosts*: *Clematis subumbellata*, *Hevea brasiliensis*—(Phookamsak et al. 2019; this study).

*Distribution*: Thailand—(Phookamsak et al. 2019; this study).

*GenBank accession numbers*: LSU: MT214589; SSU: MT226702; ITS: MT310634; *tef1*: MT394647; *rpb2*: MT394703.

*Notes*: *Neorousoella heveae* was described from a twig of *Hevea brasiliensis* from northern Thailand (Phookamsak et al. 2019). Isolate MFLUCC 17–2069, recorded on *Clematis* formed a close relationship (100% ML/1.00 BYPP) with the type strain of *N. heveae* (MFLUCC 17–0338). Our collection (MFLU 17–1477, Fig. 69) had larger conidia than the type ( $128\text{--}263 \times 142\text{--}304$  vs  $90\text{--}130 \times 115\text{--}180 \mu\text{m}$ ). Comparison of ITS sequence data revealed only 1 base pair difference between our isolate and the type. Unfortunately, the *tef1* region of the type strain is not available for comparison.

*Neorousoella heveae* was evaluated for potential secondary metabolite production in the same manner as *N. clematidis*. Isolate MFLUCC 17–2069 demonstrated weak inhibitory activities against *Bacillus subtilis*.

***Pararousoella*** Wanas., E.B.G. Jones & K.D. Hyde

*Pararousoella* (type species *P. rosarum*) was introduced for species that are distantly related to the type species of *Rousoella* (*R. nitidula* Sacc. & Paol.). The genus is characterized by having immersed and globose ascomata with minute black dots of ostioles, cellular pseudoparaphyses, central, papillate, black ostioles, cylindrical to oblong asci, and uniseriate, brown to dark brown ascospores, with irregular, longitudinal striations (Wanasinghe et al. 2018). Multi-gene phylogenetic analysis (Fig. 66) showed that *Rousoella mangrovei* is related to *Pararousoella*, thus a new combination is proposed.

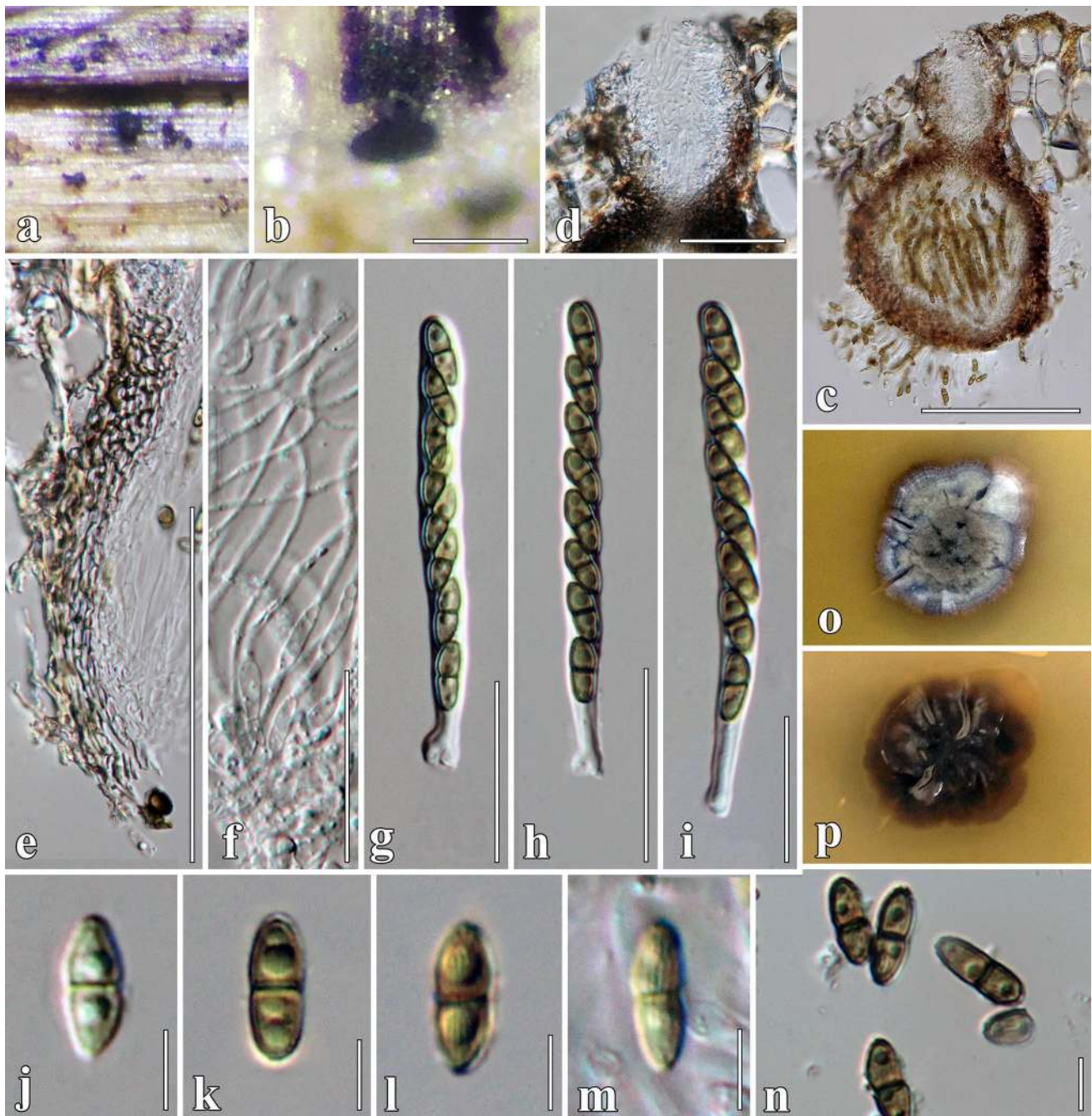
***Pararousoella mangrovei*** (Phukhams. & K.D. Hyde) Phukhams. & K.D. Hyde, **comb. nov.**

$\equiv$  *Rousoella mangrovei* Phukhams. & K.D. Hyde, in Hyde et al., Mycosphere 9(2): 339 (2018)

*Index Fungorum number*: IF557372; *Facesoffungi number*: FoF 03923

*Notes*: *Pararousoella mangrovei* formed a related lineage with other *Rousoella* species. Karunarathna et al. (2019) showed that the strain is related to *Pararousoella* (Fig. 66). Therefore, *Rousoella mangrovei* is transferred to *Pararousoella*.

***Pseudoneoconiothyrium*** Wanas., Phukhams., Camporesi & K.D. Hyde



**Fig. 67** *Neorousoella clematidis* (MFLU 17–1467, holotype). **a, b** Appearance of ascomata on *Clematis subumbellata*. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Vertical section through

peridium. **f** Trabeculate pseudoparaphyses. **g–i** Asci. **j–n** Ascospores. **o, p** Culture characters on MEA. Scale bars: **b** = 200  $\mu$ m, **c** = 100  $\mu$ m, **d–e** = 50  $\mu$ m, **f–i** = 20  $\mu$ m, **j–n** = 5  $\mu$ m

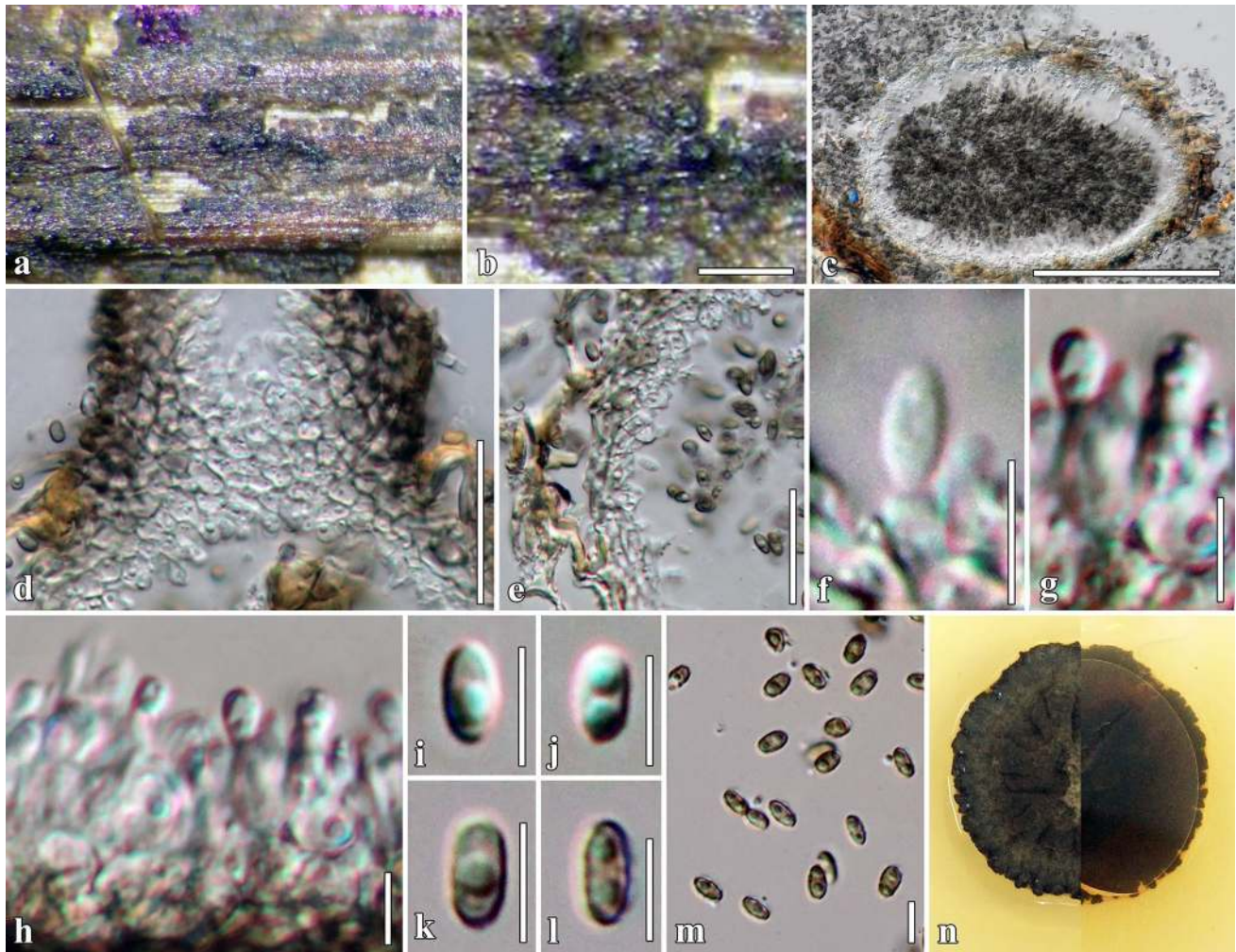
*Pseudoneoconiothyrium* was described as *Neoconiothyrium* for fungi associated with Rosaceae plants (Wanasinghe et al. 2018). *Neoconiothyrium* has been assigned for the fungal strains in Coniothyriaceae (Crous et al. 2017; Hawksworth et al. 2018). Thus, *Pseudoneoconiothyrium* was proposed for *Neoconiothyrium* species in Roussoellaceae

and is typified by *P. rosae* (Phukhams., et al.) Phukhams., Camporesi & K.D. Hyde (Fig. 66).

***Pseudoneoconiothyrium euonymi*** (Crous & Akulov) Phukhams. & K.D. Hyde, **comb. nov.**

$\equiv$  *Rousoella euonymi* Crous & Akulov, in Crous et al., Fungal Systematics and Evolution 1: 204 (2018)





**Fig. 68** *Neorousoella fulvicomae* (MFLU 17–1481, **holotype**). **a** Appearance of conidiomata on *Clematis fulvicoma*. **b** Close up of conidioma on host substrate. **c** Vertical section through conidioma. **d**

Ostiolar canal. **e** Section of conidioma wall. **f–h** Conidiogenous cells and conidia. **i–m** Conidia. **n** Culture characteristics on MEA. Scale bars: **b** = 200  $\mu$ m, **c** = 100  $\mu$ m, **d**, **e** = 20  $\mu$ m, **f–m** = 5  $\mu$ m

*Index Fungorum number*: IF557605; *Facesoffungi number*: FoF 07331

*Notes*: *Pseudoneoconiothyrium euonymi* originally clustered with *Pararousoella mukdahanensis* ( $\equiv$  *Rousoella mukdahanensis*). Based on the phylogenetic analysis (Fig. 66), *Pseudoneoconiothyrium euonymi* is transferred as a second species of *Pseudoneoconiothyrium*.

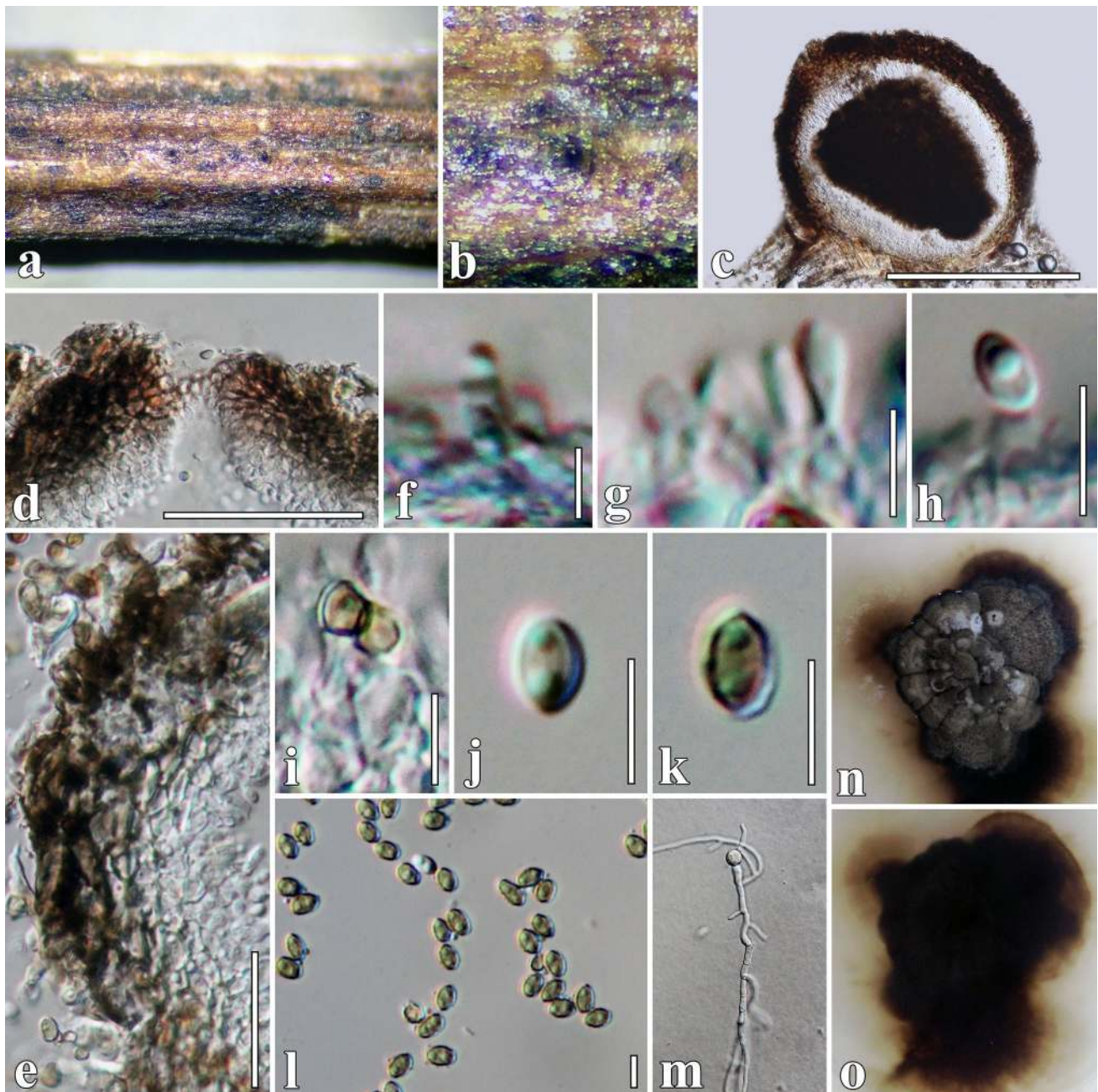
#### *Pseudorousoella* Mapook & K.D. Hyde

Mapook et al. (2020) introduced *Pseudorousoella* as a separate lineage for *P. euonymi* and *P. chromolaenae*. Phylogenetic analyses (Fig. 66) coupled with morphological characters of collections on *Clematis* species revealed a first record of *P. chromolaenae* and *P. euonymi* on *Clematis* species (Figs. 70, 71).

***Pseudorousoella chromolaenae*** Mapook & K.D. Hyde, Mapook et al., Fungal Divers (2020), **new host record**

*Index Fungorum number*: IF557116; *Facesoffungi number*: FoF 07332, Fig. 70.

*Saprobic* on dead stems of *Clematis subumbellata*. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 86–100  $\times$  107–112  $\mu$ m ( $\bar{x}$  = 90  $\times$  110  $\mu$ m,  $n$  = 5), pycnidial, solitary, sometimes aggregated, uniloculate, immersed, with black shiny ostioles visible, globose to subglobose, dark brown to brown, coriaceous, thick-walled, ostiolate. *Ostioles* 55–70  $\times$  50–67  $\mu$ m, central, papillate, ovoid. *Conidiomatal wall* 6–14(–27)  $\mu$ m wide, outer layer composed of 8–10 layers of brown to light brown cells of *textura angularis*, lined with a hyaline layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 3–7  $\times$  1.5–3  $\mu$ m ( $\bar{x}$  = 4.5  $\times$  2  $\mu$ m,  $n$  = 30), enteroblastic, phialidic, determinate, discrete, cylindrical



**Fig. 69** *Neorousoella heveae* (MFLU 17–1477). **a** Appearance of conidiomata on *Clematis subumbellata* specimen. **b** Close up of conidioma on host substrate. **c** Vertical section of conidioma. **d** Ostiolar canal. **e** Section of conidioma wall. **f–i** Conidiogenous cells

and conidia. **j–l** Conidia. **m** Germinated conidium. **n, o** Cultures characters on MEA. Scale bars: **c**=200  $\mu\text{m}$ , **d**=100  $\mu\text{m}$ , **e**=20  $\mu\text{m}$ , **f–l**=5  $\mu\text{m}$

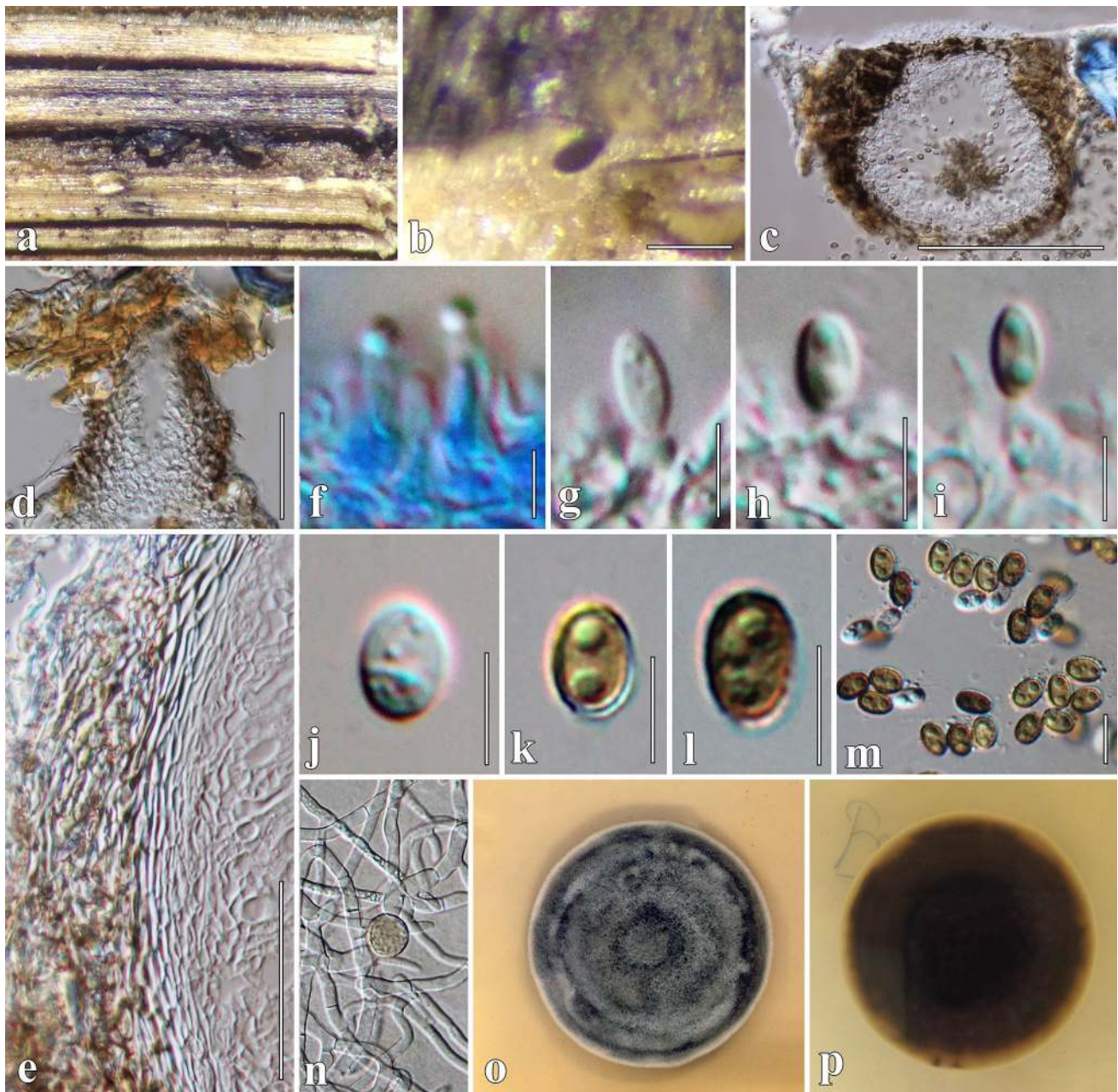
to sub-cylindrical, smooth-walled, hyaline. *Conidia* 4.5–8  $\times$  3–5  $\mu\text{m}$  ( $\bar{x}$ =6  $\times$  4  $\mu\text{m}$ ,  $n$ =50), oval, hyaline when immature, yellowish brown at maturity, slightly curved, with 1(–2) guttules in each cell, aseptate, smooth-walled.

*Culture characters*: Colonies on MEA reaching 20 mm diam. after 2 weeks at 25  $^{\circ}\text{C}$ . Cultures from above, greyish brown, dense, circular, umbonate, fluffy, covered with

white aerial mycelium; reverse dark brown at the central, mycelium cream radiating outwardly.

*Material examined*: Thailand, Chiang Rai Province, on dead branches of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH04 (MFLU 17–1468); living culture, MFLUCC 17–2062.

*Hosts*: *Chromolaena odorata*, *Clematis subumbellata*—(Mapook et al. 2020; this study).



**Fig. 70** *Pseudorousoella chromolaenae* (MFLU 17–1468). **a** Appearance of conidiomata on *Clematis subumbellata*. **b** Close up of conidioma on host substrate. **c** Vertical section through conidioma. **d** Ostiolar canal. **e** Section of conidioma wall. **f–i** Conidiogenous cells

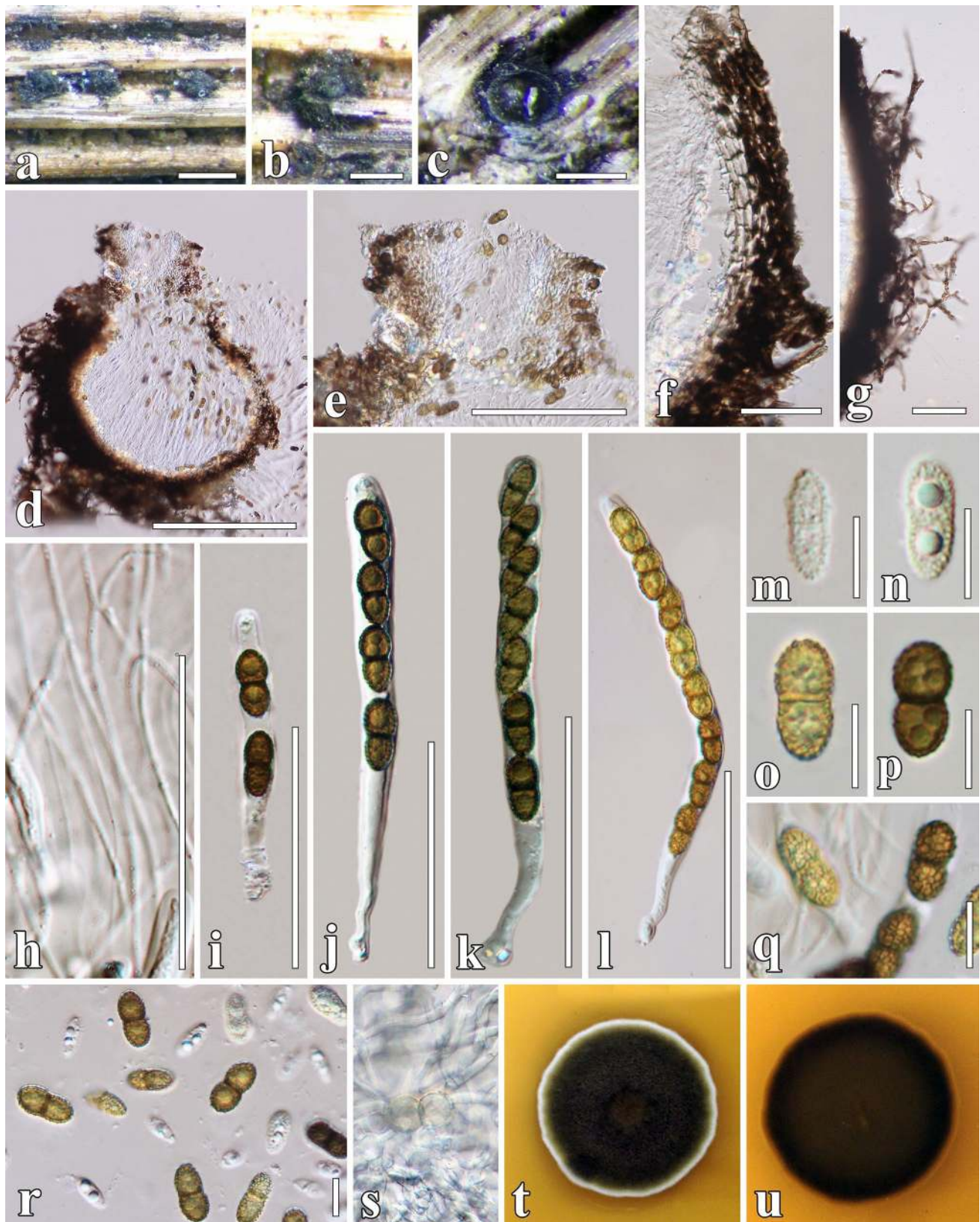
and conidia (**f** conidiogenous cells in cotton blue). **j–m** Conidia **n** Geminated conidium. **o, p** Cultures characters on MEA. Scale bars: **b** = 500  $\mu$ m, **c** = 200  $\mu$ m, **d** = 100  $\mu$ m, **e** = 20  $\mu$ m, **f–m** = 5  $\mu$ m

**Distribution:** Thailand—(Mapook et al. 2020; this study).

**GenBank accession numbers:** LSU: MT214590; SSU: MT226703; ITS: MT310635; *tef1*: MT394648; *rpb2*: MT394704.

**Notes:** Phylogenetic analysis (Fig. 66) has shown that the newly collected strain (MFLUCC 17–2062), is related to the ex-type strain of *P. chromolaenae* (MFLUCC 17–1492) with strong support (100% ML/1.00 BYPP, Fig. 66). The

conidiomata of our collection (Fig. 70) are slightly smaller than the ex-type strain (90  $\times$  110 vs 165  $\times$  195  $\mu$ m). Pair-wise comparison of the ITS region (including 5.8S region) showed 100% similarity, while the *tef1* region had two base pair differences; these findings are not significantly distinct for the introduction of the strain as a new species (Jeewon and Hyde 2016). *Pseudorousoella chromolaenae* strain MFLUCC 17–2062 was evaluated for secondary metabolite



**Fig. 71** *Pseudorousoella elaicola* (MFLU 17–1465). **a–c** Appearance of ascomata on *Clematis subumbellata*. **d** Vertical section through ascoma. **e** Ostiolar canal. **f, g** Section of peridium. **h** Pseudoparaphyses. **i–l** Asci. **m–r** Ascospores (**q** verruculose surface). **s**

Germinated ascospore. **t, u** Culture characters on MEA. Scale bars: **a**=500  $\mu$ m, **b–d**=200  $\mu$ m, **e**=100  $\mu$ m, **f, g**=20  $\mu$ m, **h–l**=50  $\mu$ m, **m–r**=10  $\mu$ m

production. The collection showed weak inhibitory activities against the growth of *Bacillus subtilis*.

***Pseudorousoella elaeicola*** (Konta & K.D. Hyde) Mapook & K.D. Hyde, **new host record**

*Index Fungorum number*: IF555291; *Facesoffungi number*: FoF 07333, Fig. 71.

*Saprobic* on dead stems of *Clematis subumbellata*. **Sexual morph**: *Ascomata* 375–554 × 375–462 μm ( $\bar{x}$  = 453 × 430 μm, n = 10), on the surface of the host, solitary, gregarious, erumpent, semi-immersed, dark brown hyphae radiating outwards from the peridium wall, globose to depressed-globose, black to dark brown, coriaceous, rough-walled, ostiolar. *Ostioles* 149–157 × 123–140 μm, central, dark brown to black, papillate, opening by a pore, ostiolate with periphyses. *Peridium* 17–54 (–83 μm at apex) wide, outer layer composed of 8–10 layers of dark brown to light brown cells of *textura angularis*, the inner layer comprising thin, hyaline layers. *Hamathecium* of dense, 0.8–1.5 μm wide ( $\bar{x}$  = 1.3 μm, n = 50), filiform, branches, anastomosing above asci, septate, pseudoparaphyses. *Asci* 60–132 × 7–10 μm ( $\bar{x}$  = 103 × 8 μm, n = 20), (2–)8-spored, bitunicate, fissitunicate, cylindrical to cylindrical-clavate, apically rounded with ocular chamber. *Ascospores* 10–18 × 5–10 μm ( $\bar{x}$  = 13 × 7 μm, n = 50), uniseriate, sometimes partially overlapping, oval with round ends, brown to yellowish brown, uni-septate, constricted at septum, with guttules in each cell, verruculose, with mucilaginous sheath. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Culture above dark green radiating, edge white, dense, circular, flattened, umbonate, edge entire, fluffy; reverse light brown at the middle, dark brown at the edge.

*Material examined*: Thailand, Chiang Rai Province, on dead branches of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH01 (MFLU 17–1465); living culture, MFLUCC 17–2059.

*Hosts*: *Chromolaena odorata*, *Clematis subumbellata*, *Elaeis guineensis*—(Phookamsak et al. 2019; Mapook et al. 2020; this study).

*Distribution*: Thailand—(Phookamsak et al. 2019; Mapook et al. 2020; this study).

*GenBank accession numbers*: LSU: MT214591; SSU: MT226704; ITS: MH744730; *tefl*: MH750239; *rpb2*: MT394705.

*Notes*: *Pseudorousoella elaeicola* ( $\equiv$  *Rousoella elaeicola*) was collected from *Elaeis guineensis* (Phookamsak et al. 2019). Mapook et al. (2020) recorded this fungus on *Chromolaena odorata*. In the phylogenetic analysis, *R. elaeicola* formed a separate clade from the type species of *Rousoella* (*R. nitidula* Sacc. & Paol.), thus Mapook et al. (2020) synonymized *R. elaeicola* under *Pseudorousoella*

*elaicola*. Our new isolate (MFLUCC 17–2059) grouped with another collections reported from *Chromolaena odorata* and *Elaeis guineensis* (97% ML/0.99 BYPP, Fig. 70). Our collection is identical to the other collections of this species (Fig. 71). Comparison of the ITS region revealed only one base pair difference between our isolate and the ex-type strain (MFLUCC 15–0276). The pairwise comparison of the available *tefl* region with MFLUCC 17–1483 also showed one base pair difference. Therefore, we introduce our collection as a new host record.

*Pseudorousoella elaeicola* (MFLUCC 17–2059) was evaluated for the potential of secondary metabolite production. The isolate showed inhibitory activity on biofilm formation by *Staphylococcus aureus* and showed weak cytotoxicity on L929 murine fibroblasts and human KB3-1 cancer cells (Phukhamsakda et al. 2018).

### **Sulcatisporaceae** Tanaka & K. Hirayama

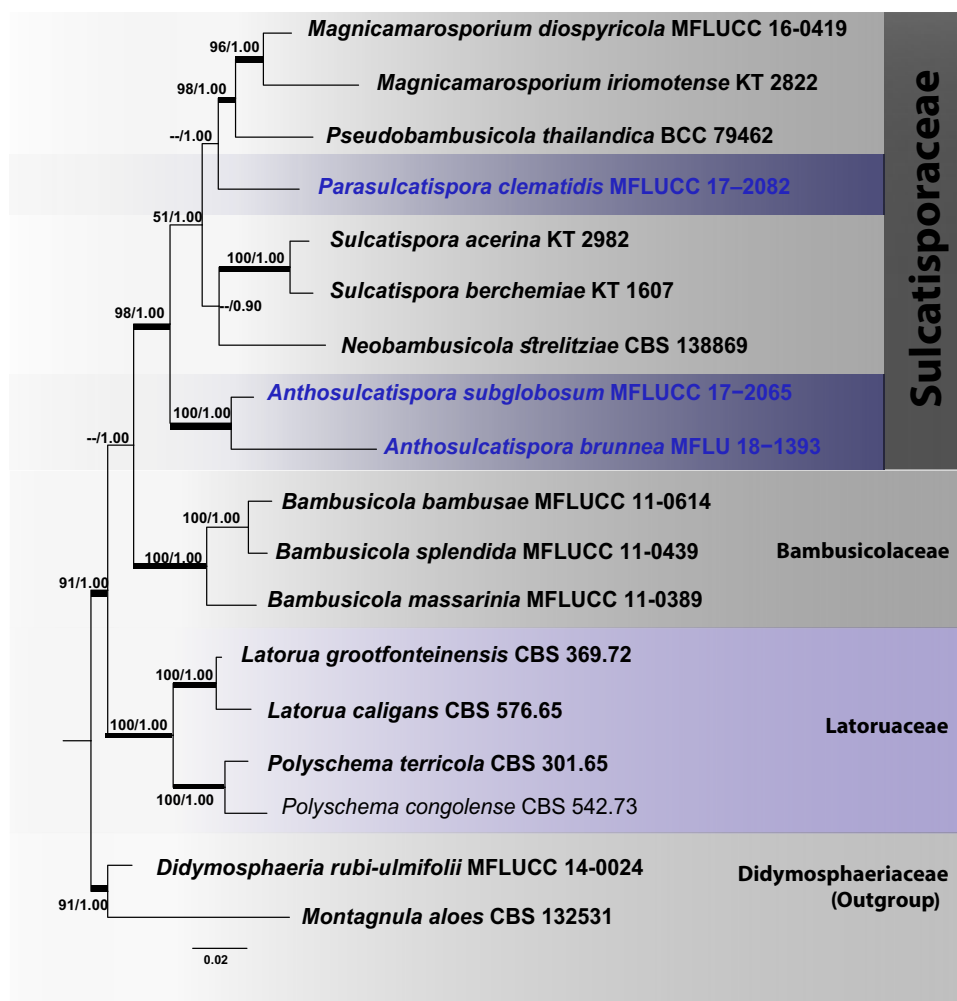
Sulcatisporaceae was erected for a well-separated clade which included *Magnicamarosporium*, *Neobambusicola* and *Sulcatispora* in Pleosporales (Tanaka et al. 2015). Rucpic et al. (2018) added *Pseudobambusicola* to Sulcatisporaceae which was collected from a twig of an unidentified plant in Thailand based on the morphology and phylogeny analyses. Sulcatisporaceae is characterized by immersed to erumpent, subglobose to hemispherical ascomata, short ostiolar neck, trabecular pseudoparaphyses (Liew et al. 2000), clavate, short pedicellate asci, and broadly fusiform, hyaline, septate ascospores with mucilaginous appendages (Tanaka et al. 2015). Asexual morph is pycnidial conidiomata with various conidia characters (Tanaka et al. 2015; Phukhamsakda et al. 2017a; Rucpic et al. 2018). We provide an updated tree of Sulcatisporaceae based on the combined dataset of LSU, ITS, SSU and *tefl* sequence data and propose two new genera from *Clematis* species collected in Thailand. Additionally, the biological activity of their secondary metabolites is preliminarily reported (Fig. 72).

### ***Anthosulcatispora*** Phukhams. & K.D. Hyde, **gen. nov.**

*Index Fungorum number*: IF557201; *Facesoffungi number*: FoF 07340, Fig. 73.

*Etymology*: Anthos-meaning flower, *Anthosulcatispora* referring to the occurrence on flowering plants.

*Saprobic* on dead stems of herbaceous plants. **Sexual morph**: *Ascomata* semi-immersed, blackish, irregular, scattered, uniloculate, glabrous, ostiolate, apapillate. *Ostioles* dark, circular and sunken. *Peridium* two-layered, outer layer irregular, comprising dark brown cells of *textura angularis* and inner layer irregular comprising light brown cells. *Hamathecium* composed of numerous, filamentous, branched or simple, septate, cellular pseudoparaphyses, embedded in a hyaline gelatinous matrix. *Asci* 4- or 8-spored, bitunicate, cylindrical to cylindrical-clavate, short pedicellate, apically



**Fig. 72** Bayesian 50% majority-rule consensus phylogram based on combined LSU, ITS, SSU and *tef1* sequence data for Sulcatissporaceae. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted with members of the Didymosphaeriaceae. Eighteen strains were included in the combined analyses which comprised 3390 characters (843 characters for LSU, 1025 characters for SSU, 585 characters for ITS, 937 characters for *tef1*, including gap regions). The best scoring RAxML tree had a final likelihood value of  $-11220.180237$ . The matrix had 709 distinct alignment patterns, with 31.84% of undetermined characters and gaps. Estimated base frequencies were as fol-

lows; A=0.238159, C=0.264197, G=0.261408, T=0.236236; substitution rates AC=0.958607, AG=2.023708, AT=1.033715, CG=0.741292, CT=5.717037, GT=1.000000; gamma distribution shape parameter  $\alpha=0.485427$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study are indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95)

rounded, with an ocular chamber. *Ascospores* 1-seriate, brown to dark brown, oblong to ellipsoidal, with rounded ends, 1-septate, slightly constricted at the septum, smooth-walled (Phookamsak et al. 2019). **Asexual morph:** *Conidiomata* pycnidial, solitary or gregarious, unilocular, scattered, immersed or erumpent, base flattened, subglobose to compressed, coriaceous, dark brown to reddish brown, with or without ostioles. *Pycnidial wall* multilayered with cells *textura angularis*, inner layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, phialidic, determinate, elongated

cylindrical or truncate, smooth-walled, hyaline. *Conidia* oblong, ends rounded, hyaline, aseptate, guttulate, smooth-walled, with mucilaginous sheath.

*Type species: Anthosulcatisspora subglobosa* Phukhams. & K.D. Hyde

*Notes: Anthosulcatisspora* is introduced for a collection occurring on stems of herbaceous plants (Phookamsak et al. 2019; this study). The genus formed a basal lineage in Sulcatissporaceae with strong support (98% ML/1.00 BYPP, Fig. 72). The sexual morph of *Anthosulcatisspora* was described as *Neobambusicola brunnea* (Phookamsak et al.

2019). Asexual morphs in Sulcatissporaceae comprised *Magnicamarosporium*, *Neobambusicola*, *Pseudobambusicola* and *Sulcatisspora* (Tanaka et al. 2015; Phukhamsakda et al. 2017a; Rupcic et al. 2018). *Anthosulcatisspora* is distinct within Sulcatissporaceae in having brown ascospores, while the sexual morph in *Sulcatisspora* has hyaline, broadly fusiform ascospores with an entire sheath (Tanaka et al. 2015). The asexual morph of *Anthosulcatisspora* shares similar characters with *Neobambusicola* and *Pseudobambusicola*. They all have solitary, unilocular pycnidial, phialidic conidiogenesis and hyaline conidia. *Anthosulcatisspora* is distinct from *Neobambusicola* and *Pseudobambusicola* in its subglobose conidiomata, elongated cylindrical or truncate conidiogenous cells and oblong and aseptate conidia, while *Neobambusicola* and *Pseudobambusicola* have globose conidiomata with two types of conidia (Crous et al. 2014b; Rupcic et al. 2018).

***Anthosulcatisspora brunnea*** (Chen & C. Norphanphoun) Phukhams. & K.D. Hyde, **comb. nov.**

*Index Fungorum number*: IF557202; *Facesoffungi number*: FoF 05708

*Basionym*: *Neobambusicola brunnea* Chen & Norphanphoun, in Phookamsak et al., Fungal diversity 95:1–273 (2019)

*Notes*: *Neobambusicola brunnea* (MFLU 18–1393) was described based on phylogenetic analysis of a combined LSU and ITS dataset (Phookamsak et al. 2019). The strain formed a clade with the type species of *Neobambusicola strelitziae* (CBS 138869) with moderate support (87% ML). In our study, the phylogenetic analysis based on the combined LSU, SSU ITS, and *tefl* sequence data showed different topology. The strain formed a separate clade from *N. strelitziae* (CBS 138869) and clustered with our new species *Anthosulcatisspora subglobosa* with strong support (100% ML/1.00 BYPP, Fig. 72). Therefore, *Neobambusicola brunnea* is transferred to a new genus.

*Hosts*: Dead stem of herbage—(Phookamsak et al. 2019).

*Distribution*: China—(Phookamsak et al. 2019).

***Anthosulcatisspora subglobosa*** Phukhams. & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557203; *Facesoffungi number*: FoF 07341, Fig. 73.

*Etymology*: Refers to the subglobose conidiomata.

*Holotype*: MFLU 17–1473.

*Saprobic* on dried stems of *Clematis subumbellata*.

**Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 160–210 × 265–345 μm ( $\bar{x}$  = 178 × 291 μm, n = 5), pycnidial, solitary, unilocular, scattered, shiny, immersed or erumpent, flattened base, subglobose to compressed, coriaceous, dark brown to reddish brown, lacking ostioles. *Pycnidial wall* 18–32 μm wide, thick, multilayered,

composed of 10–14 brown layers of *textura angularis*, inner layer subhyaline, lining bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 5–17(–30) × 2–3.5 μm ( $\bar{x}$  = 13 × 2.5 μm, n = 30), enteroblastic, phialidic, determinate, elongated cylindrical or truncate, smooth-walled, hyaline. *Conidia* 7–10 × 3–4 μm ( $\bar{x}$  = 8 × 4 μm, n = 50), oblong, rounded ends, hyaline, aseptate, with 1–3 guttules in each cell, smooth-walled, with mucilaginous sheath.

*Cultural characters*: Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Cultures from above, cream at the centre, radiating, dense, circular, edge lobate, umbonate, papillate, fairly fluffy, covered with white aerial mycelium, black oil drops produced on the surface of cultures; reverse dark brown at the centre, faintly zonate, white at the edge.

*Material examination*: Thailand, Phayao Province, Phu Sang District, dead stems of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH09 (MFLU 17–1473, **holotype**); ex-type living culture, MFLUCC 17–2065.

*Host*: *Clematis subumbellata*—(This study).

*Distribution*: Thailand—(This study).

*GenBank accession numbers*: LSU: MT214592; SSU: MT226705; ITS: MT310636; *tefl*: MT394649; *rpb2*: MT394706.

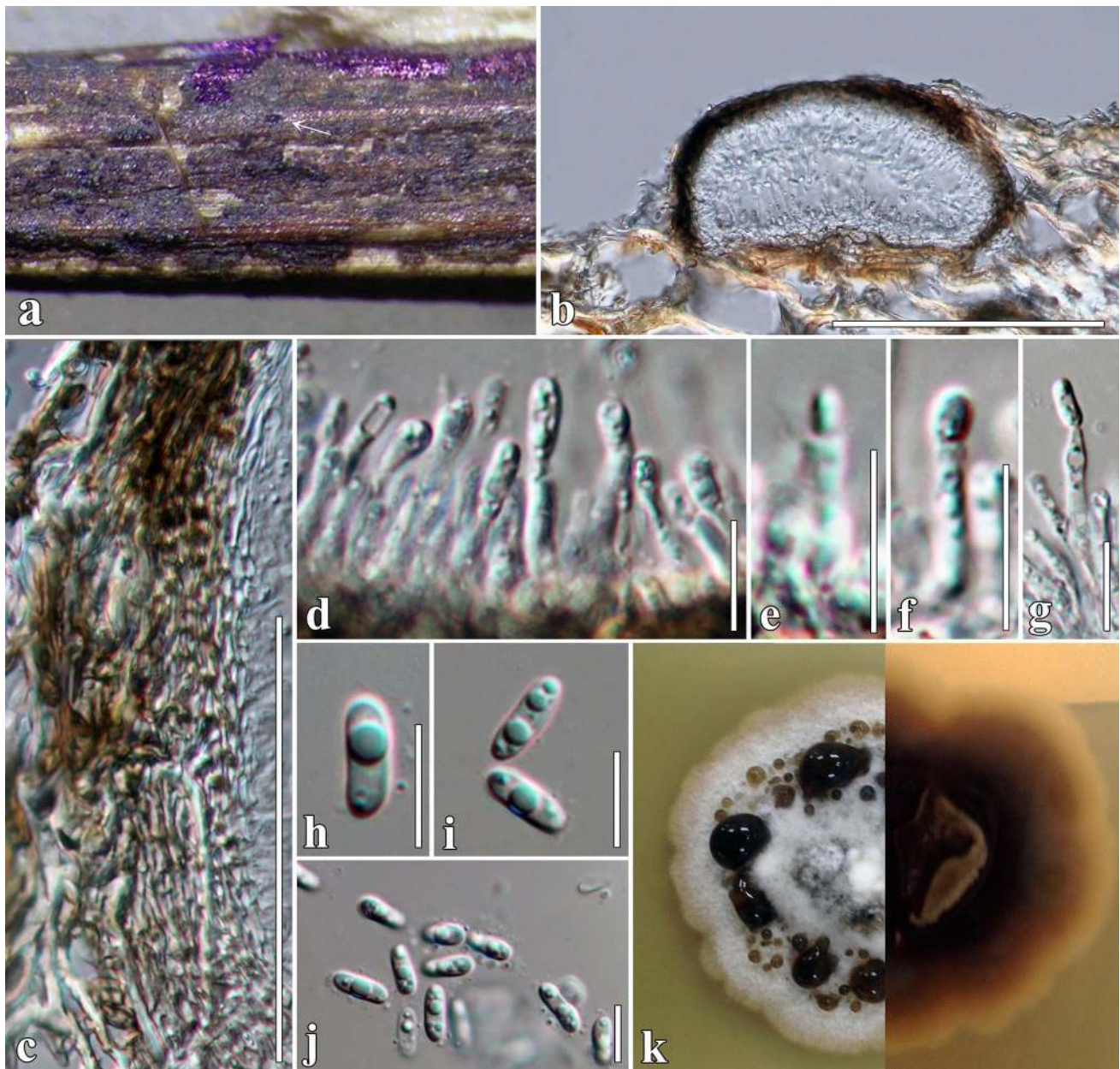
*Notes*: In a BLASTn search of GenBank, the closest match for LSU sequence of *Anthosulcatisspora subglobosa* MFLUCC 17–2065 was *Neobambusicola strelitziae* (strain CBS 138869), (NG\_058125) with 97% similarity, while the closest match for the ITS sequence of MFLUCC 17–2065 was *Magnicamarosporium iriomotense* (strain HHUF 30125), (NR\_153445) with 89% similarity. Pairwise comparison of the ITS region showed that *A. subglobosa* differs from *A. brunnea* with 12% differences (76 base pairs difference of 585 base pairs, with gaps). The collection has oblong, hyaline, guttulate, and aseptate, with rounded ends conidia but microconidia were not observed in culture (Crous et al. 2014b; Rupcic et al. 2018). We also noted the formation of oil droplets in culture as a notable character for *A. subglobosa* (Fig. 73).

Strain MFLUCC 17–2065 was evaluated for potential secondary metabolite production with *Bacillus subtilis*, *Escherichia coli*, *Mucor plumbeus* and *Schizosaccharomyces pombe* as test organisms. The isolate showed moderate growth inhibitory activities against *Bacillus subtilis* and *Mucor plumbeus*, and is thus a suitable candidate for further evaluation.

***Parasulcatisspora*** Phukhams. & K.D. Hyde, **gen. nov.**

*Index Fungorum number*: IF557204; *Facesoffungi number*: FoF 01686, Fig. 74.

*Etymology*: Refers to the characteristic features similar to *Sulcatisspora*.



**Fig. 73** *Anthosulcatipora subglobosa* (MFLU 17–1473, holotype). **a** Appearance of conidiomata on *Clematis subumbellata*. **b** Vertical section through conidioma. **c** Section of conidioma wall. **d–g** Conid-

igenous cells and conidia. **h–j** Conidia. **k** Culture characteristics on MEA. Scale bars: **b** = 100  $\mu$ m, **c** = 50  $\mu$ m, **d–j** = 10  $\mu$ m

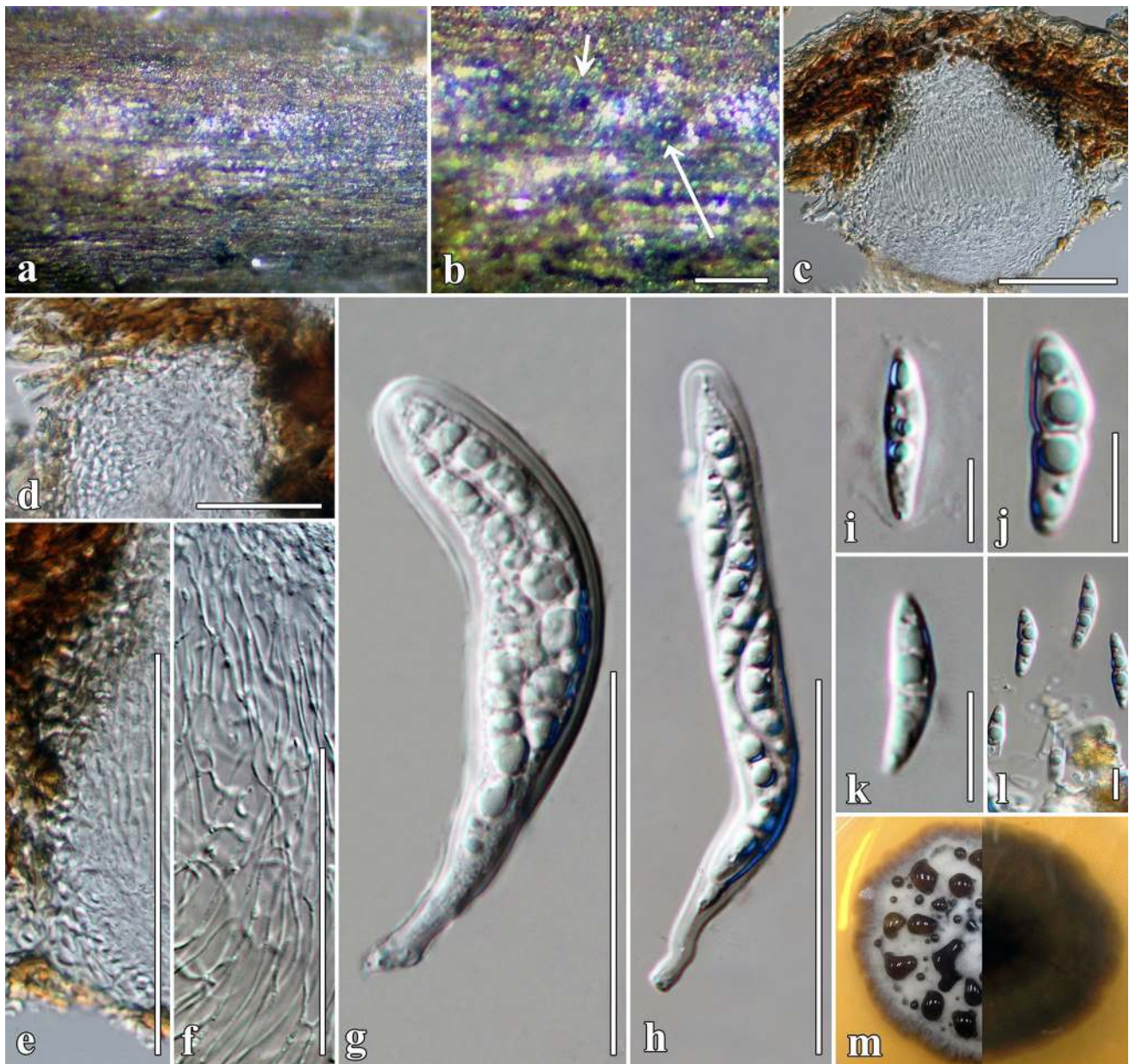
*Saprobic* on dried stem of herbaceous plants. **Sexual morph:** *Ascomata* solitary, gregarious, semi-immersed to erumpent, subglobose to compressed, coriaceous, dark brown to black, ostiolate. *Ostioles* central, filled with hyaline periphyses. *Peridium* thin, uniform of flattened cells of *textura angularis*, thin-walled, cells towards the inside lighter, inner layer composed of thin, hyaline, gelatinous layer. *Hamathecium* composed of numerous, filamentous, branched, septate, anastomosing, trabeculate pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate,

cylindrical-clavate to clavate, asymmetric, with furcate pedicel, apically rounded, with an ocular chamber. *Ascospores* biseriate or partially overlapping, broad fusiform, hyaline, tapering towards the ends, 1-euseptate, constricted at the septum, smooth-walled, with guttules in each cell, with mucilaginous sheath. **Asexual morph:** Undetermined.

*Type species: Parasulcatipora clematidis* Phukhams. & K.D. Hyde

*Notes:* *Parasulcatipora* is established as a monotypic genus. In a BLASTn search of GenBank, the closest match





**Fig. 74** *Parasulcatispora clematidis* (MFLU 17–1490, holotype). **a** Appearance of ascomata on host surface. **b** Close up of ascoma on host substrate. **c** Vertical section through ascoma. **d** Ostiolar canal.

**e** Section of peridium. **f** Trabeculate pseudoparaphyses. **g–h** Asci. **i–l** Ascospores. **m** Culture characteristics on MEA. Scale bars: **b**=200  $\mu$ m, **c**=100  $\mu$ m, **d**=20  $\mu$ m, **e–h**=50  $\mu$ m, **i–l**=10  $\mu$ m

for LSU and ITS sequences of MFLUCC 17–2082 is *Sulcatispora berchemiae* (HHUF 29097, NG\_059390) with 98% similarity, while the ITS sequence had 88% similarity to NR\_153444. Based on the multi-gene (LSU, SSU, ITS and *tef1* regions) analyses, MFLUCC 17–2082 formed a separate lineage within Sulcatisporaceae (Fig. 72). The genus matches Sulcatisporaceae species in having immersed, subglobose to hemispherical ascomata, short ostioles, trabeculate pseudoparaphyses (Liew et al. 2000), and fusiform, hyaline, septate ascospores with mucilaginous appendages

(Tanaka et al. 2015). *Parasulcatispora* is similar to *Sulcatispora* but lacks a pseudoclypeus and has small flattened ascospores with narrower asci and ascospores. The asexual morph failed to develop in culture. We introduce the new genus based on morphology and phylogenetic evidence.

***Parasulcatispora clematidis*** Phukhams. & K.D. Hyde, **sp. nov.**

*Index Fungorum* number: IF557205; *Facesoffungi* number: FoF 07342, Fig. 74.

**Etymology:** The specific name “*clematidis*” refers to the host substrate.

**Holotype:** MFLU 17–1490.

**Saprobic** on dead stems of *Clematis fulvicoma*.

**Sexual morph:** *Ascomata* 160–230 × 230–320 μm ( $\bar{x}$  = 190 × 305 μm, n = 5), solitary, gregarious, semi-immersed to erumpent, subglobose to compressed, coriaceous, dark brown to black, only ostiole visible, ostiolate. *Ostioles* 84–115 × 116–133 μm ( $\bar{x}$  = 100 × 124 μm, n = 5), shiny, central, smooth-walled, filled with hyaline periphyses. *Peridium* 22–37 μm wide ( $\bar{x}$  = 19 μm, n = 20), uniform, wider at the apex, composed of 3–4 layers of somewhat flattened cells of *textura angularis*, thin-walled, cells towards the inside lighter, inner layer composed of thin hyaline gelatinous layer. *Hamathecium* composed of numerous, dense, 2–3 μm wide, filamentous, branched, septate, anastomosing, trabecular pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 53–88 × 8–17 μm ( $\bar{x}$  = 72 × 13 μm, n = 30), 8-spored, bitunicate, fissitunicate, cylindrical-clavate to clavate, asymmetric, with furcate pedicel, apically rounded, with an ocular chamber. *Ascospores* 16–21 × 4–6 μm ( $\bar{x}$  = 17 × 5 μm, n = 40), biseriate or partially overlapping, broad fusiform with acute ends, tapering towards the ends, 1-euseptate, constricted at the septum, upper cell broader than lower cell, smooth-walled, with two guttules in each cell, hyaline, with 4–7 μm wide mucilaginous appendages. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Culture from above, greyish brown, covered with grey fluffy mycelia, dense, circular, umbonate, dull, undulate, radially furrowed, yellow oil droplets formed in the middle of cultures; reverse grey, radiating outwardly.

**Material examined:** Thailand, Chiang Rai Province, Mae Sai District, dried stems of *Clematis fulvicoma*, 20 March 2017, C. Phukhamsakda, CMTH28 (MFLU 17–1490, **holotype**); ex-type living culture, MFLUCC 17–2082.

**Host:** *Clematis fulvicoma*—(This study).

**Distribution:** Thailand—(This study).

**GenBank accession numbers:** LSU: MT214593; ITS: MT310637; *tefl*: MT394650.

**Notes:** *Parasulcatispora clematidis* can be distinguished morphologically (Fig. 74) and is supported by the phylogenetic analyses of combined LSU, SSU, ITS and *tefl* data (Tanaka et al. 2015, Fig. 72). The isolate MFLUCC 17–2082 also produces oil droplets in culture, and was further evaluated for the possibility of bioactive secondary metabolite production. The isolate MFLUCC 17–2082 inhibited the growth of *Bacillus subtilis* and partially inhibited conidial production of *Mucor plumbeus*, but did not reach significant values (data not shown).

## Teichosporaceae Barr

Teichosporaceae is characterized by erumpent to superficial ascomata, that are obpyriform, obovoid, or globose, with blunt ostioles filled with pseudoparaphyses or periphyses, and a multilayered peridium (Barr 2002; Jaklitsch et al. 2016). The family was treated under Floricolaceae (Thambugala et al. 2015; Wijayawardene et al. 2016), but Jaklitsch et al. (2016) synonymized Floricolaceae under Teichosporaceae. Wijayawardene et al. (2018) accepted twelve genera in Teichosporaceae. Based on molecular analyses and morphological comparisons, four of our collections from *Clematis* clustered in Teichosporaceae (Fig. 75).

### *Floricola* Kohlm. & Volkm.-Kohlm.

*Floricola* was introduced with *F. striata* as the type species. This genus is characterized by immersed, ostiolate, pycnidial conidiomata with a peridium of thick-walled cells with 5–6 cell layers, phialidic conidiogenesis cells, and septate, brown conidia (Ariyawansa et al. 2015a; Thambugala et al. 2015). The genus occurs on old inflorescences (Kohlmeyer and Volkmann-Kohlmeyer 2000; Ariyawansa et al. 2015a). We introduce *F. clematidis* on *Clematis vitalba* from Italy (Fig. 76).

### *Floricola clematidis* Phukhams., Camporesi & K.D. Hyde, sp. nov.

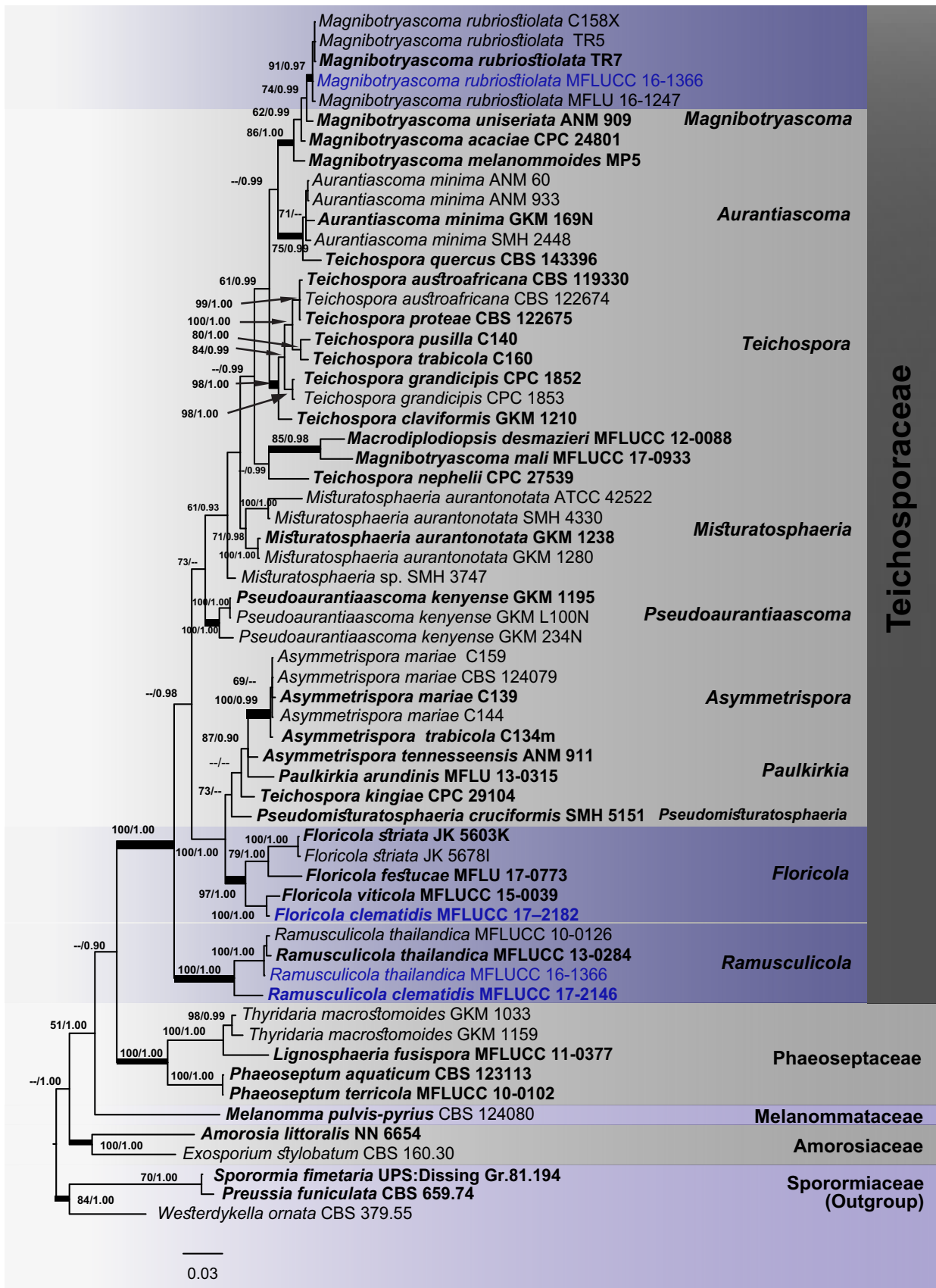
**Index Fungorum number:** IF557180; **Facesoffungi number:** FoF 07343, Fig. 76.

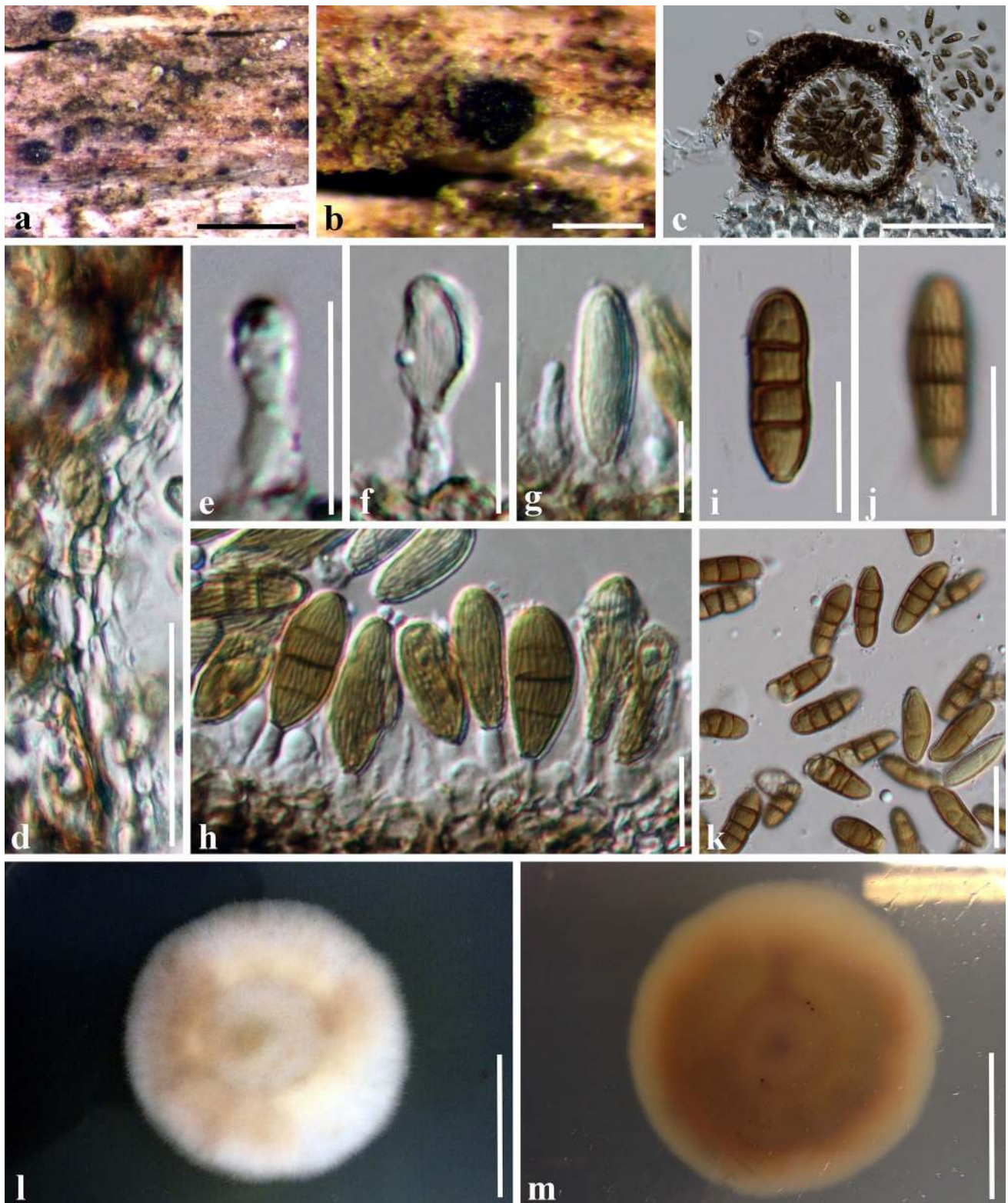
**Etymology:** The specific name “*clematidis*” refers to the host.

**Holotype:** MFLU 17–1535.

**Saprobic** on dead stems of *Clematis vitalba*. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata*

**Fig. 75** Best scoring RAXML tree with a final likelihood value of  $-21485.053484$  based on combined LSU, ITS, *tefl* and *rpb2* sequence data for Teichosporaceae. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted with members of the Sporormiaceae. Sixty-one strains were included in the combined gene sequence analyses which comprised 3719 characters (1084 characters for LSU, 626 characters for ITS, 924 characters for *tefl* and 1085 characters for *rpb2*, including gap regions). The tree from the maximum likelihood analysis had similar topology to the Bayesian 50% majority-rule consensus phylogram. The matrix had 1574 distinct alignment patterns with 42.64% undetermined characters and gaps. Estimated base frequencies were as follows; A = 0.240047, C = 0.261812, G = 0.279530, T = 0.218610; substitution rates AC = 1.269781, AG = 3.374707, AT = 1.851379, CG = 1.247283, CT = 9.170687, GT = 1.000000; gamma distribution shape parameter  $\alpha = 0.475238$ . In our analysis, GTR + I + G model was used for each partition in Bayesian posterior analysis. The species determined in this study are indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95)





**Fig. 76** *Floricola clematidis* (MFLU 17–1535, holotype). **a, b** Appearance of conidiomata on *Clematis vitalba*. **c** Vertical section through conidioma. **d** Section of conidioma wall. **e–h** Conidiog-

enous cells and conidia. **i–k** Conidia. **l, m** Culture characteristic on MEA. Scale bars: **a** = 500  $\mu$ m, **b** = 200  $\mu$ m, **c** = 100  $\mu$ m, **d, k** = 20  $\mu$ m, **e–j** = 5  $\mu$ m, **l, m** = 25 mm

119–135 × 133–166 µm ( $\bar{x}$  = 127 × 146 µm, n = 10), pycnidial, solitary, aggregated, uniloculate, flat, semi-immersed, covered by a pseudoclypeus, globose, coriaceous, thick-walled, dark brown to reddish brown, without ostioles. *Conidiomatal wall* 11–24 µm wide, uniform, outer layer composed 4–5 layers of reddish brown cells of *textura angularis*, lined with a thin, hyaline layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 4–12 × 2–4 µm ( $\bar{x}$  = 7 × 3 µm, n = 30), enteroblastic, phialidic, determinate, discrete, truncate or doliiform, hyaline. *Conidia* 13–21 × 5–7 µm ( $\bar{x}$  = 17 × 6 µm, n = 50), broad oblong or ellipsoid, slightly curved towards the ends, ends rounded, bud scars or disjunctors present at the site of attachment, hyaline when immature, reddish brown at maturity, with longitudinal striations visible even while attached to conidiogenous cells, 3-euseptate, with prominent longitudinal striations, smooth-walled.

**Culture characters:** Colonies on MEA reaching 20 mm diam. after 2 weeks at 25 °C. Cultures from above, cream to orangish-white, dense, circular, umbonate, papillate, fluffy, slightly radiating in the lower part; reverse orange at the centre, light brown, radiating outwardly.

**Material examined:** Italy, Forlì-Cesena Province, Cabelli-Santa Sofia, dead aerial branch of *Clematis vitalba*, 23 January 2014, E. Camporesi, IT 1668 (MFLU 17–1535, **holotype**); ex-type living culture, MFLUCC 17–2182.

**Host:** *Clematis vitalba*—(This study).

**Distribution:** Italy—(This study).

**GenBank accession numbers:** LSU: MT214594; SSU: MT226706; ITS: MT310638; *tef1*: MT394651.

**Notes:** *Floricola clematidis* constitutes a strongly supported clade (100% ML/1.00 BYPP) with *F. viticola* (MFLUCC 15–0039) (Fig. 75). *Floricola clematidis* is similar to *F. viticola*. Both species are similar in having semi-immersed conidiomata, but *F. clematidis* has smaller conidiomata (127 × 146 vs 237 × 177 µm) and larger, broad oblong or ellipsoid conidia (17 × 6 vs 9 × 4 µm). *Floricola clematidis* also has prominent, longitudinal striations on the conidia (Ariyawansa et al. 2015a, Fig. 76). A BLASTn search of GenBank showed ITS sequence had 99.26% similarity to *F. viticola* (7 nucleotide difference in 626 nucleotides).

### *Magnibotryascoma* Thambug. & K.D. Hyde

*Magnabotrioscoma*, typified by *M. uniseriatum* was introduced to accommodate a separate lineage of *Misturatosphaeria uniseriata* in Teichosporaceae. *Magnibotryascoma* is characterized by erumpent to superficial ascomata with short ostioles, and fusiform to elliptical, septate, guttulate ascospores. The asexual morph is pycnidial with aseptate and brown conidia (Crous et al. 2015b; Jaklitsch et al. 2016). We redefine the Teichosporaceae, based on multigene phylogenetic analyses, as four species formed a strongly supported

clade with *Magnibotryascoma* sensu stricto (Fig. 75). We introduce the first report of *Magnibotryascoma rubriostiolata* on *Clematis vitalba* and document the characters of the asexual morph (Fig. 77).

### *Magnibotryascoma rubriostiolata* (Jaklitsch & Voglmayr) Phukhams., E.B.G. Jones & K.D. Hyde, **comb. nov. and new host record**

**Index Fungorum number:** IF557181; **Facesoffungi number:** FoF 07344, Fig. 77

**Basionym:** *Teichospora rubriostiolata* Jaklitsch & Voglmayr, Mycological Progress 15(31):13 (2016)

**Saprobic** on dead stems of *Clematis vitalba*. **Sexual morph:** Described in Jaklitsch et al. 2016. **Asexual morph:** *Conidiomata* 110–188 × 93–164 µm ( $\bar{x}$  = 160 × 131 µm, n = 5), pycnidial, solitary, aggregated, uniloculate, immersed, globose to subglobose, coriaceous, dark brown to brown, with papilla ostiolate. *Ostioles* 25 × 19 µm, central, papillate. *Conidiomatal wall* 15–29 µm wide, thick, multilayered, outer layer composed of 6–7 layers of light brown to brown cells of *textura angularis*, lined with a hyaline layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 4–11 × 2–3 µm ( $\bar{x}$  = 7 × 2 µm, n = 30), enteroblastic, discrete, integrated, oblong, cylindrical, hyaline, arising from the inner layer of pycnidium wall. *Conidia* 3–5 × 1.8–3 µm ( $\bar{x}$  = 3.7 × 2.5 µm, n = 50), subglobose, oval, with guttule in each cell, hyaline when immature, pale brown at maturity, aseptate, smooth-walled.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 2 weeks at 25 °C. Cultures from above, greenish-brown, dense, circular, umbonate, papillate, fluffy, covered with white aerial mycelium, droplets formed at room temperature; reverse orange at the centre, brown radiating outwardly.

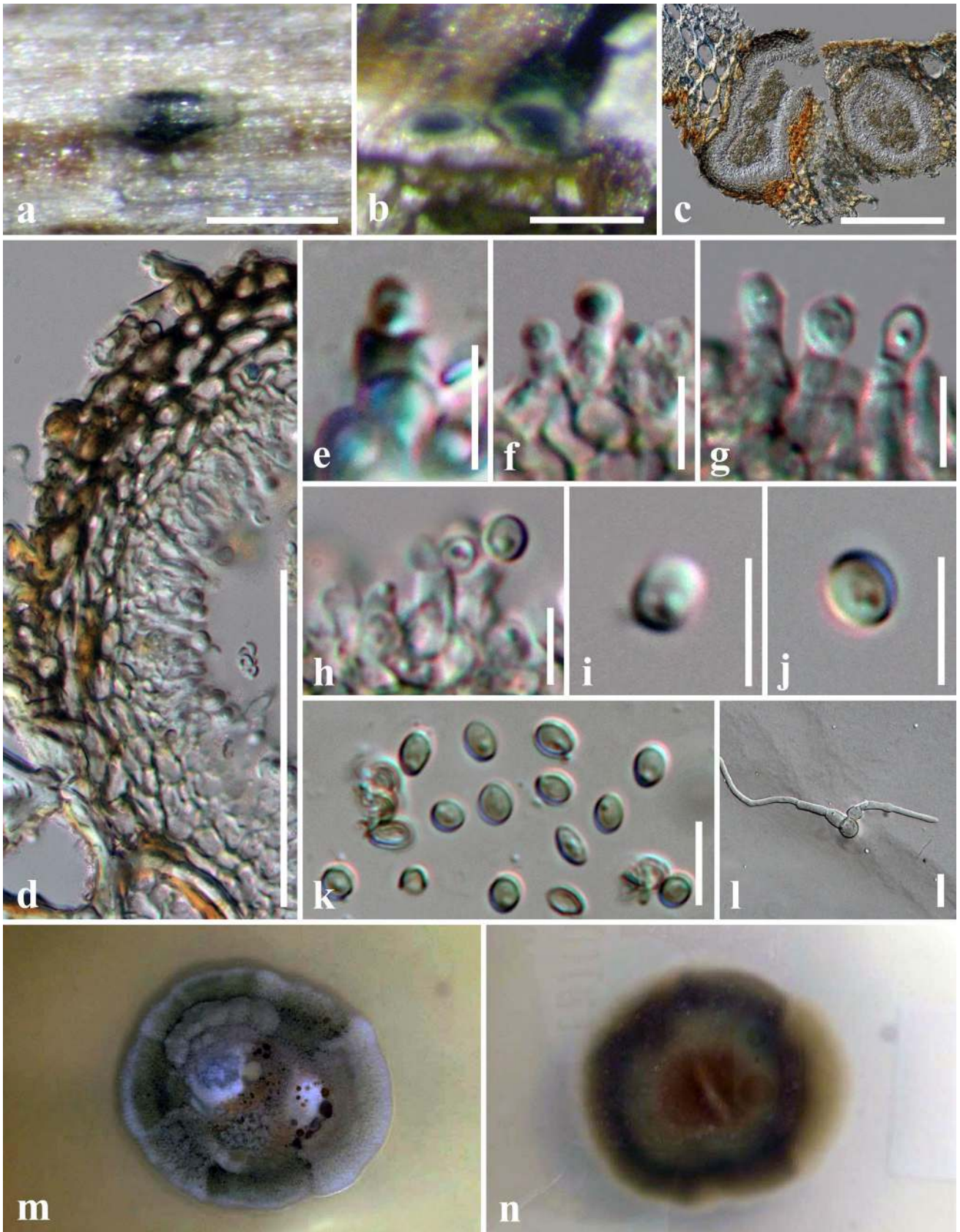
**Material examined:** UK, Carmarthenshire, Laugharne, on dead stems of *Clematis vitalba*, 29 July 2016, E. B. G. Jones, GJ 311 (MFLU 17–1539); living culture, MFLUCC 16–1366.

**Hosts:** *Clematis vitalba*, *Ribes sanguineum*, *Robinia pseudoacacia*, *Vaccinium myrtillus*—(Jaklitsch et al. 2016; this study).

**Distribution:** Belgium, Germany, Norway, UK (England)—(Jaklitsch et al. 2016; this study).

**GenBank accession numbers:** LSU: MT214595; ITS: MT310639.

**Notes:** Isolate MFLUCC 16–1366 formed a strongly supported clade (91% ML/0.97 BYPP) with four isolates of *Magnibotryascoma rubriostiolata* (Fig. 75). *Magnibotryascoma rubriostiolata* was introduced as *Teichospora rubriostiolata* by Jaklitsch et al. (2016) on bark and wood of trees, shrubs, and vineyard poles distributed in Europe. A collection on twigs of *Ribes sanguinea* was recorded as coniothyrium-like, but without a full description, although



**Fig. 77** *Magnibotryascoma rubriostiolata* (MFLU 17–1539). **a**, **b** Appearance of conidiomata on *Clematis vitalba*. **c** Vertical section through conidiomata. **d** Section of conidioma wall. **e–h** Conidiogenous cells and conidia. **i–k** Conidia. **l** Germinating conidium. **m**, **n** Culture characteristics on MEA. Scale bars: **a**, **b** = 200  $\mu\text{m}$ , **c** = 100  $\mu\text{m}$ , **d** = 50  $\mu\text{m}$ , **e–l** = 5  $\mu\text{m}$

Jaklitsch et al. (2016) illustrated the sexual morph. A comparison of the ITS and *tef1* DNA sequences of our isolate (MFLUCC 16–1366) with four isolates showed 100% similarity. This is the first record of *M. rubriostiolata* on *Clematis* with illustration of their asexual morph (Fig. 77). We also provide sequence data and phylogenetic analyses (Fig. 75).

#### *Ramusculicola* Thambug. & K.D. Hyde

*Ramusculicola* is typified by *R. thailandica* Thambug. & K.D. Hyde. The genus is characterized by semi-immersed to partially erumpent, coriaceous ascomata, short pedicellate asci, and fusiform to cylindrical, hyaline ascospores with polar appendages with blunt ends, and a lateral pad-like structure of sheath that surrounds the ascospores (Thambugala et al. 2015). Based on phylogenetic analyses our collection isolated from *Clematis* species from Thailand revealed a new species, *Ramusculicola clematidis* (Fig. 75).

#### *Ramusculicola clematidis* Phukhams. & K.D. Hyde, sp. nov.

*Index Fungorum number*: IF557183; *Facesoffungi number*: FoF 07345, Fig. 78.

*Etymology*: The specific name “*clematidis*” refers to the host.

*Holotype*: MFLU 17–1503

*Saprobic* on dead stem of *Clematis sikkimensis*.

**Sexual morph**: *Ascomata* 155–205  $\times$  135–200  $\mu\text{m}$  ( $\bar{x}$  = 175  $\times$  160  $\mu\text{m}$ ,  $n$  = 5), solitary or scattered, uniloculate, semi-immersed to erumpent, ampulliform to compressed subglobose, coriaceous, black to dark brown, ostiolate. *Ostioles* 75–95  $\times$  110–130  $\mu\text{m}$ , central, composed of 6–10 layers of *textura prismatica*, papillate, with variable walls, with pore-like opening, filled with hyaline periphyses. *Peridium* 15–30  $\mu\text{m}$  wide ( $\bar{x}$  = 20  $\mu\text{m}$ ,  $n$  = 20), uniform, multilayered, composed of 7(–9) layers of *textura prismatica*, cells towards the inside lighter, somewhat flattened, inner layer composed of thin hyaline gelatinous layer. *Hamathecium* composed of numerous, dense, 1.5–3.5  $\mu\text{m}$  ( $\bar{x}$  = 2  $\mu\text{m}$ ,  $n$  = 30), wide, filamentous, branched, septate, anastomosing, cellular pseudoparaphyses, situated between and above the asci, embedded in a gelatinous matrix. *Asci* 55–100  $\times$  8–13  $\mu\text{m}$  ( $\bar{x}$  = 80  $\times$  10  $\mu\text{m}$ ,  $n$  = 30), 8-spored, bitunicate, fissionate, oblong to cylindrical-clavate, short pedicellate, apically rounded, with an ocular chamber, arising from the basal ascoma. *Ascospores* 20–30  $\times$  4–8  $\mu\text{m}$  ( $\bar{x}$  = 25  $\times$  6  $\mu\text{m}$ ,  $n$  = 50), biserial or partially overlapping, broad-fusiform, tapering

towards the ends, rounded at upper end, acute towards the lower end, (1–)2(–3)-transversely euseptate, strongly constricted at the septa, with (1–)2 guttules in each cell, hyaline, swollen near median septum, with 3–8  $\mu\text{m}$  of sheath drawn out at both ends, forming polar appendages with blunt ends, with a lateral pad-like structure, up to 2  $\mu\text{m}$  wide at the sides. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA reaching 20 mm diam. after 2 weeks at 25 °C. Culture from above, brownish grey, dark green in the middle, forming cream, fluffy mycelium at the edge, dense, umbonate, raised with concave edge, rough, dull, lobate, radially furrowed, slight brown pigment diffused into the agar, oil droplets formed in the middle of cultures; reverse dark brown radiating light brown with white margin.

*Material examined*: Thailand, Chiang Rai Province, Mae Sai District, dead stems of *Clematis sikkimensis*, 24 June 2017, C. Phukhamsakda & M. van de Bult, CMTHDT16 (MFLU 17–1503, **holotype**); ex-type living culture, MFLUCC 17–2146.

*Host*: *Clematis sikkimensis*—(This study).

*Distribution*: Thailand—(This study).

*GenBank accession numbers*: LSU: MT214596; SSU: MT226707; ITS: MT310640; *tef1*: MT394652; *rpb2*: MT394707.

*Notes*: Based on phylogenetic analysis, *Ramusculicola clematidis* (MFLUCC 17–2146) clustered with *R. thailandica* with strong support (100% ML/1.00 BYPP, Fig. 77). *Ramusculicola clematidis* can be distinguished by its broad-fusiform ascospores, tapering towards the ends, rounded at the upper end and acute towards the lower end, while *R. thailandica* has ascospores that are acute at both ends (Thambugala et al. 2015; this study). Comparison of 551 nucleotides (without gaps) in the ITS region revealed 23 bp (4.1%) differences, while the *tef1* showed 27/920 bp (3%) differences from *R. thailandica*, respectively. Therefore, *R. clematidis* is introduced as a new species (Fig. 78).

#### *Ramusculicola thailandica* Thambug. & K.D. Hyde, in Fungal Diversity 74: 251 (2015), new host record

*Index Fungorum number*: IF551265; *Facesoffungi number*: FoF 01092, Fig. 79.

*Saprobic* on dead stem of *Clematis sikkimensis*. **Sexual morph**: *Ascomata* 174–180  $\times$  170–215  $\mu\text{m}$ , solitary or scattered, uniloculate, semi-immersed to erumpent, ampulliform to compressed globose, coriaceous, dark brown to black, ostiolate. *Ostioles* 43–62  $\times$  79–120  $\mu\text{m}$ , central, thick, composed of 20 layers of *textura prismatica*, papillate, with variable walls, with pore-like opening, filled with hyaline periphyses. *Peridium* 15–48  $\mu\text{m}$  wide ( $\bar{x}$  = 26  $\mu\text{m}$ ,  $n$  = 20), uniform, multilayered, composed of 13(–15) layers of *textura angularis*, thick-walled cells lighter towards



**Fig. 78** *Ramusicicola clematidis* (MFLU 17–1503, holotype). **a** Appearance of ascomata on host surface. **b** Close up of ascoma on host substrate. **c** Vertical section of ascoma. **d** Ostiolar canal. **e** Sec-

tion of peridium. **f** Pseudoparaphyses. **g–i** Asci. **j–n** Ascospores. Scale bars: **b**=200  $\mu$ m, **c**=100  $\mu$ m, **d**=50  $\mu$ m, **e–i**=20  $\mu$ m, **j–n**=10  $\mu$ m



the inside, somewhat flattened, inner layer composed of thin hyaline gelatinous layer. *Hamathecium* composed of numerous, dense, 1.6–3 µm wide, filamentous, branched, septate, cellular pseudoparaphyses, situated between and above the asci, embedded in a gelatinous matrix. *Asci* 60–91 × 8–13 µm ( $\bar{x}$  = 74 × 10 µm, n = 30), 8-spored, bitunicate, fissitunicate, oblong to cylindrical-clavate, short pedicellate, apically rounded, with an ocular chamber. *Ascospores* 18–30 × 4–7 µm ( $\bar{x}$  = 24 × 6 µm, n = 50), biseriate or partially overlapping, broad-fusiform, tapering towards the ends, ends acute, 1-transversely euseptate, strongly constricted at the septum, with 2–3(–4) guttules in each cells, hyaline, swollen near median septum, with 3–6 µm of sheath drawn out at both ends forming polar appendages, with a lateral pad-like structure, up to 2 µm wide at the sides. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 40 mm diam. after 4 weeks at 25 °C. Culture from above, brownish grey, forming zonate greyish orange, fluffy mycelium at the edge, dense, umbonate, raised with concave edge, rough, dull, lobate, radially furrowed, brown pigment slightly diffused in the agar, oil droplets formed in the middle of culture; reverse dark brown, radiating, light orange outwardly.

**Material examined:** Thailand, Chiang Rai Province, Mae Sai District, dead stems of *Clematis sikkimensis*, 24 June 2017, C. Phukhamsakda & M. van de Bult, CMTHDT13 (MFLU 17–1502); living culture, MFLUCC 17–2093.

**Hosts:** Fallen twig of deciduous tree, *Clematis sikkimensis*—(Thambugala et al. 2015; this study).

**Distribution:** Thailand—(Thambugala et al. 2015; this study).

**GenBank accession numbers:** LSU: MT214597; SSU: MT226708; ITS: MT310641; *tefl*: MT394653; *rpb2*: MT394708.

**Notes:** *Ramusculicola thailandica* was first recorded by Thambugala et al. (2015). The comparison of morphological characters and pairwise comparisons in the ITS and *tefl* regions confirm the similarity of MFLUCC 17–2093 with collections reported in Thambugala et al. (2015). This is the first record of *R. thailandica* on *Clematis* species (Fig. 79). We also provide sequence data and phylogenetic analyses (Fig. 75).

### Thyridariaceae Tian & K.D. Hyde

We follow Hyde et al. (2013) and Tibpromma et al. (2017) to treat Thyridariaceae as a separate family in *Pleosporales*. Seven genera are listed in Thyridariaceae (Hyde et al. 2013; Tibpromma et al. 2017; Devadatha et al. 2018; Wanasinghe et al. 2018; Mapook et al. 2020). The phylogenetic tree, based on combined LSU, ITS, *tefl* and *rpb2* sequence data is presented in Fig. 80.

### *Parathyridaria* Jaklitsch & H. Voglmayr

*Parathyridaria* is typified with *P. ramulicola*. *Parathyridaria* is characterized by immersed and globose ascomata, a pseudoparenchymatous peridium, papilla with or without orange colouration and fusoid, septate, hyaline to greyish brown ascospores, with a pycnidial asexual morph (Jaklitsch and Voglmayr 2016; Wanasinghe et al. 2018). The concatenated LSU, ITS, *tefl*, and *rpb2* dataset showed that fungal collections from *Clematis* species grouped with the type strain of *Parathyridaria* with moderate support (51% ML/1.00 BYPP, Fig. 80). The new species *Parathyridaria clematidis*, *P. serratifoliae* and *P. virginiana* are introduced from *Clematis* collections from the European continent.

### *Parathyridaria clematidis* Phukhams., Camporesi & K.D. Hyde, sp. nov.

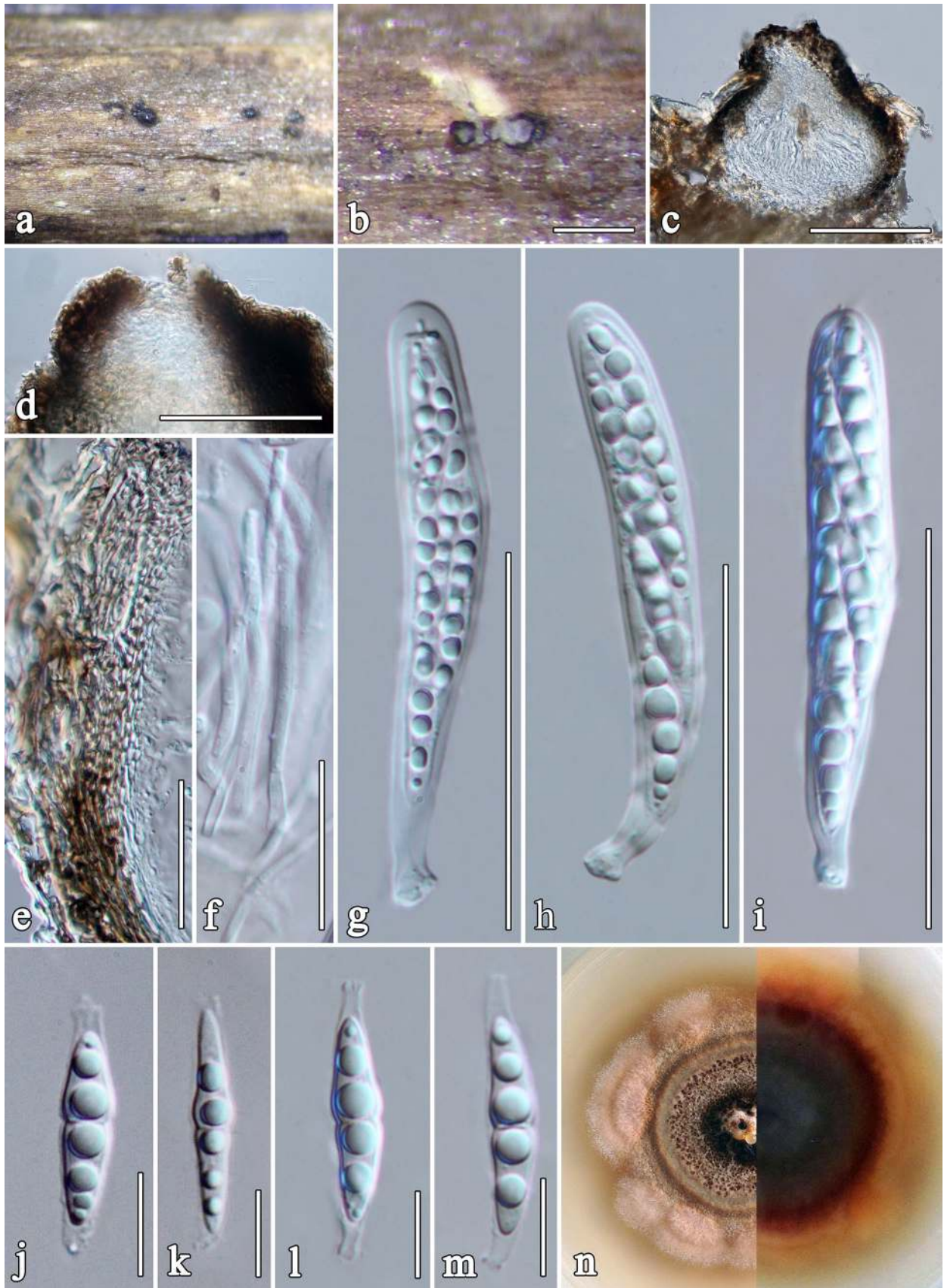
**Index Fungorum number:** IF557206; **Facesoffungi number:** FoF 07347, Fig. 81.

**Etymology:** The epithet refers to the host plant, *Clematis*.

**Holotype:** MFLU 16–0061

**Saprobic** on dead stems of *Clematis*. **Sexual morph:** *Ascomata* 180–310 × 205–330 µm ( $\bar{x}$  = 253 × 263 µm, n = 15), on the surface of the host, solitary, gregarious, immersed, only orange ostioles are visible, globose to depressed-globose, coriaceous, black to dark brown, rough-walled, papillate, ostiolate. *Ostioles* 67–107 × 122–183 µm, central, black to reddish brown, papillate, opening by a pore, filled with paraphyses, orange around pore. *Peridium* 17–33(–50 µm at apex) wide, multilayered, outer layer composed of 7–10 layers of dark brown to light brown cells of *textura angularis*, heavily pigmented at outer layer with thin, hyaline inner layer. *Hamathecium* composed of numerous, dense, 0.5–1.3 µm wide ( $\bar{x}$  = 1 µm, n = 60), filiform, branched, septate, trabeculate pseudoparaphyses. *Asci* 77–112 × 12–15 µm ( $\bar{x}$  = 93 × 14 µm, n = 20), (4–)8-spored, bitunicate, fissitunicate, broad fusiform, clavate, apically rounded with an ocular chamber. *Ascospores* 16–24 × 5–8 µm ( $\bar{x}$  = 20 × 6 µm, n = 50), biseriate, partial overlapping, oval to broad fusiform, sometimes inequilateral, with rounded ends, hyaline, 1(–2)-septate, constricted at septa, cell above median septum enlarged, granulate in each cell, rough-walled, with mucilaginous sheath. **Asexual morph:** Coelomycetous, pycnidia produced on mycelium in MEA. *Conidiomata* 135–170 × 102–195 µm diam., pycnidial, dark brown to black, covered by dense vegetative hyphae, superficial, uniloculate, solitary to scattered, globose. *Conidiomatal wall* 15–17 µm wide, thin, black to brown, with cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 4–8(–18) × 2–6 µm ( $\bar{x}$  = 7 × 3 µm, n = 30), enteroblastic, annellidic, sometimes a sympodial. *Conidia* 5–7 × 3–5 µm ( $\bar{x}$  = 6 × 4 µm, n = 100), ellipsoid, pale brown, aseptate, guttulate, smooth-walled.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Culture from above, cream



**Fig. 79** *Ramusculicola thailandica* (MFLU 17–1502). **a** Appearance of ascomata on host surface. **b** Close up of ascoma on host substrate. **c** Vertical section of ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Pseudoparaphyses. **g–i** Asci. **j–m** Ascospores. **n** Culture characters on MEA. Scale bars: **b**=200  $\mu$ m, **c**=100  $\mu$ m, **d**, **g–i**=50  $\mu$ m, **e–f**=20  $\mu$ m, **j–m**=10  $\mu$ m

with lilac in the middle, white edge, dense, radially striated with lobate edge, flattened, umbonate, fluffy; reverse cream. Asexual morph formed in culture similar to those occurring on the host substrate.

**Material examined:** Italy, Forlì-Cesena Province, near Meldola, on dead aerial stems of *Clematis vitalba*, 27 December 2015, E. Camporesi, IT 2759 (MFLU 16–0061, **holotype**); ex-type living culture, MFLUCC 17–2185; Belgium, Flemish Brabant, Meise Botanic Garden, Bouchout Domain, on dead stems of *C. viticella*, 13 June 2017, D. Ertz & C. Gerstmans, BRCV6 (MFLU 17–1518, paratype); ex-paratype culture, MFLUCC 17–2160; *ibid.*, BRCV3 (MFLU 17–1515, paratype); ex-paratype culture, MFLUCC 17–2157; on dead stems of *C. serratifolia*, 13 June 2017, D. Ertz & C. Gerstmans, BRCS3 (MFLU 17–1511, paratype); ex-paratype culture, MFLUCC 17–2154.

**Hosts:** *Clematis serratifolia*, *C. vitalba*, *C. viticella*—(This study).

**Distribution:** Belgium, Italy—(This study).

**GenBank accession numbers:** MFLUCC 17–2185: LSU: MT214598; SSU: MT226709; ITS: MT310642; *tef1*: MT394654; *rpb2*: MT394709. MFLUCC 17–2160: LSU: MT214599; SSU: MT226710; ITS: MT310643; *tef1*: MT394655; *rpb2*: MT394710. MFLUCC 17–2157: LSU: MT214600; SSU: MT226711; ITS: MT310644; *tef1*: MT394656; *rpb2*: MT394711. MFLUCC 17–2154: LSU: MT214601; SSU: MT226712; ITS: MT310645; *tef1*: MT394657; *rpb2*: MT394712.

**Notes:** Four isolates of *Parathyridaria clematidis* were recovered from *Clematis* species collected from Europe. Morphological characters such as immersed ascomata, an orange ostiolar canal, trabeculate pseudoparaphyses and fusoid ascospores matches the concept of *Parathyridaria* (Jaklitsch and Voglmayr 2016). *Parathyridaria clematidis* is similar to *P. robiniae* in having hyaline ascospores, however, *P. robiniae* has larger ascomata (450–470  $\times$  255–270 vs 182–310  $\times$  205–329  $\mu$ m, Table 4) with one septate ascospores (Tibpromma et al. 2017). Our four isolates of *P. clematidis* grouped together with strong statistical support (96% ML/1.00 BYPP, Fig. 80). A comparison of the *tef1* region of *P. robiniae* (MFLUCC 14–1119) with our new strains revealed seven base pair differences, therefore, we identify our isolates as a new species, *Parathyridaria clematidis* (Fig. 81).

***Parathyridaria serratifoliae*** Phukhams., Ertz, Gerstmans & K.D. Hyde, **sp. nov.**

**Index Fungorum number:** IF557208, **Facesoffungi number:** FoF 07348, Fig. 82.

**Etymology:** The species epithet refers to a species of the host species *Clematis serratifolia*.

**Holotype:** MFLU 17–1512

**Saprobic** on dead stems of *Clematis serratifolia*. **Sexual morph:** *Ascomata* 288–342  $\times$  235–301  $\mu$ m ( $\bar{x}$  = 322  $\times$  265  $\mu$ m,  $n$  = 10), on the surface of the host, solitary, gregarious, immersed, orange ostioles visible, globose to depressed-globose, coriaceous, dark brown to brown, rough-walled, papillate, ostioles central. *Ostioles* 72–103  $\times$  106–142  $\mu$ m ( $\bar{x}$  = 83  $\times$  124  $\mu$ m,  $n$  = 5), central, black to reddish brown, papillate, opening by a pore, filled with periphyses. *Peridium* 17–30(–50  $\mu$ m at apex) wide, outer layer composed of 5–7 layers of reddish brown cells of *textura angularis*, heavily pigmented at outer layer, the inner layer composed of 7 layers of hyaline and thin-walled cells. *Hamathecium* of dense, 0.9–1.5  $\mu$ m wide ( $\bar{x}$  = 1.3  $\mu$ m,  $n$  = 50), filiform, branched, septate, trabeculate pseudoparaphyses. *Asci* 97–145  $\times$  12–19  $\mu$ m ( $\bar{x}$  = 110  $\times$  17  $\mu$ m,  $n$  = 20), 8-spored, bitunicate, fissitunicate, broad fusiform, cylindrical-clavate, apically rounded with ocular chamber. *Ascospores* 20–38  $\times$  5–9  $\mu$ m ( $\bar{x}$  = 24  $\times$  7  $\mu$ m,  $n$  = 50), biseri-ate, partially overlapping, ellipsoid to broad fusiform, sometimes inequilateral, with rounded ends, hyaline, (1–)3-septate, constricted at the septa, cell above median septum enlarged, granulate in each cell, rough-walled, with mucilaginous sheath. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Culture from above, cream, brown in the middle, edge white, dense, flattened, umbonate, floccose; reverse cream, thin, with flat parchment-like sheets.

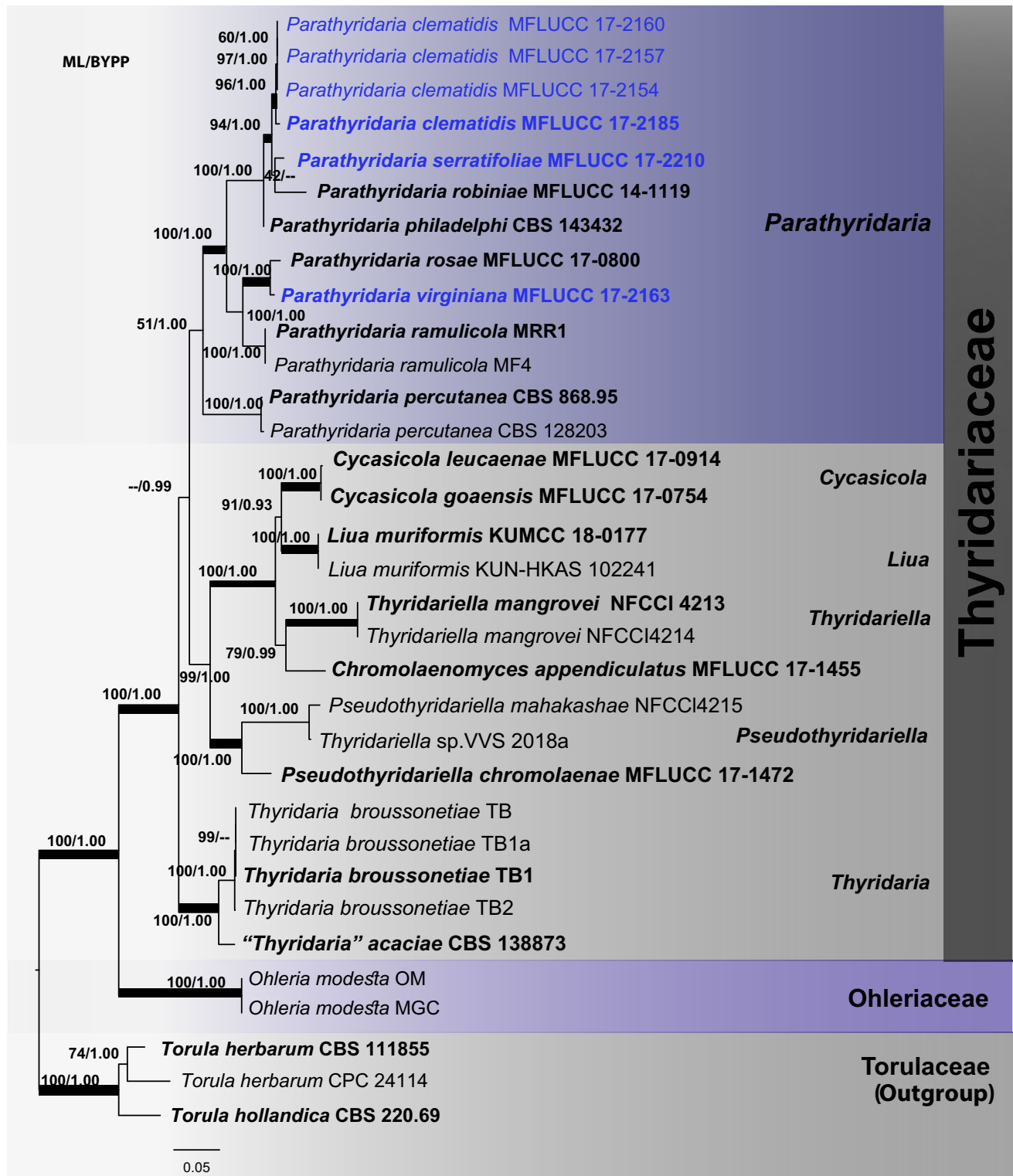
**Material examined:** Belgium, Flemish Brabant, Meise Botanic Garden, Bouchout Domain, on dead stems of *Clematis serratifolia*, 13 June 2017, D. Ertz & C. Gerstmans, BRCS4 (MFLU 17–1512, **holotype**); ex-type living culture, MFLUCC 17–2210.

**Host:** *Clematis serratifolia*—(This study).

**Distribution:** Belgium—(This study).

**GenBank accession numbers:** LSU: MT214602; SSU: MT226713; ITS: MT310646; *tef1*: MT394658; *rpb2*: MT394713.

**Notes:** In our phylogenetic analysis from LSU, ITS, *tef1*, and *rpb2* sequence data, *Parathyridaria serratifoliae* formed a basal clade with *P. clematidis* and *P. robiniae* with strong support (94% ML/1.00 BYPP, Fig. 80). *Parathyridaria serratifoliae* can be distinguished from *P. clematidis* and *P. robiniae* based on spore dimensions and having 3-septate ascospores (Table 4). To further support the establishment of the new taxon as per the guidelines of Jeewon and Hyde (2016), we checked the nucleotide differences. Within the



*tef1* regions there were 10 base pair differences from *P. robiniae*. Therefore, *P. serratifoliae* on *Clematis serratifolia* is introduced as a new species (Fig. 82).

*Parathyridaria virginianae* Phukhams., Ertz, Gerstmanns & K.D. Hyde, **sp. nov.**

*Index Fungorum* number: IF557209; *Facesoffungi* number: FoF 07349, Fig. 83.

**Fig. 80** The best scoring RAxML tree with a final likelihood value of  $-16490.277529$  based on combined LSU, ITS, *tef1* and *rpb2* sequence data for Thyrideriaceae. The tree is rooted with members of the Torulaceae. Thirty-three strains were included in the combined genes sequence analyses which comprised 3466 characters (890 characters for LSU, 534 characters for ITS, 964 characters for *tef1* and 1078 characters for *rpb2*, including gap regions). The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree from the maximum likelihood analysis had similar topology to the Bayesian 50% majority-rule consensus phylogram. The matrix had 1267 distinct alignment patterns, with 27.03% of undetermined characters and gaps. Estimated base frequencies were as follows; A=0.243395, C=0.269803, G=0.270965, T=0.215837; substitution rates AC=1.430051, AG=3.703624, AT=1.723186, CG=1.129553, CT=9.368763, GT=1.000000; gamma distribution shape parameter  $\alpha=0.456291$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study are indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95)

*Etymology:* The epithet refers to *Clematis virginiana*.

*Holotype:* MFLU 17–1521

*Saprobic* on dead branches of *Clematis virginiana*. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* 145–207  $\times$  204–259  $\mu\text{m}$  ( $\bar{x}=175 \times 230 \mu\text{m}$ ,  $n=5$ ), pycnidial, solitary, aggregated, uniloculate, immersed, covered by a pseudoclypeus, globose to subglobose, coriaceous, thick-walled, dark brown to brown, papillate, ostiolate. *Ostioles* 31  $\times$  50  $\mu\text{m}$ , central. *Conidiomatal wall* 13–24  $\mu\text{m}$  wide, outer layer composed of 5–7 layers of brown to light brown cells of *textura angularis*, lined with a hyaline layer bearing conidiogenous cells. *Conidiophores* single, short, densely aggregated, straight or flexuous, cylindrical, hyaline, erect, septate, 1–3-septate, smooth. *Conidiogenous cells* 4–21  $\times$  2–4  $\mu\text{m}$  ( $\bar{x}=7 \times 3 \mu\text{m}$ ,  $n=30$ ), enteroblastic, phialidic, annellidic, hyaline, discrete, arising from the inner layer of pycnidium wall. *Conidia* 3–5  $\times$  1.8–3  $\mu\text{m}$  ( $\bar{x}=4 \times 2.5 \mu\text{m}$ ,  $n=50$ ), oval or subglobose, slightly curved, with 1(–2) guttules in each cell, hyaline when immature, pale brown at maturity, aseptate, smooth-walled.

*Culture characters:* Colonies on MEA reaching 20 mm diam. after 2 weeks at 25 °C. Cultures from above, white, dense, circular, umbonate, covered with white aerial mycelium; reverse yellow at the central, cream.

*Material examined:* Belgium, Flemish Brabant, Meise Botanic Garden, Bouchout Domain, on dead stems of *Clematis virginiana* L., 13 June 2017, D. Ertz & C. Gerstmans, BRVir3 (MFLU 17–1521, **holotype**); ex-type living culture, MFLUCC 17–2163.

*Hosts:* *Clematis virginiana*—(This study).

*Distribution:* Belgium—(This study).

*GenBank accession numbers:* LSU: MT214603; SSU: MT226714; ITS: MT310647; *tef1*: MT394659; *rpb2*: MT394714.

*Notes:* According to morphology and phylogenetic analysis (Fig. 83), *Parathyridaria virginiana* (MFLUCC 17–2163) is closely related to *P. rosae* (MFLUCC 17–0800) which was collected from *Rosa* sp. in the USA (Wanasinghe et al. 2018). The asexual morph in *Parathyridaria* has pycnidia, with phialidic conidiogenous cells and ellipsoid, unicellular, hyaline to pale brown conidia (Crous et al. 2018), which resembles our collection. *Parathyridaria rosae* was reported as a sexual morph whilst our collection showed only the asexual morph, thus the morphology could not be compared. Pairwise comparison of ITS (including 5.8S region) region showed five base pair differences (of 534 base pairs), however, the *tef1* and *rpb2* regions are not available for comparison. Phylogenetic analyses show strong support for the two species (100% ML/1.00 BYPP, Fig. 80), thus, we introduce a new species, *P. virginiana*.

### Torulaceae Corda

Torulaceae, typified by *Torula* was introduced by Sturm (1829) for an asexual morph with mononematous conidiophores (Seifert et al. 2011). Placement of Torulaceae was verified in Crous et al. (2015a) with two accepted genera, *Dendryphion* and *Torula*. Subsequently, *Sporidesmioides*, *Neotorula* and *Rostriconidium* were introduced to the family based on modern taxonomic classification. Torulaceae currently contains five genera which are asexual morphs (Crous et al. 2015a; Hyde et al. 2016; Su et al. 2016; Tibpromma et al. 2018). In this study, a combined dataset of LSU, ITS, *tef1*, and *rpb2* sequence data was used to follow the recent treatment in Su et al. (2016), with first report of *Dendryphion europaeum* and *Torula chromolaenae* on *Clematis* (Fig. 84).

### Dendryphion Wallr.

*Dendryphion* (typified with *D. comosum*) is commonly associated with dead stems of herbaceous plants and decaying wood, but also recorded from freshwater (Ellis 1971; Su et al. 2016). The genus is distinguishable from other members in Torulaceae in having apically branched, polytretic or enteroblastic conidiophores with dark pores, septate, brown conidia in chains with bud scars (Seifert et al. 2011; Crous et al. 2014a; Su et al. 2016). Based on morphological characters and phylogenetic results, we report the first record of *Dendryphion europaeum* on *Clematis* species (Fig. 85).

***Dendryphion europaeum*** Crous & R.K. Schumacher, in Persoonia 32: 243 (2014), **new host records**

*Index Fungorum number:* IF808927; *Facesoffungi number:* FoF 07346, Fig. 85.



**Fig. 81** *Parathyridaria clematidis* (MFLU 16–0061, holotype). **a**, **b** Appearance of ascomata on *Clematis vitalba*. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Trabeculate pseudoparaphyses. **g–i** Asci. **j–o** Ascospores (**i** Arrow indicated mucilaginous sheath). **p** Germinated ascospore. **q** Culture character-

istics on MEA. **r** Conidiomata forming on agar on rice straw media after 8 weeks. **s–x** Conidiogenous cells and developing conidia (**t** Conidiogenous cells). **y** Conidia. Scale bars: **a**, **b**, **r**=500  $\mu$ m, **c**=200  $\mu$ m, **d**=100  $\mu$ m, **e–i**=50  $\mu$ m, **j–o**, **t**=10  $\mu$ m, **s**, **u–y**=5  $\mu$ m, **q**=10 cm

**Table 4** Synopsis of sexual morph of *Parathyridaria* species

Species	Ascomata ( $\mu\text{m}$ )	Asci ( $\mu\text{m}$ )	Ascospores			Host	References
			Size ( $\mu\text{m}$ )	Septa	Colour		
<i>Parathyridaria clematidis</i> MFLUCC 17–2185	182–310×205–329	77–112×12–15	16–24×5–8	1(–2)	Hyaline	<i>Clematis</i> sp.	This study
<i>P. serratifoliae</i> MFLUCC 17–2210	288–342×235–301	97–145×12–19	20–38×5–9	(1–)3	Hyaline	<i>Clematis serratifolia</i>	This study
<i>P. ramulicola</i> MRR1	(200–)260–400(–460)×(200–)210–335(–375)	(67–)75–89(–96)×10.0–11.7	14.0–16.2(–19.5)×4.8–5.6	3(–4) transverse, sometimes 1-vertical	Brown	<i>Ribes rubrum</i> and <i>Sambucus nigra</i>	Jaklitsch and Voglmayer (2016)
<i>P. robiniae</i> MFLUCC 14–1119	450–470×255–270	105–115×10–15	15–20×4–7	1	Hyaline	<i>Robinia pseudacacia</i>	Tibpromma et al. (2017)
<i>P. rosae</i> MFLUCC 17–0800	300–400×150–200	80–100×12.5–16	18–24×6–8	1	Pale brown	<i>Rosa</i> sp.	Wanasinghe et al. (2018)

*Saprobic* on dead stems of *Clematis* species. **Sexual morph:** Undetermined. **Asexual morph:** Colonies on the natural substrate effused, scattered, hairy, dark brown. *Mycelium* semi-immersed to superficial, composed of pale brown, septate hyphae. *Conidiophores* 198–310×9–19.6  $\mu\text{m}$  ( $\bar{x}$  = 240×13  $\mu\text{m}$ , n = 20), macronematous, mononematous, single or in groups of 2–4, branched at the apex, stipes straight or flexuous, subcylindrical to cylindrical, erect, septate, smooth, dark brown to reddish brown, 10–19-septate, with 3–5 primary branches, irregularly branched at the upper parts, brown, smooth or verruculose. *Conidiogenous cells* 10–16×6–7  $\mu\text{m}$  ( $\bar{x}$  = 15×6  $\mu\text{m}$ , n = 20), monotretic or polytretic, integrated, terminal on conidiophores, doliform to oblong, pale brown. *Conidia* 14–26(–28)×2.5–8  $\mu\text{m}$  ( $\bar{x}$  = 26×7  $\mu\text{m}$ , n = 20), phragmosporous, in branched chains, acrogenous, cylindrical to oblong, 2–5 septa, deeply constricted at septa, dark brown to reddish brown, verrucose, rounded ends, bud scars or disjunctors present at the site of attachment, easily separating.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 2 weeks at 25 °C. Cultures from above, cream to orangish-white, medium, circular, umbonate, fluffy, slightly radiating outwardly, reverse dark brown at the centre, cream, radiating outwardly.

**Material examined:** Belgium, Flemish Brabant, Meise Botanic Garden, Bouchout Domain, on dead stems of *Clematis virginiana*, 13 June 2017, D. Ertz & C. Gerstmans, BRCVir2 (MFLU 17–1520); living culture, MFLUCC 17–2162; UK, Botley wood, Hampshire, on dead stems of *C. vitalba*, 16 April 2016, E.B.G. Jones, GJ 265 (MFLU 17–1462); living culture, MFLUCC 16–1003.

**Hosts:** *Clematis virginiana*, *C. vitalba*, *Hedera helix*, *Heracleum sphondylium*—(Crous et al. 2014a; this study).

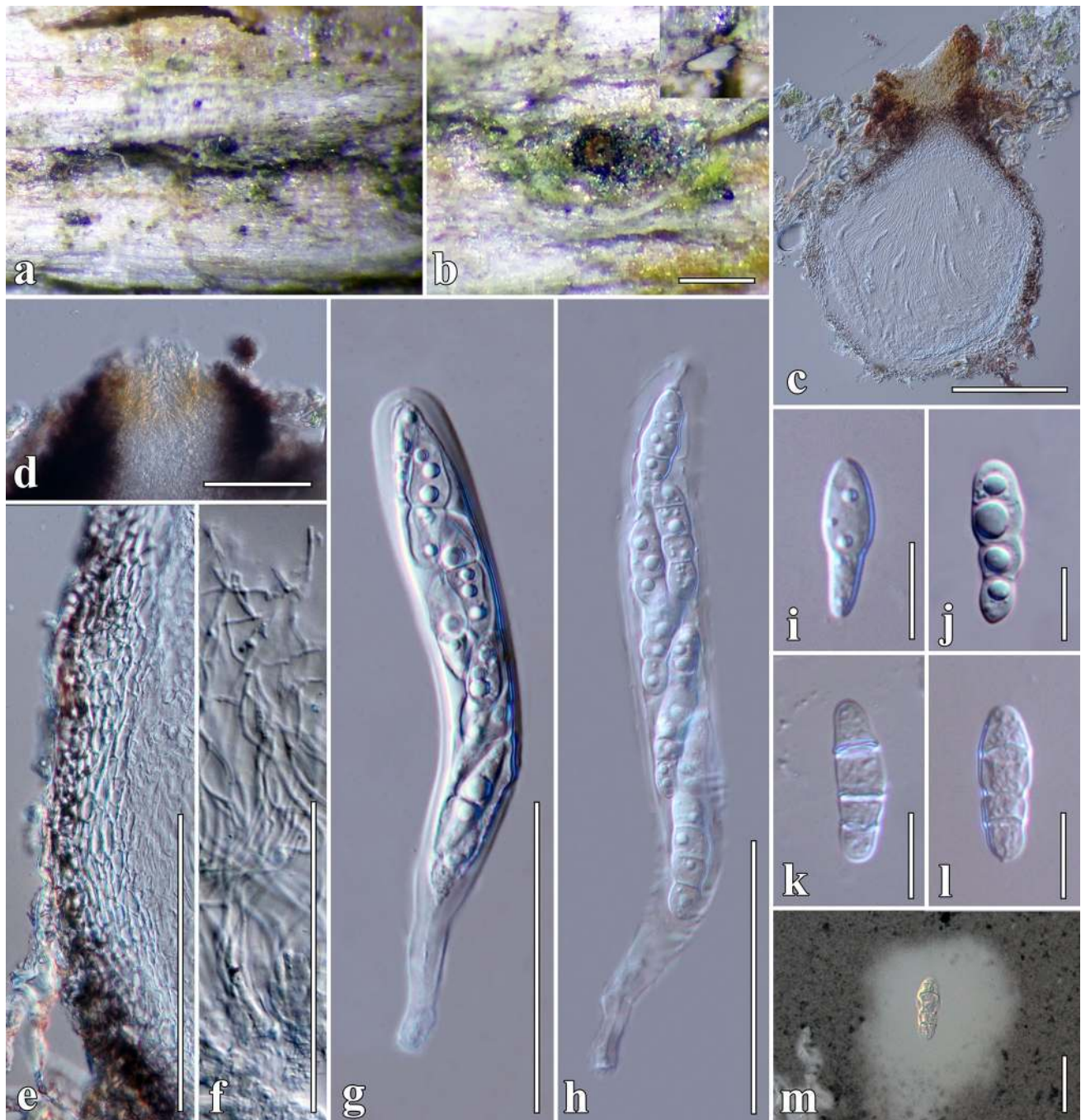
**Distribution:** Belgium Germany, Netherlands, UK (England)—(Crous et al. 2014a; this study).

**GenBank accession numbers:** MFLUCC 17–2162: LSU: MT214604; SSU: MT226715; ITS: MT310648; *tefl*: MT394660; *rpb2*: MT394715. MFLUCC 16–1003: LSU: MT214605; SSU: MT226716; ITS: MT310649; *tefl*: MT394661.

**Notes:** *Dendryphion europaeum* was described by Crous et al. (2014a) from *Hedera helix* (Araliaceae) and *Heracleum sphondylium* (Apiaceae). A phylogram generated from multi-locus phylogeny analysis of our collections on *Clematis* is shown in Fig. 84. Isolates MFLUCC 16–1003 and MFLUCC 17–2162 formed a strongly supported clade with the ex-type strain of *D. europaeum* (92% ML/1.00 BYPP). Based on morphological and geological comparisons, our isolates are identical to those reported by Crous et al. (2014a). A pairwise comparison of the ITS region of MFLUCC 16–1003 showed two nucleotide difference from the other isolates that clustered in the same clade (2/497 base pairs, 0.4%). According to the recommendation for new taxon establishment proposed by Jeewon and Hyde (2016), this is regarded as not statistically significant. Therefore, we name our new isolates as new host records (Fig. 85).

#### *Torula* Pers.

*Torula* is typified by *T. herbarum* (= *T. monilis*) (Scott et al. 2007). *Torula* species are characterized by asexual morph characters having monoblastic or polyblastic, intact or cupulate conidiogenous cells, and acropetal chains of dark phragmoconidia (Persoon 1795; Scott et al. 2007). More



**Fig. 82** *Parathyridaria serratifoliae* (MFLU 17–1512, **holotype**). **a**, **b** Appearance of ascomata on *Clematis serratifolia*. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Trabec-

ulate pseudoparaphyses. **g**, **h** Asci. **i–l** Ascospores. **m** Ascospore in 10% Indian ink. Scale bars: **b**, **c** = 100  $\mu$ m, **d–h** = 50  $\mu$ m, **i–l** = 10  $\mu$ m, **m** = 20  $\mu$ m

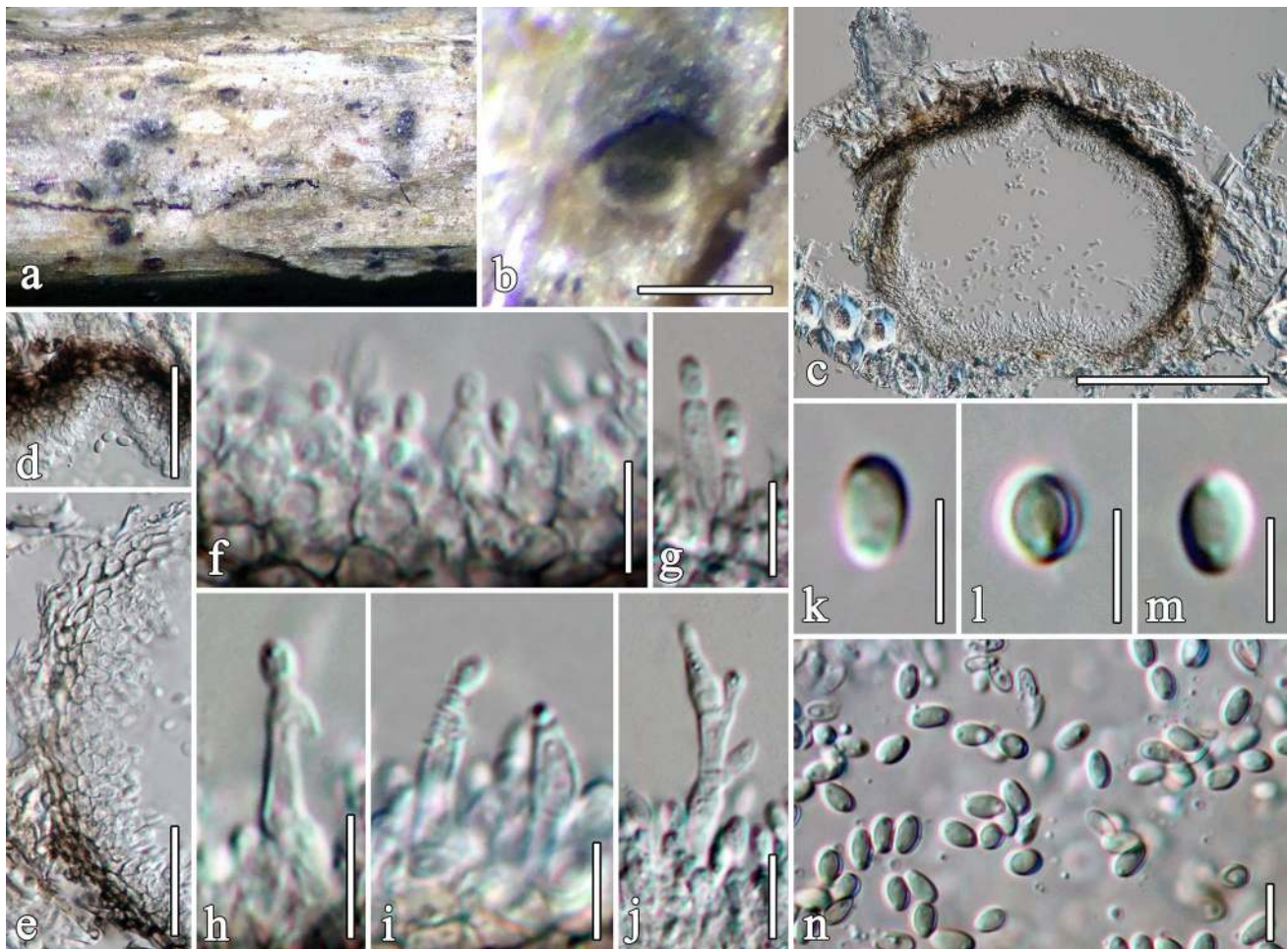
than 500 epithets are listed in Index Fungorum (2020). We report *T. chromolaenae* from *Clematis fulvicoma* in Thailand (Fig. 86).

***Torula chromolaenae*** Li, Phookamsak, Mapook & K.D. Hyde, in Mycological Progress 16 (4): 454 (2017), **new host record**

*Index Fungorum* number: IF819536; *Facesoffungi* number: FOF 02713, Fig. 86.

**Saprobic** on dried stems of *Clematis fulvicoma*. **Sexual morph:** Undetermined. **Asexual morph:** Colonies on the natural substrate effuse, scattered, hairy, powdery, dark brown to black. *Mycelium* 1.7  $\mu$ m wide, immersed to superficial, initially hyaline, later pale brown, septate, branched





**Fig. 83** *Parathyridaria virginianae* (MFLU 17–1521, holotype). **a** Appearance of conidiomata on *Clematis virginiana*. **b** Close up of conidioma on host substrate. **c** Vertical section through conidioma. **d**

Ostiolar canal. **e** Section of conidioma wall. **f–j** Conidiogenous cells and conidia. **k–n** Conidia. Scale bars: **b**=200  $\mu$ m, **c**=100  $\mu$ m, **d**, **e**=20  $\mu$ m, **f–n**=5  $\mu$ m

hyphae. *Conidiophores* up to 5  $\mu$ m long, micronematous, reduced to conidiogenous cells, with hyaline to pale brown ampulliform supporting cell. *Conidiogenous cells* 1.15–4  $\times$  1.5–2  $\mu$ m ( $\bar{x}$  = 2.5  $\times$  2.3  $\mu$ m, n = 20) diam., mono to polyblastic, solitary, cylindrical, integrated, terminal on conidiophores, hyaline to pale brown. *Conidia* 9.5–14  $\times$  4–6.5  $\mu$ m ( $\bar{x}$  = 11  $\times$  5  $\mu$ m, n = 50), phragmosporous, in short branched chains, acrogenous, dry, cylindrical, 2–3-septate, deeply constricted at septa, brown to reddish brown, verrucose, rounded at both ends, bud scars or disjunctors present at the site of attachment, easily separating into segments.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 25  $^{\circ}$ C. Cultures from above, olive brown at the centre, faintly zonate edge, fluffy, grey, dense, lobate, raised with concave edge, convex surface, downy, brown radiating to the media; reverse dark brown at the centre, burnt umber radiating outwardly.

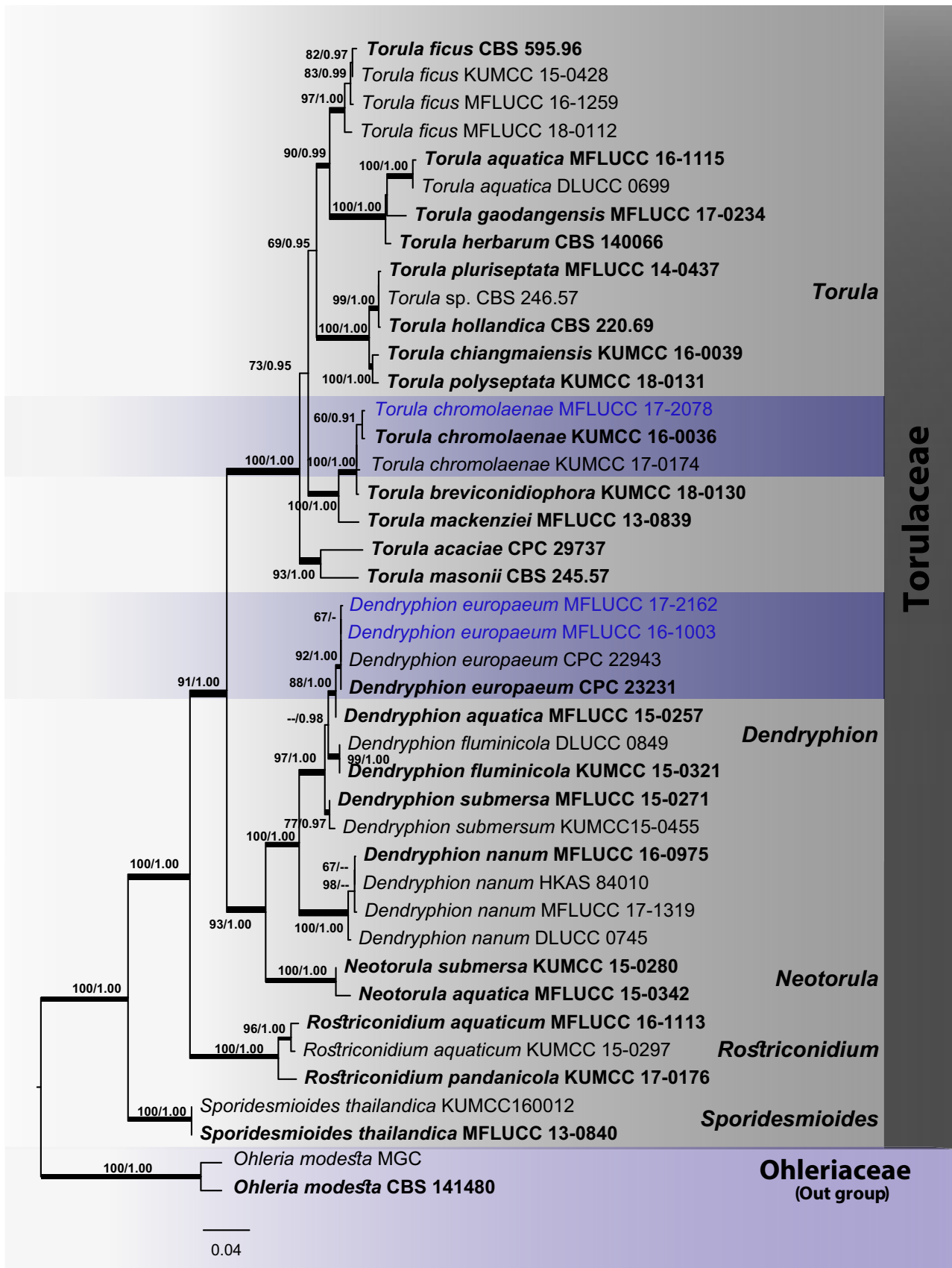
**Material examined:** Thailand, Nan Province, on dead stems of *Clematis fulvicoma*, 20 March 2017, C. Phukham-sakda, CMTH23 (MFLU 17–1486); living culture, MFLUCC 17–2078.

**Hosts:** *Chromolaena odorata*, *Clematis fulvicoma*, *Pandanus tectorius*—(Li et al. 2017; Tibpromma et al. 2018; this study).

**Distribution:** China, Thailand—(Li et al. 2017; Tibpromma et al. 2018; this study).

**GenBank accession numbers:** LSU: MT214606; SSU: MT226717; ITS: MT310650; *tefl*: MT394662; *rpb2*: MT394716.

**Notes:** *Torula chromolaenae* was described from *Chromolaena odorata* collected from Thailand (Li et al. 2017). Subsequently, Tibpromma et al. (2018) recorded the species from *Pandanus tectorius* in China. Based on the phylogenetic analyses (Fig. 84), isolate MFLUCC 17–2078 formed a strongly supported clade with *T. chromolaenae* KUMCC 16–0036 and KMUCC 17–0174. Our collection is similar



**Fig. 84** The best scoring RAxML tree with a final likelihood value of  $-12765.550785$  based on combined LSU, ITS, *tefl* and *rpb2* sequence data for Torulaceae. The topology and clade stability of the combined gene analyses was compared with the single gene analyses. The tree is rooted with members of the Ohleriaceae. Forty-two strains were included in the combined genes sequence analyses which comprised 3000 characters (808 characters for LSU, 497 characters for ITS, 854 characters for *tefl* and 841 characters for *rpb2*, including gap regions). The tree from the maximum likelihood analysis had similar topology to the Bayesian 50% majority-rule consensus phylogram. The matrix had 907 distinct alignment patterns, with 29.34% of undetermined characters and gaps. Estimated base frequencies were as follows; A=0.240459, C=0.270154, G=0.275197, T=0.214190; substitution rates AC=2.096044, AG=4.720144, AT=2.201665, CG=1.169889, CT=12.100709, GT=1.000000; gamma distribution shape parameter  $\alpha=0.542434$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study are indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95)

to the type species of *T. chromolaenae* (KUMCC 16-0036) but differ in size of the conidia ( $23 \times 8$  vs  $11 \times 5$   $\mu\text{m}$ ) (Tibpromma et al. 2018). A pairwise comparison of DNA sequences of ITS and *tefl* regions of our isolate (MFLUCC 17-2078) with *T. chromolaenae* strains (KUMCC 16-0036 and KMUCC 17-0174) do not show significant differences. Therefore, we name our collection as a new host record.

Isolate MFLUCC 17-2078 was further evaluated for bioactive secondary metabolite production. The strain inhibited *Bacillus subtilis* and partially inhibited conidia production of *Mucor plumbeus*, but did not reach significant values (data not shown).

#### Dothideomycetes, family *incertae sedis*

##### Dyfrulomycetales Pang, Hyde & E.B.G. Jones

We follow the treatment by Hyde et al. (2013) and Wijayawardene et al. (2017).

#### Pleurotremales Watson

Pleurotremales was introduced for *Pleurotrema polysemum*, a species lacking fissitunicate dehiscence asci in Sordariomycetes (Watson 1929; Barr 1994). The isotype specimens of *P. polysemum* were re-examined by Maharachchikumbura et al. (2016). Based on morphological evidence, *P. polysemum* has similar characters with species of *Saccardoella* and *Dyfrulomyces* in Dyfrulomycetales (Dothideomycetes). Subsequently, *P. polysemum* was excluded from Sordariomycetes and transferred to Dyfrulomycetales (Dothideomycetes). Dyfrulomycetales is currently synonymized under Pleurotremales (Maharachchikumbura et al. 2016). Three genera accepted in Pleurotremales are *Dyfrulomyces*, *Melomastia* and *Pleurotrema* (Wijayawardene et al. 2017).

#### *Melomastia* Nitschke ex Sacc.

Norphanphoun et al. (2017) verified the phylogenetic placement of *Melomastia*. *Melomastia* is accommodated in Pleurotremales with more than 30 epithets recorded in Index Fungorum (2020). Based on combined LSU, SSU and *tefl* sequence data (Fig. 87), we introduce two novel species, *M. clematidis* and *M. fulvicoma*, from *Clematis* species in Thailand.

#### *Melomastia clematidis* Phukhams., & K.D. Hyde, sp. nov.

*Index Fungorum number*: IF557210; *Facesoffungi number*: FoF 07334, Fig. 88.

*Etymology*: Refers to the host genus, *Clematis*.

*Holotype*: MFLU 17-1500

*Saprobic* on dead stems of *Clematis sikkimensis*. **Sexual morph**: *Ascomata* 300–510  $\times$  260–445  $\mu\text{m}$  ( $\bar{x}=420 \times 400$   $\mu\text{m}$ ,  $n=10$ ), only ostioles visible at the surface of host, solitary, gregarious, semi-immersed to immersed, globose to compressed globose, carbonaceous, dark brown to black, rough-walled, ostiolate. *Ostioles* 80–160  $\times$  90–116  $\mu\text{m}$ , central, oblong, carbonaceous, dark brown to black, papillate, periphyses filling the ostiolar canal. *Peridium* 24–50(–90 at apex)  $\mu\text{m}$  wide, outer layer carbonaceous, composed of 10–15 layers of dark brown cells of *textura prismatica* mixed with cells of *textura angularis*, inner layer comprising thin hyaline layers. *Hamathecium* composed of numerous, dense, 1.9–3  $\mu\text{m}$  ( $\bar{x}=2.5$   $\mu\text{m}$ ,  $n=40$ ), filiform, unbranched, septate, cellular pseudoparaphyses. *Asci* 115–160  $\times$  4–7  $\mu\text{m}$  ( $\bar{x}=140 \times 6$   $\mu\text{m}$ ,  $n=20$ ), 8-spored, bitunicate, broad filiform, apically rounded with simple and short pedicel, ocular chamber visible. *Ascospores* 13–20  $\times$  3.8–5  $\mu\text{m}$  ( $\bar{x}=15 \times 4.7$   $\mu\text{m}$ ,  $n=50$ ), uniseriate, partially overlapping, broad fusiform with acute ends, hyaline, 3-septate, constricted at septa, guttulate, smooth-walled, with thin mucilaginous sheath. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Culture from above, white, radiating outwardly, with yellow ring in the middle, oil droplets produced in the cultures, medium dense, circular, umbonate, dull, edge erose, downy, covered with fluffy cream mycelium; reverse brown in the middle with orange ring, dark yellow, radiating outwardly.

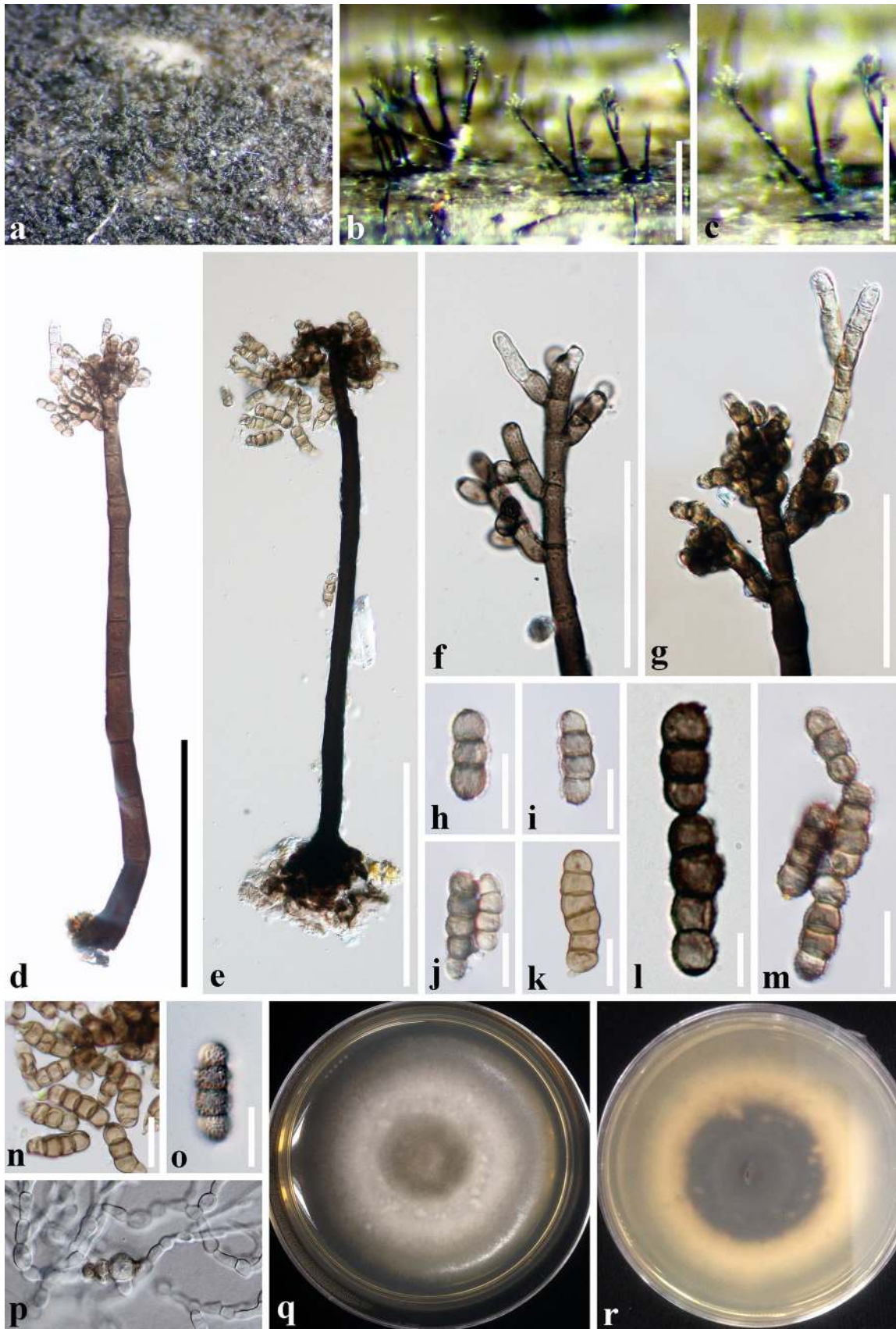
*Material examined*: Thailand, Chiang Rai Province, on dead branches of *Clematis sikkimensis*, 24 June 2017, C. Phukhamsakda & M. van de Bult, CMTHDT09 (MFLU 17-1500, **holotype**); ex-type living culture, MFLUCC 17-2092.

*Host*: *Clematis sikkimensis*—(This study).

*Distribution*: Thailand—(This study).

*GenBank accession numbers*: LSU: MT214607; SSU: MT226718; ITS: MT310651; *tefl*: MT394663.

*Notes*: In the phylogenetic analysis (Fig. 87), *Melomastia clematidis* formed a close relationship with *M. fulvicoma*



◀**Fig. 85** *Dendryphion europaeum* (MFLU 17–1520). **a–c** Appearance of conidiophores on *Clematis*. **d, e** Mononematous conidiophore. **f, g** Conidiogenous cells and conidia. **h–o** Conidia. **p** Germinated conidia. **q, r** Culture characters on MEA. Scale bars: **b, c** = 200  $\mu\text{m}$ , **d, e** = 100  $\mu\text{m}$ , **f, g** = 50  $\mu\text{m}$ , **h–o** = 10  $\mu\text{m}$

(MFLUCC 17–2083), *M. italica* (MFLUCC 15–0160) and *M. maolanensis* (GZCC 16–0102). Morphologically, our collection matched with the generic concept of *Melomastia* in having large ascomata with cylindrical, bitunicate asci and hyaline, symmetrical and septate ascospores (Pang et al. 2013). Our collection is distinct in having large ascomata with 3-septate ascospores. In a BLASTn search of GenBank, the closest match for the LSU region of strain MFLUCC 17–2092 is *D. thailandica* (MFLUCC 15–0945) with 96.79% similarity (NG\_059714). The closest match for the *tef1* region is *M. maolanensis* strain GZCC 16–0102 with 93.74% similarity (KY814762). Therefore, we introduce *M. clematidis* as a new species.

*Melomastia clematidis* strain MFLUCC 17–2083 was evaluated for secondary metabolite production. However, the isolate did not show inhibitory activity against the tested strains.

***Melomastia fulvicomae*** Phukhams., & K.D. Hyde, **sp. nov.**

*Index Fungorum* number: IF557211; Facesoffungi number: FoF 07335, Fig. 89. *Etymology*: The epithet refers to the species of the host substrate, *Clematis fulvicoma*.

*Holotype*: MFLU 17–1491

*Saprobic* on dead stems of *Clematis fulvicoma*. **Sexual morph**: *Ascomata* 275–375  $\times$  130–250  $\mu\text{m}$  diam. ( $\bar{x}$  = 310  $\times$  185  $\mu\text{m}$ ,  $n$  = 5), only ostioles visible on the surface of host, solitary, gregarious, semi-immersed, globose, subcarbonaceous, black to rust brown, rough-walled, ostiolate. *Ostioles* 140–180  $\times$  90–110  $\mu\text{m}$ , central, oblong, carbonaceous, dark brown to black, papillate, periphyses filling ostiole. *Peridium* 15–30(–40  $\mu\text{m}$  at apex) wide, outer layer composed of 5–7 layers of black cells of *textura prismatica*, the inner layer comprised of hyaline cells and thin. *Hamathecium* composed of numerous, 1.5–2.5  $\mu\text{m}$  ( $\bar{x}$  = 2  $\mu\text{m}$ ,  $n$  = 40), dense, filiform, unbranched, septate, cellular pseudoparaphyses. *Asci* 70–90  $\times$  4–6  $\mu\text{m}$  ( $\bar{x}$  = 85  $\times$  5  $\mu\text{m}$ ,  $n$  = 30), 8-spored, bitunicate, cylindrical, apically rounded, with simple and short pedicel, ocular chamber visible when young. *Ascospores* 9–15  $\times$  3.5–5.5  $\mu\text{m}$  ( $\bar{x}$  = 13  $\times$  4  $\mu\text{m}$ ,  $n$  = 50), uniseriate, partial overlapping, broad fusiform with rounded ends, ends acute, hyaline, 2–3-septate, constricted at the septa, with guttules in each cell, smooth-walled with mucilaginous sheath. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA reaching 30 mm diam. after 4 weeks at 25  $^{\circ}\text{C}$ . Culture from above, yellowish radiating outwardly, with yellow ring in the middle, medium dense, circular in shape, umbonate, dull, edge erose, downy,

covered with fairly cream mycelium; reverse: brown in the middle with orange ring, dark yellow radiating outwardly.

*Material examined*: Thailand, Chiang Rai Province, on dead branches of *Clematis fulvicoma*, 20 March 2017, C. Phukhamsakda, CMTH29 (MFLU 17–1491, **holotype**); ex-type living culture, MFLUCC 17–2083.

*Hosts*: *Clematis fulvicoma*—(This study).

*Distribution*: Thailand—(This study).

*GenBank accession numbers*: LSU: MT214608; SSU: MT226719; ITS: MT310652; *tef1*: MT394664.

*Notes*: *Melomastia fulvicomae* (MFLUCC 17–2146) was associated with *Clematis fulvicoma* in Thailand. *Melomastia fulvicomae* can be distinguished from *M. clematidis* by its smaller ascomata (310  $\times$  185 vs 420  $\times$  400  $\mu\text{m}$ ) and asci (85  $\times$  5 vs 140  $\times$  6  $\mu\text{m}$ ). The ascospores of *M. fulvicomae* are 2-septate, while those of *M. clematidis* are 3-septate (Fig. 89). Multi-gene analysis showed that *M. fulvicomae* (MFLUCC 17–2083) clustered with other *Melomastia* species with moderate statistical support (67% ML/1.00 BYPP, Fig. 87).

The new strain was evaluated for secondary metabolite production with *Bacillus subtilis*, *Escherichia coli*, *Mucor plumbeus* and *Schizosaccharomyces pombe*. Isolate MFLUCC 17–2083 had moderate growth inhibitory activity against *Bacillus subtilis* and partial development inhibition against *Mucor plumbeus*. This strain is a suitable candidate for further evaluation.

**Class Lecanoromycetes** Erikss. & K. Winka

**Subclass Ostropomycetidae** Reeb, Lutzoni & Cl. Roux  
**Ostropales** Nannf.

Taxa of *Ostropales* are highly diverse and contain lichenized, lichenicolous and non-lichenized clades of fungi. Ascomata are apothecial or perithecial (Nannfeldt 1932; Lumbsch et al. 2007).

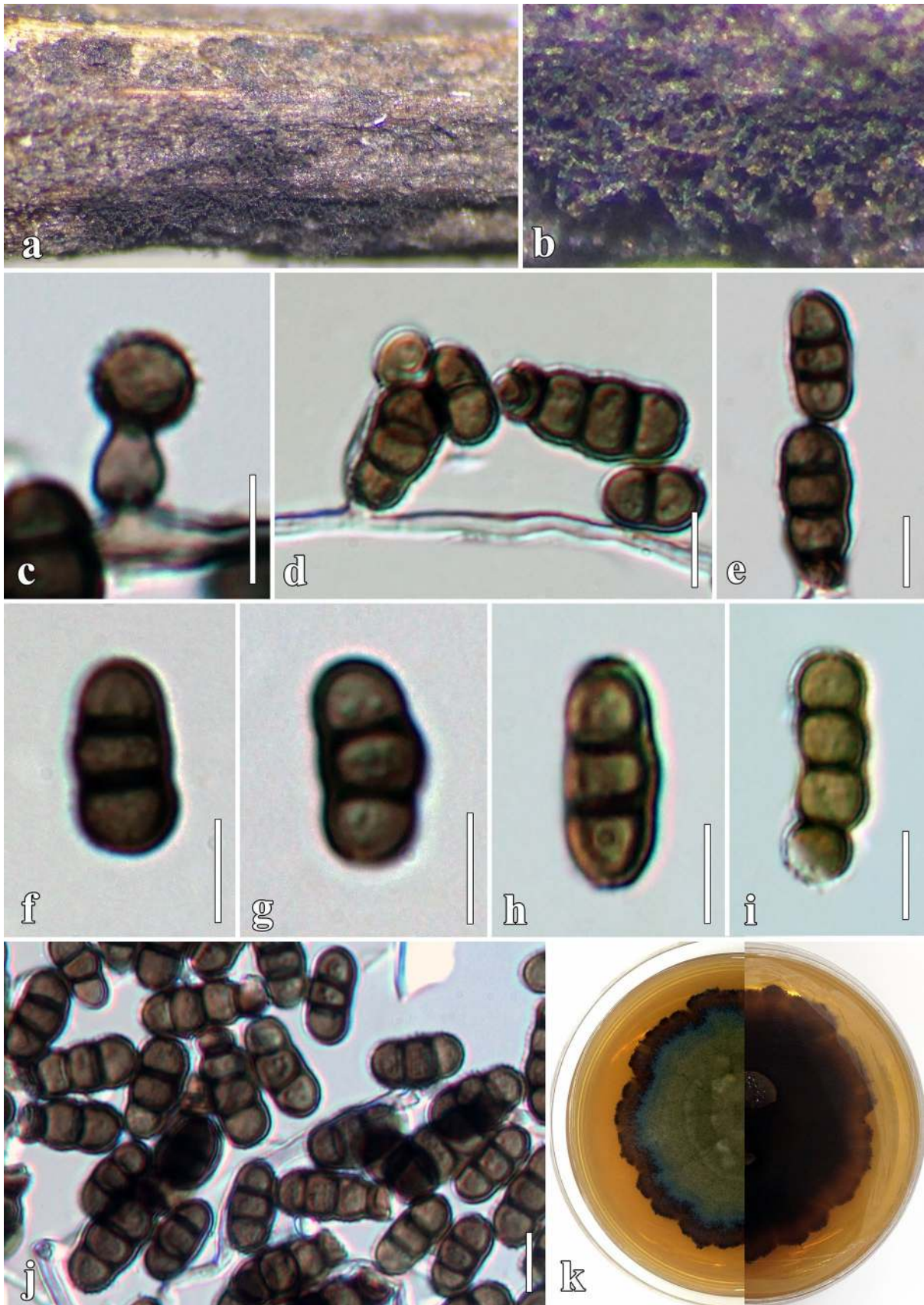
**Stictidaceae** Fr. [as ‘Sticteti’]

Taxa of Stictidaceae are parasitic, lichenized or lichenicolous and characterized by crustose thalli with chlorococoid photobionts, apothecial ascomata or perithecia, filiform unbranched paraphyses, J-, cylindrical asci and ellipsoid to filiform ascospores and, sometimes formed by fragmentation (Crous et al. 2017).

***Fitzroyomyces*** Crous

*Fitzroyomyces* is a monotypic genus (Fig. 90). To date, sexual morphs have not been recorded, however, we found the sexual morph on *Clematis*. Asexual morphs are characterised by pycnidial conidiomata (Crous et al. 2017).

***Fitzroyomyces cyperacearum*** Crous, Persoonia 39:389 (2017), **new host record**



◀**Fig. 86** *Torula chromolaenae* (MFLU 17–1486). **a, b** Appearance of conidiophores on *Clematis fulvicoma*. **c, d** Conidiophores and conidia attached to conidiogenous cells. **e–j** Conidia. **k** Culture characters on MEA. Scale bars: **e–j** = 5  $\mu$ m

*Index Fungorum number*: IF 823923; *Facesoffungi number*: FoF 05807, Fig. 91.

*Saprobic* on dead stem of *Clematis subumbellata*. **Sexual morph**: *Apothecia* 201–260  $\times$  210–310  $\mu$ m ( $\bar{x}$  = 230  $\times$  260  $\mu$ m,  $n$  = 10), arising singly or in small groups, sessile, immersed in substrate, under the clypeus, cupulate. *Hypothecium* convex. *Disc* 270–370  $\mu$ m wide ( $\bar{x}$  = 320  $\mu$ m,  $n$  = 10), whitish to cream. *Margins* white. *Hymenium* hyaline, enclosed in a thick gelatinous matrix. *Epitecium* absent. *Excipulum* 17–70  $\mu$ m wide, composed of cells of *textura intricata*. *Paraphyses* 1.3–3  $\mu$ m wide ( $\bar{x}$  = 2  $\mu$ m,  $n$  = 50) at the apex, numerous, filiform, aseptate, unbranched. *Asci* 110–150  $\times$  10–20  $\mu$ m ( $\bar{x}$  = 130  $\times$  12  $\mu$ m,  $n$  = 10), 8-spored, long, broad cylindrical, rounded at the apex, short sessile. *Ascospores* 100–145  $\times$  2.5–3.5  $\mu$ m, fasciculate, spiraled in ascus, thread-like, filiform, ends rounded, 17–21 transversely euseptate, slightly constricted at the septa, hyaline. **Asexual morph**: Undetermined.

*Culture characters*: Colonies on MEA reaching 20 mm diam. after 4 weeks at 25 °C. Culture from above, brown, radiating, yellowish towards the edge, erumpent, spreading, surface folded, margins lobate, dense, circular, flat, dull, fimbriate, radially furrowed, and slightly covered with white aerial mycelia; reverse pale orange, with radiating cream mycelia.

*Material examined*: Thailand, Chiang Rai Province, on dead stems of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH16 (MFLU 17–1480); living culture, MFLUCC 17–2072.

*Hosts*: woody stem, *Epilobium* (= *Chamaenerion*) *angustifolium*, *Clematis subumbellata*—(Crous et al. 2017; this study).

*Distribution*: Australia, Thailand—(Crous et al. 2017; this study).

*GenBank accession numbers*: LSU: MT214609; SSU: MT226720; ITS: MT310653; *tef1*: MT394665.

*Notes*: *Fitzroyomyces cyperacearum* (MFLUCC 17–2072) was found on the stem of *Clematis subumbellata* in Thailand. The strain formed a clade with *F. cyperacearum* from Australia and the UK (Fig. 90). Isolate MFLUCC 17–2072 clustered with three *Fitzroyomyces* strains with strong support and the ITS data of our strain was 99% similar to the ITS data of the type species of *F. cyperacearum* (CBS 143170). *Fitzroyomyces* was introduced based on its pycnidial characters (Crous et al. 2017). However, the asexual morph was not observed in our collection, therefore it could not be compared to the asexual morph of the type strain. Taking into consideration the genetic similarity and phylogenetic

results, we name our collection as the sexual morph of *F. cyperacearum*. This is the first record of *F. cyperacearum* from Thailand and on *Clematis* (Fig. 91).

*Fitzroyomyces cyperacearum* (MFLUCC 17–2072) was evaluated for secondary metabolite production with *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* and *Schizosaccharomyces pombe*. It showed weak inhibitory activities against *Bacillus subtilis* and completely inhibited the development of *Aspergillus niger* with an inhibition zone diameter of 17 mm. This strain is a suitable candidate for further evaluation.

***Neostictis* Ekanayaka, Camporesi & K.D. Hyde, gen. nov.**

*Index Fungorum number*: IF557307; *Facesoffungi number*: FoF 07338, Fig. 92.

*Etymology*: Name refers to the similarity to *Stictis*.

*Saprobic* on decaying wood material or herbaceous plant in terrestrial habitats. **Sexual morph**: *Apothecia* arising singly or in small groups, sessile, immersed in substrate, under the clypeus, cupulate. *Hypothecium* convex. *Disc* blackish. *Margins* brownish. *Hymenium* hyaline, enclosed in a thick gelatinous matrix. *Excipulum* composed of multi-layer of cells of *textura intricata*. *Paraphyses* numerous, filiform, aseptate. *Asci* 8-spored, short sessile, broad cylindrical to oblong, rounded at the apex. *Ascospores* fasciculate, thread-like, spiraled, filiform, rounded ends, multi-septate, transversely euseptate, hyaline, sometimes breaking into small fragments. **Asexual morph**: Undetermined.

*Type species*: *Neostictis nigricans* Ekanayaka, Phukhams., Camporesi & K.D. Hyde

*Notes*: Our collection MFLU 17–1540, from Italy, formed a well-supported clade close to *Phacidiella podocarpi*. Our new species is morphologically similar to *Fitzroyomyces cyperacearum* by having immersed apothecia, long cylindrical asci and filiform ascospores (Crous et al. 2017). However, our new species differs by having blackish margins, smaller disc and asci and ascospores (Fig. 92). The ascospores of our collection failed to germinate and therefore, we were unable to compare the asexual morph characters of our collection with *Fitzroyomyces cyperi* and *Phacidiella podocarpi*.

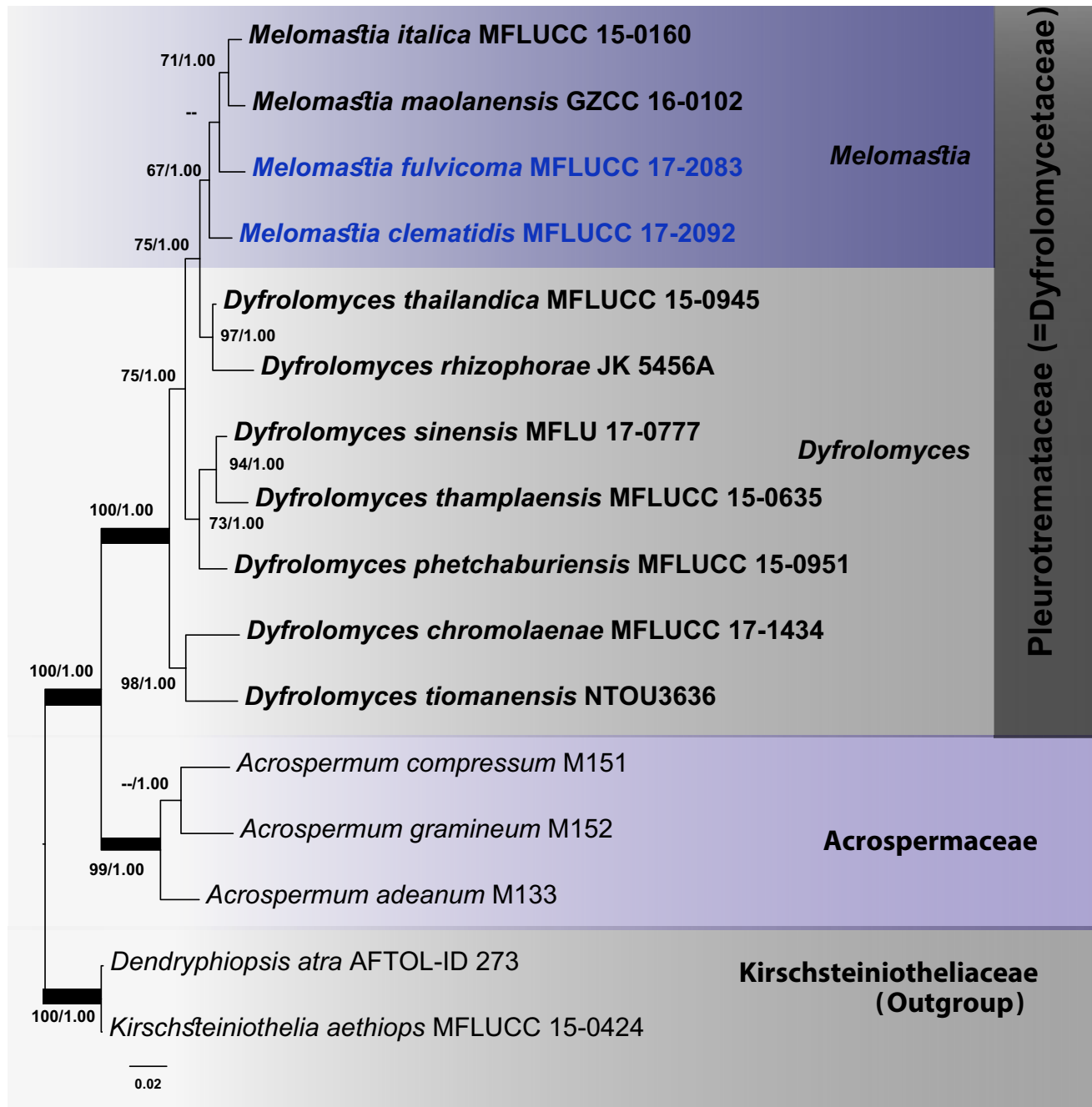
***Neostictis nigricans* Ekanayaka, Phukhams., Camporesi & K.D. Hyde, sp. nov.**

*Index Fungorum number*: IF557308; *Facesoffungi number*: FoF 07339, Fig. 92.

*Etymology*: The epithet refers to the apothecial morphology. The disc and the margins of the apothecium is black.

*Holotype*: MFLU 17–1540

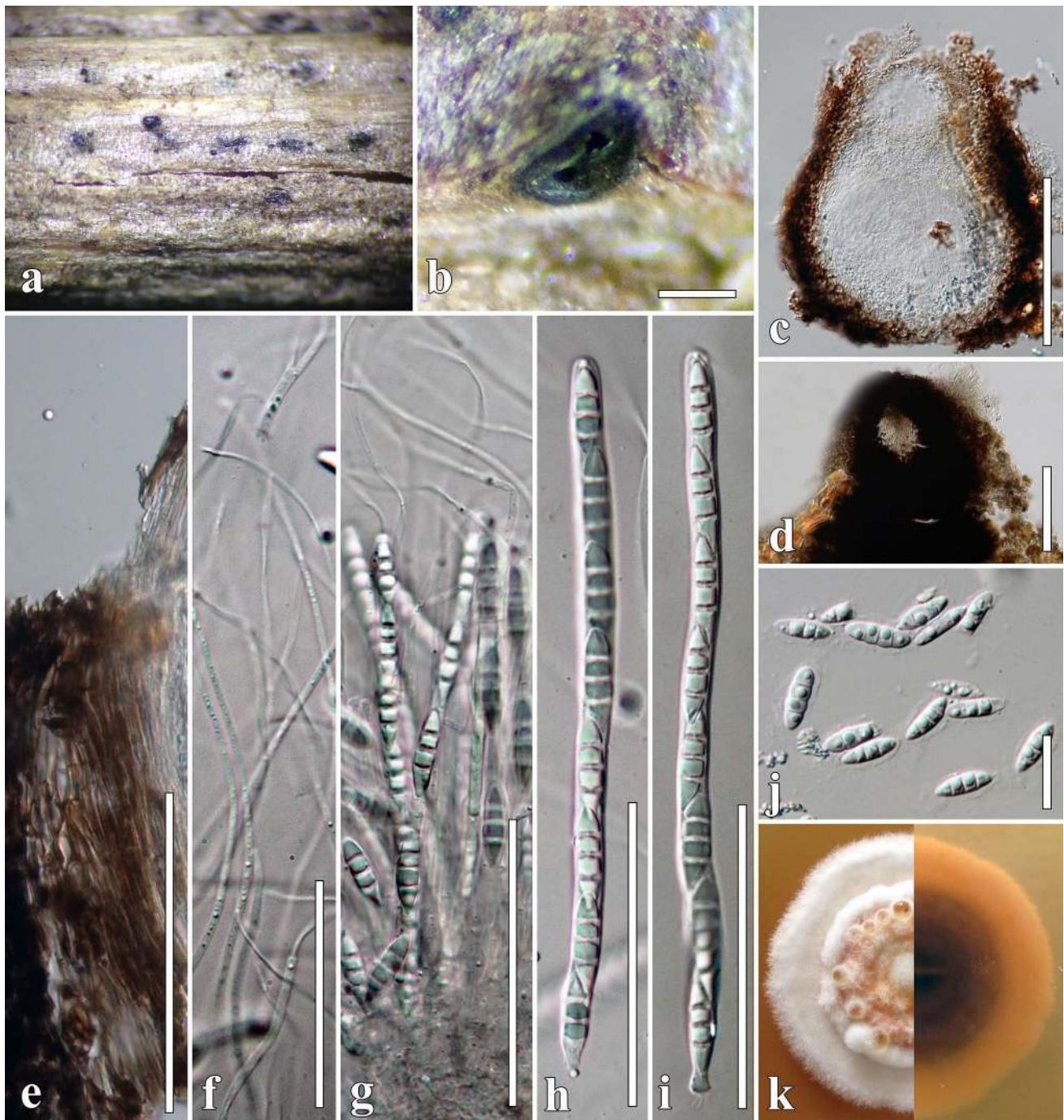
*Saprobic* on dead stem of *Clematis vitalba*. **Sexual morph**: *Apothecia* 0.4–1 mm wide, arising singly or in small groups, sessile, immersed in substrate, under the clypeus, cupulate. *Hypothecium* convex. *Disc* blackish. *Margins* blackish. *Hymenium* hyaline, enclosed in a thick



**Fig. 87** The Bayesian 50% majority-rule consensus phylogram based on combined LSU, SSU and *tefl* sequence data for Pleurotremataceae and related taxa. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted with members of the Kirschsteinioteliaceae. Sixteen strains were included in the combined genes sequence analyses which comprised 3269 characters (1297 characters for LSU, 1049 characters for SSU, 923 characters for *tefl*, including gap regions). The tree from the maximum likelihood analysis had similar topology to the Bayesian analyses. The best scoring RAxML tree had a final likelihood value of  $-8012.999545$ . The matrix had 707 distinct alignment patterns, with 31.71% of undetermined characters and gaps. Estimated

base frequencies were as follows; A=0.236800, C=0.268407, G=0.273839, T=0.220954; substitution rates AC=0.626404, AG=2.767488, AT=0.626269, CG=1.143373, CT=7.006679, GT=1.000000; gamma distribution shape parameter  $\alpha=0.740235$ . In our analysis, GTR+I + G model was used for each partition in Bayesian posterior analysis. The species determined in this study are indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses at family level (BS  $\geq$  70%/BYPP  $\geq$  0.95)





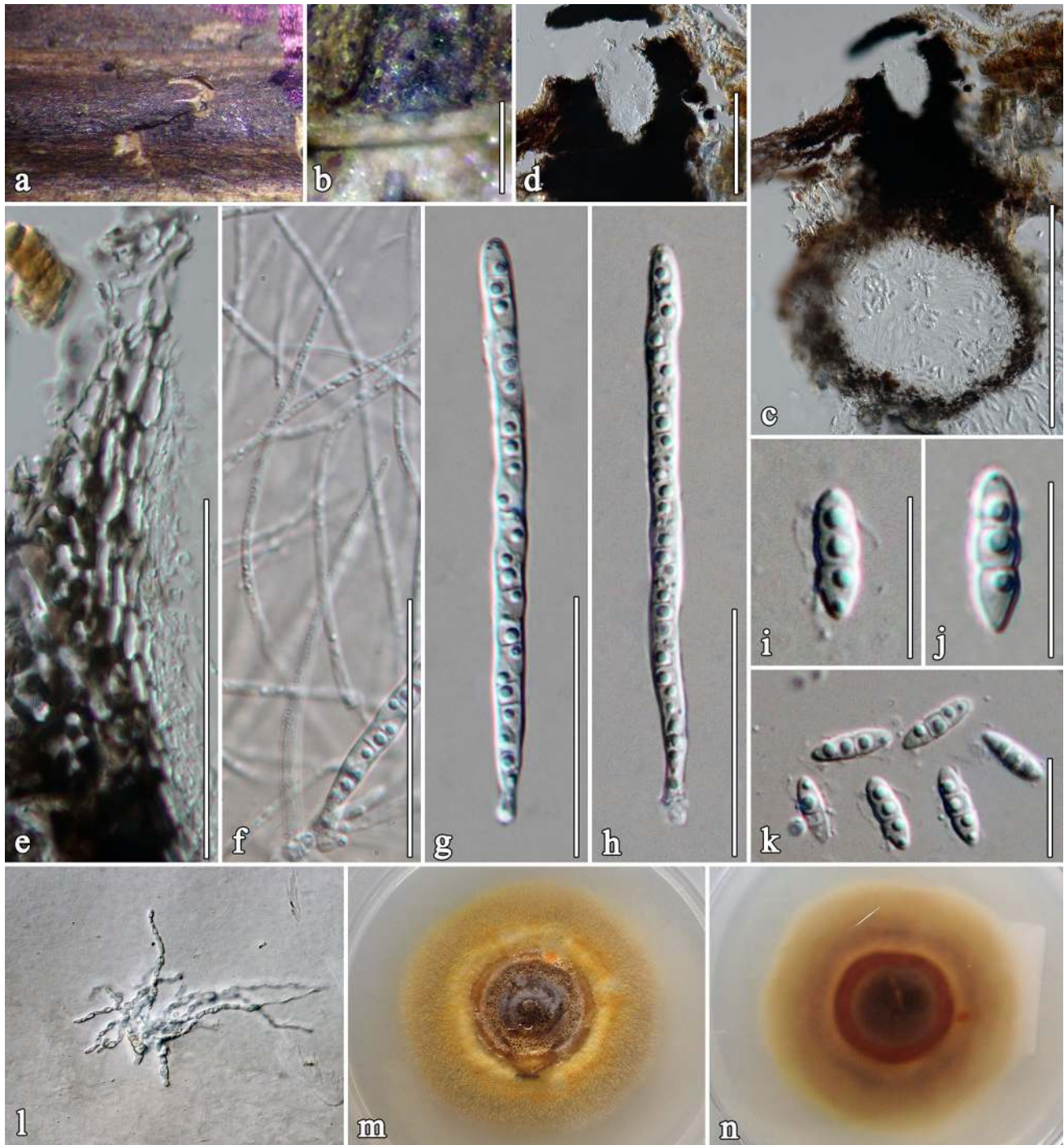
**Fig. 88** *Melomastia clematidis* (MFLU 17–1500, holotype). **a, b** Appearance of ascomata on *Clematis sikkimensis*. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Pseudo-

paraphyses. **g–i** Asci. **j** Ascospores. **k** Culture characteristics on MEA. Scale bars: **b, c** = 200  $\mu$ m, **d–i** = 50  $\mu$ m, **j** = 10  $\mu$ m

gelatinous matrix. *Excipulum* 25–35  $\mu$ m wide, composed of multi-layer cells of *textura intricata*. *Paraphyses* 1.3–2  $\mu$ m wide at the apex, numerous, filiform, aseptate, unbranched. *Asci* 80–100  $\times$  8–12  $\mu$ m, 8-spored, short sessile, broad cylindrical to oblong, rounded at the apex. *Ascospores* 35–50  $\times$  2.5–3.5  $\mu$ m, fasciculate, weakly spiral, thread-like,

filiform, ends rounded, multi-septate, approximately 32-transversely euseptate, hyaline, sometimes breaking into small fragments. **Asexual morph:** Undetermined.

*Material examined:* Italy, Forli-Cesena Province, Fiumicello di Premilcuore, Bouchout Domain, dead aerial branch of *Clematis vitalba*, 18 January 2014, E. Camporesi, IT1655



**Fig. 89** *Melomastia fulvicomae* (MFLU 17–1491, **holotype**). **a, b** Appearance of ascomata on *Clematis fulvicoma*. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Section of peridium. **f** Pseudo-

paraphyses. **g, h** Asci. **i–k** Ascospores. **l** Germinated ascospore. **m, n** Culture characteristics on MEA. Scale bars: **b** = 500  $\mu$ m, **c** = 200  $\mu$ m, **d, e** = 50  $\mu$ m, **f–h** = 20  $\mu$ m, **i–k** = 10  $\mu$ m

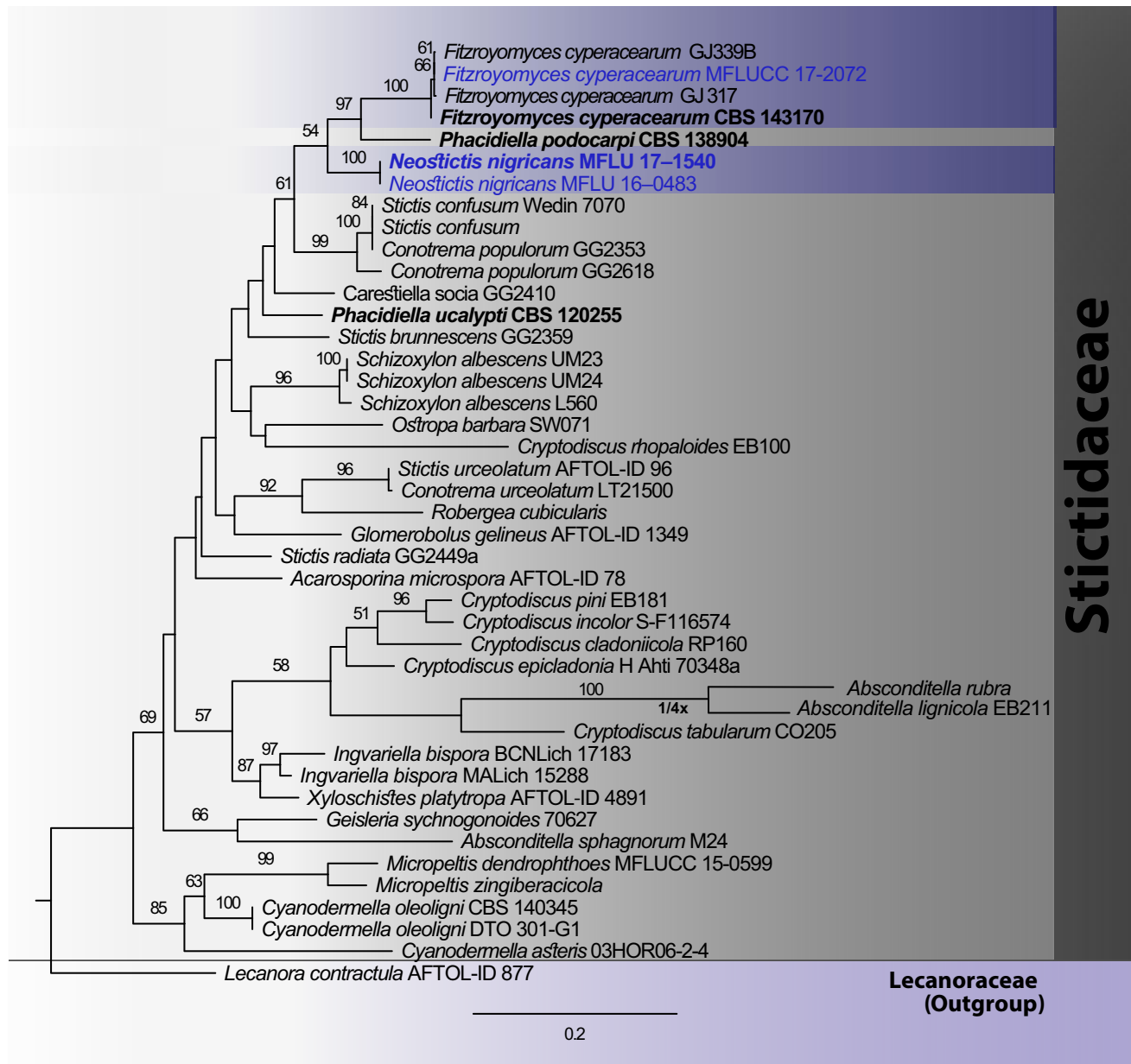
(MFLU 17–1540, **holotype**); *ibid.*, 10 October 2013 (MFLU 16–0483 = MFLU 16–0595).

*Host*: *Clematis vitalba*—(This study).

*Distribution*: Italy—(This study).

*GenBank accession numbers*: LSU: MT214610; SSU: MT226721; ITS: MT310654; *tefl*: MT394666.

*Notes*: See note under *Neostictis*.



**Fig. 90** The best scoring RAxML tree with a final likelihood value of  $-13518.083608$  for ITS and LSU sequence data. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted with *Lecanora contractula* (AFTOL-ID 877). The matrix had 958 distinct alignment patterns with 48.65% undetermined characters and gaps. Estimated base frequencies were as follows; A=0.252, C=0.244, G=0.276, T=0.228;

substitution rates AC=1.173341, AG=1.808670, AT=1.760640, CG=0.888202, CT=5.267472, GT=1.000000; gamma distribution shape parameter  $\alpha=0.339348$ . Ex-type strains are in bold black and the species determined in this study are indicated in blue. Bootstrap values (BT) (over 50% BT) from maximum likelihood are given at the nodes

### Class Sordariomycetes Erikss. & K. Winka

We follow Hyde et al. (2020b) which is the latest treatment of this class.

### Subclass: Sordariomycetidae Erikss. & K. Winka

#### Chaetosphaeriales Huhndorf, Mill. & F.A. Fernández

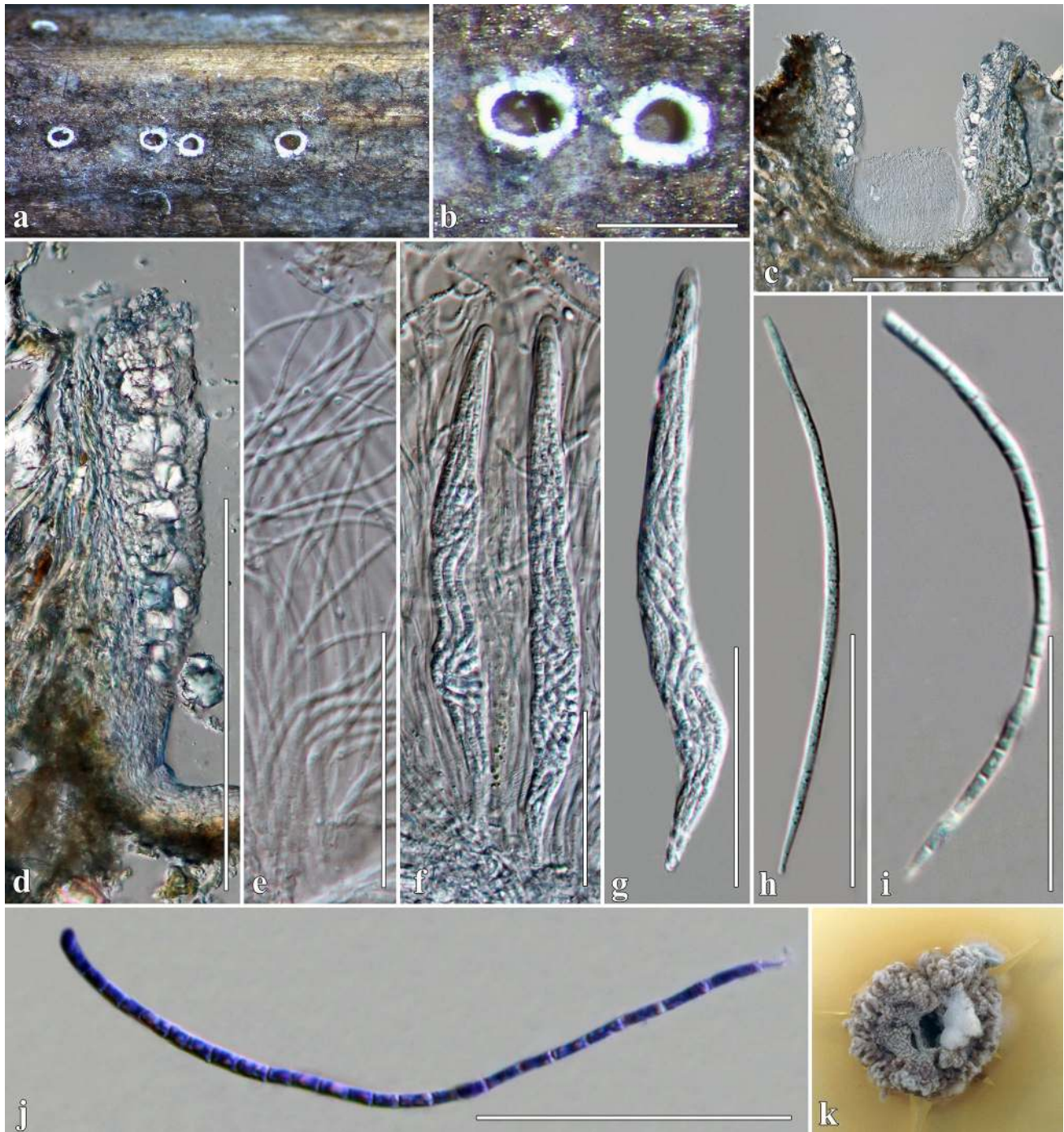
#### Chaetosphaeriales, genera incertae sedis

We follow the treatment of the order of Hyde et al. (2020b).

### *Neoleptospora* Phukhams. & K.D. Hyde, gen. nov.

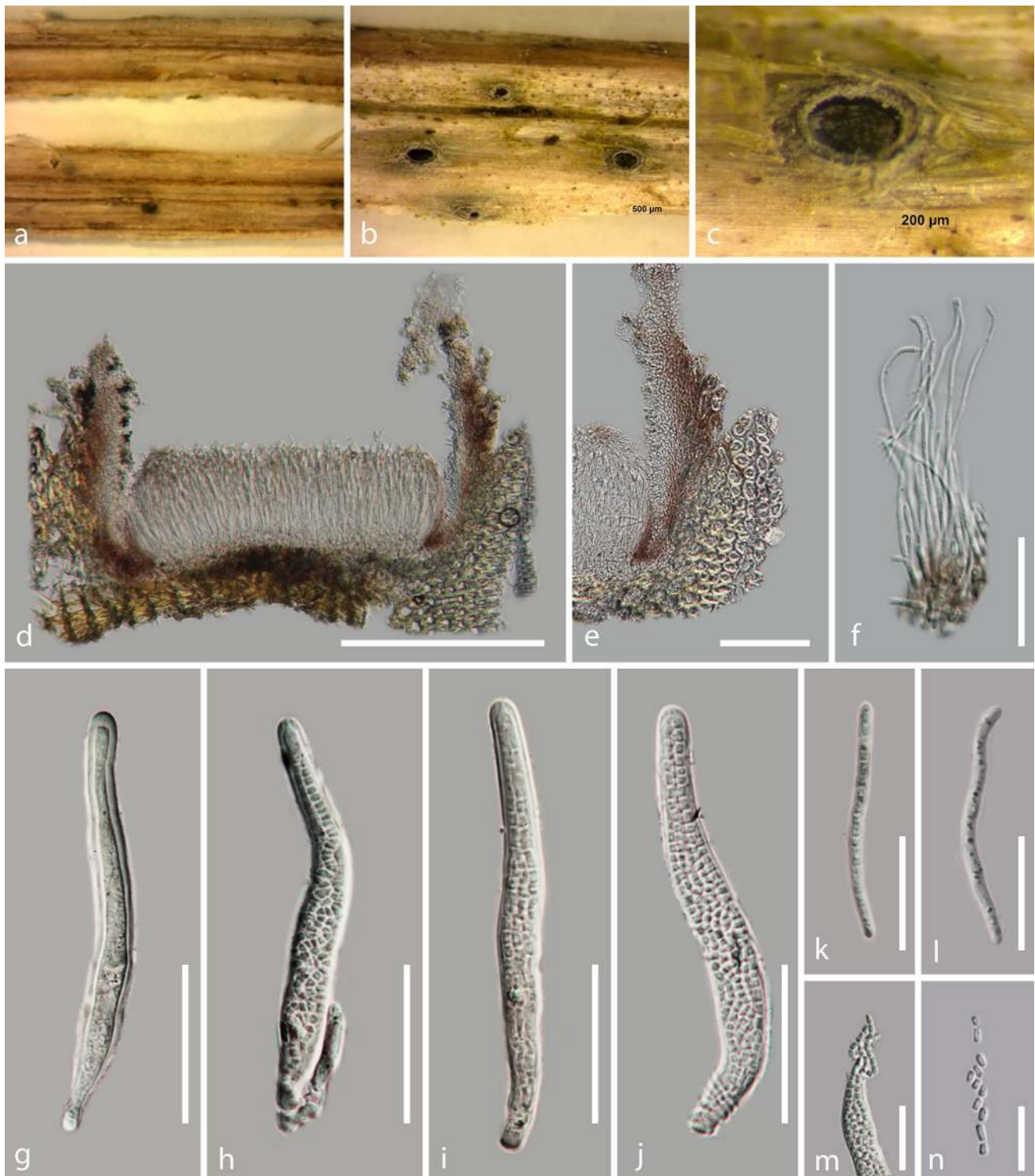
*Index Fungorum* number: IF557081; *Facesoffungi* number: FoF 07246, Fig. 94.

*Etymology*: The generic epithet refers to its similarity with *Leptospora*.



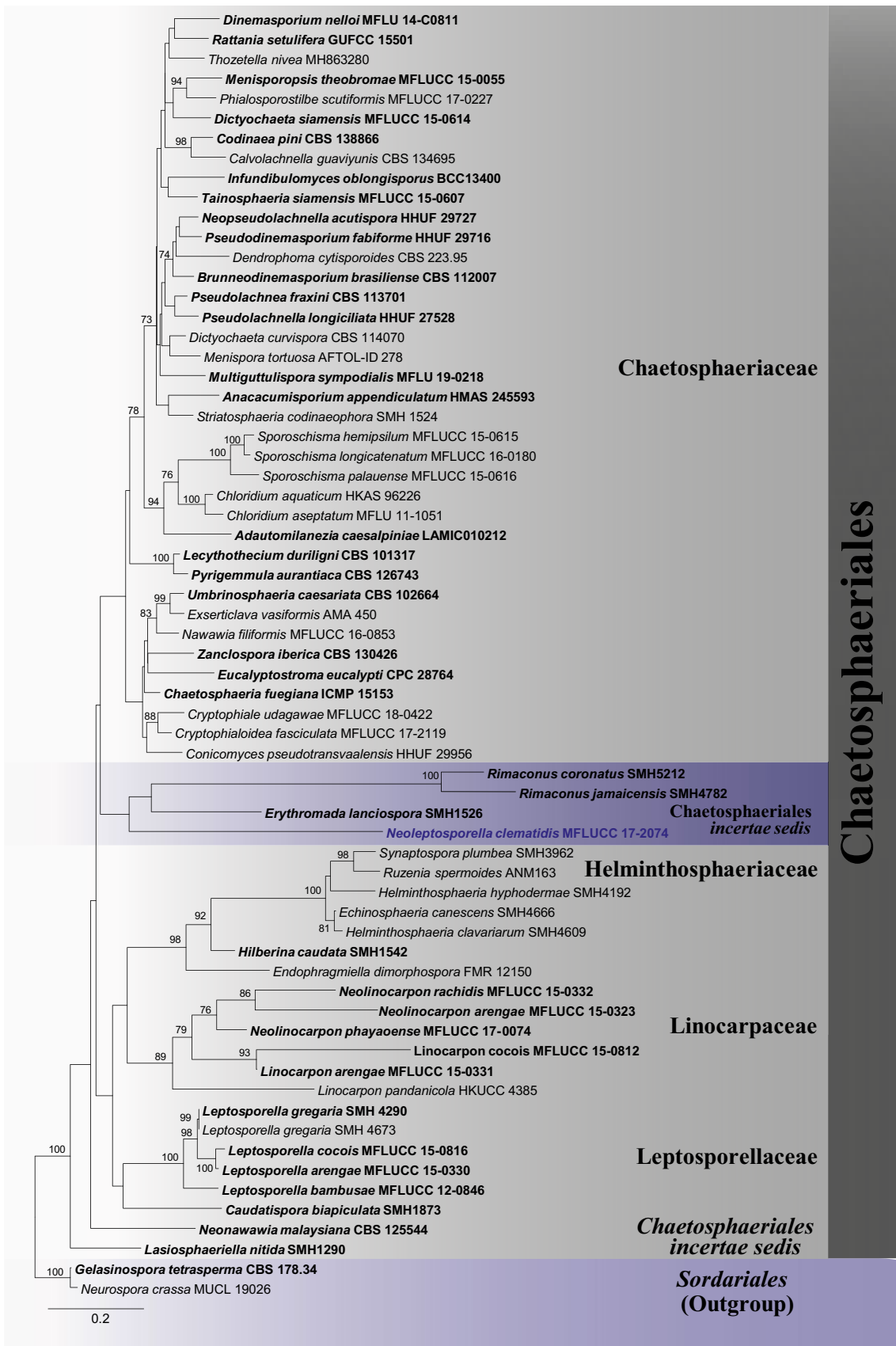
**Fig. 91** *Fitzroyomyces cyperacearum* (MFLU 17–1480). **a** Appearance of apothecia on *Clematis subumbellata*. **b** Close up of apothecia on host substrate. **c** Vertical section through an apothecium. **d** Excipulum layers. **e** Aseptate paraphyses. **f, g** Cylindrical asci. **h–j**

Fusiform ascospores (**j** Ascospore in cotton blue). **k** Culture characteristics on MEA. Scale bars: **b**=500  $\mu$ m, **c**=250  $\mu$ m, **d**=100  $\mu$ m, **e–j**=50  $\mu$ m



**Fig. 92** *Neostictis nigricans* (MFLU 17–1540, holotype). **a** Appearance of apothecia on *Clematis vitalba*. **b, c** Close up of apothecia on substrate. **d** Vertical section of an apothecium. **e** Vertical section of the peridium. **f** Aseptate paraphyses. **g–j** Asci. **k, l** Needle-

like ascospores. **m** Ascospores released from an ascus. **n** Secondary ascospores. Scale bars: **b**=500  $\mu\text{m}$ , **c**=200  $\mu\text{m}$ , **d**=300  $\mu\text{m}$ , **e**=100  $\mu\text{m}$ , **f–j**=30  $\mu\text{m}$ , **k–n**=20  $\mu\text{m}$



Chaetosphaeriales

**Fig. 93** The best scoring RAxML tree with a final likelihood value of  $-19621.398134$  of LSU and ITS sequence data. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. Sixty-five strains were included in the combined genes sequence analyses which comprised 1655 characters (1060 characters for LSU and 595 characters for ITS, including gap regions). This tree includes selected taxa of Chaetosphaeriales and rooted with *Gelasinospora tetrasperma* (CBS 178.34) and *Neurospora crassa* (MUCL 19026) in Sordariales. The matrix had 990 distinct alignment patterns, with 30.05% of undetermined characters and gaps. Estimated base frequencies were as follows; A=0.230333, C=0.267483, G=0.312066, T=0.190118; substitution rates AC=1.241984, AG=1.768569, AT=1.330285, CG=1.057213, CT=5.757832, GT=1.000000; gamma distribution shape parameter  $\alpha=0.462145$ . Ex-type strains are in bold black and the species determined in this study is indicated in blue. Bootstrap values (over 70% BT) from maximum likelihood are given at the nodes

*Saprobic* on stem of terrestrial herbaceous plants. **Sexual morph:** *Ascomata* solitary, immersed, only black shiny ostioles visible, immersed beneath small clypeus, appear as disc around the neck, coriaceous, subglobose to depressed globose, as raised blister-like areas, ostiolate. *Ostioles* central, carbonaceous, black, filled with periphyses. *Peridium* outer cells merging with the host epidermal cells, dark brown to black cells of *textura angularis*. *Paraphyses* comprising numerous, hyaline, branched, septate. *Asci* 8-spored, unitunicate, broad cylindrical, long-pedicellate, with a J-, wedge-shaped, subapical ring. *Ascospores* fasciculate, fusiform, straight or curved, C-shaped or sigmoid, aseptate, ends acute, without polar appendages, smooth-walled, with guttules in each cell. **Asexual morph:** Undetermined.

*Type species: Neoleptospora clematidis* Phukhams., Konta & K.D. Hyde

*Notes: Neoleptospora* is introduced for a taxon on *Clematis subumbellata* and is distinguishable from *Leptospora* in having immersed ascomata which are partial carbonaceous at the apex, the lower part is coriaceous, subglobose to depressed globose, not flattened at the base and without the cover of a pseudoclypeus. The apical part of the asci is wedge-shaped, and J-, with fusiform, aseptate ascospore with acute ends (Huhndorf and Miller 2011; Dai et al. 2017; Konta et al. 2017; Hyde et al. 2020a). The strain forms a lineage with three strains that have uncertain placement in Chaetosphaeriales, but received low statistical support (less than 75% ML, Fig. 93).

*Neoleptospora clematidis* Phukhams., Konta & K.D. Hyde, **sp. nov.**

*Index Fungorum number:* IF557060; *Facesoffungi number:* FoF 07246, Fig. 94.

*Etymology:* named after the host genus, *Clematis*.

*Holotype:* MFLU 17–1482.

*Saprobic* on dead branches of *Clematis subumbellata*. **Sexual morph:** *Ascomata* (including neck)  $150\text{--}200 \times 100\text{--}120 \mu\text{m}$  ( $\bar{x} = 170 \times 115 \mu\text{m}$ ,  $n = 10$ ), solitary,

immersed, only black shiny ostioles visible, immersed beneath small clypeus, appearing as a disc around the neck, coriaceous, subglobose to depressed globose, as raised blister-like areas, ostiolate. *Ostioles* central,  $60 \times 55 \mu\text{m}$ , carbonaceous, black, filled with periphyses. *Peridium*  $5\text{--}10\text{--}(22) \mu\text{m}$  wide, outer cells merging with the host epidermal cells, composed of 3–4 (–8 at apex) layers of dark brown to black cells of *textura angularis*. *Paraphyses* of numerous,  $6\text{--}16 \mu\text{m}$  wide ( $\bar{x} = 4 \mu\text{m}$ ,  $n = 10$ ), hyaline, branched, septate, longer than asci. *Asci*  $60\text{--}86 \times 6\text{--}10 \mu\text{m}$  ( $\bar{x} = 75 \times 8 \mu\text{m}$ ,  $n = 30$ ), 8-spored, unitunicate, broad cylindrical, long-pedicellate, with a J-, wedge-shaped, subapical ring. *Ascospores*  $32\text{--}50 \times 2\text{--}4 \mu\text{m}$  ( $\bar{x} = 38 \times 3 \mu\text{m}$ ,  $n = 50$ ), filiform, straight or curved, C-shaped or sigmoid, aseptate, acute ends, without polar appendages, smooth-walled, with minute guttules in each cell. **Asexual morph:** Undetermined.

*Culture characters:* Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C, Culture from above, cream, radiating, wrinkled, folded in the middle, dense, lobate, flattened, umbonate, edge irregular, fluffy; reverse black in the middle and cream at the edge, radially striate with lobate edge.

*Material examined:* Thailand, Chiang Rai Province, on dead stems of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH18 (MFLU 17–1482, **holotype**); ex-type living culture, MFLUCC 17–2074.

*Host: Clematis subumbellata*—(This study).

*Distribution:* Thailand—(This study).

*GenBank accession numbers:* LSU: MN628626; *tefl1*: MN629286; *rpb2*: MN628628.

*Notes:* In the phylogenetic analysis, *Neoleptospora clematidis* (strain MFLUCC 17–2074) formed a distinct clade from other *Leptospora* species (Fig. 93). The asexual morph of our taxon was not obtained from culture. In a BLASTn search of GenBank, the closest match of the LSU sequence of MFLUCC 17–2074 is *Leptospora bambusae* with 85% similarity to strain MFLUCC 12–0846 (NG\_059674). This is the first record of Leptosporaceae on *Clematis* (Fig. 94).

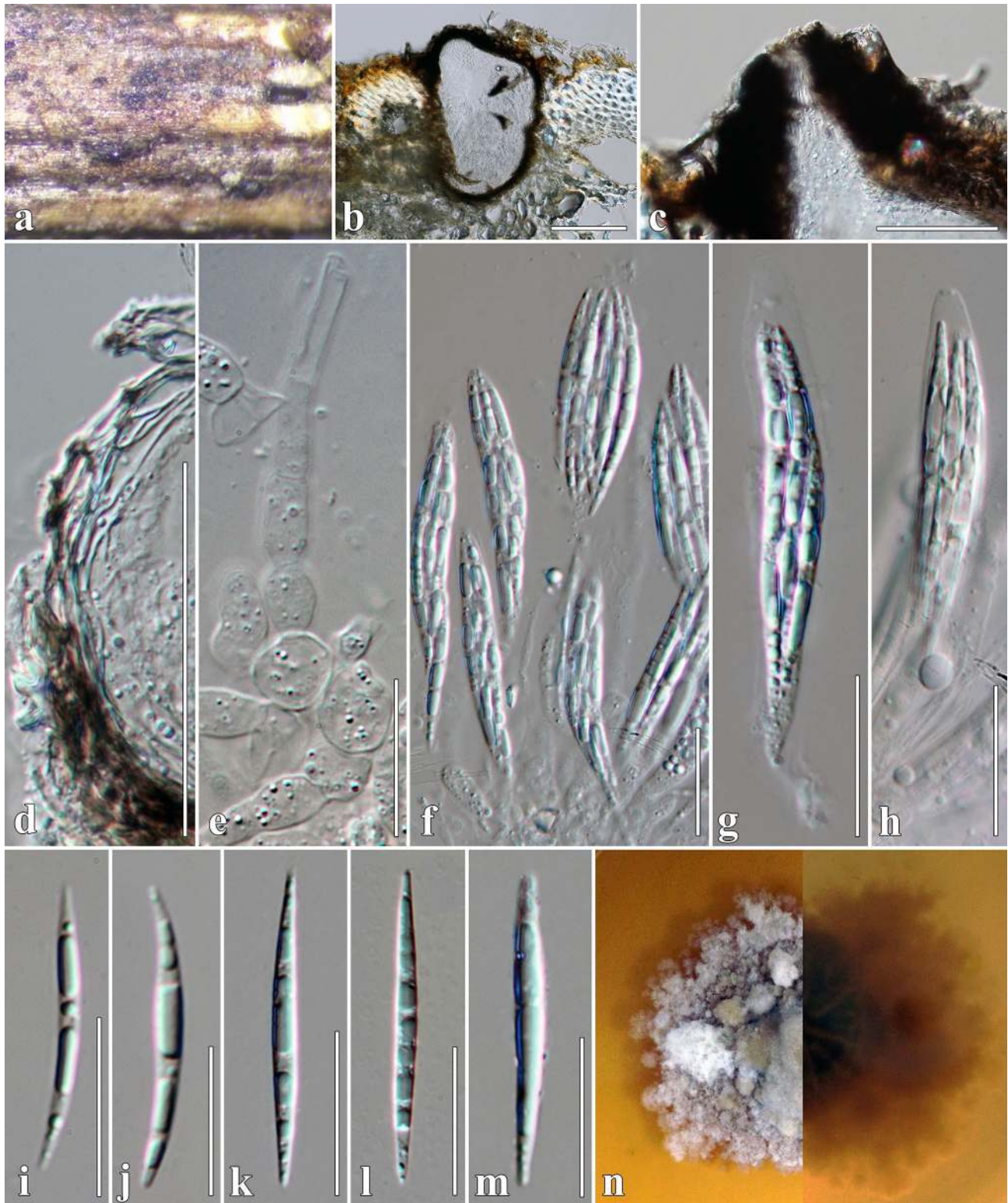
Isolate MFLUCC 17–2074 was evaluated for bioactive secondary metabolite production. The strain inhibits the growth of *Bacillus subtilis* but not at significant values (data not shown).

**Sordariales** Chadeff. ex Hawksw. & O.E. Erikss.

This order comprises three families Chaetomiaceae, Sordariaceae and Lasiosphaeriaceae sensu lato (Zhang et al. 2006; Maharachchikumbura et al. 2016).

**Chaetomiaceae** Winter

Chaetomiaceae is a highly diverse family that can be found in several environments as fungicolous, saprobes, or parasites in plant or animals (Mukerji and Manoharachary



**Fig. 94** *Neoleptospora clematidis* (MFLU 17–1482, holotype). **a** Appearance of ascomata on *Clematis subumbellata*. **b** Vertical section through ascoma. **c** Ostiole. **d** Peridium. **e** Paraphyses. **f–h** Asci

(**h** asci in 5% KOH presenting a J-reaction of apical ring, wedge-shaped). **i–m** Ascospores. **n** Culture characteristics on MEA. Scale bars: **b** = 100  $\mu$ m, **c**, **d** = 50  $\mu$ m, **e–h** = 20  $\mu$ m, **i–m** = 10  $\mu$ m



2010; Ahmed et al. 2016). Wang et al. (2019) made a major revision for Chaetomiaceae, accepting 37 genera.

### *Dichotomopilus* Wang, Samson & Crous

*Dichotomopilus* was erected by Wang et al. (2016) for a group characterised by dichotomously branched terminal ascomatal hairs. *Dichotomopilus* is typified by *D. indicus*, with 12 species listed in Index Fungorum (2020). Based on both morphological and phylogenetic analysis of combined ITS, LSU, *tef1* and *rpb2* sequence data (Fig. 95), we introduce the first record of *Dichotomopilus ramosissimum* on *Clematis* (Fig. 96).

### *Dichotomopilus ramosissimum* (X. Wei Wang & L. Cai) Wei Wang & Samson, Studies in Mycology 84: 217 (2016) **new host record**

*Basionym*: *Chaetomium ramosissimum* X. Wei Wang & L. Cai, Mycol. Prog. 13: 725. (2014).

*Index Fungorum number*: IF 801734; *Facesoffungi number*: FoF 07245, Fig. 96.

*Saprobic* on dead branches of *Clematis vitalba*. **Sexual morph**: *Ascomata* 70–225 × 80–220 μm ( $\bar{x}$  = 150 × 140 μm, n = 5), erumpent to superficial, with thick aerial hyphae or exposed ostiolate, covered with black ascomatal hairs, sphaerical or ovate, without papilla. *Peridium* reddish brown to brown, composed of hypha-like cells, *textura intricata* mixed with *textura epidermoidea* in surface view. *Terminal hairs* erect, 120–290 × 3–6 μm ( $\bar{x}$  = 170 × 5 μm, n = 20), rigid, dark brown, copiously dichotomously branched more than four times at the side, straight angles, starting from the lower half, punctulate or verrucose. *Lateral hairs* 50–100 × 3–4 μm ( $\bar{x}$  = 70 × 4 μm, n = 20), unbranched, setae-like, tapering towards the end. *Asci* 30–40 × 8–15 μm ( $\bar{x}$  = 35 × 10 μm, n = 10), 8-spored, spatulate, long pedicellate, apically rounded. *Ascospores* 5–8 × 2.5–5 μm ( $\bar{x}$  = 7 × 4 μm, n = 50), overlapping, ovate to subglobose, ends acute, hyaline, becoming brown with age, aseptate, verruculose, with slit-like germ pores at maturity. **Asexual morph**: Undetermined.

*Material examined*: UK, Hampshire, Botley wood, Hampshire, on dead stems of *Clematis vitalba*, 16 April 2016, E.B.G. Jones, GJ264 (MFLU 16–2137).

*Hosts*: The rhizosphere of *Panax notoginseng*, soil sample, *Clematis vitalba*—(Wang et al. 2014; this study).

*Distribution*: China, UK—(Wang et al. 2014; this study).

*GenBank accession numbers*: LSU: MT214611; SSU: MT226722; ITS: MT310655; *tef1*: MT394667.

*Notes*: *Dichotomopilus ramosissimum* was described as *Chaetomium ramosissimum*. Later, with more taxon sampling of Chaetomiaceae, the species formed a separate lineage and was synonymized under *Dichotomopilus*. Our collection formed a close relationship with the type strain of *D. ramosissimum* (CGMCC 3.14183, 100% ML/1.00 BYPP).

The morphological characters of our collection are similar to those reported by Wang et al. (2014). In a BLASTn search of GenBank, the closest match of the ITS sequence of strain MFLU 16–2137 was *D. erectus* strain CBS 140.56 with 98.5% similarity (MH857548), while the closest match of the *tef1* sequence was *D. ramosissimum* strain CGMCC 3.14183 (KC485021) with 100% similarity.

### Sordariaceae Winter

Sordariaceae is typified by *Sordaria*, and species in this family mainly occur as coprophilous or saprobes (Cai et al. 2006; Zhang et al. 2006; Arif and Saleem 2017). Sordariaceae is characterized by coriaceous ascomata and hyaline or brown ascospores with sheaths (Maharachchikumbura et al. 2016). Eight genera are accepted in this family (Maharachchikumbura et al. 2016; Wijayawardene et al. 2017).

### *Sordaria* Ces. & De Not

*Sordaria* is typified with *Sordaria fimicola*. There are more than 50 epithets listed in Index Fungorum (2020), but only 15 strains have sequence data (Cai et al. 2006). Most species are reported from dung, soil, and seed pods (Furtado 1969; Watanabe 1989; Mungai et al. 2012). *Sordaria* is characterized by perithecioid, semi-immersed to superficial, coriaceous ascomata, paraphyses, cylindrical asci, with a lobate pedicel, and a prominent apical ring, and uniseriate ascospores that are ellipsoidal to ovoid, surrounded by a gelatinous sheath. Based on both morphological and phylogenetic analysis of combined LSU, ITS and *tub* sequence data (Fig. 97), we introduce the first record of *Sordaria*, *S. clematidis* on *Clematis* species (Fig. 98).

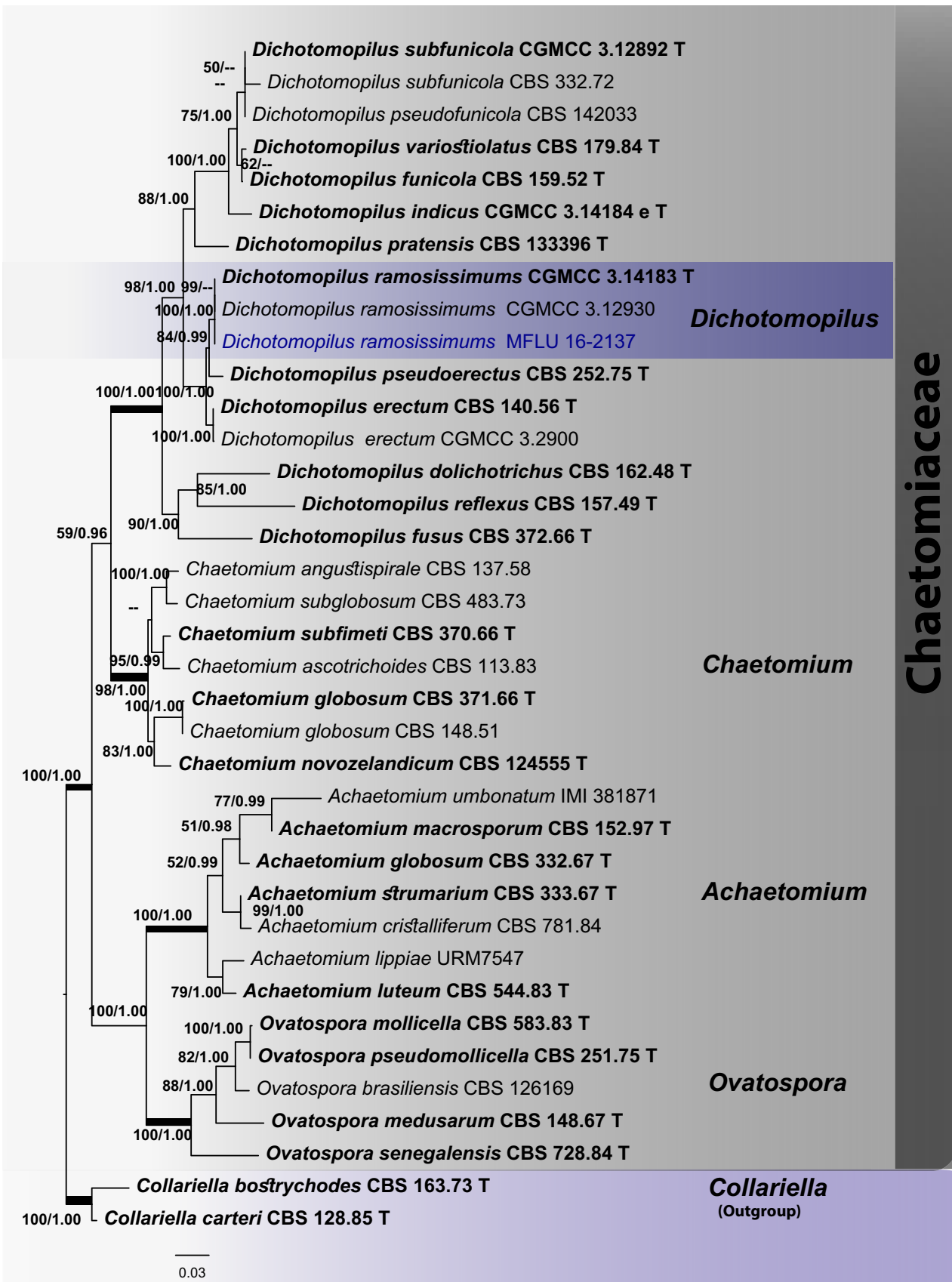
### *Sordaria clematidis* Phukhams. & K.D. Hyde, sp. nov.

*Index Fungorum number*: IF557306; *Facesoffungi number*: FoF 07337, Fig. 98.

*Etymology*: Refers to the host genus, *Clematis*.

*Holotype*: MFLU 16–2138

*Saprobic* on dead stems of *Clematis vitalba*. **Sexual morph**: *Ascomata* 220–315 × 200–350 μm ( $\bar{x}$  = 270 × 300 μm, n = 5), perithecial, single, superficial, solitary, scattered, ampulliform or pyriform, globose, with a sphaerical body, coriaceous, smooth or nearly, often glossy and black, ostiolate. *Ostioles* central, oblong, dark brown to black, papillate, periphyses filling ostioles. *Peridium* 20–30 μm wide, composed of 6–7 layers of *textura angularis* of thin-walled, brown cells, inner layer lined with thin hyaline layers. *Paraphyses* of numerous, 10–17 μm ( $\bar{x}$  = 14 μm, n = 40), catenophyses, branched, transversely septate, hyaline. *Asci* 115–160 × 4–7 μm ( $\bar{x}$  = 180 × 20 μm, n = 20), unitunicate, oblong, 8-spored, with a thin but rather persistent wall, with a disc and J-ring at the truncate apex, simple pedicel. *Ascospores* 21–26 × 11–15 μm ( $\bar{x}$  = 25 × 13 μm, n = 20), uniseriate, ovate, aseptate, smooth-walled, hyaline or pale



## Chaetomiaceae

**Fig. 95** Phylogram generated from maximum likelihood analysis based on combined ITS, LSU, *tef1* and *rpb2* sequence data representing related taxa in Chaetomiaceae. Related sequences were taken from Wang et al. (2019) and 33 strains were included in the combined analyses which comprised 2972 characters (581 characters for ITS, 855 characters for LSU, 936 characters for *tef1*, 600 characters for *rpb2*, including gap regions). *Collariella bostrychodes* (CBS 163.73) and *Collariella carteri* (CBS 128.85) are used as the outgroup taxa. The tree from the maximum likelihood analysis had similar topology to the Bayesian analyses. The best sorting RaxML tree had a final likelihood value of  $-12501.711733$  is presented. The matrix had 710 distinct alignment patterns with 6.40% undetermined characters or gaps. Estimated base frequencies were as follows: A=0.235341, C=0.273490, G=0.285063, T=0.206106; substitution rates AC=1.347902, AG=3.116716, AT=1.526141, CG=1.846122, CT=7.547565, GT=1.000000; gamma distribution shape parameter  $\alpha=1.039485$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study is indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses at the genus level (BS  $\geq$  70%/BYPP  $\geq$  0.95)

brown, guttulate, with 3–7  $\mu\text{m}$  thick mucilaginous sheath with upper pore. **Asexual morph:** Undetermined.

**Material examined:** UK, Hampshire, Botley Wood, on dead branches of *Clematis vitalba*, 25 May 2016, E.B.G. Jones, GJ289 (MFLU 16–2138, **holotype**).

**Hosts:** *Clematis vitalba*—(This study).

**Distribution:** UK—(This study).

**GenBank accession numbers:** LSU: MT214612; SSU: MT226723; ITS: MT310656; *tef1*: MT394668; *rpb2*: MT394717.

**Notes:** *Sordaria clematidis* (Fig. 98) has characters typical of *Sordaria* in having single perithecial, superficial, coriaceous ascomata with papillate ostioles, unitunicate, oblong asci with uniseriate, ovate, aseptate ascospores with a mucilaginous sheath. In the phylogenetic analysis (Fig. 97), *S. clematidis* formed a close relationship with the generic type *S. fimicola* (CBS 508.50), but received relatively low statistical support. *Sordaria* species are similar in their morphological characters, however, *S. clematidis* is unique in having pale yellow ascospores with upper pore in mucilaginous sheath and a germ-slit is absent (Maharachchikumbura et al. 2016). In a BLASTn search of GenBank, the closest match of the LSU sequence of strain MFLU 16–2138 is *S. fimicola* (strain CBS 485.64) with 98.00% similarity (MH870123). The closest match with the ITS sequence was *Sordaria fimicola* strain CBS 485.64 with 99.06% similarity (MH858489). Therefore, we introduce *S. clematidis* as a new species.

**Subclass Diaporthomycetidae** Senan., Maharachch. & K.D. Hyde

**Diaporthales** Nannf.

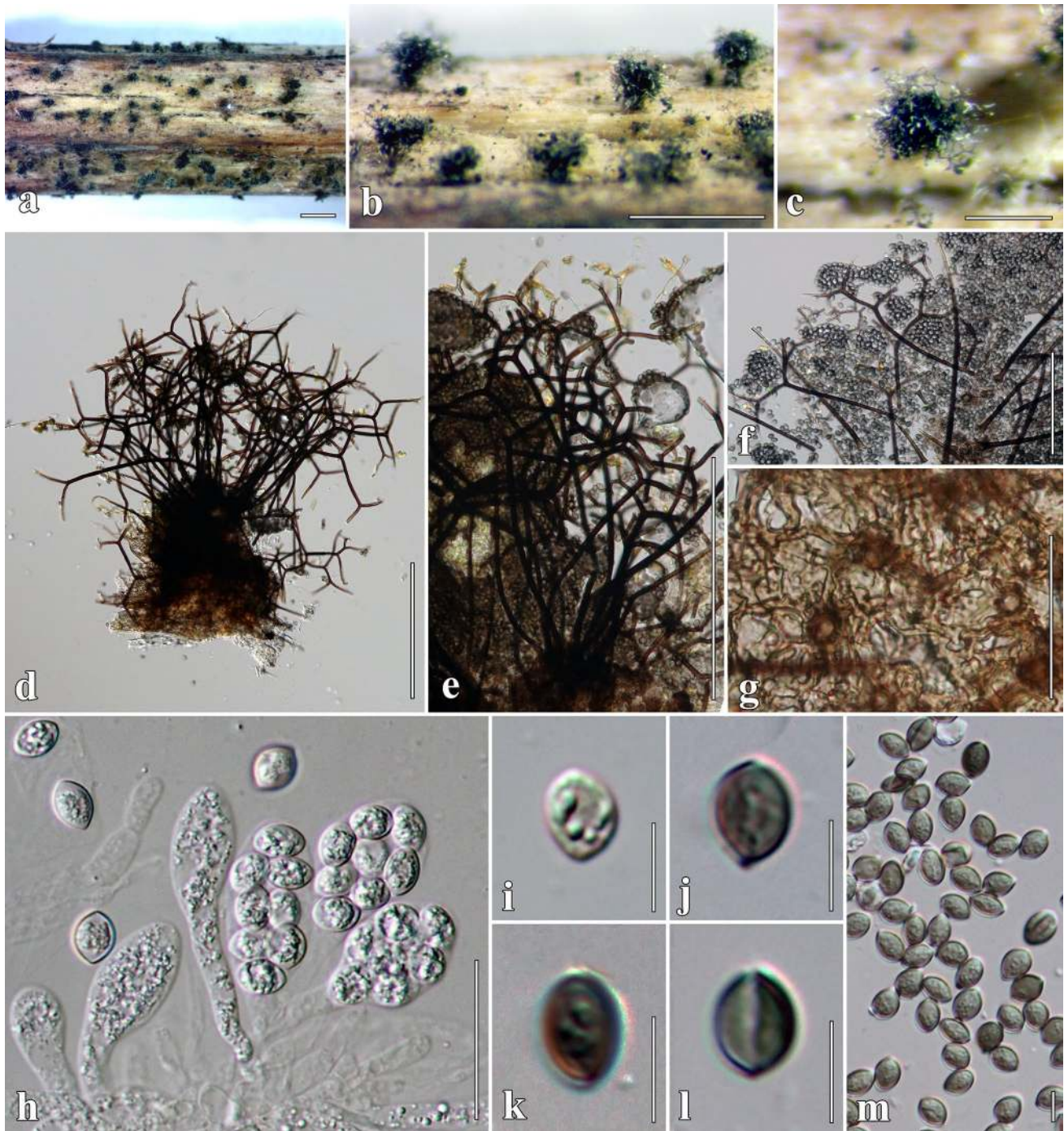
Diaporthales is one of the most diverse orders within Diaporthomycetidae and comprises 24 families (Senanayake et al. 2017; Guterres et al. 2019). The order includes plant-associated fungi, some of which are economically important pathogens, parasites, others are endophytes or saprobes on various substrates (Alvarez et al. 2016). Diaporthales taxa are characterized by having pseudo- or ascostromata, brown to black perithecial fruiting bodies, asci with J-, refractive apical ring and hyaline to brown ascospores. The asexual states are usually coelomycetous with conidiophores arising from the inner side of the peridium layer, enteroblastic, holoblastic, phialidic or annellidic conidiogenous cells, and hyaline to brown conidia (Sogonov et al. 2008; Senanayake et al. 2017).

**Diaporthaceae** Höhn. ex Wehm.

Diaporthaceae taxa have a cosmopolitan distribution occurring as pathogens, saprobes or endophytes of terrestrial plants (Udayanga et al. 2014; Rossman et al. 2017; Thambugala and Hyde 2018). The sexual morphs of this family are characterized by perithecial, immersed to erumpent, papillate ascomata, 8-spored, asci with J-, refractive apical ring, and 1 septum, hyaline or dark to blackish brown ascospores with or without polar appendages. Asexual morphs are characterized by acervular or pycnidial conidiomata, ampulliform, cylindrical, septate conidiophores, enteroblastic, phialidic, determinate conidiogenous cells and 0–2-septate, hyaline or brown conidia sometimes with short appendages (Voglmayr and Jaklitsch 2014; Dissanayake et al. 2017; Senanayake et al. 2017, 2018). The family comprises 14 genera accepted by Wijayawardene et al. (2018).

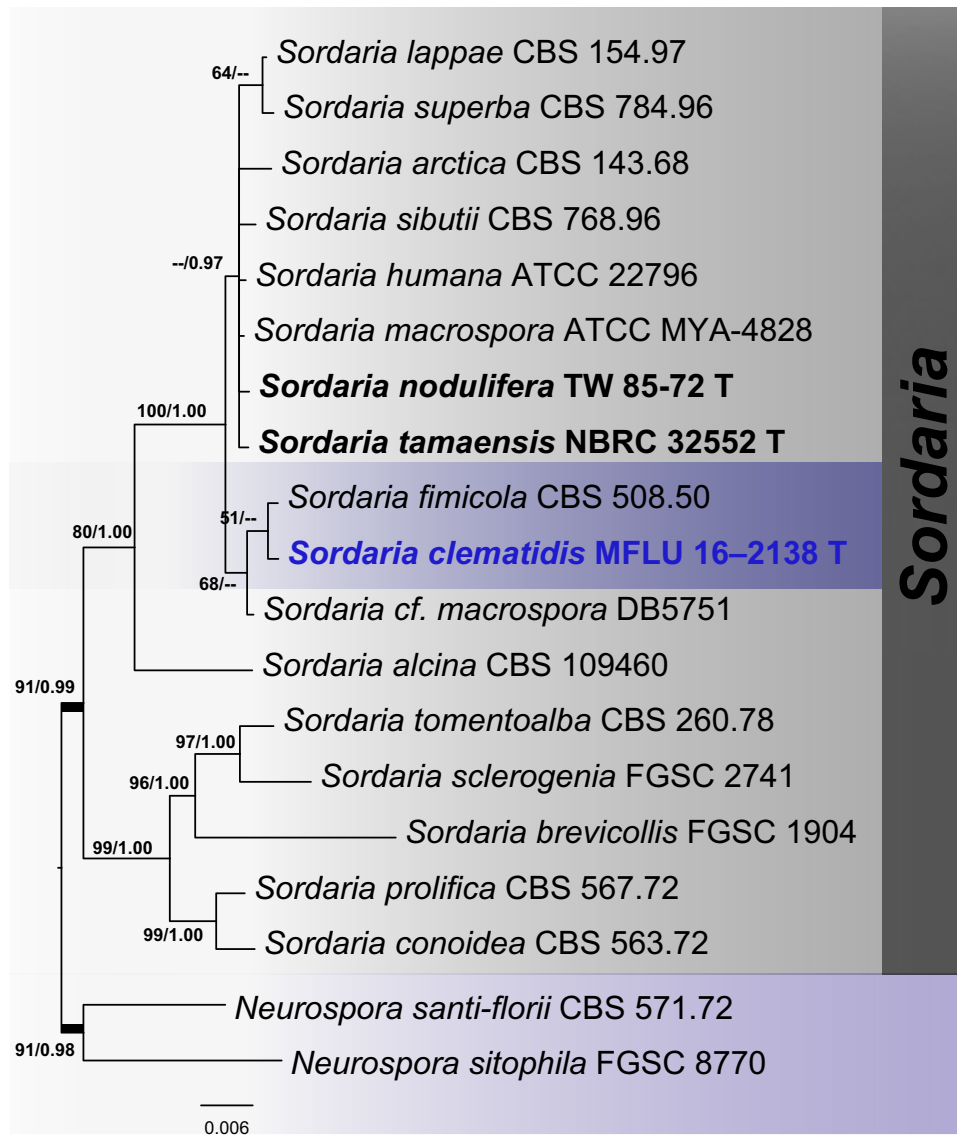
**Diaporthe** Nitschke

*Diaporthe* (= *Phomopsis*) is an economically important plant pathogen that also includes endophytes or saprobes on a broad range of plant hosts (Udayanga et al. 2014; Hyde et al. 2016). Over 1000 epithets are listed under *Diaporthe* and most were introduced based on morphological characters and host association (Aa et al. 1990). In view of the genetic diversity and complexity of the known species, identifications of *Diaporthe* species currently relies on multi-locus phylogenetic analyses (Senanayake et al. 2018; Manawasinghe et al. 2019). Phylogenetic analysis resulting from combined ITS, *tef1*, *cal* and *tub2* sequence data of selected *Diaporthe* species are presented in Figs. 99 and 101. The ambiguous species that are related to the newly described species were analysed separately using Genealogical Concordance Phylogenetic Species Recognition (GCPSR) (Taylor et al. 2000) by performing a pairwise homoplasy index ( $\Phi_w$ ) test and the results are shown in Figs. 100 and 103.



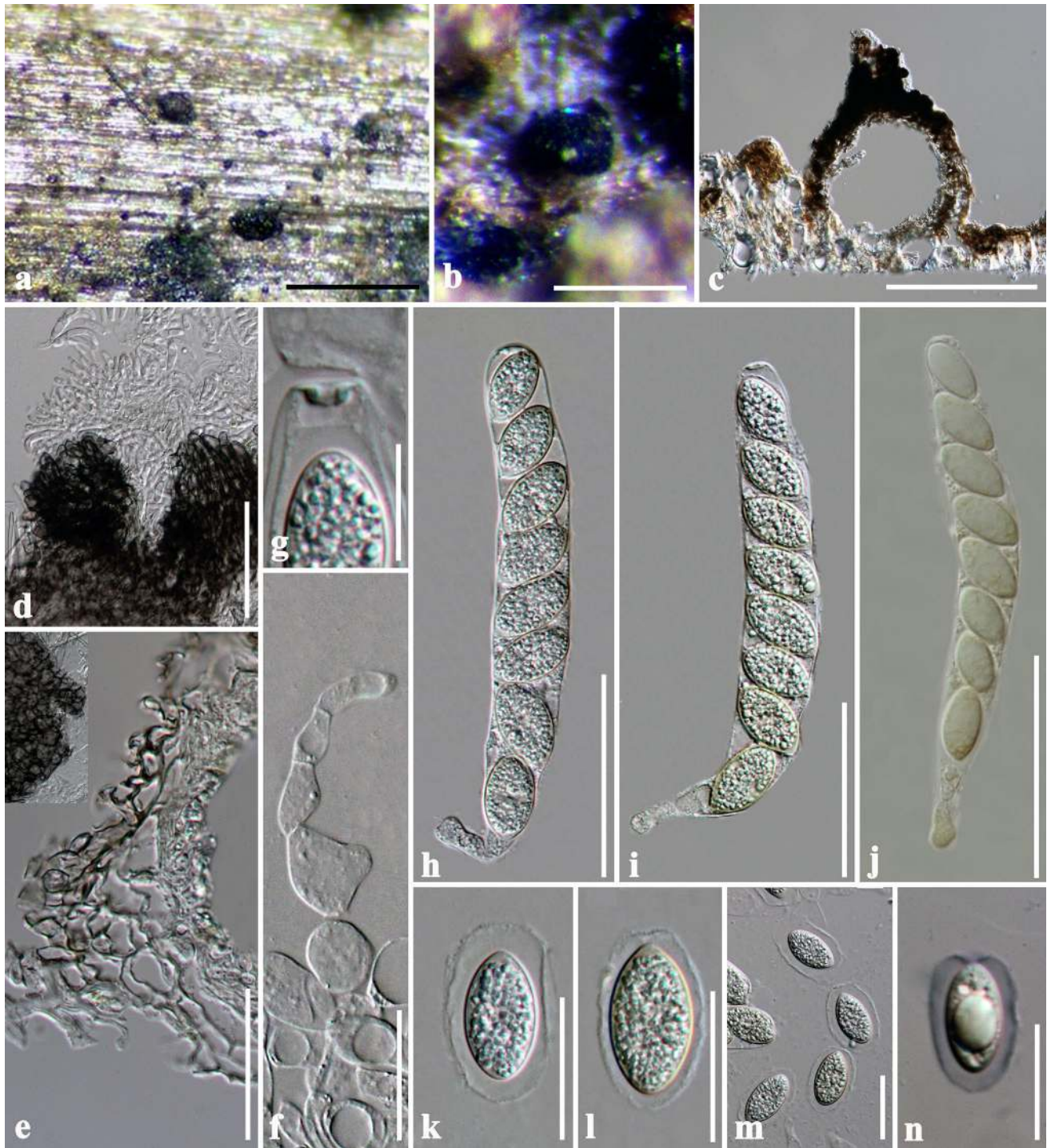
**Fig. 96** *Dichotomopilus ramosissimum* (MFLU 16–2137). **a** Appearance of perithecia on *Clematis vitalba*. **b, c** Close up of ascomata on host surface. **d** Ascoma. **e, f** Dichotomously branched terminal hairs

and setae-like lateral hairs. **g** Structure of peridium in surface view. **h** Asci. **i–m** Ascospores. Scale bars: **b** = 200  $\mu$ m, **c, d** = 100  $\mu$ m, **e, f** = 50  $\mu$ m, **g, h** = 20  $\mu$ m, **i–m** = 5  $\mu$ m



**Fig. 97** The Bayesian 50% majority-rule consensus phylogram for *Sordaria* members based on combined LSU, ITS and *tub* sequence data. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted with *Neurospora santi-florii* (CBS 571.72) and *Neurospora sitophila* (FGSC 8770). Nineteen strains were included in the combined analyses which comprised 2436 characters (833 characters for LSU, 601 characters for ITS, 1002 characters for *tub*, including gap regions). The tree from the maximum likelihood analysis had similar topology to the Bayesian analyses. The best scoring RAxML tree had a final likelihood value of  $-5155.259712$ . The matrix had 275 distinct alignment patterns with 36.05% undetermined characters and

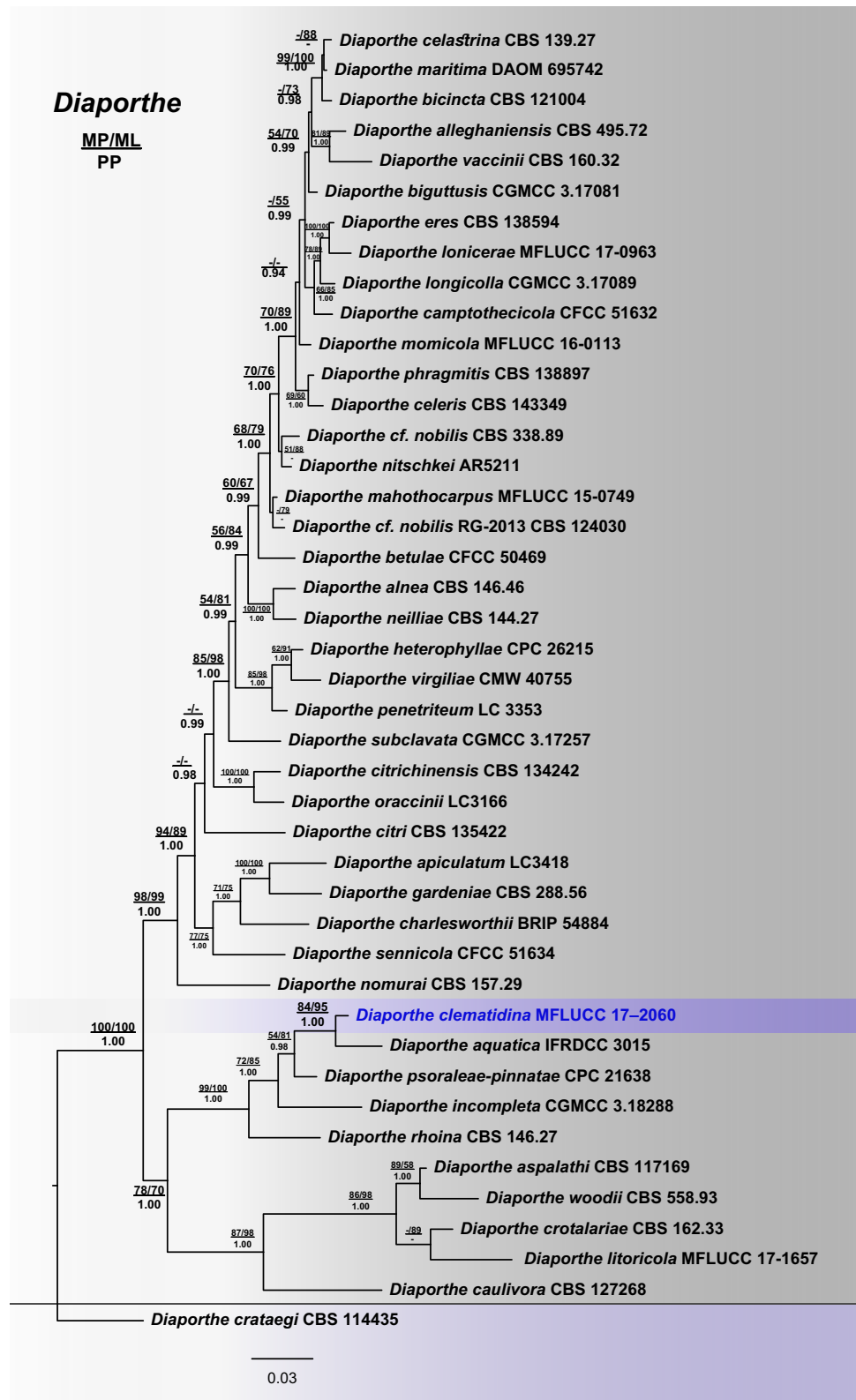
gaps. Estimated base frequencies were as follows; A=0.239918, C=0.256198, G=0.278423, T=0.225461; substitution rates AC=1.231551, AG=4.410425, AT=1.886486, CG=1.711684, CT=9.592785, GT=1.000000; gamma distribution shape parameter  $\alpha=1.270759$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study is indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95) at the genus level



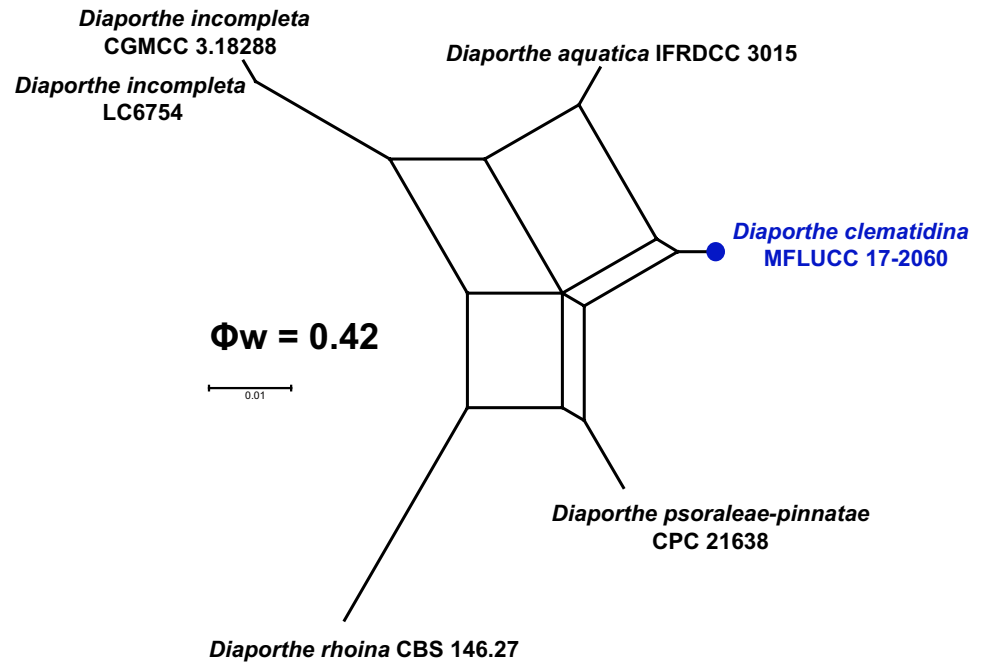
**Fig. 98** *Sordaria clematidis* (MFLU 16–2138, **holotype**). **a, b** Appearance of ascomata on *Clematis vitalba*. **c** Vertical section through ascoma. **d** Ostioles. **e** Partial peridium part. **f** Paraphyses. **g** Presenting of apical ring. **h–j** Asci (**j** Ascus in 5% KOH with J-reac-

tion of wedge-shaped apical ring. **k–n** Ascospores (**n** Ascospore in 10% Indian ink). Scale bars: **a**=500  $\mu$ m, **b**=200  $\mu$ m, **c**=100  $\mu$ m, **d–f**, **k–n**=20  $\mu$ m, **g**=10  $\mu$ m, **h–j**=50  $\mu$ m

**Fig. 99** Phylogram generated from maximum parsimony analysis based on combined ITS, *tef1*, *cal* and *tub2* sequence data for selected members of *Diaporthe*. Related sequences are taken from Hyde et al. (2019) and retrieved from GenBank. The species tree included all members of *Diaporthe* and was performed for placement determination. Forty-two strains were included in the analysis of the combined loci and comprised 2359 characters (598 characters for ITS, 393 characters for *tef1*, 541 characters for *cal*, 827 characters for *tub2*, including alignment gaps). The tree was rooted with *Diaporthe crataegi* (CBS 114435). Maximum parsimony analysis of 590 parsimony informative characters resulted in a single most parsimonious tree (CI=0.585, RI=0.681, RC=0.398, HI=0.415). The best scoring RAxML tree had a final likelihood value of  $-14560.846477$ . GTRGAMMA bootstrapping model was applied to the matrix which had 1152 distinct alignment patterns, with 27.96% of undetermined characters and gaps. Estimated base frequencies were: A=0.218108, C=0.320856, G=0.237387, T=0.223649; substitution rates AC=1.282937, AG=3.695471, AT=1.225056, CG=1.103739, CT=4.789247, GT=1.000000; gamma distribution shape parameter  $\alpha=0.888152$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. Bootstrap values (BS) from maximum parsimony (MP, left), maximum likelihood (ML, right) higher than 50% BS and Bayesian posterior probabilities (BYPP, below) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. The ex-type strains are in bold and black. The newly generated sequence is in bold and blue



**Fig. 100** The splits graph from the pairwise homoplasy index (PHI) test generated from the concatenated gene set of ITS, *tef1*, *cal* and *tub2* sequence data of closely related species using both LogDet transformation and splits decomposition. In the PHI test results ( $\Phi_w$ ) < 0.05 indicates significant recombination within the dataset. The strain determined in this study is in bold and blue



***Diaporthe clematidina*** Phukhams., M.V. de Bult & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF557300; *Facesoffungi number*: FoF 07262, Fig. 101.

*Etymology*: Named after the host genus, *Clematis*.

*Holotype*: MFLU 17–1466.

*Saprobic* on *Clematis subumbellata*. **Sexual morph**: Ascumata 280–367 × 167–296 μm ( $\bar{x}$  = 325 × 250 μm, n = 5), perithecial, solitary to aggregated, immersed, papillate only visible on the host substrate, obpyriform to globose, coriaceous, brown to dark brown, heavily pigmented at apex, papillate. *Ostioles* 125–221 × 47–50 μm ( $\bar{x}$  = 180 × 50 μm, n = 5), papillate, central or eccentric, broad oblong, filled with periphyses. *Peridium* 12–31 μm wide ( $\bar{x}$  = 21 μm, n = 20), composed of 2–3 layers of thin-walled cells of *textura globosa* mixed with *textura angularis*, brown to reddish brown, thin at inner layer, hyaline. *Paraphyses* dense, 2–3 μm ( $\bar{x}$  = 2.5 μm, n = 30), septate, constricted at septa, broad filiform, tapering above asci. *Asci* 32–54 × 7–10 μm ( $\bar{x}$  = 46.5 × 9 μm, n = 20), 8-spored, unitunicate, oblong to broad oblong, thin-walled, short or apedicellate, with a refractive, J-, apical ring. *Ascospores* 12–15 × 3–5 μm ( $\bar{x}$  = 14 × 4 μm, n = 40), partially overlapping, oblong-ellipsoidal, hyaline, uniseptate, with two guttules in each cell. **Asexual morph**: Pycnidial on PDA media. *Conidiomata* 140–350 × 100–220 μm ( $\bar{x}$  = 225 × 155 μm, n = 5), globose, eustromatic, multilocular, gregarious, erumpent or superficial, covered by dense vegetative hyphae, occasionally with ostiolate necks. *Pycnidial wall* parenchymatous, consisting of brown, thick-walled cells of *textura angularis*. *Conidiophores* short, hyaline, smooth, densely aggregated,

cylindrical, straight. *Conidiogenous cells* 8–14 × 8–12 μm, phialidic, cylindrical, terminal, with slight tapering towards apex, hyaline, formed from the inner layer of pycnidial wall. *Alpha conidia* 5–8 × 2–3 μm ( $\bar{x}$  = 6 × 2.5 μm, n = 20), aseptate, hyaline, smooth, ovate to ellipsoidal, biguttulate. *Beta conidia* 17.5–30 × 1–2 μm ( $\bar{x}$  = 25 × 1.7 μm, n = 5), aseptate, hyaline, smooth, fusiform, tapering towards both ends, apex conical, base subtruncate. *Gamma conidia* not observed.

*Culture characters*: Colonies growing on PDA reaching 20 mm within 4 weeks at 25 °C. Culture from above, white, radiating, to the edge, faintly zonate, margin undulate, dense, flat or umbonate; reverse light brown, white radiating outwardly.

*Material examined*: Thailand, Chiang Rai Province, on dead stems of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH2 (MFLU 17–1466, **holotype**); ex-type living culture, MFLUCC 17–2060.

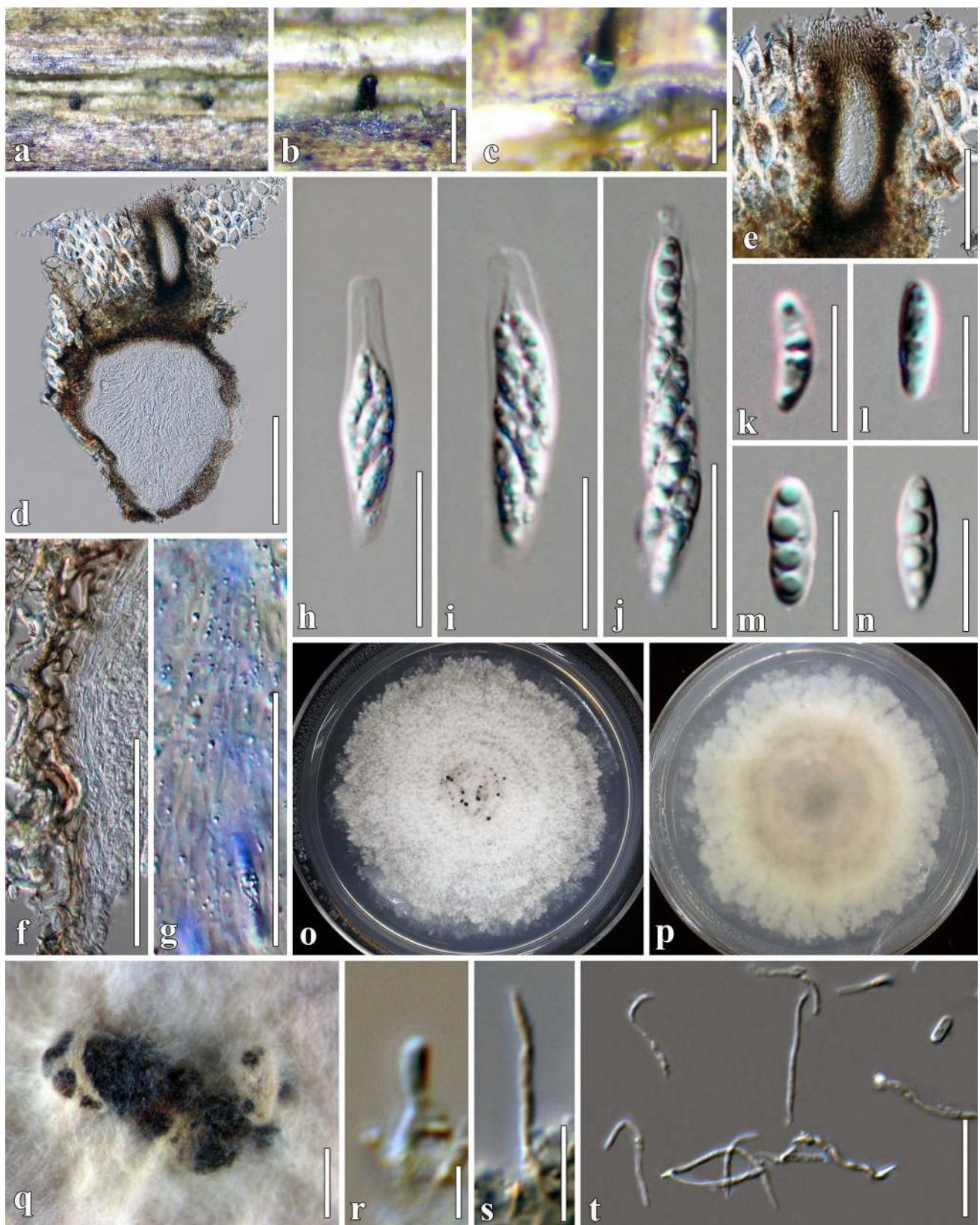
*Host*: *Clematis subumbellata*—(This study).

*Distribution*: Thailand—(This study).

*GenBank accession numbers*: LSU: MT214613; SSU: MT226724; ITS: MT310657; *tef1*: MT394669, MT394625; *rpb2*: MT394718; *cal*: MT394624; *tub2*: MT394623.

*Notes*: Phylogenetic analysis of *Diaporthe clematidina* based on the combined ITS, *tef1*, *cal* and *tub2* sequence data, shows that the strain clusters in a well-supported clade (84% MP, 95% ML support and 1.00 BYPP) sister to *D. aquatica* (IFRDCC 3015) as shown in Fig. 99. *Diaporthe clematidina* differs from *D. aquatica* in its shorter neck (1100–2250 × 80–120 vs 125–221 × 47–50 μm), and broad oblong ascospores with conical ends. *Diaporthe clematidina* was found in a terrestrial habitat, while *D. aquatica* was

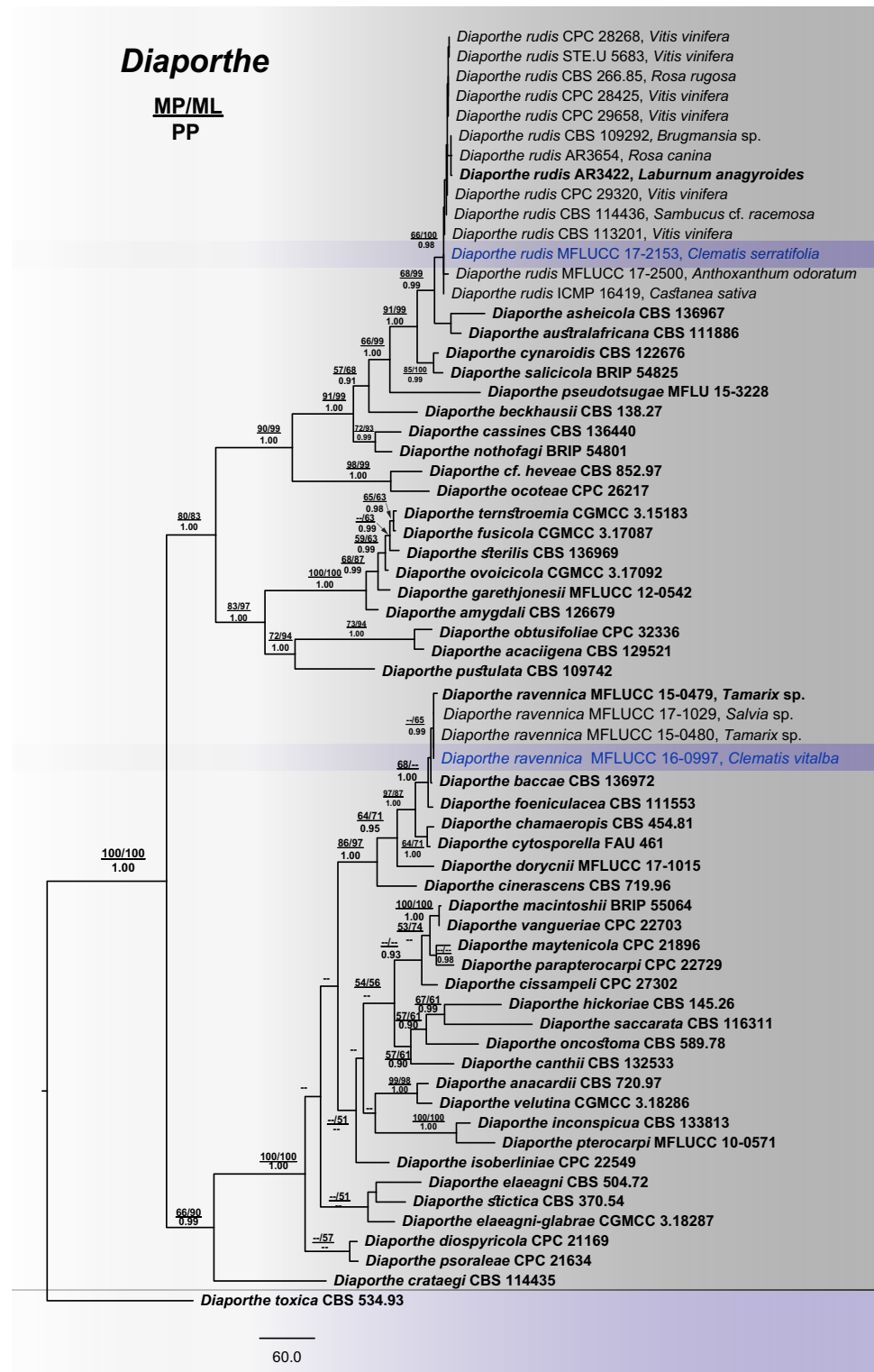


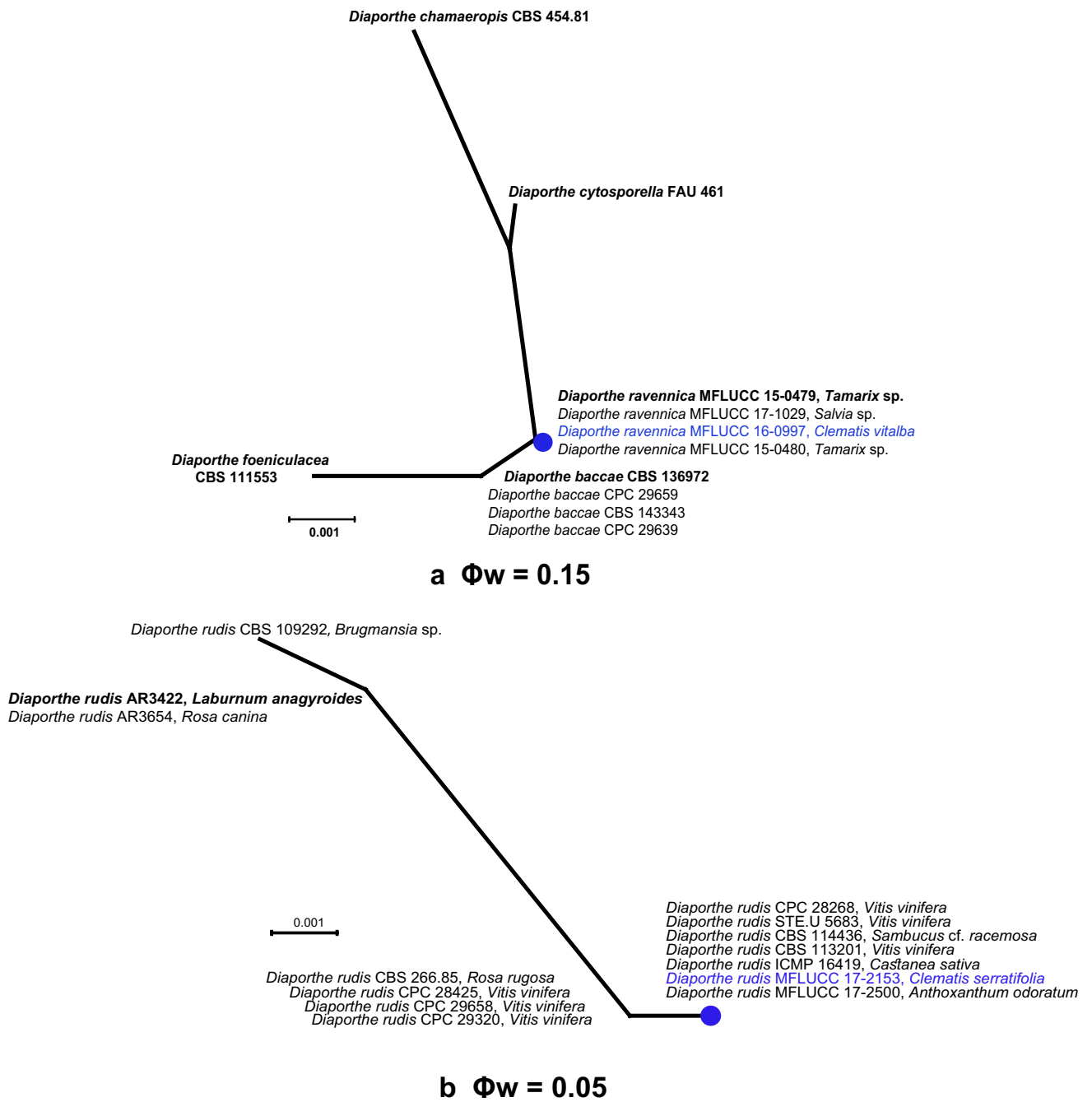


**Fig. 101** *Diaporthe clematidina* (MFLU 17–1466, holotype). **a**, **b** Appearance of ascomata on *Clematis subumbellata*. **c** Close up of ascoma on the host. **d** Vertical section through ascoma. **e** Ostiolar canal. **f** Peridium. **g** Paraphyses. **h–j** Asci. **k–n** Ascospores. **o**,

**p** Culture characteristics on PDA. **q** Pycnidia produced in culture. **r** Conidiogenous cell with an alpha conidium. **s** Conidiogenous cell with a beta conidium. **t** Alpha and beta conidia. Scale bars: **b–d**, **q**=200  $\mu$ m. **e**=100  $\mu$ m. **f**, **g**=50  $\mu$ m. **h–j**=20  $\mu$ m. **k–n**, **r–t**=10  $\mu$ m

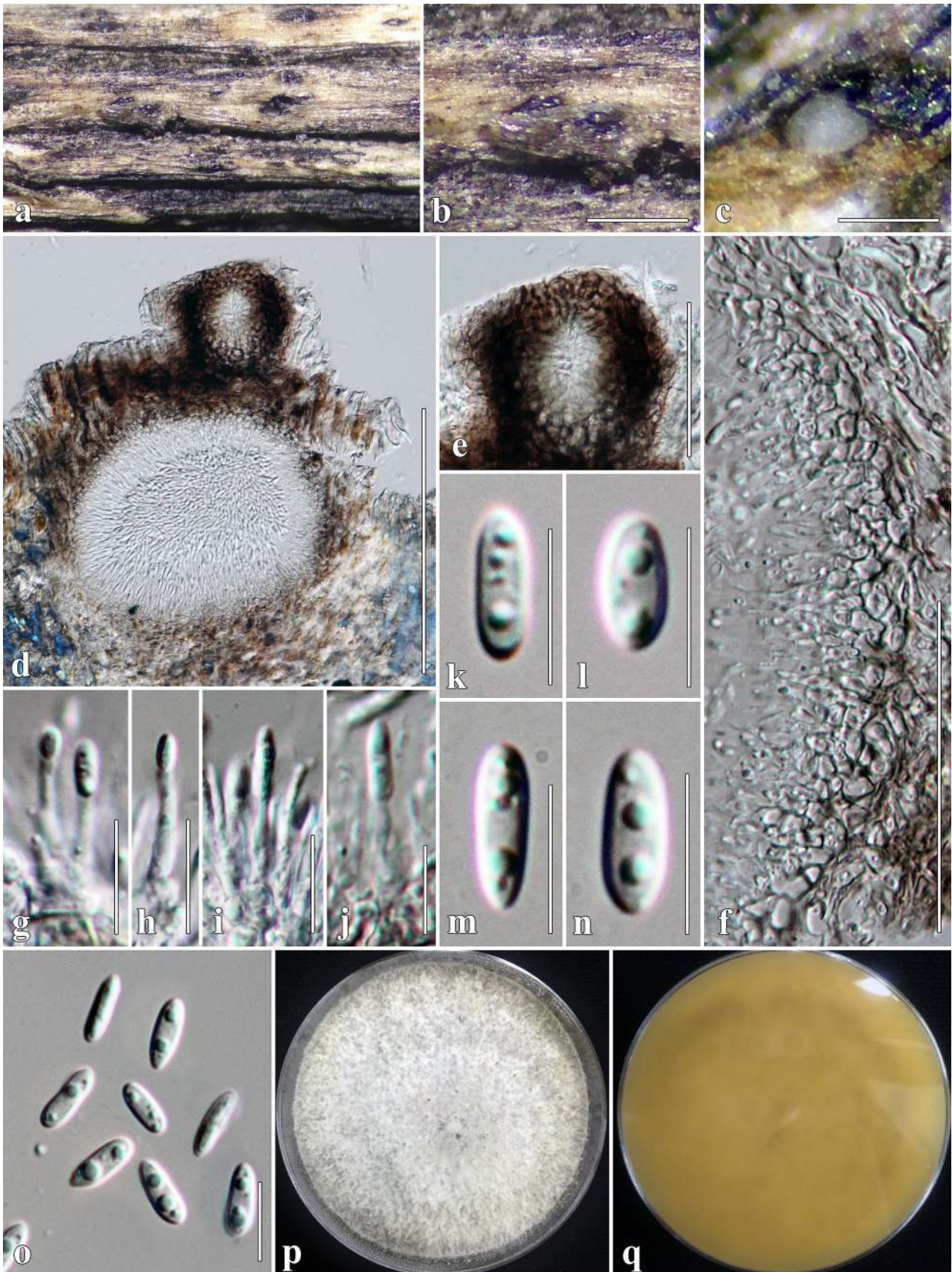
**Fig. 102** Phylogram generated from maximum parsimony analysis based on combined ITS, *tef1*, *cal* and *tub2* sequence data for selected *Diaporthe* species. Related sequences were selected from Hyde et al. (2019) and retrieved from GenBank. The species tree was performed for placement determination. Sixty-four strains were included in the combined analyses, which comprised 2380 characters (603 characters for ITS, 409 characters for *tef1*, 542 characters for *cal*, 826 characters for *tub2*, including alignment gaps). The tree was rooted with *Diaporthe toxica* (CBS 534.93). Maximum parsimony analysis of 736 parsimony informative characters resulted in a single most parsimonious tree (CI=0.555, RI=0.815, RC=0.453, HI=0.445). The best scoring RAXML tree had a final likelihood value of  $-18803.562906$ . The matrix had 1329 distinct alignment patterns, with 32.77% of undetermined characters and gaps. Estimated base frequencies were: A=0.224328, C=0.306223, G=0.237767, T=0.231682; substitution rates AC=1.005463, AG=2.446938, AT=0.976921, CG=0.744774, CT=3.4857627, GT=1.000000; gamma distribution shape parameter  $\alpha=0.765064$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study are indicated in blue. Bootstrap values (BS) from maximum parsimony (MP, left), maximum likelihood (ML, right) higher than 50% BS and Bayesian posterior probabilities (BYPP, below) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. The ex-type strains are in bold and black





**Fig. 103** The splits graph from the pairwise homoplasy index (PHI) test generated from the concatenated gene set of closely related *Diaporthe* species **a** *D. ravennica*, **b** *D. rudis* using both LogDet transfor-

mation and splits decomposition. The PHI test ( $\Phi_w$ ) < 0.05 indicates significant recombination within the dataset. The strains determined in this study are in blue



associated with submerged wood in a small ditch (Hu et al. 2012). *Diaporthe clematidina* is phylogenetically related, but differentiated from *D. aquatica* by 49 nucleotides in ITS. However, the other gene regions for *D. aquatica* are not available for nucleotide comparisons. When applying the GCPSR concept to fulfil the genetic differentially independence of the combined dataset within the closely related taxa (Fig. 99), we detected no significant recombination between these isolates ( $\Phi_w = 0.42$ , Fig. 100). Thus, *D. aquatica* and *D. clematidina* represents two distinct species.

***Diaporthe ravennica*** Thambug., Camporesi & K.D. Hyde, Fungal Diversity 82: 296 (2016), **new host record**

*Index Fungorum number*: IF552100; *Facesoffungi number*: FoF02171, Fig. 104.

*Saprobic* on *Clematis vitalba*. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 250–450 × 220–300  $\mu\text{m}$  ( $\bar{x} = 330 \times 250 \mu\text{m}$ ,  $n = 5$ ), scattered, uniloculate or multi-loculate, immersed to slightly erumpent, gregarious, globose, occasionally with ostiolate necks. *Ostioles* 75–190 × 52–170  $\mu\text{m}$  ( $\bar{x} = 121 \times 124 \mu\text{m}$ ,  $n = 5$ ), papillate, central or eccentric, broad oblong, lined with periphyses. *Pycnidial wall* 12–20  $\mu\text{m}$  ( $\bar{x} = 14.6 \mu\text{m}$ ,  $n = 20$ ), parenchymatous, walls consisting of dark brown, thick, walled cells of *textura angularis*. *Paraphyses* not observed. *Conidiophores* 5–14 × 1.5–2.5  $\mu\text{m}$  ( $\bar{x} = 8.5 \times 2.0 \mu\text{m}$ ,  $n = 30$ ), long, hyaline, smooth, densely aggregated, unbranched, cylindrical, straight. *Conidiogenous cells* 1.8–2 × 2–3  $\mu\text{m}$ , phialidic, cylindrical, terminal, slightly tapering towards apex, hyaline, formed from the inner layer of pycnidium wall. *Alpha conidia* 8–11.5 × 2–4  $\mu\text{m}$  ( $\bar{x} = 9.5 \times 2.8 \mu\text{m}$ ,  $n = 40$ ), aseptate, hyaline, smooth, ovate to ellipsoidal, biguttulate or multi-guttulate. *Beta* and *gamma conidia* not observed on *Clematis vitalba* or in culture.

*Culture characters*: Colonies growing on PDA reaching 40 mm within 4 weeks at 16 °C. Culture from above, white, radiating to the edge, margin undulate, medium dense, flat or umbonate; reverse, cream, radiating white outwardly.

*Material examined*: Italy, Forlì-Cesena Province, San Lorenzo in Noceto-Forlì, on dead aerial branch *Clematis vitalba*, 20 March 2016, E. Camporesi, IT2893C (MFLU 20–0422); living culture, MFLUCC 16–0997.

*Hosts*: *Clematis vitalba*, *Tamarix* sp., *Salvia* sp.—(Thambugala et al. 2017; Dissanayake et al. 2017; this study).

*Distribution*: Italy—(Thambugala et al. 2017; Dissanayake et al. 2017; this study).

*GenBank accession numbers*: LSU: MT214614; SSU: MT226725; ITS: MT310658; *tef1*: MT394670.

*Notes*: *Diaporthe ravennica* (MFLUCC 16–0997) clusters with other *D. ravennica* isolates from *Tamarix* sp. and *Salvia* sp. (Thambugala et al. 2017; Dissanayake et al. 2017). The description of *D. ravennica* by Thambugala et al. (2017) stated that the strain produced beta conidia in natural substrates, but beta conidia were not observed in the strain associated with *Clematis vitalba* branches or in culture. A phylogenetic tree derived from an alignment of combined ITS, *tef1*, *cal* and *tub2* sequence data showed that the *D. ravennica* clade received 65% support from ML and 0.99 BYPP (Fig. 102). The GCPSR concept was applied to the concatenated gene set of the closely related taxa (*D. baccae*, *D. chamaeropsis*, *D. foeniculacea*, *D. ravennica*) are presented (Fig. 103). A phi-test of the four loci detected non-significant recombination between these isolates ( $\Phi_w = 0.15$ , Fig. 103a). Thus, the four isolates of *D. ravennica* including our collection are clearly demarcated and delimited from the other taxa (Fig. 104).

***Diaporthe rudis*** (Fr.) Nitschke, in Pyrenomycetes Germanici 2: 282 (1870), **new host record**

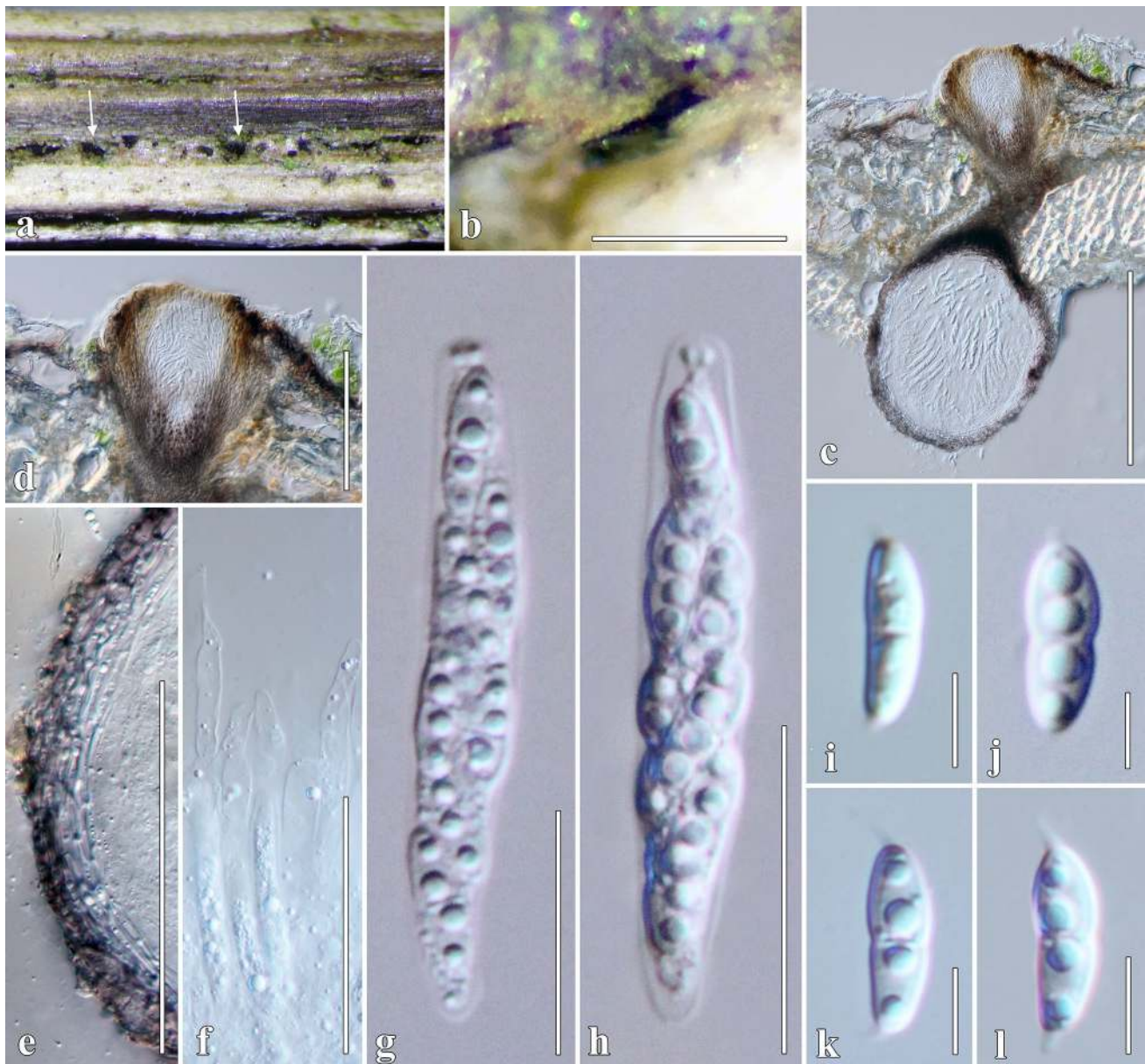
*Index Fungorum number*: IF552100; *Facesoffungi number*: FoF02171, Fig. 105.

*Saprobic* on *Clematis serratifolia*. **Sexual morph**: *Ascomata* 320–420 × 190–275  $\mu\text{m}$  ( $\bar{x} = 376 \times 246 \mu\text{m}$ ,  $n = 5$ ), perithecial, solitary to aggregated, immersed, only papilla visible on the host substrate, obpyriform to globose, coriaceous, dark brown to black, lighter pigment at apex, papillate. *Ostioles* 130–255 × 120–135  $\mu\text{m}$  ( $\bar{x} = 205 \times 125 \mu\text{m}$ ,  $n = 5$ ), papillate, central or eccentric, broad oblong, lined with periphyses. *Peridium* 18–30(–45  $\mu\text{m}$  at apex) wide, composed of 2–3 layers of thin-walled cells of *textura angularis*, brown to reddish brown, thin at inner layer, hyaline. *Paraphyses* sparse, 5–8  $\mu\text{m}$  ( $\bar{x} = 6 \mu\text{m}$ ,  $n = 30$ ), septate, constricted at septa, broad filiform, tapering above asci. *Asci* 50–72 × 9–13  $\mu\text{m}$  ( $\bar{x} = 60 \times 10 \mu\text{m}$ ,  $n = 20$ ), 8-spored, unitunicate, oblong to broad oblong, thin-walled, short or apedicellate, with a refractive, J-, apical ring. *Ascospores* 7.5–13 × 2–5  $\mu\text{m}$  ( $\bar{x} = 7 \times 3 \mu\text{m}$ ,  $n = 40$ ), partially overlapping, biseriolate, oblong-ellipsoidal, hyaline, uniseptate, with two guttules in each cell, with approximately 2.5  $\mu\text{m}$  long polar appendages. **Asexual morph**: See Udayanga et al. (2014).

*Culture characters*: Colonies growing on PDA reaching 20 mm within 4 weeks at 25 °C. Culture from above, white, radiating outwardly to the edge, circular, dense, flat or umbonate; reverse cream, white, radiating outwardly.

*Material examined*: Belgium, Flemish Brabant; Meise Botanic Garden, Bouchout domain, on dead branch of

◀ **Fig. 104** *Diaporthe ravennica* (MFLU 20–0422). **a, b** Appearance of conidiomata on *Clematis vitalba*. **c** Close up of conidioma on host substrate. **d** Vertical section through conidioma. **e** Ostiolar canal. **f** Peridium. **g–j** Conidiogenous cells and developing states of conidia. **k–o** Alpha conidia. **p, q** Culture characteristics on PDA. Scale bars: **b** = 500  $\mu\text{m}$ , **c, d** = 200  $\mu\text{m}$ , **e, f** = 50  $\mu\text{m}$ , **g–o** = 10  $\mu\text{m}$



**Fig. 105** *Diaporthe rudis* (MFLU 17–1510). **a** Appearance of ascomata on *Clematis serratifolia*. **b** Close up of ascoma on host substrate. **c** Vertical section through ascoma. **d** Ostiolar canal. **e** Perid-

ium. **f** Paraphyses. **g–h** Asci. **i–l** Ascospores. Scale bars: **b** = 500  $\mu$ m, **c** = 200  $\mu$ m, **d**, **e** = 100  $\mu$ m, **f** = 50  $\mu$ m, **g**, **h** = 20  $\mu$ m, **i–l** = 5  $\mu$ m

*Clematis serratifolia*, 13 June 2017, D. Ertz & C. Gerstmanns, BRCS1 (MFLU 17–1510); living culture, MFLUCC 17–2153.

**Hosts:** *Acer* sp., *Anthoxanthum odoratum*, *Asphodelus albus*, *Aucuba japonica*, *Brugmansia*, *Carlina vulgaris*, *Caragana arborescens*, *Castanea* sp., *Clematis serratifolia*, *Cornus* sp., *Corylus* sp., *Dioscorea communis*, *Dipsacus ful-lonum*, *Epilobium* sp., *Eucalyptus* sp., *Fagus* sp., *Fraxinus* sp., *Genista* sp., *Holcus* sp., *Hydrangea* sp., *Ileostylis* sp., *Laburnum* sp., *Lupinus*, *Malus* sp., *Protea* sp., *Pyrus* sp., *Rosa* sp., *Sambucus* cf. *racemosa*, *Salix* sp., *Vaccinium* sp.,

*Vitis vinifera*—(Dissanayake et al. 2017; Guarnaccia and Crous 2017; Rossman et al. 2017; Guterres et al. 2019; Farr and Rossman 2020; this study).

**Distribution:** Australia, Austria, Belgium, Canada, Chile, Czech Republic, France, Germany, Italy, Latvia, Japan, Poland, Portugal, Spain, Sweden, Switzerland, Netherlands, New Zealand, South Africa, UK, Ukraine, USA—(Dissanayake et al. 2017; Guarnaccia and Crous 2017; Rossman et al. 2017; Guterres et al. 2019; Farr and Rossman 2020; this study).

**GenBank accession numbers:** LSU: MT214615; SSU: MT226726; ITS: MT310659; *tef1*: MT394671; *rpb2*: MT394719.

**Notes:** *Diaporthe rudis* has been reported from a broad range of hosts such as cultivated crops, ornamental plants and is associated with grapevine trunk diseases worldwide (Lombard et al. 2014; Guarnaccia and Crous 2017). Phylogenetic analysis represented by 14 isolates of *D. rudis* from ten different hosts and 14 countries show species variation of host and geographic ranges (Dissanayake et al. 2017). The monophyletic clade has strong support with 66% MP and 100% ML support and 1.00 BYPP (Fig. 102). *Diaporthe rudis* was described as *Sphaeria rudis* on *Laburnum anagyroides* collected in France (Udayanga et al. 2014; Guarnaccia and Crous 2017). Our strain (Fig. 105), which was isolated from *Clematis serratifolia* in Belgium, differs from the holotype by having a thinner peridium (Udayanga et al. 2014). Due to the genetic complexity especially in the ITS region of *D. rudis*, the recombination parameters among selected taxa were calculated as shown in Figs. 103b. The GCPSR result of the concatenated gene set showed borderline insignificant recombination between the isolates ( $\Phi_w = 0.05$ , Fig. 103b). Thus, we confirm the status of *D. rudis* according to pairwise homoplasy index and the phylogenetic results, which highlights the high degree of genetic variation and distribution in a wide host range (Quaedvlieg et al. 2014; Guarnaccia and Crous 2017).

#### **Phomatosporales** Senan., Maharachch & K.D. Hyde

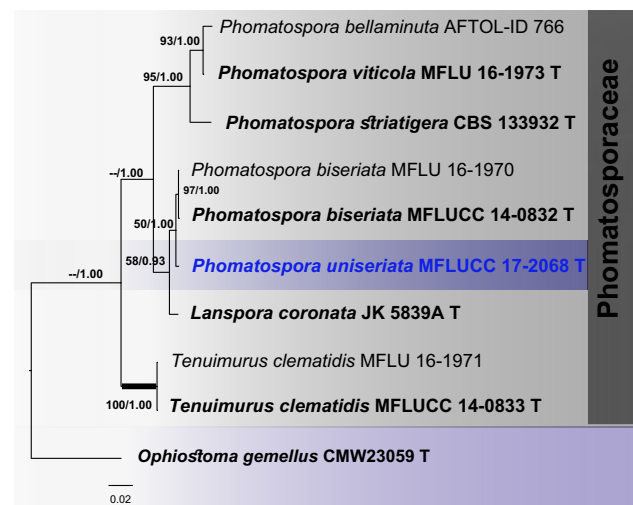
Senanayake et al. (2016) introduced *Phomatosporales* for fungal strains that formed a distinct lineage in Diaporthomycetidae. The order comprises one family and the phylogeny of Phomatosporaceae is shown in Fig. 106.

#### **Phomatosporaceae** Senan. & K.D. Hyde

Phomatosporaceae was formally reinstated by Senanayake et al. (2016) and comprises three genera, *Lanspora*, *Phomatospora* and *Tenuimurus* (Fig. 106). Phomatosporaceae is typified by *Phomatospora*, which was placed in Sordariomycetes genera *incertae sedis* by Lumbsch and Huhndorf (2007). We establish a novel species of *Phomatospora* from *Clematis* in Thailand based on molecular data.

#### **Phomatospora** Sacc.

*Phomatospora* can be an endophyte or saprobe in both aquatic and terrestrial habitats (Fallah and Shearer 1998; Senanayake et al. 2016; Guarnaccia and Crous 2017). *Phomatospora* is typified by *P. berkeleyi* Sacc. and is characterized by immersed, globose or subglobose ascomata, pseudoparenchymatous cells forming a *textura angularis* to *textura prismatica* peridium cell type, a paraphyses of hypha-like, filamentous structures, cylindrical or oblong-fusiform, and short pedicellate, unitunicate asci, with J-apical ring,



**Fig. 106** The Bayesian 50% majority-rule consensus phylogram based on combined LSU, SSU and ITS sequence data for Phomatosporaceae members. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted with *Ophiostoma gemellus* (CMW23059). Ten strains were included in the combined analyses which comprised of 2467 characters (883 characters for LSU, 1049 characters for SSU, 535 characters for ITS, including gap regions). The tree from the maximum likelihood analysis had similar topology to the Bayesian analyses. The best scoring RAxML tree had a final likelihood value of  $-5571.410827$ . The matrix had 292 distinct alignment patterns, with 31.28% of undetermined characters and gaps. Estimated base frequencies were as follows; A=0.253538, C=0.239531, G=0.293422, T=0.213509; substitution rates AC=3.147737, AG=3.686222, AT=2.035694, CG=1.607623, CT=10.739856, GT=1.000000; gamma distribution shape parameter  $\alpha=0.540032$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study is indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 70% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95) in genus level

ellipsoidal to fusiform, hyaline, aseptate to multi-septate ascospores, with a rounded bipolar gelatinous caps, and sporothrix-like asexual morphs reported from culture (Fallah and Shearer 1998; Fournier and Lechat 2010). Based on the phylogenetic results of a combined LSU, SSU and ITS dataset, the fungus collected on *Clematis* in Thailand clustered with other *Phomatospora* species (Fig. 106).

#### **Phomatospora uniseriata** Phukhams., M.V. de Bult & K.D. Hyde, **sp. nov.**

**Index Fungorum number:** IF557302; **Facesoffungi number:** FoF 06577, Fig. 107.

**Etymology:** Based on the uniseriate arrangement of the ascospores.

**Holotype:** MFLU 17-1476

*Saprobic* on *Clematis subumbellata*. **Sexual morph:** *Ascomata* 185–220 × 115–185 µm ( $\bar{x}$  = 195 × 175 µm, n = 5), solitary to aggregated, immersed and becoming erumpent with age, obpyriform to subglobose, coriaceous, brown to dark brown, heavily pigmented at apex, ostiolate. *Ostioles* 50–60 × 50–55 µm ( $\bar{x}$  = 57 × 53 µm, n = 5), papillate, central or eccentric, broadly conical, filled with periphyses. *Peridium* 8–12 µm wide ( $\bar{x}$  = 10 µm, n = 10), composed of 5–6 layers of thin-walled cells of *textura prismatica* mixed with *textura angularis*, brown to reddish brown, comprising inner, hyaline, thick-walled cells. *Paraphyses* sparse, 1.6–3.4 µm ( $\bar{x}$  = 2 µm, n = 30), hypha-like, thin-walled, septate, constricted at septa, tapering above and shorter than the asci. *Asci* 90–135 × 3–5 µm ( $\bar{x}$  = 103 × 4 µm, n = 20), 8-spored, unitunicate, cylindrical, thin-walled, pedicellate, with a refractive, J-, apical ring. *Ascospores* 5.5–9 × 2–4 µm ( $\bar{x}$  = 7 × 3 µm, n = 40), uniseriate, partially overlapping, oblong-ellipsoidal, hyaline, pale brown when mature, unicellular, bi-guttulate, guttules located at the ends of the cell, longitudinally striate. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 20 mm within 4 weeks at 25 °C. Culture from above, cream, radiating to the edge, wrinkled, folded, margin undulate, medium dense, flat or umbonate, and covered with sparse grey aerial mycelium; reverse white radiating outwardly.

**Material examined:** Thailand, Chiang Rai Province, on dead stems of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH12 (MFLU 17–1476, **holotype**); ex-type living culture, MFLUCC 17–2068.

**Host:** *Clematis subumbellata*—(This study).

**Distribution:** Thailand—(This study).

**GenBank accession numbers:** LSU: MT214616; SSU: MT226727; ITS: MT310660; *tef1*: MT394672; *rpb2*: MT394720.

**Notes:** *Phomatospora uniseriata* (MFLUCC 17–2068) formed a close relationship with *P. biseriata* which was also recorded on *Clematis vitalba* in Italy (Senanayake et al. 2016). *Phomatospora biseriata* is similar to *P. uniseriata* in having unitunicate, cylindrical, thin-walled, pedicellate asci, with J-apical ring, overlapping uniseriate as ellipsoid, hyaline, unicellular, bi-guttulate and longitudinally striate ascospores (Senanayake et al. 2016). *Phomatospora uniseriata* has uniseriate, oblong ascospores that become pale brown at senescence (Fig. 107). *Phomatospora uniseriata* is distinguished from *P. biseriata* by its smaller asci and ascospores (Table 5).

In the phylogenetic analysis, *P. uniseriata* (MFLUCC 17–2068) formed a close relationship with *P. biseriata* (50% ML/1.00 BYPP, Fig. 106). A comparison of the ITS region (including of the 5.8S region) showed 1.5% nucleotide (eight nucleotide difference from 535 base pairs). This justifies these two isolates as being two distinct taxa.

**Diaporthomycetidae**, family *incertae sedis*

**Distoseptisporaceae** Hyde & McKenzie

Distoseptisporaceae contains *Distoseptispora* Hyde, E. McKenzie & S. Maharachchikumbura as the generic type (Fig. 108). We follow the latest treatment and updated accounts of Distoseptisporaceae by Maharachchikumbura et al. (2016) and Wijayawardene et al. (2017).

***Distoseptispora*** Hyde & E. McKenzie & S. Maharachchikumbura

*Distoseptispora* is characterized by macronematous, septate, unbranched, short olivaceous to brown conidiophores, monoblastic, determinate, terminal conidiogenous cells and acrogenous, brown, euseptate or distoseptate conidia with a basal cell with cross walls and a basal scar (Yang et al. 2018b). There are 16 species listed in *Distoseptispora* (Hyde et al. 2016; Su et al. 2016; Tibpromma et al. 2017; Luo et al. 2018; Yang et al. 2018b). The combined gene phylogenetic analyses revealed a new species, *Distoseptispora clematidis*, which constitutes the first record of *Distoseptispora* on *Clematis* from Thailand (Fig. 108).

***Distoseptispora clematidis*** Phukhams., M.V. de Bult & K.D. Hyde, **sp. nov.**

**Index Fungorum number:** IF557301; **Facesoffungi number:** FoF 07261, Fig. 109.

**Etymology:** In reference to the host genus, *Clematis*.

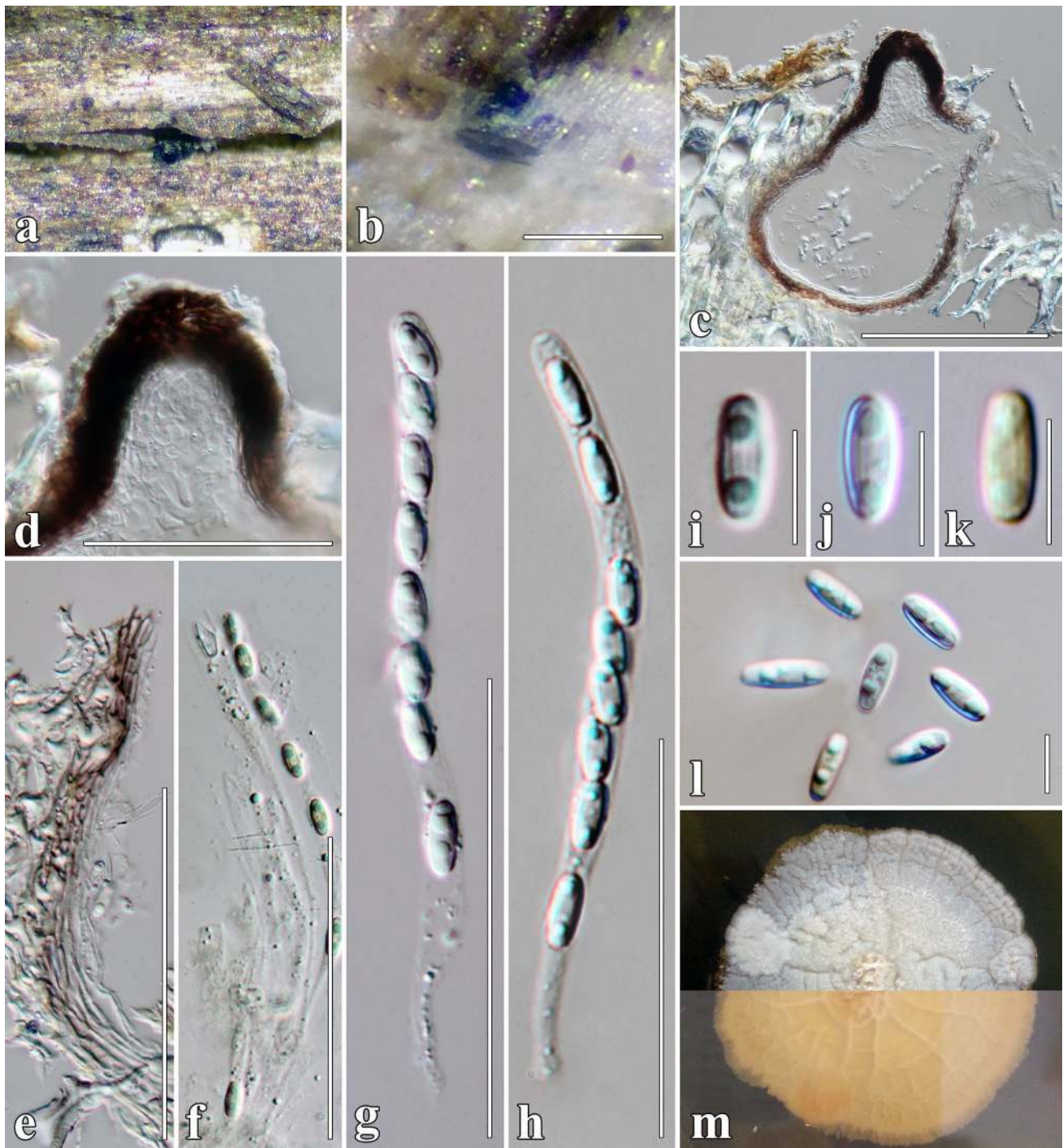
**Holotype:** MFLU 17–1501

*Saprobic* on dried branches of *Clematis sikkimensis*. **Colonies** on the substratum superficial, effuse, scattered, hairy or velvety, black. **Mycelium** immersed, composed of branched, septate, smooth, dark brown hyphae. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiophores* 22–40 × 4–10 µm ( $\bar{x}$  = 25 × 7 µm, n = 10), macronematous, mononematous, septate, unbranched, single or in groups of 2 or 3, erect, straight or flexuous, 3–5-septate, smooth, dark brown to brown, cylindrical, robust at the base. *Conidiogenous cells* 3–10 × 2–8 µm ( $\bar{x}$  = 6 × 5 µm, n = 20), monoblastic, integrated, determinate, terminal, cylindrical, light brown. *Conidia* 120–210 × 12–20 µm ( $\bar{x}$  = 175 × 17 µm, n = 20), acrogenous, solitary, dry, oblong, obclavate, cylindrical or rostrate, elongated, straight or curved, truncate at the base, rounded at the apex, 28–35-distoseptate, smooth, brown with green tinge, lighter towards the apex, thick-walled, bud scars or disjunctors present at the site of attachment.

**Culture characters:** Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Cultures from above, dark brown, medium dense, circular, umbonate, fluffy; reverse dark brown at the centre, black, radiating outwardly.

**Material examined:** Thailand, Chiang Rai Province, Doi Tung, on dried stem of *Clematis sikkimensis*, 2 May 2017, C. Phukhamsakda & M.V. de Bult, CMTHDT12 (MFLU





**Fig. 107** *Phomatospora uniseriata* (MFLU 17–1476, **holotype**). **a** Appearance of ascomata on *Clematis subumbellata*. **b** Close up of ascoma on host substrate. **c** Vertical section through ascoma. **d** Osti-

olar canal. **e** Peridium. **f** Paraphyses. **g, h** Asci **i–l** Ascospores. **m** Culture characteristics on MEA. Scale bars: **b** = 100  $\mu$ m, **c–h** = 50  $\mu$ m, **i–l** = 5  $\mu$ m

**Table 5** A synopsis comparison of *Phomatosporaceae* species discussed in this study

Species	Ascomata ( $\mu\text{m}$ )	Asci ( $\mu\text{m}$ )	Ascospores ( $\mu\text{m}$ )	Host	Location/reference
<i>Phomatospora biseriata</i> (MFLU 16–1970)	115–170 $\times$ 125–200, solitary, immersed, globose to subglobose, coriaceous	200–230 $\times$ 19–23, unitunicate, cylindrical to fusiform, J-apical ring	25–29 $\times$ 9–11.5, overlapping biseriata, ellipsoidal, hyaline, unicellular, longitudinally striate	<i>Clematis vitalba</i>	Italy (Senanayake et al. 2016)
<i>P. uniseriata</i> (MFLU 17–1476)	185–220 $\times$ 115–185, solitary, immersed, obpyriform to subglobose, coriaceous	90–135 $\times$ 3–4, unitunicate, cylindrical, J-apical ring	5.5–9 $\times$ 2–4, overlapping, unicellular, oblong-ellipsoidal, hyaline, pale brown at senescent states, longitudinally striate	<i>Clematis subumbellata</i>	Chiang Rai, Thailand (this study)
<i>Lanspora coronata</i> (AFTOL-ID 736)	160–320 $\times$ 200–360, solitary, globose to subglobose, coriaceous	20–46 $\times$ 7–11, unitunicate, cylindrical or oblong-ventricose, short pedicellate	10–15 $\times$ 2.5–4, uni or biseriata, ellipsoidal, 1-celled, hyaline, with longitudinal wall striations, crown-like appendage at both ends	Driftwood	Mare Anglaise beach, Seychelles (Hyde and Jones 1986)

**Fig. 108** Best scoring RAxML tree with a final likelihood value of  $-16025.131561$  based on combined LSU, SSU, ITS and *rpb2* sequence data for Distoseptisporaceae. The tree is rooted with *Crytodelphia* members. Thirty-seven strains were included in the combined analyses which comprised 3444 characters (905 characters for LSU, 1022 characters for SSU, 454 characters for ITS, 1063 characters for *rpb2*, including gap regions). The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree from the maximum likelihood analysis had similar topology to the Bayesian 50% majority-rule consensus phylogram. The matrix had 1081 distinct alignment patterns, with 40.90% undetermined characters and gaps. Estimated base frequencies were as follows; A=0.252755, C=0.239940, G=0.285179, T=0.222126; substitution rates AC=1.420436, AG=2.672673, AT=1.211428, CG=1.349930, CT=6.537630, GT=1.000000; gamma distribution shape parameter  $\alpha=0.699416$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study is indicated in blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95) at genus and family level

17–1501, **holotype**); ex-type living culture, MFLUCC 17–2145.

*Host*: *Clematis sikkimensis*—(This study).

*Distribution*: Thailand—(This study).

*GenBank accession numbers*: LSU: MT214617; SSU: MT226728; ITS: MT310661; *rpb2*: MT394721.

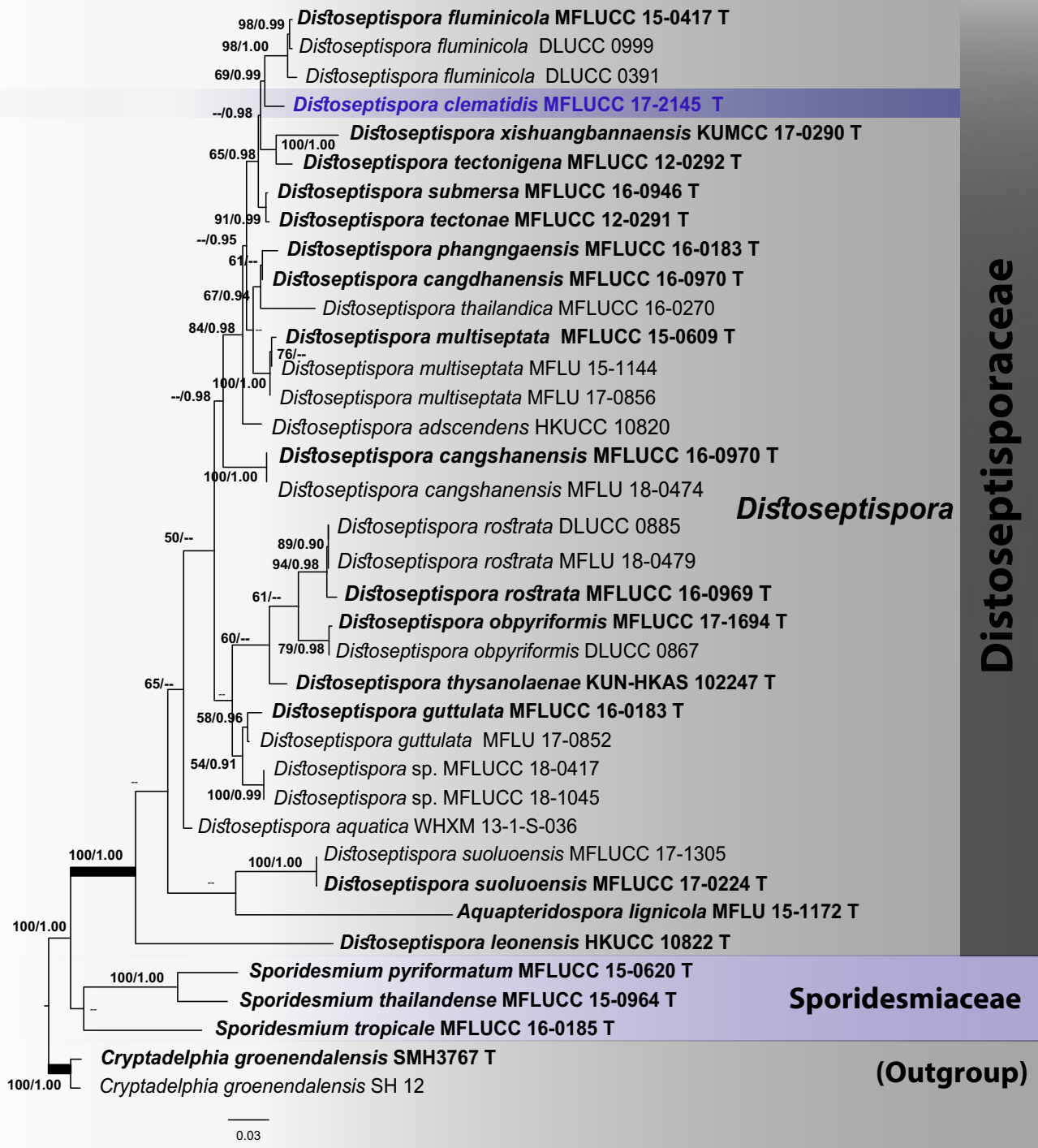
*Notes*: *Distoseptispora clematidis* (MFLUCC 17–2145) is introduced based on both morphological (Fig. 109) and phylogenetic evidence (Fig. 108). The morphology of *D. clematidis* is similar to *D. xishuangbannaensis*, but distinguishable by the characters of the conidia. *Distoseptispora clematidis* has smaller conidia (175  $\times$  17 vs 244  $\times$  11.5  $\mu\text{m}$ ) and up to 35 septa, while *D. xishuangbannaensis* has 40 septa (Tibpromma et al. 2017). In the phylogenetic analysis, *D. clematidis* forms a related lineage with *D. fluminicola*, *D. xishuangbannaensis* and *D. tectonigena* with moderate support (69% ML/0.99 BYPP). In a BLASTn search of GenBank, the closest match of the LSU sequence of MFLUCC 17–2145 was *D. tectonigena* (MFLUCC 12–0292) with 99.26% similarity to NG\_059144, while the closest match with the ITS sequence was 97.36% similarity to NR\_154018. This is the first record of *Distoseptispora* on *Clematis*.

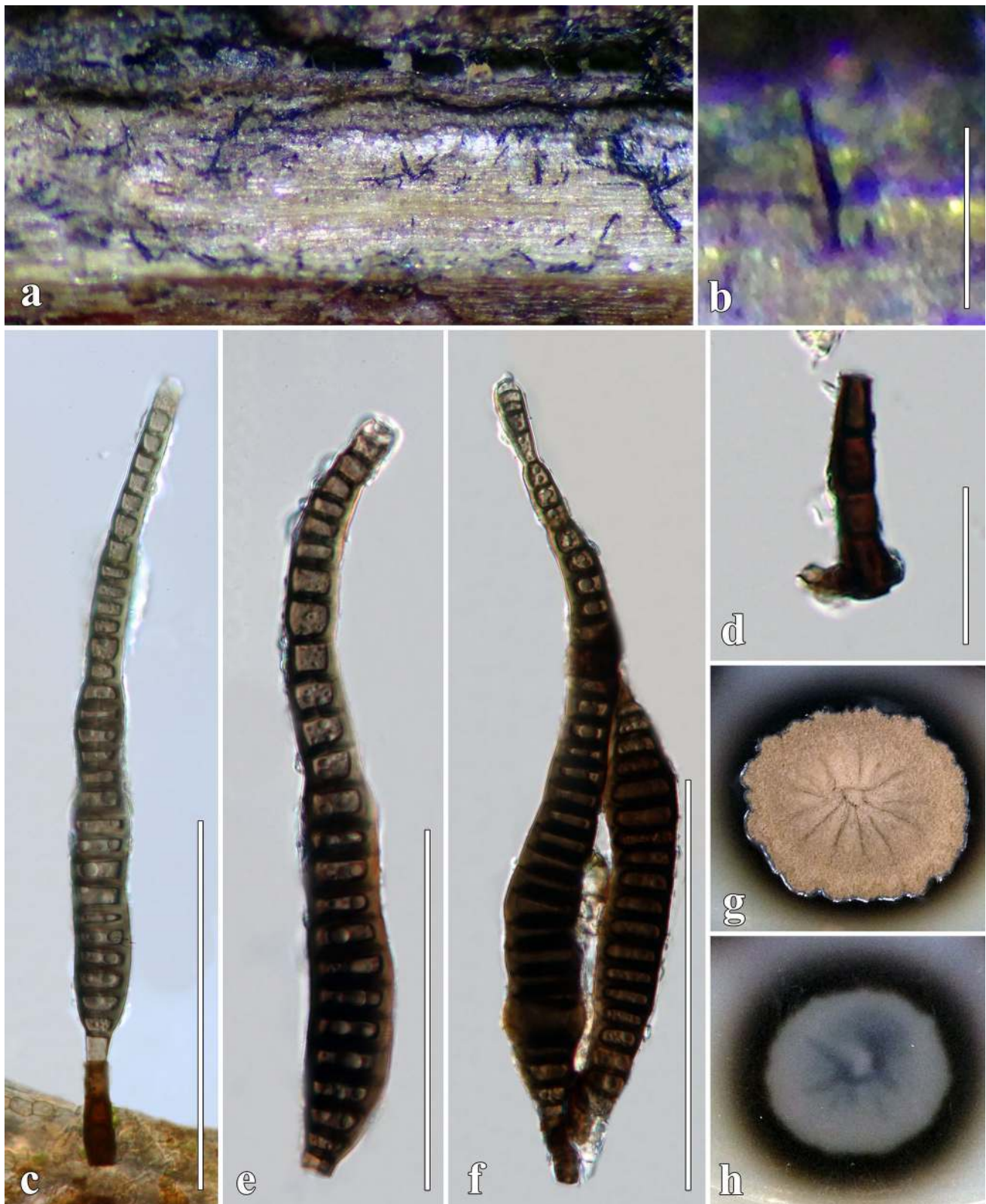
**Subclass: Xylariomycetidae** Erikss. & W. Winka

The latest treatment of subclass Xylariomycetidae is by Senanayake et al. (2015).

**Amphisphaeriales** Hawksw. & O.E. Erikss.

Several studies treated Amphisphaeriales as a synonym of Xylariales. Senanayake et al. (2015) revised the taxonomic relationship within Amphisphaeriales, and currently, 14 families are accepted by Hyde et al. (2020).





**Fig. 109** *Distoseptispora clematidis* (MFLU 17–1501, holotype). **a, b.** Colonies on the substratum. **c** Conidiophore and conidia. **d** Conidiophore. **e, f** Conidia. **g, h** Culture characteristics on MEA. Scale bars: **b** = 200  $\mu$ m, **c, e–f** = 100  $\mu$ m, **d** = 20  $\mu$ m

**Sporocadaceae** Corda [as “Sporocadeae”], *Icon. Fung. (Prague)* 5: 34. 1842

Sporocadaceae was resolved by Liu et al. (2019) and comprises 30 genera. Members of Sporocadaceae are saprobic or pathogenic on various plants (Senanayake et al. 2015). The family is recognised by having immersed ascomata and uniseriate, brown, ovoid to elliptic, straight or inequilateral, septate ascospores. The asexual morph characters of some Sporocadaceae are usually termed “pestaloid”, referring to acervular or pycnidial conidiomata, ellipsoid to clavate, or fusiform conidia that are hyaline, pale olivaceous or brown, septate, with polar appendages (Guba 1961; Maharachchikumbura et al. 2014; Senanayake et al. 2015; Liu et al. 2019).

#### ***Pestalotiopsis* Stewart**

*Pestalotiopsis* is characterized by acervular conidioma and fusoid to oval, 4-euseptate, pigmented conidia, usually two to five apical appendages arising as tubular extensions from the apical cell, with a centric basal appendage (Maharachchikumbura et al. 2014). *Pestalotiopsis* occurs as an endophyte or saprobe in various plants and often causes disease symptoms on leaves and fruit in many plants (Liu et al. 2006; Maharachchikumbura et al. 2012; Chen et al. 2018). *Pestalotiopsis verruculosa* was associated with *Clematis vitalba* branches in Italy. The multigene phylogenetic analysis of ITS and *tub* sequence data for *Pestalotiopsis* is shown in Fig. 110.

***Pestalotiopsis verruculosa* Maharachch. & K.D. Hyde**, in Maharachchikumbura et al. *Fungal Diversity* 56(1): 123 (2012), **new host record**

*Index Fungorum number*: 800527; *Facesoffungi number*: FoF 07336, Fig. 111.

*Saprobic* on dead stem of *Clematis vitalba*. *Conidiomata* pycnidial in culture on PDA, globose to oval, solitary or aggregated in clusters, semi-immersed, black, 120–320 µm diam., exuding globose, black, glistening, conidial masses. *Conidia* 21–33 × 6–15 µm ( $\bar{x}$  = 27 × 9 µm, n = 40), fusiform or clavate-fusiform, straight or slightly curved, 4-septate, concolorous; basal cell conical, hyaline, thin and verruculose, 2–5 µm long ( $\bar{x}$  = 3.4 µm, n = 15), three median cells doliiiform, olivaceous, concolorous, verruculose, 14–16 µm long, septa and periclinal walls darker than the rest of the cell (second cell from base 2–6 µm; third cell 5–9 µm; fourth cell 3.4–6.9 µm long); apical cell 1.6–5.4 µm long, hyaline, cylindrical to subcylindrical with 3–6 tubular apical appendages (mostly 3), arising from crown of the apical cell,

unbranched, filiform 11–34 µm ( $\bar{x}$  = 20 µm, n = 60) long; single, unbranched, filiform basal appendage present, 6–10 µm long ( $\bar{x}$  = 7.5 µm, n = 60).

*Culture characters*: Colonies on PDA reaching 30 mm diam. after 7 days at 25 °C. Culture from above, edge undulate, white to pale yellow, aerial mycelium with black fruiting bodies, concentric; reverse of culture, yellow to pale orange.

*Material examined*: Italy, Forlì-Cesena Province, Santa Sofia–near Corniolo, on dead and terrestrial stem of *Clematis vitalba*, 5 February 2013, E. Camporesi (MFLU 14–0624), living culture MFLUCC 14–1091.

*Host*: *Clematis vitalba*, *Rhododendron* sp.—(Maharachchikumbura et al. 2012; this study).

*Distribution*: China, Italy—(Maharachchikumbura et al. 2012; this study).

*GenBank accession numbers*: ITS: MT186199; *tub2*: MT199995; *tef1*: MT199996.

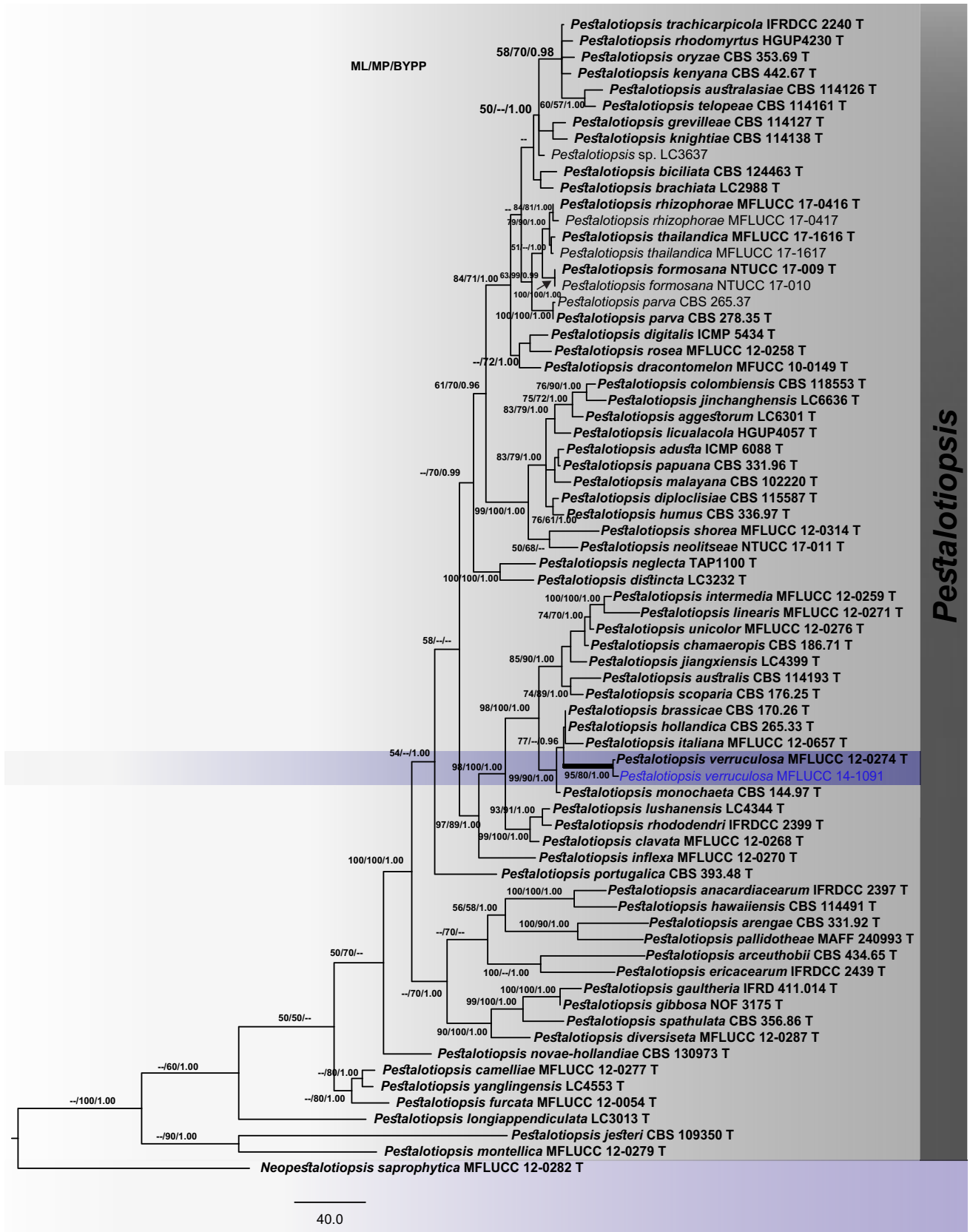
*Notes*: This strain of *Pestalotiopsis verruculosa* has somewhat smaller conidia (21–33 × 6–15 µm) when compared with the type strain of *P. verruculosa* (28–35 × 9–11 µm) and shorter apical appendages (11–34 vs 25–40 µm) (Maharachchikumbura et al. 2012). In the phylogenetic analyses, our strain clustered with the type strain of *P. verruculosa* (Fig. 111). *Pestalotiopsis verruculosa* has been recorded only from *Rhododendron* sp. in China as an endophyte (Maharachchikumbura et al. 2012). This study provides the first report of *P. verruculosa* from *Clematis vitalba*.

#### **Xylariales Nannf.**

The Xylariales is a large order in Sordariomycetes, with 20 accepted families. We follow the treatments by Maharachchikumbura et al. (2016), Li et al. (2017), and Hyde et al. (2020).

#### **Diatrypaceae Nitschke**

Species of Diatrypaceae are highly diverse and widespread on woody plants in terrestrial, aquatic or marine (Trouillas and Gubler 2010; Abdel-Wahab et al. 2014; Liu et al. 2015; Dayarathne et al. 2016; Senwana et al. 2017; Shang et al. 2017). Based on the latest treatment and updated accounts of Diatrypaceae, the family contains 20 genera (Maharachchikumbura et al. 2015, 2016; Shang et al. 2017; Phookamsak et al. 2019). Diatrypaceae is characterized by perithecial ascomata, with poorly or well-developed ascotromata, immersed to erumpent in the host substrates, with papillate ascomata (Fuckel 1870). Based on phylogenetic analysis of a combined ITS and *tub* dataset (Fig. 112), we



Pestalotiopsis

◀**Fig. 110** One of the 1000 most parsimonious trees obtained from a heuristic search of combined ITS and *tub* sequence data for *Pestalotiopsis*. The tree is rooted at *Neopestalotiopsis saprophytica* (MFLUCC 12-0282). Maximum parsimony and maximum likelihood support  $\geq 50\%$ , Bayesian posterior probabilities  $\geq 0.90$  (ML/MP/BYPP) are given at the nodes. The species obtained in this study is in blue. Ex-type (T) taxa from other studies are in bold black

report a new record of *N. baoshanensis* on *Clematis vitalba* from Europe.

***Neoeutypella*** Raza, Shang, Phookamsak & L. Cai

*Neoeutypella* was introduced as a monotypic genus for eutypella-like strains that clustered among *Diatrypella* species but are distantly related to *Eutypella* sensu stricto (Dayarathne et al. 2016). *Neoeutypella* is characterized by entostromatic, carbonaceous ascostromata, perithecial, black, immersed to semi-immersed, papilla filled with periphyses, thick peridium, 8-spored spindle-shaped, and long pedicellate. Asexual morph of *Neoeutypella* produce hyphae-like, branched, filiform, hyaline or pale brown conidia in culture conditions (Crous et al. 2016; Phookamsak et al. 2019). Phylograms generated from maximum likelihood and Bayesian probability analyses based on combined ITS and *tub* sequence data (Fig. 112) showed that our collection from *Clematis vitalba* clustered with the type species of *Neoeutypella*. Thus, the new host record of *N. baoshanensis* is reported (Fig. 113).

***Neoeutypella baoshanensis*** Raza, Shang, Phookamsak & L. Cai, **new host record**

*Synonym:* *Eutypella caricae* (De Not.) Berl.: [1] (1902)

*Index Fungorum number:* IF555372; *Facesoffungi number:* FoF 04928, Fig. 113.

*Saprobic* on dead stem of *Clematis vitalba*. **Sexual morph:** Stromata poorly developed, flask-shaped, multiloculate, with 8–12 locules forming groups in stromata, black, erumpent or semi-immersed in the surface layers of the host tissue. *Ascomata* partially or deeply immersed in wide-spread stroma, 550–620 × 350–600  $\mu\text{m}$  ( $\bar{x}$  = 585 × 500  $\mu\text{m}$ ,  $n$  = 10), globose, reddish brown to black, single or gregarious and sometimes confluent, ostiolate. *Ostioles* 110–140 × 90–125  $\mu\text{m}$  ( $\bar{x}$  = 130 × 110  $\mu\text{m}$ ,  $n$  = 10), oblong-conical, with papillate, immersed in a wide-spread entostroma which protrudes above the substrate surface, periphyses. *Peridium* 13–40 (–80  $\mu\text{m}$  at apex) wide, comprising an outer, brown, thick-walled layer of polygonal melanized cells, interspersed with cells of the substrate and several inner, hyaline, thick-walled cell layers of *textura angularis*. *Paraphyses* composed of numerous, 1.8–3.5  $\mu\text{m}$  wide, aseptate, paraphyses, narrowing and tapering towards the apex. *Asci* 75–120 × 7–12  $\mu\text{m}$  ( $\bar{x}$  = 100 × 8  $\mu\text{m}$ ,  $n$  = 20), multi-spored, unitunicate, with narrow, thin-walled pedicel, 40–70  $\mu\text{m}$  long, with cylindrical, swollen upper portion,

apex flat, with J-, cylindrical, conspicuous ring. *Ascospores* 10–15 × 2–4  $\mu\text{m}$  ( $\bar{x}$  = 13 × 3  $\mu\text{m}$ ,  $n$  = 50), hyaline or pale reddish brown to brown, allantoid, straight or slightly curved, unicellular, thin-walled with 2 small globules in each cell, smooth-walled. **Asexual morph:** Undetermined.

*Culture characters:* Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Culture from above, white, yellowish towards the edge, erumpent, spreading, surface folded, margins lobate, medium dense, circular, flat, dull, fimbriate, radially furrowed and slightly covered with white aerial mycelium; reverse yellowish with radiating cream mycelium.

*Material examined:* UK, Hampshire, Botley Wood, on dead stems of *Clematis vitalba*, 16 April 2016, E.B.G. Jones, GJ 279 (MFLU 17–1461); living culture, MFLUCC 16–1002.

*Hosts:* *Clematis vitalba*, *Ficus carica*, *Pinus armandii*—(Acero et al. 2004; Phookamsak et al. 2019; this study)

*Distribution:* China, France, UK—(Acero et al. 2004; Phookamsak et al. 2019; this study)

*GenBank accession numbers:* LSU: MT214618; SSU: MT226729; ITS: MT310662; *tef1*: MT394673.

*Notes:* Our new isolate MFLUCC 16–1002 clustered with the type species of *Neoeutypella baoshanensis* strain LC 12111 (Fig. 112) with moderate support (98% ML/0.95 BYPP). Isolate MFLUCC 16–1002 is similar to the type species reported by Phookamsak et al. (2019), Fig. 113. In the phylogenetic analysis, *Diatrype macowaniana* strain CBS 214.87 clustered among *Neoeutypella baoshanensis* strains. Although Acero et al. (2004) mentioned that *N. baoshanensis* (= *Eutypella caricae*) and *Diatrype macowaniana* have no sequence divergence; morphological comparison of *Diatrype macowaniana* and *N. baoshanensis* were distinct in having smaller asci (32–36 × 6 vs 77.5 × 8  $\mu\text{m}$ ) and hyaline ascospores (10.8 × 2.4 vs 5–7 × 1.5–2) (Saccardo 1882). Thus, we maintain the strain as *Diatrype macowaniana* until the paratype specimen is re-collected. *Diatrypella banksiae* CPC 29054 and CPC 29118 formed a strongly supported clade with *Neoeutypella*. However, *Diatrypella banksiae* is only present as an asexual morph, thus we maintain the strain under the current name (Crous et al. 2016).

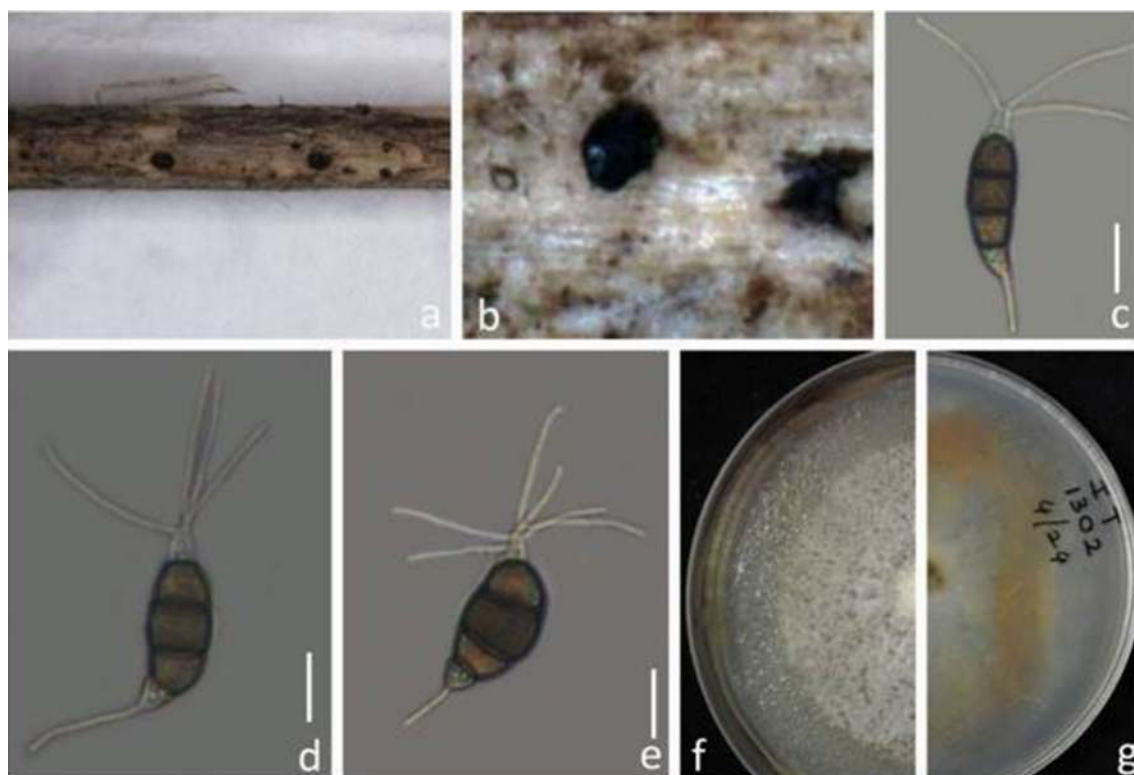
**Hypocreomycetidae** Erikss. & K. Winka

**Glomerellales** Chadeff. ex Réblová et al.

Glomerellales includes Australiascaceae, Glomerellaceae, Malaysiascaceae, Plectosphaerellaceae and Reticulascaceae (Réblová et al. 2011; Maharachchikumbura et al. 2016; Tibpromma et al. 2018).

**Plectosphaerellaceae** Gams, Summerb. & R. Zare

Plectosphaerellaceae was introduced by Zare et al. (2007) to accommodate a monophyletic lineage within



**Fig. 111** *Pestalotiopsis verruculosa* (MFLU 14-0624) **a, b** Appearance of conidiomata on *Clematis vitalba*. **c–e** Conidia with concolorous median cells. **f** Colony on PDA (upper view) **g** Colony on PDA (reverse view). Scale bars: **c–e** = 10  $\mu$ m

*Glomerellales*. *Plectosphaerellaceae* taxa are fungicolous, insecticolous, plant pathogens, saprobic, and cause soil-borne diseases (Cannon et al. 2012; Duc et al. 2009; Grum-Grzhimaylo et al. 2016; Giraldo et al. 2019). The family is mainly represented by asexual morphs with only a few sexual morphs described by Carlucci et al. (2012), Grum-Grzhimaylo et al. (2013, 2016) and Phookamsak et al. (2019). Phylogenetic relationships of the intra-members of *Plectosphaerellaceae* were analysed based on a LSU, ITS, *tef1* and *rpb2* dataset (Fig. 114). Combining morphological and molecular data, one new genus *Xenoplectosphaerella* and one new combination *Fuscohypha kunmingensis* are introduced below.

***Fuscohypha*** Giraldo López & P. Crous

*Fuscohypha* is typified by *F. expansa* Giraldo & Crous. The genus is characterized by branched conidiophores with ellipsoidal to cylindrical conidia, pale brown in mass, with slimy heads. *Fuscohypha expansa* was isolated from soil and tuber of *Dioscorea*. In our study, *Fuscohypha* formed a separate clade basal to *Plectosphaerella* and *Brunneochlamydosporium* (Fig. 114).

***Fuscohypha kunmingensis*** (Phookamsak, J.F. Li & K.D. Hyde) Jayaward., Phukhams., & K.D. Hyde, **comb. nov.**

*Index Fungorum number*: IF557607; *Facesoffungi number*: FoF 05716

*Basionym*: *Plectosphaerella kunmingensis* Phookamsak, J.F. Li & K.D. Hyde, in Phookamsak et al., *Fungal Diversity* 95:1–273(2019)

*Notes*: *Fuscohypha kunmingensis* was described as *Plectosphaerella kunmingensis* based on the morphological comparison and the strain formed a basal clade to *Plectosphaerella* species (Phookamsak et al. 2019). Phylogenetic inference in this study places the ex-type strain of *Fuscohypha kunmingensis* (KMUCC 18–0181) in a close relationship with the type species of *Fuscohypha* (*F. expansa*) with good support (81% ML/100% MP/0.99 BYPP, Fig. 114). *Fuscohypha kunmingensis* was a contaminant in the laboratory. The strain is similar to *Fuscohypha* as they both lack hyphal coils and hyaline to yellowish brown conidiophores (Giraldo et al. 2019).

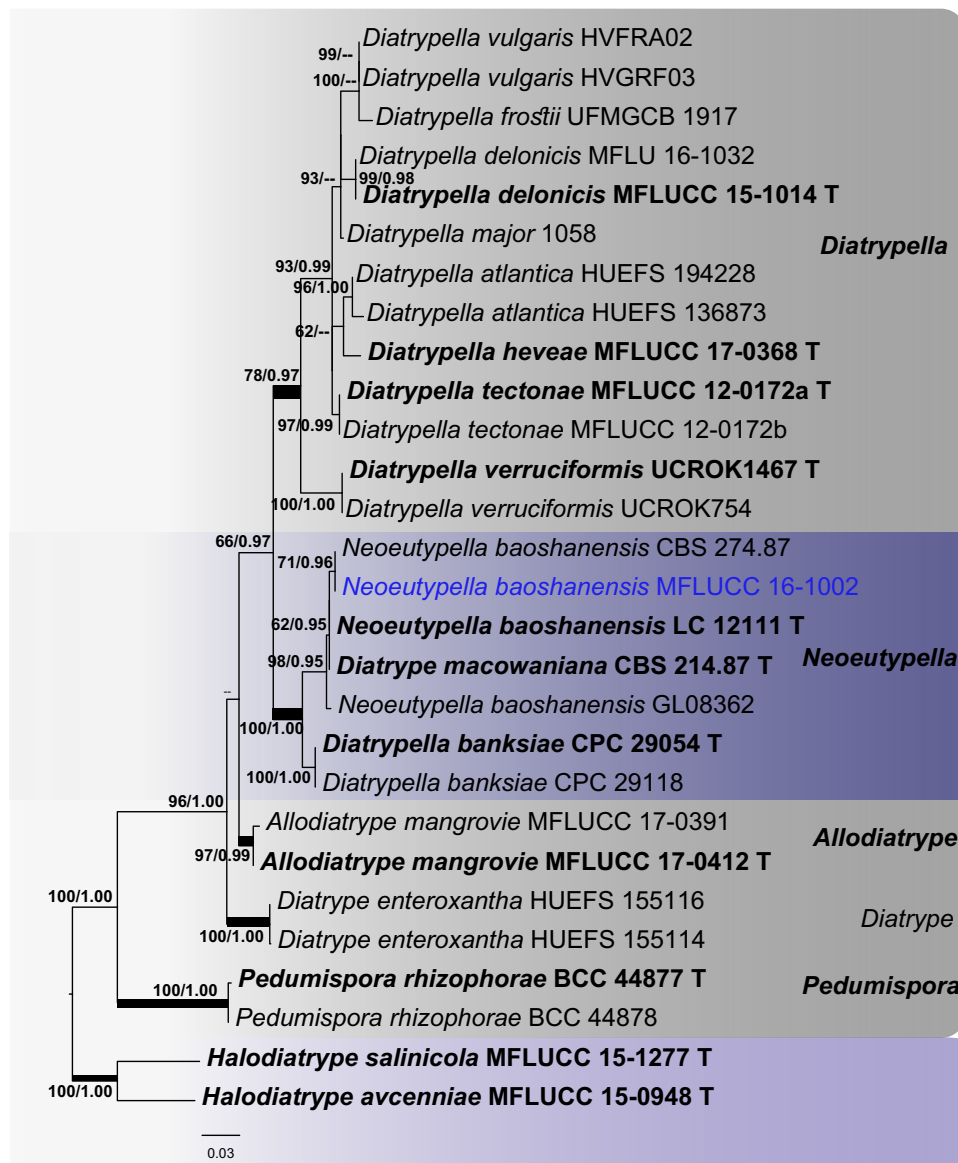
*Host*: Air-borne fungus—Phookamsak et al. (2019).

*Distribution*: China—Phookamsak et al. (2019).

***Xenoplectosphaerella*** Jayaward., Phukhams., & K.D. Hyde, **gen. nov.**

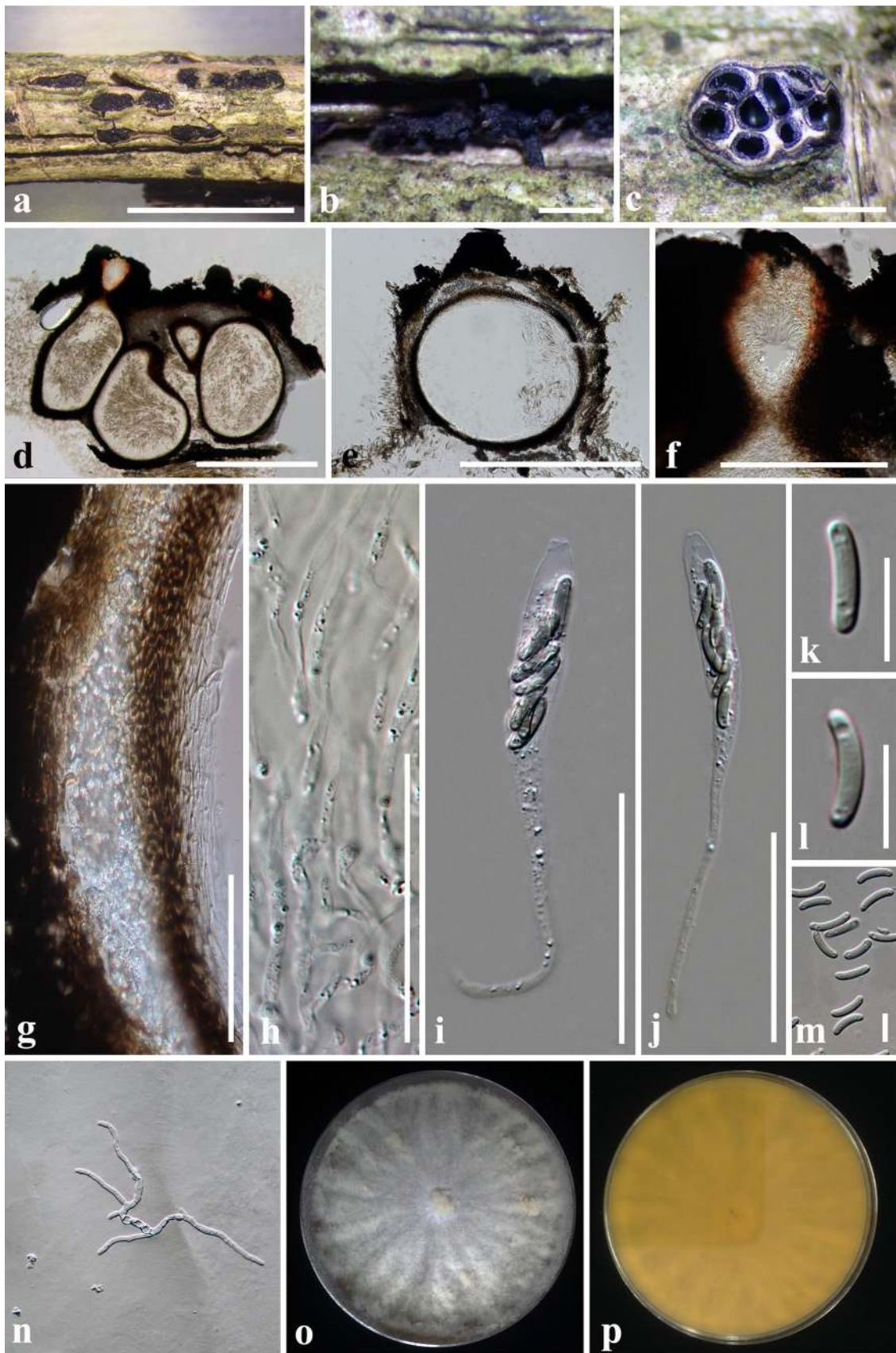
*Index Fungorum number*: IF557303; *Facesoffungi number*: FOF 01334, Fig. 115.





**Fig. 112** Best scoring RAxML tree with a final likelihood value of  $-4428.491036$  based on combined ITS and *tub* sequence data of related species. The tree is rooted with *Halodiatrype avcenniae* (MFLUCC 15-0948) and *H. salinicola* (MFLUCC 15-1277) in Diatrypaceae. Twenty-eight strains were included in the combined gene analyses which comprised 1393 characters (623 characters for ITS, 770 characters for *tub*, including gap regions). The tree from the maximum likelihood analysis had similar topology to the Bayesian analysis. The matrix had 396 distinct alignment patterns with 40.26% undetermined characters or gaps. Estimated base frequencies were as follows: A=0.224095, C=0.268057, G=0.236876, T=0.270973;

substitution rates AC=1.104749, AG=3.483519, AT=0.878988, CG=1.108511, CT=4.234084, GT=1.000000; gamma distribution shape parameter  $\alpha=0.276482$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study is indicated in bold blue. Bootstrap values (BS) greater than 50% BS (ML, left) and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses at genus level (BS  $\geq$  70%/BYPP  $\geq$  0.95)



**Fig. 113** *Neoeutypella baoshanensis* (MFLU 17–1461). **a** Appearance of stromata on *Clematis vitalba*. **b** Close up of stromata on host substrate. **c** Horizontal section through stroma. **d** Vertical section through stroma. **e** Vertical section through ascoma. **f** Ostiolar canal. **g** Peridium. **h** Aseptate paraphyses. **i, j** Asci. **k–m** Ascospores. **n** Germinated ascospore. **o, p** Culture characteristics on MEA. Scale bars: **a** = 1 mm, **b–e** = 500  $\mu\text{m}$ , **f** = 200  $\mu\text{m}$ , **g–j** = 50  $\mu\text{m}$ , **k–m** = 10  $\mu\text{m}$

**Etymology:** Name refers to the similarity of its morphology to *Plectosphaerella*.

**Saprobic** on herbaceous plant in terrestrial habitats. **Sexual morph:** *Ascomata* single or gregarious, erumpent or immersed under the host epidermal layer, visible as black spot on host substrate, papillate. *Papilla* filled with periphyses. *Peridium* composed of thin-walled cells of *textura angularis* mixed with *textura globosa*, multilayered, inner layers, hyaline. *Paraphyses* numerous, septate. *Asci* unitunicate, broad clavate to spatulate, with simple pedicel, conspicuous apical or subapical, J-, ring. *Ascospores* biseriolate, overlapping, fusiform-elliptical to ellipsoid, 1 septum, hyaline, constricted at the septum, straight or slightly curved towards the apex. **Asexual morph:** Undetermined.

**Type species:** *Xenoplectosphaerella clematidis* Jayaward., Phukhams., & K.D. Hyde

**Notes:** *Xenoplectosphaerella* is introduced as a monotypic genus in Plectosphaerellaceae (Fig. 115). The genus was associated with a herbaceous plant in Thailand and formed obpyriform, coriaceous ascomata with papilla, with paraphyses, and uniquely spatulate asci (Carlucci et al. 2012; Grum-Grzhimaylo et al. 2016; Giraldo et al. 2019). In the BLASTn search of GenBank, the closest match of the LSU region of the new species was *Musciellium theobromae* (CBS 968.72) with 97.22% similarity (accession number LR025907). The closest match of the ITS region of MFLUCC 17–2067 was also *M. theobromae* (CBS 968.72) with 90.83% similarity (accession number NR\_156205). However, *Musciellium* is phylogenetically distant from our new isolate (Fig. 114). Phylogenetic analyses of sequence data of taxa within Plectosphaerellaceae resolves *Xenoplectosphaerella* as basal to *Musciellium* and *Paramusciellium*, which are strains isolated from soil and monocotyledons or the mushroom genus *Lactarius* sp. (Giraldo et al. 2019). We name our collection as *Xenoplectosphaerella clematidis* MFLUCC 17–2067, a new genus which formed a distinct lineage from other genera in the family (Fig. 114).

*Xenoplectosphaerella clematidis* Jayaward., Phukhams., & K.D. Hyde, **sp. nov.**

**Index Fungorum number:** IF557304; **Facesoffungi number:** FoF 07326, Fig. 115.

**Etymology:** Name refers to the host plant, *Clematis*.

**Holotype:** MFLU 17–1475

**Saprobic** on dead stems of *Clematis subumbellata*. **Sexual morph:** *Ascomata* 90–100  $\times$  90–110  $\mu\text{m}$  ( $\bar{x}$  = 98  $\times$  105  $\mu\text{m}$ ,  $n$  = 5), single or gregarious, immersed under the host epidermal layer, visible as black spots on host substrate, obpyriform, coriaceous with minute papilla. *Ostioles* papillate, filled with periphyses. *Peridium* 7–12  $\mu\text{m}$  wide, composed of 7–10 layers of thin-walled cells of *textura angularis* mixed with *textura globosa*, inner layers, hyaline, thick-walled. *Paraphyses* numerous, 1.5–3.5  $\mu\text{m}$  ( $\bar{x}$  = 2.5  $\mu\text{m}$ ,  $n$  = 30), septate, narrowed and tapering towards the apex. *Asci* 31–50  $\times$  10–15  $\mu\text{m}$  ( $\bar{x}$  = 38  $\times$  12  $\mu\text{m}$ ,  $n$  = 20), unitunicate, broad clavate to broad fusiform, simple pedicel, apex flat, with J-, conspicuous apical or subapical ring. *Ascospores* 10–17  $\times$  4–5  $\mu\text{m}$  ( $\bar{x}$  = 13  $\times$  4  $\mu\text{m}$ ,  $n$  = 50), biseriolate, overlapping, fusiform-elliptical to ellipsoid, hyaline, 1 septum, constricted at the median septum, straight or slightly curved towards the apex, thin-walled, with two guttules in each cell, smooth-walled. **Asexual morph:** Undetermined.

**Culture characters:** Colonies on MEA reaching 20 mm diam. after 4 weeks at 25  $^{\circ}\text{C}$ . Culture from above, black, radiating, wrinkled, folded, margin undulate, dense, flat or umbonate, and slightly covered with grey aerial mycelium; reverse black with radiating, brown mycelium.

**Material examined:** Thailand, Chiang Rai Province, on dead stems of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH11 (MFLU 17–1475, **holotype**); ex-type living culture, MFLUCC 17–2067.

**Host:** *Clematis subumbellata*—(This study).

**Distribution:** Thailand—(This study).

**GenBank accession numbers:** LSU: MT214619; SSU: MT226730; ITS: MT310663; *tefl*: MT394674; *rpb2*: MT394722.

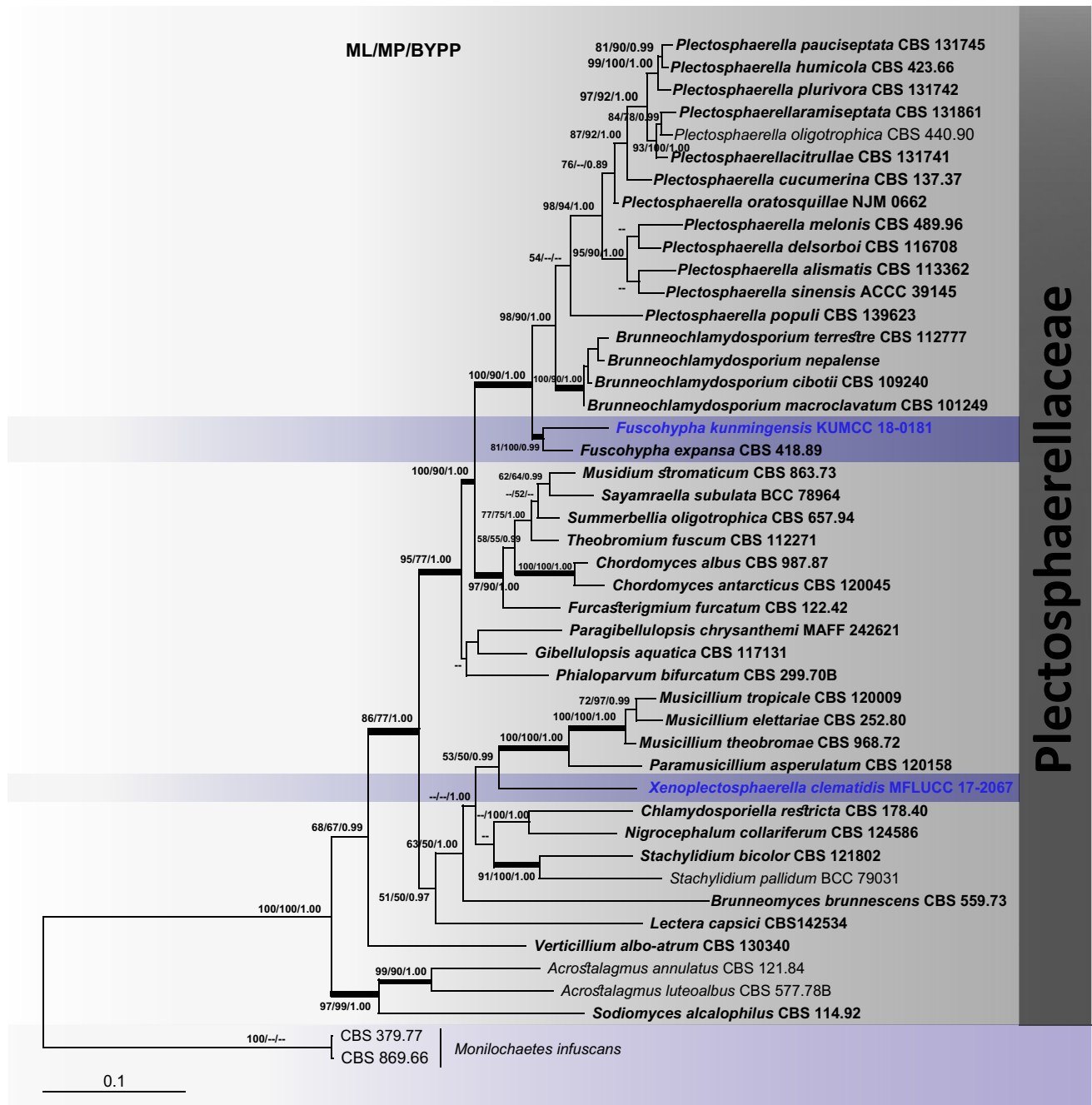
**Notes:** The novel species resembles other genera in Plectosphaerellaceae (e.g. *Fuscohypha* and *Plectosphaerella*) by its coriaceous ascomata and fusiform-elliptical to ellipsoid, 1 septum ascospores (Carlucci et al. 2012; Giraldo et al. 2019; Phookamsak et al. 2019). The species has unique characters such as paraphyses and spatulate, simple pedicel asci. Moreover, *Fuscohypha* and *Plectosphaerella* are phylogenetically not closely related (Fig. 114).

## Hypocreales Lindau

We followed recent treatment by Hyde et al. (2020b).

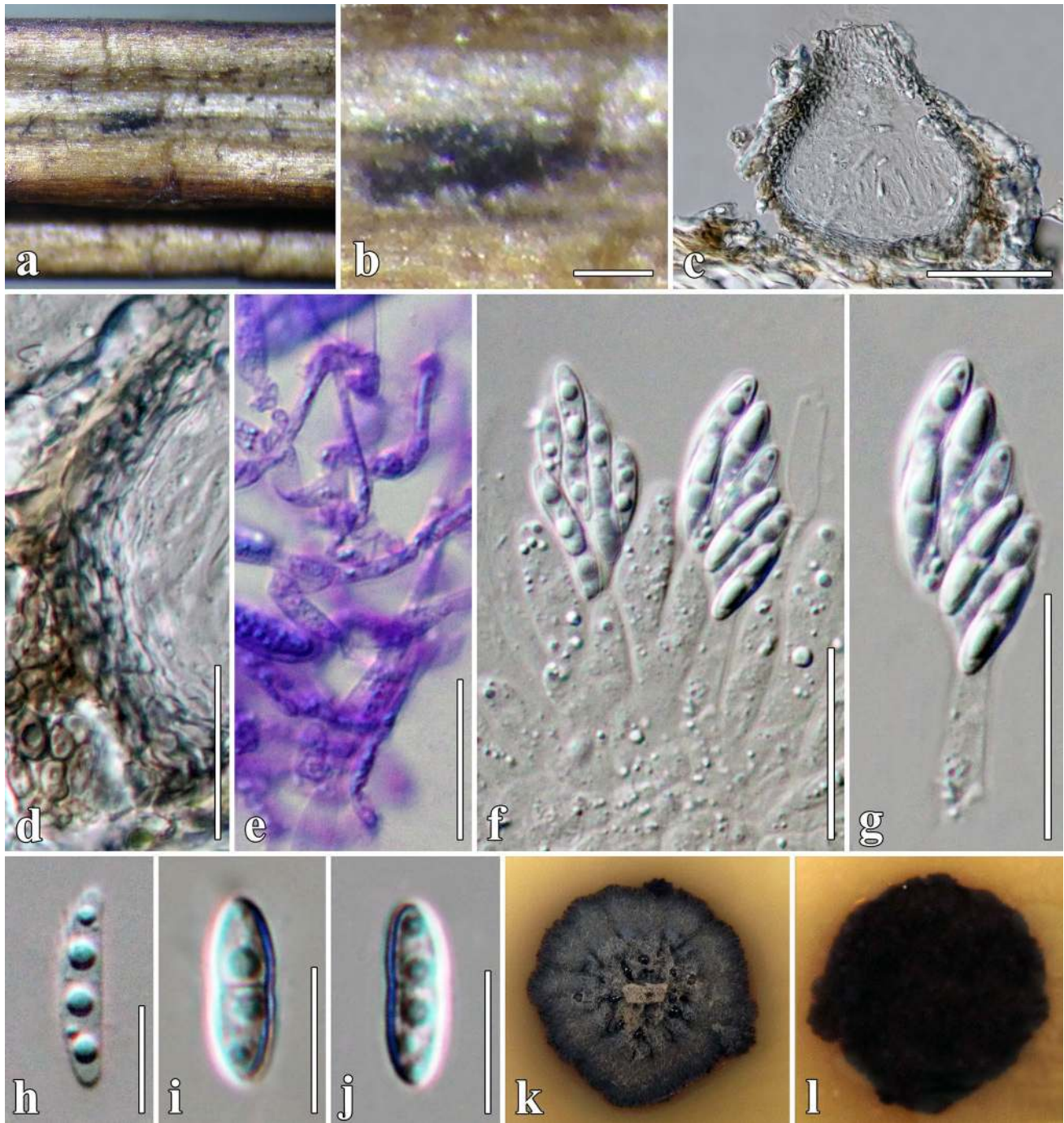
## Nectriaceae Tul. & C. Tul. [as ‘Nectriei’]

Nectriaceae is a species-rich family which includes important plant and human pathogens (Lombard et al. 2015). Members of the family are distinguishable by characters such as densely gregarious, uniloculate with yellow, orange-red to purple ascomata (Rossman et al. 1999; Lombard et al. 2015). Asexual morph are reported as synnematous, sporodochial or pycnidial with phialidic conidiogenesis (Rossman



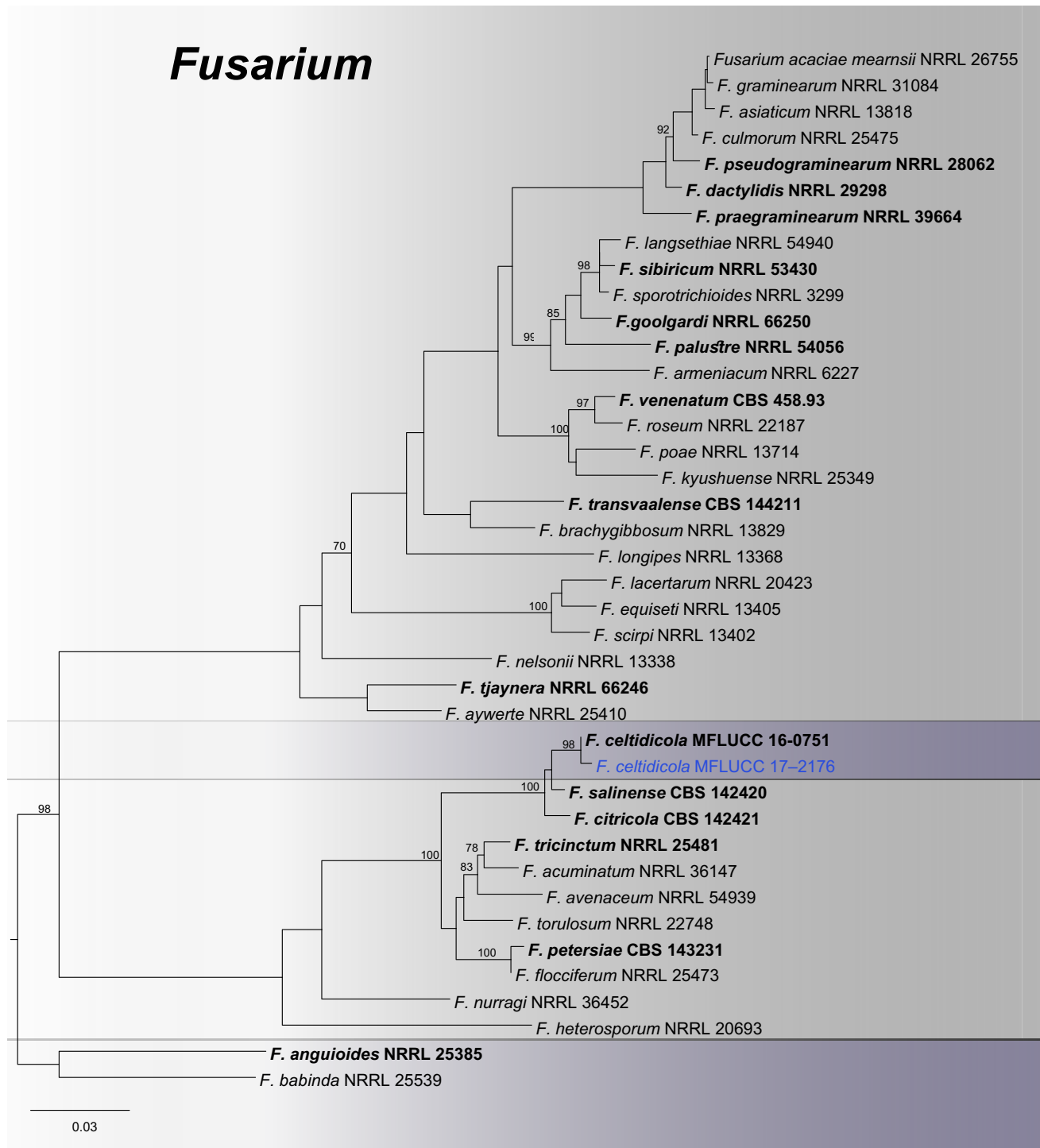
**Fig. 114** The best scoring RAxML tree with a final likelihood value of  $-23259.637010$  of ITS, LSU, *tef1* and *rpb2* sequence data for Plectosphaerellaceae. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted at *Monilochaetes infuscans* (CBS 379.77 and CBS 869.66). The matrix had 1093 distinct alignment patterns, with 13.22% of undetermined characters and gaps. Estimated base frequencies were as follows; A=0.224235, C=0.297727, G=0.281301, T=0.196738; substitution rates AC=0.820050, AG=1.988895,

AT=1.053617, CG=0.783458, CT=5.160854, GT=1.000000; gamma distribution shape parameter  $\alpha=0.2928059$ . The new species in this study are indicated in blue. Bootstrap values (BS) greater than 50% BS (maximum likelihood (left); maximum parsimony (middle)) and Bayesian posterior probabilities (BYPP) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS  $\geq$  70%/BYPP  $\geq$  0.95) at the genus level



**Fig. 115** *Xenoplectosphaerella clematidis* (MFLU 17–1475, **holotype**). **a** Appearance of ascomata on *Clematis subumbellata*. **b** Close up of ascoma on host substrate. **c** Vertical section through ascoma. **d**

Peridium. **e** Paraphyses (in cotton blue). **f, g** Asci. **h–j** Ascospores. **k, l** Culture characteristics on MEA. Scale bars: **b** = 100  $\mu$ m, **c** = 50  $\mu$ m, **d–g** = 20  $\mu$ m, **h–j** = 10  $\mu$ m



**Fig. 116** The best scoring RAxML tree with a final likelihood value of  $-52171.145500$  of *rpb1* and *rpb2* sequence data. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted with *Fusarium anguioides* (NRRL 25385) and *F. babinda* NRRL (25539). The matrix had 1722 distinct alignment patterns with 15.24% undetermined characters and gaps. Estimated base frequencies were as follows; A=0.260064,

C=0.246883, G=0.252626, T=0.240426; substitution rates AC=1.307580, AG=5.230737, AT=1.086622, CG=0.958821, CT=10.666584, GT=1.000000; gamma distribution shape parameter  $\alpha=0.761212$ . Ex-type strains are in bold black and the species determined in this study is indicated in blue. Bootstrap values (BT) (over 70% BT) from maximum likelihood are given at the nodes

et al. 1999; Lombard et al. 2015). The concept of the family was addressed by Lombard et al. (2015), Maharachchikumbura et al. (2016) and Hyde et al. (2020b) and include 69 genera (Wijayawardene et al. 2020).

#### **Fusarium** Link

*Fusarium* is a large genus in Nectriaceae, with more than 1500 epithets (Index Fungorum 2020). The genus was introduced with *Fusarium roseum* as the type species (Wijayawardene et al. 2018). Phylogenetic analysis based on *rpb1* and *rpb2* nucleotide sequences resulted in a well-supported phylogenetic clade of the newly isolated strain clustering with *F. celtidicola* (Hyde et al. 2014; Shang et al. 2018). Partial sequences of DNA-directed RNA polymerase II subunit RPB1 (*rpb1*) and DNA-directed RNA polymerase II subunit RPB2 (*rpb2*) gene regions were used for phylogenetic analysis (Fig. 116) and supported by morphological observations for species level characterization (Fig. 117).

***Fusarium celtidicola*** Shang, Camporesi & K.D. Hyde, Phytotaxa 361 (3): 255 (2018), **new host record**

*Index Fungorum number*: IF 553845; *Facesoffungi number*: FoF 02453, Fig. 117.

*Saprobic* on dead stem of *Clematis vitalba*. **Sexual morph**: *Ascomata* 210–255 × 180–280 μm ( $\bar{x}$  = 230 × 225 μm, n = 10), superficial on host substrate, single or gregarious, sometimes confluent, globose, black, ostiolate, papillate. *Papilla* 75 × 95 μm, conical, with periphysoids, with a small pore. *Peridium* 25–42 μm wide, comprising an outer purple layer, composed of 5–7 layers of thin-walled cells of *textura angularis*, inner layers, hyaline, thick-walled, several cell layers of *textura angularis*. *Catenophyses* numerous, 13–20 μm ( $\bar{x}$  = 17 μm, n = 30), septate, narrowing and tapering towards the apex. *Asci* 72–100 × 7–14 μm ( $\bar{x}$  = 70 × 10 μm, n = 20), unitunicate, simple pedicel, broad fusiform to oblong, apex flat, with J-, cylindrical, conspicuous apical ring. *Ascospores* 15–30 × 4–9 μm ( $\bar{x}$  = 17 × 7 μm, n = 50), biseriate, partial-overlapping, hyaline, oval, (1–)3-septate, constricted at the median septum, slightly constricted at the septa, straight or slightly curved towards the apex, thin-walled, with a minute guttule in each cell, smooth-walled. **Asexual morph**: See Shang et al. (2018).

*Culture characters*: Colonies on MEA reaching 30 mm diam. after 4 weeks at 25 °C. Culture from above, purple or white radiating outwardly to the edge, undulate margin, medium dense, flat, dull, fimbriate, and slightly covered with white aerial mycelium; reverse brown with radiating cream mycelium.

*Material examined*: Italy, Forlì-Cesena, Casone—Dovadola, on dead aerial stems of *Clematis vitalba*, 9 February 2013, E. Camporesi, IT1061 (MFLU 17–1537), living culture, MFLUCC 17–2176.

*Hosts*: *Celtis australis*, *Clematis vitalba*—(Shang et al. 2018; this study).

*Distribution*: Italy—(Shang et al. 2018; this study).

*GenBank accession numbers*: LSU: MT214620; SSU: MT226731; ITS: MT310664; *tefl1*: MT394675; *rpb2*: MT394723.

*Notes*: Based on the phylogenetic analysis our strain MFLUCC 17–2176 clustered with the ex-type strain of *Fusarium celtidicola* (MFLUCC 16–0526), which was recorded from Italy on *Celtis australis* (Shang et al. 2018). Our collection is morphologically identical to *F. celtidicola* (MFLUCC 16–0526) (Fig. 117).

#### **Sarocladiaceae** Lombard

Crous et al. (2018) introduced Sarocladiaceae to accommodate *Parasarocladium* and *Sarocladium*. Sarocladiaceae is characterized by its elongated phialides rising solitary on vegetative hyphae or on conidiophores that are sparsely or repeatedly branched, with elongated conidia (Giraldo et al. 2015). Phylogenetic analysis of the combined dataset of the LSU, ITS, and *act* sequences reveals a novel *Sarocladium* species (Fig. 118).

#### ***Sarocladium*** Gams & D. Hawksw.

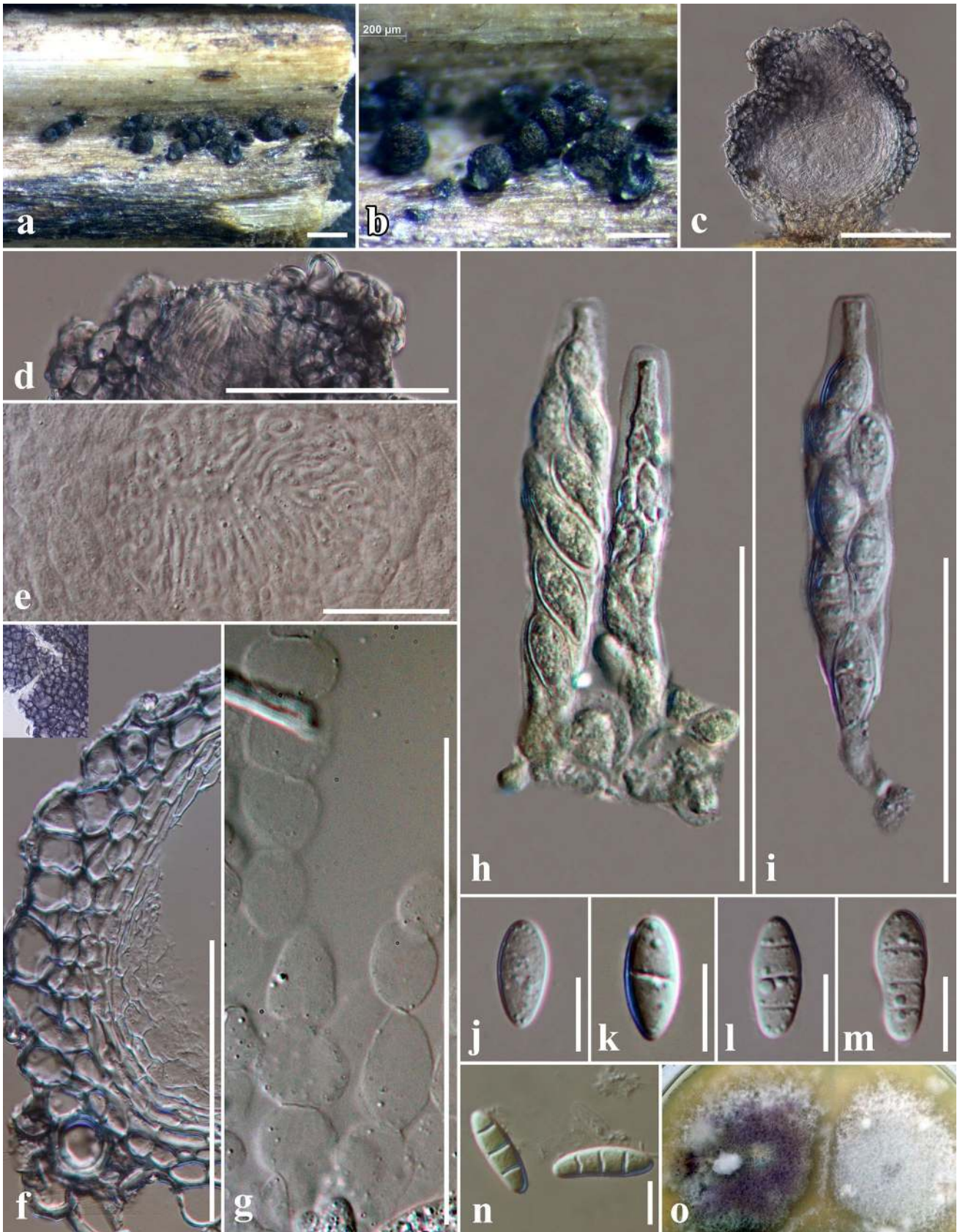
*Sarocladium* species have been reported as human pathogens, plant pathogens and saprobes (Gams 1975; Yeh and Kirschner 2014; Giraldo et al. 2015). The genus is typified by *S. oryzae*, a plant pathogen causing sheath-rot disease of rice (Ayyadurai et al. 2005; Seifert et al. 2011). Currently, 21 epithets are listed in Index Fungorum (2020). *Sarocladium* can be distinguished by its elongate phialides rising solitary on vegetative hyphae or on conidiophores that are sparsely or repeatedly branched, and cylindrical conidia. The characters of *Sarocladium* are mostly reported from culture, while our collection is associated with a dried stem of *Clematis* from Belgium (Fig. 119). The combined dataset of the LSU, ITS, and *act* sequences based on a multi-locus phylogenetic analysis revealed *Sarocladium clematidis* as a novel species (Figs. 118, 119).

#### ***Sarocladium clematidis*** Phukhams., Ertz, Gerstmanns & K.D. Hyde, **sp. nov.**

*Index Fungorum number*: IF556744; *Facesoffungi number*: FoF 06267, Fig. 119.

*Etymology*: Named after the host genus, *Clematis*.

*Holotype*: MFLU 17–1507





**Fig. 117** *Fusarium celtidicola* (MFLU 17–1461). **a** Appearance of ascomata on *Clematis vitalba*. **b** Close up of ascomata on host substrate. **c** Vertical section through an ascoma. **d, e** Ostiolar canal. **f** Peridium. **g** Catenophyses. **h, i** Asci. **j–n** Ascospores. **o** Culture characteristics on MEA. Scale bars: **a**=500  $\mu\text{m}$ , **b**=200  $\mu\text{m}$ , **c, f, g**=100  $\mu\text{m}$ , **d, h–i**=50  $\mu\text{m}$ , **e**=20  $\mu\text{m}$ , **j–n**=10  $\mu\text{m}$

*Saprobic* on dried stems of *Clematis patens*. **Sexual morph:** Undetermined. **Asexual morph:** Colonies effuse on the natural substrate, scattered, hairy, dark brown. *Mycelium* partly immersed, branched, composed of pale brown, septate hyphae. *Conidiophores* 85–320  $\times$  12–19  $\mu\text{m}$  ( $\bar{x}$ =150  $\times$  15  $\mu\text{m}$ ,  $n$ =20), macronematous, synnematos, composed of 7–12 hyphae in each stipe, tree-like, parallel and unbranched in the stipe, gregarious, or scattered, erect, stripes straight or slightly flexuous, 6–17-septate, constricted at septa, 15–30  $\mu\text{m}$  wide at base, tapering towards apex, 6–14  $\mu\text{m}$  wide at apex, irregularly branched, cylindrical, smooth, dark brown. *Conidiogenous cells* 7–15  $\times$  2–5  $\mu\text{m}$  ( $\bar{x}$ =10  $\times$  2.5  $\mu\text{m}$ ,  $n$ =20), polyphialidic, integrated or terminal, cylindrical, straight to slightly curved, slightly narrowing at apex, acropetally proliferating, hyaline to pale brown, verrucose. *Conidia* 4–9  $\times$  2–4  $\mu\text{m}$  ( $\bar{x}$ =6  $\times$  2.5  $\mu\text{m}$ ,  $n$ =40), unicellular, fusiform to broadly fusiform, aseptate, thick-walled, hyaline, verrucose, bud scars or disjunctors present at the site of attachment, 1–2-guttulate.

**Culture characters:** Colonies on PDA reaching 30 mm diam. after 4 weeks at 25 °C. Cultures from above, cream or white, medium dense, circular, umbonate, flat, at first glabrous becoming powdery at centre, wrinkled, folded, slimy in the middle, slightly radiating outwardly; reverse: cream at the centre, mycelium radiating outwardly. Asexual morph formed in culture with morphology similar to that on the natural substrates.

**Material examined:** Belgium, Flemish Brabant, Meise Botanic Garden, Bouchout Domain, on dead stems of *Clematis patens*, 13 June 2017, D. Ertz & C. Gerstmans, BRCP3 (MFLU 17–1507, **holotype**); ex-type living culture, MFLUCC 17–2150.

**Host:** *Clematis patens*—(This study).

**Distribution:** Belgium—(This study).

**GenBank accession numbers:** LSU: MN629285; SSU: MN629284; ITS: MN629287; *tef1*: MN628625; *rpb2*: MN628627.

**Notes:** *Sarocladium clematidis* formed synnemata with 7–12 hypha in each stipe on the natural substrate (Fig. 119). *Sarocladium clematidis* (MFLUCC 17–2150) is morphologically similar to *Phaeoisaria clematidis*, however they are phylogenetically distant (Yeh and Kirschner 2014). Morphological characters of *Sarocladium* have mainly been reported from culture (Seifert et al. 2011). *Sarocladium clematidis* has solitary, elongate phialides, with branched

conidiophores and fusiform conidia (Seifert et al. 2011). In phylogenetic analysis of combined sequence data (Fig. 118), *S. clematidis* formed a close relationship with *S. dejongiae* (CBS 144929) with strong support (100% ML/1.00 BYPP). In a BLASTn search of GenBank, the closest match of the ITS sequence of *S. clematidis* is *S. dejongiae* with 98% similarity to the *S. dejongiae* (511/519 with five gaps). Based on its distinct characters and the phylogenetic support, we introduce a novel species herein.

### Stachybotryaceae Lombard & P. Crous

Stachybotryaceae was introduced by Crous et al. (2014a) and comprises 36 genera (Wijayawardene et al. 2018). Species are saprobes, plant and animal pathogens, or airborne (Crous et al. 2014a; Lombard et al. 2016; Rennberger 2018). Stachybotryaceae members have sporodochial to synnematos conidiomata, phialidic conidiogenous cells, and 0–1-septate conidia (Lombard et al. 2016). In this study, phylogenetic analyses of the combined LSU, ITS, *tef1*, *rpb2* and *tub* dataset reveals a new record of *Memnoniella* from *Clematis* collected from Thailand (Fig. 120).

### Memnoniella Höhn.

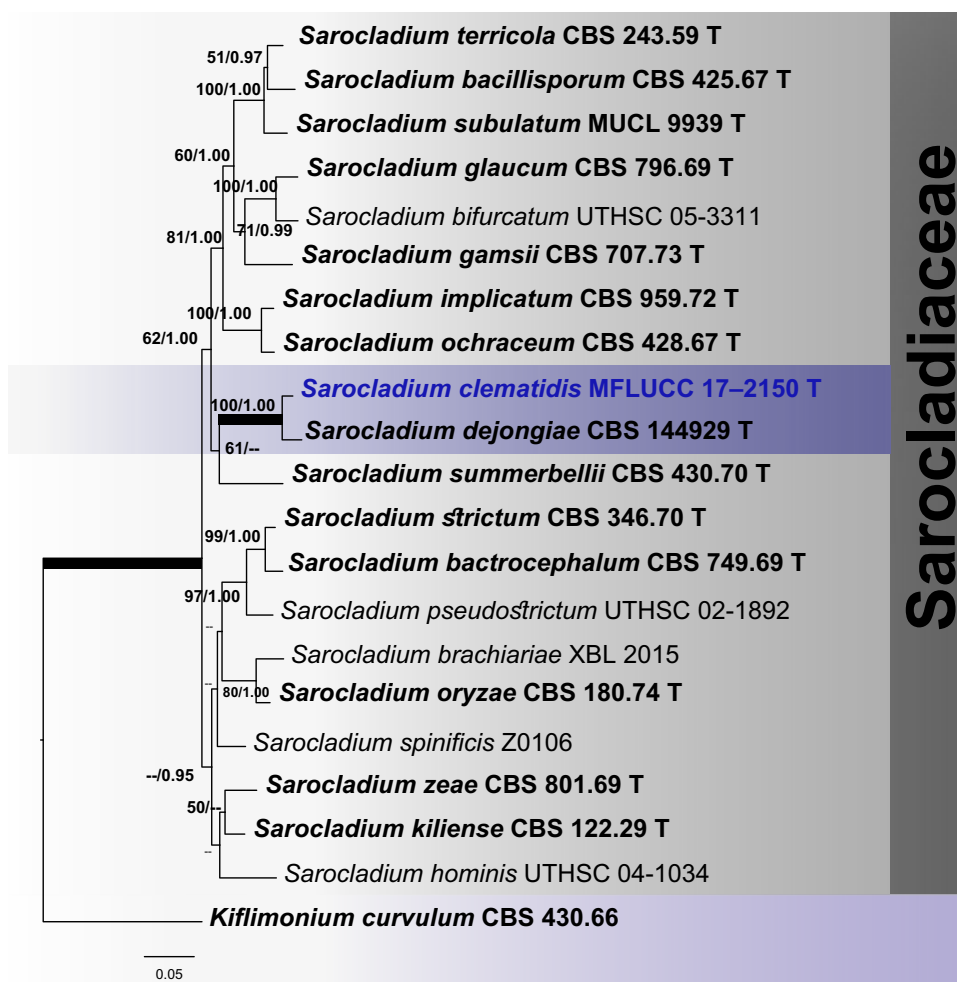
*Memnoniella* was synonymised under *Stachybotrys* (Galloway 1933). However, the distinctiveness of *Memnoniella* was confirmed by morphological characters and phylogenetic evidence (Lombard et al. 2016). *Memnoniella* is distinguished by mostly smooth, thick-walled and unbranched conidiophores giving rise to conidia in dry chains, whereas *Stachybotrys* has hyaline phialides, brightly coloured, or dark phialophores, producing 1-celled, dark conidia, accumulating in a slimy cluster (Wang et al. 2015). Some species of *Memnoniella* (e.g., *M. echinata* and *M. longistipitata*) produce dimorphic conidia (Li et al. 2003). We report a new collection of *M. oblongispora* from a *Clematis* species from Thailand which produces dimorphic conidia, a feature not known in this species (Fig. 121).

### Memnoniella oblongispora Lin, McKenzie, Wang & K.D. Hyde, new host record and dimorphic characters report

**Index Fungorum number:** IF 552085; **Facesoffungi number:** FoF 02081, Fig. 121.

*Saprobic* on dried stems of *Clematis subumbellata*. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. 1) *Colonies* of dry conidial chains, effuse on the natural substrate, scattered, hairy, dark brown. *Mycelium* semi-immersed to superficial, composed of pale brown, septate hyphae. *Conidiophores* 30–70(–120)  $\times$  3–6  $\mu\text{m}$  ( $\bar{x}$ =50  $\times$  5  $\mu\text{m}$ ,  $n$ =20), macronematous, mononematous, erect, simple, stipes straight or flexuous, mostly unbranched, but irregularly branched at the upper parts., sub-cylindrical to cylindrical, bearing a crown of phialides at apex, mammiform at the base cell, 3–4-septate, brown at the

**Fig. 118** Best scoring RAxML tree with a final likelihood value of  $-9149.858797$  of LSU, ITS, and *act* sequence data. The tree is rooted with *Kiflimonium curvulum* (CBS 430.66). The matrix had 613 distinct alignment patterns with 18.90% undetermined characters and gaps. Estimated base frequencies were as follows; A=0.223643, C=0.286812, G=0.271932, T=0.217614; substitution rates AC=1.580411, AG=2.743783, AT=2.611458, CG=0.755231, CT=8.336114, GT=1.000000; gamma distribution shape parameter  $\alpha=0.532334$ . Ex-type strains are in bold black and the species determined in this study is indicated in blue. Bootstrap values (BS) greater than 50% BS (maximum likelihood (left)) and Bayesian posterior probabilities (PP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses ( $BS \geq 70\%/BYPP \geq 0.95$ ) at the genus and family level



apex, hyaline at the base, smooth or verruculose. *Conidiogenous cells*  $5-10 \times 2.5-3.5 \mu\text{m}$  ( $\bar{x} = 8 \times 3 \mu\text{m}$ ,  $n = 10$ ), monophialidic, discrete, determinate, terminal, clustered at the apex of conidiophores, oblong, pale brown. *Conidia*  $3-5 \times 2.5-4.5 \mu\text{m}$  ( $\bar{x} = 4 \times 4 \mu\text{m}$ ,  $n = 20$ ), acrogenous, aseptate, globose, olivaceous, brown to dark brown, verrucose, thick-walled, dark brown to reddish brown, verrucose, formed in long chains, easily separated. 2) *Colonies* of slimy conidial chains produced in culture on MEA media. *Conidiophores*  $32-125 \times 4-7 \mu\text{m}$  ( $\bar{x} = 70 \times 4.5 \mu\text{m}$ ,  $n = 10$ ), macronematous, mononematous, erect, simple, straight or flexuous, unbranched, smooth, thick-walled, septate, sub-cylindrical, of elongated phialide base, apex bearing a crown of phialidic conidiogenous cells, hyaline at the base, olive grey at the apex. *Conidiogenous cells*  $8-17 \times 4.8-5.6 \mu\text{m}$  ( $\bar{x} = 10 \times 5 \mu\text{m}$ ,  $n = 20$ ), monophialidic, discrete, determinate, terminal, clustered at the apex of conidiophores, pyriform, obovate, ellipsoidal, clavate or reniform, smooth, brown. *Conidia*  $8-14 \times 4.5-7 \mu\text{m}$  ( $\bar{x} = 9 \times 5 \mu\text{m}$ ,  $n = 50$ ), aggregated in large, slimy, black and glistening heads, acrogenous, simple, spherical, oblong, immature hyaline, dark brown at

maturity, two guttules present when immature, unicellular, verrucose surface.

*Culture characters*: Colonies on MEA reaching 30 mm diam. after 2 weeks at 25 °C. Cultures from above, cream to yellowish white, medium to sparse, circular, fimbriate, wrinkled and folded, slightly radiating outwardly; reverse orange at the centre, cream radiating, dimorphic characters produced after 3 weeks of incubation, characters of dry conidial chains similar to those on natural substrates.

*Material examined*: Thailand, Phayao Province, Phu Sang District, dead stems of *Clematis subumbellata*, 20 March 2017, C. Phukhamsakda, CMTH06 (MFLU 17-1470); living culture, MFLUCC 17-2064.

*Hosts*: *Clematis subumbellata*, decaying *Quercus* leaf—(Lin et al. 2016; this study)

*Distribution*: Thailand—(Lin et al. 2016; this study)

*GenBank accession numbers*: LSU: MT214621; SSU: MT226732; ITS: MT310665; *tef1*: MT394676; *rpb2*: MT394724.

*Notes*: *Memnoniella oblongispora* (MFLUCC 17-2064) formed a clade with the type strain of *M. oblongispora*

(MFLUCC 15–1074) with moderate support (75% ML/0.92 BYPP). *Memmoniella* species may produce dimorphic asexual characters (Li et al. 2003). *Memmoniella oblongispora* was described as producing slimy conidial masses in a black and glistening head, with oblong, verrucose, olive green to black conidia (Lin et al. 2016). Our collection on a natural substrate, produced dry chains of spherical conidia. After three weeks in culture, glistening conidial masses were observed on the surfaces of white mycelia. In culture, both dry chains and stachybotrys-type slimy conidia were formed, which correlated with the morphology reported by Li et al. (2003).

The analysis (Fig. 120) showed that our collection formed a clade with the ex-type strain of *M. oblongispora* and a strain of *M. longistipitata* (CBS 136197). However, the characters of *M. longistipitata* (CBS 136197) have not been reported (Lombard et al. 2016). *Memmoniella oblongispora* (MFLUCC 17–2063) and the type strain of *M. longistipitata* (ATCC 22699) are distinguishable by conidiophores characters. *Memmoniella longistipitata* has very long conidiophores in culture ( $260\text{--}460 \times 3.6\text{--}4.7 \mu\text{m}$ ; Li et al. 2003), while *Memmoniella oblongispora* (MFLUCC 17–2064) has shorter conidiophores based on examination of their characters on natural substrates and in culture ( $32\text{--}125 \times 4\text{--}7 \mu\text{m}$ ; Lin et al. 2016; this study).

Pairwise comparison of the ITS sequence data in *Memmoniella oblongispora* (strains MFLUCC 17–2064 and MFLUCC 15–1074) are identical. Therefore, we report a new record of *M. oblongispora* from *Clematis* in Thailand, with dimorphic conidial (Fig. 121).

## Discussion

In this paper we provide data on collections of fungi associated with typically vigorous, woody, often invasive, climbing vines in Ranunculaceae, *Clematis*. The genus is distributed worldwide as a native plant and as a group that is commercially cultivated (Grey-Wilson 2000). In the present study, nine *Clematis* species were sampled from Asia (China, Thailand), Europe (Belgium, Italy, UK). These are *Clematis fulvicoma*, *C. orientalis*, *C. patens*, *C. serratifolia*, *C. sikkimensis*, *C. subumbellata*, *C. virginiana*, *C. vitalba*, *C. viticella*, additionally with *C. ligusticifolia*. Seventy-three taxa were added to the known fungi associated with *Clematis*. The classification of each species is justified by a consolidated species concept approach (Quaedvlieg et al. 2014).

### Applying a consolidated species concept to delineating taxa

The implementation of a comprehensive approach for confidently introducing new fungal taxa has been strongly

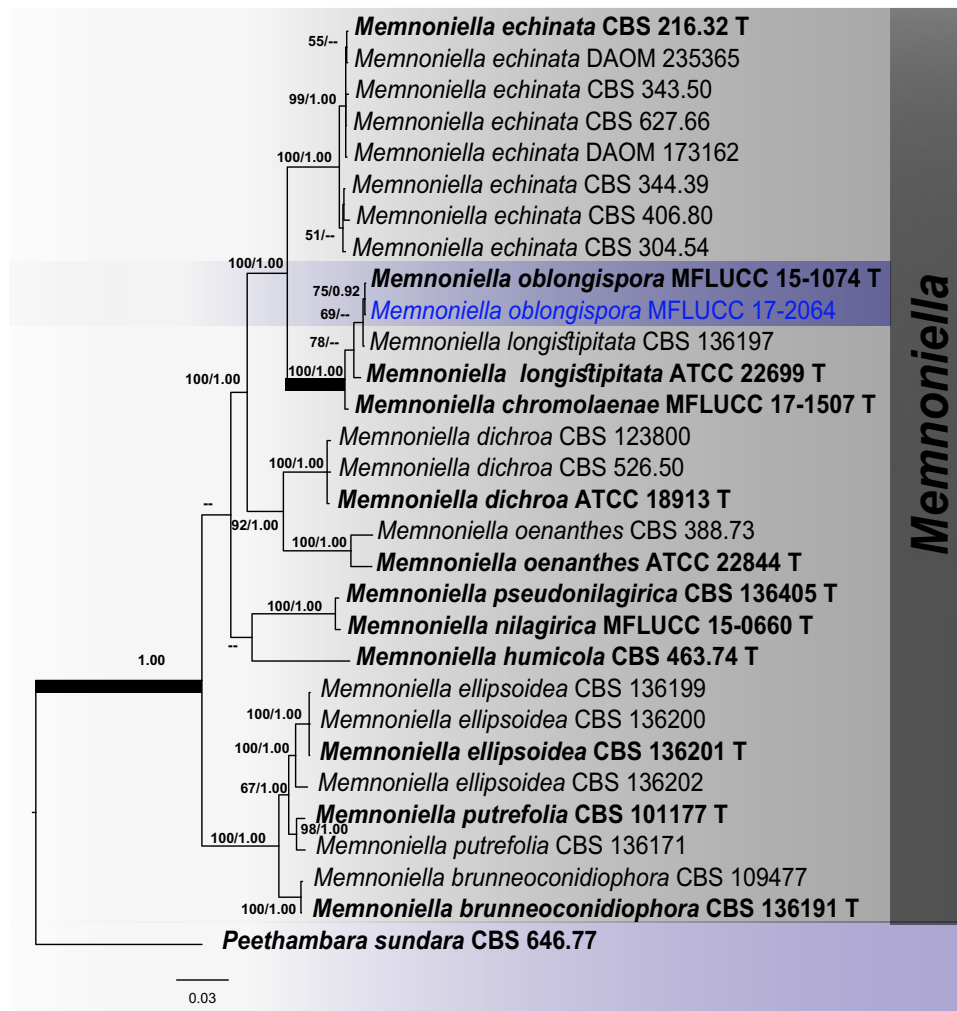
recommended in several studies. Quaedvlieg et al. (2014) introduced the “consolidated species concept” that distinguished fungal taxa based on the combination of the “biological species concept”, “ecological species concept”, “morphological species concept”, “phylogenetic species concept” and the application of “genealogical concordance phylogenetic species recognition” (Taylor et al. 2000). The approach was supplemented by performing a pairwise homoplasy index, followed by the interpretation of nucleotide difference based on its importance as highlighted by Jeewon and Hyde (2016). Phylogenetic species concept is currently regarded as an effective molecular tool to differentiate a common ancestor in the fungal community. Molecular-clock age estimates have been used as additional evidence to explore the evolution of lineages and divergence dates in fungal taxa (Hongsanan et al. 2017; Liu et al. 2017; Phukhamsakda et al. 2017b; Phillips et al. 2019; Samarakoon et al. 2019; Hyde et al. 2020b). In some complex species with high similarity in the phenotype and the evolution of these sequences cannot be described by a single tree and therefore the pairwise homoplasy index was applied to distinguish the genotype group and justify the species boundaries of phylogenetically closely related taxa (Dettman et al. 2003; Quaedvlieg et al. 2014). This method has been widely used to determine the possibility of inbreeding within the population and establish the genetic differentiation in lineages from phylogenetically closely related species (Laurence et al. 2014). Although the analysis was recommended for sexual organisms, recombination sciences have also applied this method for historical recombination (Burnett 2003; Turner et al. 2013). Diaporthaceae (Diaporthales), Hermatomycetaceae, and Lophiostomataceae (Pleosporales) are used as a case-study in the present work.

Diaporthaceae (Diaporthales): *Diaporthe* is a complex genus that presents high genetic variation within one species and can occur on a wide range of hosts. To introduce a novel species within *Diaporthe*, consolidated evidence is needed. We apply genealogical concordance phylogenetic species recognition to study the nucleotide distances of the common ancestors within a targeted clade (Fig. 100). The genealogical concordance phylogenetic species recognition is relatively reliable and can be useful in the identification of species in *Diaporthe*. *Diaporthe rudis* showed the genetic complexity especially in the ITS region and the recombination parameters among selected taxa are shown in Fig. 103b. The strains probably contain high intraspecific variation in the pseudogene of the rDNA copy (Stadler et al. 2020). The GCPSR result of the concatenated gene set shows insignificant recombination between the isolates ( $\Phi_w = 0.05$ , Fig. 103b). We demonstrate that some *D. rudis* isolates have undergone recombination of species based on the evolutionary dynamics (Quaedvlieg et al. 2014; Guarnaccia and Crous 2017).



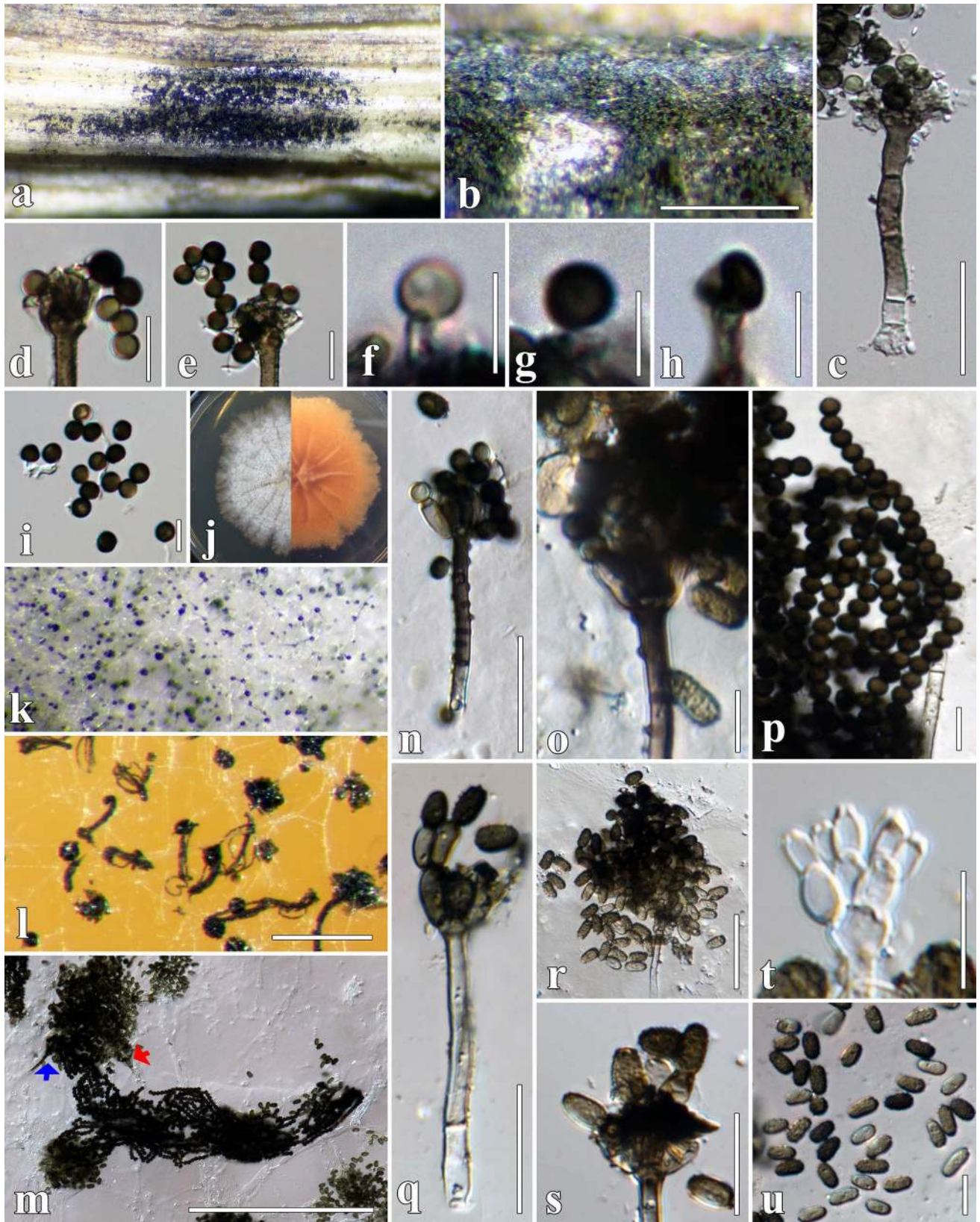
**Fig. 119** *Sarocladium clematidis* (MFLU 17–1507, holotype). **a** Appearance of synnemata on *Clematis patens*. **b, c** Close up of conidiophores. **d–g** Synnemata. **h, i** Conidiogenous cells and conidia. **j** Conidia. **k** Germinated conidia. **l, m** Culture characteristics on PDA. **n–r** Asexual morph produced in culture. Scale bars: **b**=500  $\mu$ m, **c**, **n**=50  $\mu$ m, **d**=200  $\mu$ m, **e–g**=100  $\mu$ m, **h–j, q, r**=5  $\mu$ m, **o**=20, **p**=10

Hermatomycetaceae (Pleosporales): This family lacks a sexual morph and the genealogical concordance phylogenetic species recognition result of *Hermatomyces sphaericus* (Fig. 21) contradicts the phylogeny (especially *H. biconisporus* and *H. pandanicola*). Phylogenies from maximum likelihood, maximum parsimony and Bayesian statistics did not give good support for the separate clade between *H. sphaericus* sensu stricto and *H. pandanicola*. This result is supported by the genealogical concordance phylogenetic species recognition value that does not significantly support



**Fig. 120** Bayesian 50% majority-rule consensus phylogram based on combined LSU, ITS, *tef1*, *rpb2* and *tub* sequence data for *Memnoniella* species. The topology and clade stability of the combined gene analyses was compared to the single gene analyses. The tree is rooted with *Peethambara sundara* strain CBS 646.77. Thirty strains were included in the combined genes sequence analyses which comprised 3057 characters (798 for LSU, 618 for ITS, 554 for *tef1*, 721 for *rpb2*, 366 for *tub*, including gap regions). The tree from the maximum likelihood analysis had similar topology to the Bayesian analyses. The best scoring RAxML tree had a final likelihood value of  $-11438.749144$ . The matrix had 794 distinct alignment patterns, with 19.09% of undetermined characters and gaps. Estimated

base frequencies were: A=0.236362, C=0.265886, G=0.272223, T=0.225529; substitution rates AC=1.092531, AG=3.397943, AT=1.269553, CG=0.955550, CT=9.625921, GT=1.000000; gamma distribution shape parameter  $\alpha=0.547123$ . In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. The species determined in this study is indicated in blue. Bootstrap values (BS) greater than 50% BS (maximum likelihood (left)) and Bayesian posterior probabilities (PP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS ≥ 70%/BYPP ≥ 0.95) in genus level



**Fig. 121** *Memmoniella oblongispora* (MFLU 17–1470). **a, b** Appearance of dry chains of conidiophores on *Clematis subumbellata*. **c** Conidiophores. **d–h** Conidiogenous cells and conidia. **i** Conidia. **j** Culture characteristics on MEA. **k** Slimy chains on MEA. **l–m** Dry chains and slimy chains of *M. oblongispora* (MFLUCC 17–2064) developed in the same culture on MEA media (red arrow indicates slimy chain; blue arrow indicates dry chains of conidia). **n–p** Dry chain of conidiophores, conidiogenous cells and conidia in culture. **q–u** Slimy chain with conidiophores, conidiogenous cells and conidia in culture. Scale bars: **b, l–m** = 200  $\mu$ m, **c, n, q–s** = 20  $\mu$ m, **d–e, o–p, t–u** = 10  $\mu$ m, **f–i** = 5  $\mu$ m

its speciation. *Hermatomyces sphaericus* occurs in countries that lie close to the equator, such as Cuba, Panama and Thailand where the climate is mostly hot with tropical conditions. These isolates were reported as saprobes on dicotyledonous and monocotyledonous hosts. The genus lacks a known sexual morph, thus the establishment of sexual compatibility in mating-type genes such as MAT-1 and MAT-2 genes are recommended for the determination of reproductive differentiation into a sexual morph of *Hermatomyces* (Giraud et al. 2008; Mageswari et al. 2016).

Lophiostomataceae (Pleosporales): To support the recognition of the morphological species concept and phylogenetic species concept with numerous taxa being discovered within Lophiostomataceae, GCPSR was applied to delimit species within Lophiostomataceae using data of five gene loci (Fig. 28). There are no conflicting results in this family and we provide consolidated evidence by phylogenetic relatedness in a multi-locus dataset and the interpretation of nucleotide differences (Turner et al. 2013; Quaedvlieg et al. 2014; Jeewon and Hyde 2016). We also compared our new isolates with documented fungal taxa recorded in the database and discuss the ecological species concept.

## Host–fungal interactions

It is hypothesized that modifications of the divergent plate boundaries 50 million years ago (Mya) resulting in speciation and adaptation of land plants and living organisms (Navaud et al. 2018). These caused dramatic change in continents, temperatures, peregrine, and communities of living organism and may have impacted the distribution of plant symbioses (Cawood et al. 2018). Most plants are inhabited by fungal symbionts as endophytes, mycorrhizae and saprobes. The arbuscular mycorrhizal fungi were reported to colonize the early diverging vascular plant lineages since 500 Mya (Field et al. 2012). Host–fungal interactions occurred as coevolution events with leading changes in genetic succession over generations (Naranjo-Ortiz and Gabaldón 2019). The cospeciation of the fungus with the original host consistently leads to a decrease in fitness on the original host and increased distribution of fungi species worldwide. The induction of foreign plants by global

weather changes immigration of animal and anthropogenic movement resulted in the introduction of fungal strains resident in plants to adapt and expand the range of hosts (Antonovics et al. 2002). Host jumps commonly occur in fungal species with a higher rate than the host–fungus cospeciation or coevolution (Roy 2001). This event most commonly occurs in plant pathogens that occasionally change to another lifestyle (Sillo et al. 2015). Among many examples of host–fungal interactions, we demonstrate some observed in this study.

Advances in the understanding of host–pathogen specificity and co-evolutionary interactions with specific plant genera or families has been demonstrated with Didymellaceae (Aveskamp et al. 2009, 2010; Chen et al. 2015, 2017). Cospeciation is expected to have congruent phylogenies in the host and fungal species with similar divergence times (De Vienne et al. 2013). Species of Didymellaceae associated with *Clematis* are *Calophoma clematidis-rectae* ( $\equiv$  *Phoma clematidis-rectae*), *C. clematidina* ( $\equiv$  *P. clematidina*), and *P. herbarum* (Foister 1961; Tai 1979; Woudenberg et al. 2009; Aveskamp et al. 2010). Soleimani et al. (2018) estimated the crown age of genera within Didymellaceae by using molecular dating. The molecular evolution revealed that the species of Didymellaceae diverged in the Miocene period (35.7 Mya). These results are in congruence with the relative radiation of the crown ages of *Clematis* species that had also a diversification event in the Miocene (Xie et al. 2011). However, the hypotheses of coevolution and host specificity in genera of Didymellaceae needs further addressing.

The introduction of a foreign host lends a significant accidental movement of fungi into new habitats and undergo host jumps (Herrera et al. 2016). The saprobic fungus *Nigrograna chromolaenae* was found on *Chromolaena odorata*, an invasive weed in Thailand, and was also associated with *Clematis fulvicoma* in this country (Mapook et al. 2020). Thus, the species is associated with dicotyledons plant in tropical climates. *Dictyocheirospora xishuangbannaensis* which was reported from Pandanaceae (monocots) from Yunnan Province in China was also observed from *Clematis sikkimensis* from Thailand. It can be hypothesized that *D. xishuangbannaensis* generally occur in tropical climates. *Stemphylium vesicarium* which causes leaf blight and leaf spots on several agricultural plants worldwide was associated with *Clematis vitalba* in Italy as a saprobe. Based on current trends, many fungal species in this study also have relatively broad host range with the consideration of co-factors such as environments and ecological species concept. An increasing number of fungi are likely to have an ability of switch host and then speciation might occur with this host plant.

Over 560 fungal species are listed from *Clematis* sp. worldwide (Farr and Rossman 2020). To assess the fungal biodiversity, each individual group, host, and

specific environmental factor could represent an alternative approaches to study fungal biodiversity (Mueller and Schmit 2007; Hyde et al. 2018b). Blackwell (2011) estimated the number of fungi to plants by more than 6 to 1. Therefore, there are many potential fungal habitats that need to be further studied and modern molecular methods could support the discovery of species (Hawksworth 1991, 2001).

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