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Mycosphaerellaceous fungi and new species of *Venustosynnema* and *Zasmidium* on ferns and fern allies in Taiwan

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Abstract

Selected fungi on ferns in Taiwan are described, illustrated, annotated and complemented with first DNA sequences. The ranges of distribution, host species, morphology, and the taxonomy of some species were revised. ITS rDNA sequences proved to be useful for distinguishing between species on the same host. A new species of *Venustosynnema* found on dead leaves of *Selaginella moellendorfii* differs from the other species of the genus by its small conidiomata with smooth central seta and reniform conidia. A new species of *Zasmidium* described on living fronds of *Dicranopteris linearis* is particular by its hyaline, smooth conidia. New data are derived from new collections of *Pseudocercospora athyrii*, *Ps. cyatheae*, and *Ps. pteridophytophila* hitherto only known from the type collections and of *Mycosphaerella gleicheniae*. *Ps. christellae* is considered a synonym of *Ps. pteridophytophila*. *M. gleicheniae* and *Ps. cyatheae* are new records for Taiwan.

Key words: Ascomycota, Capnodiales, *Cyclosorus*, *Deparia*, *Dicranopteris*, plant pathogens, *Sphaeropteris*

Introduction

Stevenson (1945) was probably the pioneer researcher who published an overview of fungi parasitic on ferns. The most recent overview of saprobic, parasitic and symbiotic fungi associated with ferns was published by Mehltreter (2010). The relationship between rust fungi and ferns was first to attract the attention of phylogenetic mycologists (see Sjamsuridzal *et al.* 1999 and references therein). Molecular analyses indicated that fern rusts were not basal to the rusts on seed plants (Sjamsuridzal *et al.* 1999). In contrast to the rust fungi, which are biotrophs on their host plants, evidence for parasitism by other fungi on ferns is often not clear or is only concluded from field observations. These studies mainly focused on the systematics of sexual and asexual Ascomycota including saprobic as well as presumably parasitic species (e.g. Bubák 1916, Cannon 1997, Castañeda Ruiz & Heredia 2000, Dingley 1972, Medel & Lorea-Herrnández 2008, Samuels & Rogerson 1990). The study of Ascomycota on ferns is still in the pioneer stage with biodiversity being recorded and the study of saprobic fungi on fern leaves is expected to result in new discoveries (Mehltreter 2010). Among the Ascomycota, mycosphaerellaceous fungi, including sexual species of *Mycosphaerella* and related genera, as well as their corresponding cercosporoid anamorphs, are particularly species-rich and in most cases are considered to be highly specific to host genera and families of vascular plants (Crous & Braun 2003).

Taiwan has one of the highest density of ferns and is, therefore, particularly suitable for studying the fungi on ferns. Given approximately 700 species of ferns known each for Taiwan, Japan, and Thailand, the species density is 0.019, 0.003, and 0.001 taxa/km^2, respectively (calculated from Lu & Yang 2005). Because of the high population and species density of ferns, fungi preferring ferns as substrate are also likely to be found frequently in Taiwan.

Approximately 30 species of *Pseudocercospora* are known from ferns (Braun *et al.* 2013), and six species were described from and are only known from Taiwan (Hsieh & Goh 1990, Kirschner & Chen 2007). A worldwide study on species of the cercosporoid genus *Periconiella* on ferns was published by Braun (2004); two of the species were later recorded from Taiwan (Kirschner & Chen 2007, 2010). Braun *et al.* (2013) compiled information concerning cercosporoid hyphomycetes on ferns worldwide based on the literature and herbaria. Cercosporoid fungi are considered comparatively host-specific and are identified to species mainly based on morphological

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comparison of congeneric species known from members of the same host genus or family. The conspectus of *Mycosphaerella* by Aptroot (2006) contains more than 30 taxa on ferns and fern-allies, although several species on different ferns were morphologically identical with *M. filicum* (Desm.) Starbäck (Aptroot 2006).

Classification of ferns, however, has been unsettled over the last decades including frequent changes to concepts of families and genera (Christenhusz *et al.* 2011, Frey *et al.* 2009, Kramer *et al.* 1990, followed in Knapp 2011, Rothfels *et al.* 2012, Smith *et al.* 2006). In contrast to fungi parasitic on specific seed plants, which can often be conveniently identified based on the knowledge of the host genera and families, this approach might be misleading for the fungi on ferns.

Several species of fern-inhabiting cercosporoid fungi are known only from the type collection and lack cultures and DNA data. It is, therefore, difficult to rely only on the original morphological descriptions for identification. In particular, conidiophore lengths can vary considerably between different specimens of the same species (Kirschner 2013). The aim of the study was to increase our knowledge of the fungal diversity of fungi on ferns from Taiwan by providing collections of species previously being unknown or only known from the type, revising their morphology, and depositing cultures and DNA sequences as far as possible.

TABLE 1. Collection data and GenBank accession numbers of species of *Mycosphaerella*, *Pseudocercospora*, and *Zasmidium* from Taiwan with ITS rDNA sequences newly generated in this study.

Species	Host	Collection no., strain no.[1]	Collection data in Taiwan	GenBank no.
M. gleicheniae T.S. Ramakr. & K. Ramakr.	*Dicranopteris linearis* (Burm. f.) Underw. var. *linearis* (Gleicheniaceae)	RoKi 3613	New Taipei City, Yingge, trail to Yingge Rock, 100–250 m, 11 April 2012	KJ201929
M. gleicheniae	*Dicranopteris linearis* var. *linearis* (Gleicheniaceae)	RoKi 3945	Taoyuan County, Dasi (Daxi) Township, Weiliao Old Trail, ca. 150 m, 29 September 2013	KJ201930
Ps. athyrii Goh & W.H. Hsieh	*Deparia longipes* (Ching) Shinohara (Dryopteridaceae)	RoKi 3943	Taoyuan County, Dasi (Daxi) Township, Weiliao, ca. 150 m, 29 September 2013	KJ201932
Ps. camelliicola U. Braun & C.F. Hill	*Camellia japonica* cultivar (Theaceae)	RoKi 3774, BCRC FU30031	Hsinchu County, Naluo, 12 October 2012	KJ201933
Ps. chrysanthemicola (J.M. Yen) Deighton	*Chrysanthemum* sp. (Asteraceae)	RoKi 3708, BCRC FU30006	Taoyuan County, Kuanyin Township, Lotus Garden Leisure Farm, 4 August 2012	KJ201934
Ps. cyatheae C. Nakash. & S. Inaba	*Sphaeropteris lepifera* (J. Sm. ex Hook.) R.M. Tryon (Cyatheaceae)	RoKi 3808, BCRC FU30106	Taipei City, Yangmingshan, ca. 450 m, 7 December 2012	KJ201935
Ps. ebulicola (W. Yamam.) Deighton	*Sambucus javanica* Blume (Adoxaceae)	RoKi 3933	Taoyuan County, Shihmen Dam, 10 September 2013	KJ201936
Ps. jussiaeae-repentis (Sawada) Goh & W.H. Hsieh	*Ludwigia octovalvis* (Jacq.) P.H. Raven (Onagraceae)	RoKi 3793, FU30038	New Taipei City, Yingge, ca. 50 m, 29 October 2012	KJ201937
Ps. lindericola (W. Yamam.) Goh & W.H. Hsieh	*Lindera megaphylla* Hemsl. (Lauraceae)	RoKi 3605, BCRC 34946	Taoyuan County, Shihmen Dam, ca. 300 m, 1 April 2012	KJ201931
Ps. pteridophytophila Goh & W.H. Hsieh	*Cyclosorus parasiticus* (L.) Farw. (Thelypteridaceae)	RoKi 3602	New Taipei City, Yingge, trail to Yingge Rock, 100–250 m, 25 March 2012	KJ201938
Ps. puerariicola (W. Yamam.) Deighton	*Pueraria montana* (Lour.) Merr. (Fabaceae)	RoKi 3769, BCRC FU30030	Hsinchu County, Smangus Bridge, ca. 900 m, 12 October 2012	KJ201939
Ps. wedeliae (A.K. Kar & M. Mandal) Deighton[2]	*Sphagneticola trilobata* (L.) Pruski (Asteraceae)	RoKi 3588, BCRC 34945	Yilan County, Beiguan Tidal Park at the coast, 12 March 2012	KJ201940
Z. dicranopteridis R. Kirschner	*Dicranopteris linearis* (Burm. f.) Underw. var. *linearis* (Gleicheniaceae)	RoKi 3953	Taipei City, Wenshan District, Maokong, ca. 300 m, 20 October 2013	KJ201941

[1] RoKi=R. Kirschner, dried specimen deposited in TNM, BCRC=Bioresource Collection and Research Center, Hsinchu, Taiwan, strain number; [2] Described in detail in Kirschner (2013).

Materials and methods

Specimens of ferns, fern-allies, and seed plants were collected in northern Taiwan during the years 2012–2013. The host plants were identified using the Flora of Taiwan (Huang & Editorial Committee of the Flora of Taiwan 1993–2003). The monographs by Knapp (2011) and Chang *et al.* (2012) were also consulted. The type specimen of *Pseudocercospora pteridophytophila* was loaned from the herbarium of the Plant Pathology Department, National Chung Hsing University, Taichung, Taiwan (NCHUPP). Fern leaf spots and dead leaves were examined under a dissecting microscope for the presence of fungi. Fungi were identified using Hsieh & Goh (1990) and original publications of the species. Morphology, cultivation and DNA techniques were applied as described in Kirschner & Okuda (2013).

Leaf fragments with mature ascomata of a *Mycosphaerella* species were fixed beneath the lid of a Petri dish containing 2% malt extract agar. Ascospores shot from the ascomata onto the agar were allowed for germination for 1 day and then investigated microscopically. Isolates were grown on 2% malt extract agar and corn meal agar with and without 0.2% chloramphenicol and cultivated at approx. 23° C in diffuse daylight. Dried specimens are deposited at the herbarium of the National Museum of Natural Science, Taichung, Taiwan (TNM) and cultures are deposited at Bioresource Collection and Research Center, Hsinchu, Taiwan (BCRC). DNA sequences are deposited at GenBank. Collection data with specimen and strain numbers and newly generated GenBank accession numbers of ITS sequences are given in Table 1.

Sequences were aligned with those of closely related species retrieved with BLAST search from GenBank with MUSCLE implemented in MEGA6. Two species of *Teratosphaeria* were included as outgroup. The alignment was not manually manipulated, but only the uneven ends were cut off resulting into a block of 518 positions. The best model found with MEGA6 was the Kimura 2-parameter model with gamma distributed rate of nucleotide substitution (Tamura *et al.* 2013). Maximum likelihood with 1,000 bootstrap replicates was used for estimating the unrooted phylogenetic tree shown in Fig. 1.

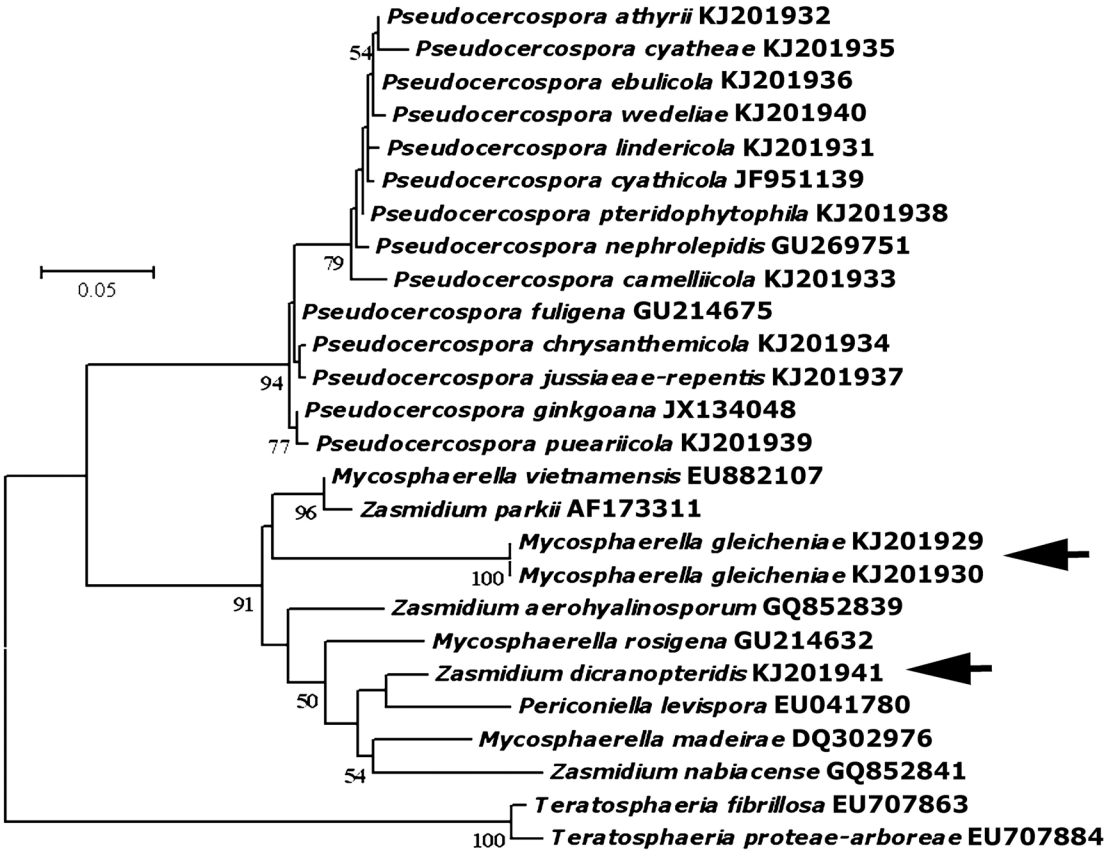

FIGURE 1. Unrooted Maximum Likelihood tree showing estimated relationships among mycosphaerellaceous species on ferns and other plants based on 5.8S-ITS rDNA sequences (GenBank accession numbers given behind the species names). Bootstrap above 50% (1,000 replicates) are indicated below the nodes. Arrows indicate the positions of *Mycosphaerella gleicheniae* and *Zasmidium dicranopteridis* from the same host.

Results

Six fungal taxa were found on ferns and fern-allies during collection in northern Taiwan in the years 2012–2013 and are described and illustrated herein. The host plants belonged to the orders Cyatheales, Gleicheniales, Polypodiales, and Selaginellales. Thirteen ITS sequences were generated for 12 species of mycosphaerellaceous fungi for the first time. All ITS sequences obtained in this study differed at least for 2 bp from all other available sequences in GenBank and were useful tools for identifying species of mycosphaerellaceous fungi on ferns. The ITS sequences from a sexual *Mycosphaerella* species and an asexual state on the same host, *Dicranopteris linearis*, clustered in different clades. The asexual state is therefore described as new species. Congeneric species on ferns appeared to be dispersed among species from seed plants in the phylogenetic analysis. A species of *Venustosynnema* on dead leaves of a lycophyte could not be cultivated, but its morphology was sufficient for distinguishing it from the other known species and is also described as new. The fungi which could be successfully isolated in pure culture remained sterile.

Taxonomy

Mycosphaerella gleicheniae T.S. Ramakr. & K. Ramakr., Proc. Indian Acad. Sci., Sect. B, 32: 205 (1950) (Figs 2, 3)

Leaf spots amphigenous on living pinnules, pale to dark brown with purplish margin or limited by the main vein and margins of the pinnule, 2–10 × 2 mm. *Hyphae* internal, pale brown, 1–4 µm wide. *Ascomata* amphigenous, but more frequent on the adaxial side, globose to subglobose, 65–70 µm diam. or 55–90 µm high and 45–80 µm wide (then 10–20 µm higher than wide), wall composed of an outer 2–3-layered part of brown cells and an inner hyaline layer whose cells gradually become flattened and break down during maturity, 5–20 µm thick, cells up to 13 × 10 µm, sometimes some pigmented cells connecting walls of neighboring ascomata, but not forming a compact stroma. *Asci* approx. 9–16 per ascoma, pyriform, 35–40 × 13–15 µm. *Ascospores* in the fresh stage in KOH cylindrical, straight or slightly curved, with one septum at or slightly distal to the center, with dense fine guttulation of the slightly greenish cytoplasm, wall hyaline, smooth, 15.5–20 × 3–3.5 µm, germinating at the ends and the septum. Anamorph unknown. *Cultures* dark brown, almost black, almost without aerial mycelium.

Known hosts and distribution:—On *Dicranopteris linearis* (Gleicheniaceae), India, Malaysia (Aptroot 2006, Ramakrishnan & Ramakrishnan 1950), Taiwan (new record).

Material examined:—All on spots of living leaves of *Dicranopteris linearis* (Burm. f.) Underw. var. *linearis* (Gleicheniaceae), TAIWAN. New Taipei City, Yingge, trail to Yingge Rock, 100–250 m, 11 April 2012, *R. Kirschner* 3613 (TNM); Taipei City, Nankang District, ca. 50 m, Academia Sinica, 14 December 2013, *R. Kirschner* 3981 (TNM); Taipei City, Wenshan District, Maokong, ca. 300 m, 20 October 2013, *R. Kirschner* 3954 (TNM); Taoyuan County, Dasi (Daxi) Township, Weiliao Old Trail, ca. 150 m, 29 September 2013, *R. Kirschner* 3945 (TNM).

Notes:—Ascospore sizes were given as 12–15 × 3 µm by Ramakrishnan & Ramakrishnan (1950). Aptroot (2006) could not find information about the type specimen and for a specimen from Malaysia stated that it was indistinguishable from *M. filicum* (Desm.) Starbäck with ascospore sizes as 13–16 × 3.5–4.5 µm. The slightly larger ascospores in the Taiwanese specimens might be attributed to measurements of fresh mature spores. Aptroot (2006) also found morphological identity with *M. filicum* for seven further species of *Mycosphaerella* on different ferns belonging to six families. As long as detailed morphological characters such as ascospore germination and asexual states for these species, as well as DNA data are lacking, we prefer to maintain the species on different host families as distinct. In Taiwan, the only fern on which a *Mycosphaerella* species has been found is *D. linearis*, which might indicate host specificity. Ascospore germination is considered an important characteristic for distinguishing species of *Mycosphaerella* (Crous & Wingfield 1996) and is reported for a fern-inhabiting *Mycosphaerella* species here for the first time as a base for future comparison with other species from ferns. Matches up to 98% similarity (at least 11 different bp) with ITS sequences of *Mycosphaerella* species from non-fern hosts were found using BLAST searches.

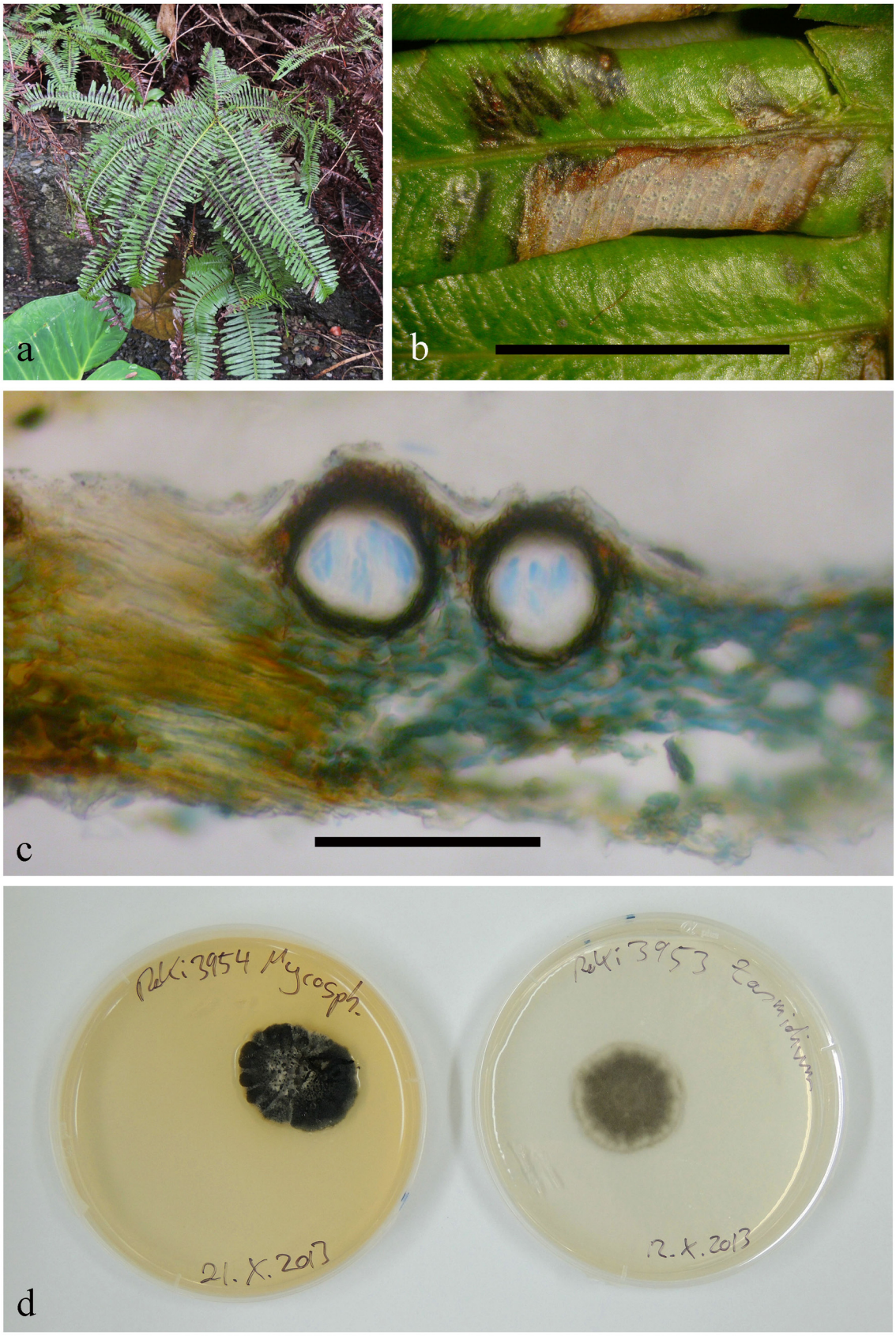

FIGURE 2. a–d. *Mycosphaerella gleicheniae* and *Zasmidium dicranopteridis*. a–c. *Mycosphaerella gleicheniae*, habitus of fresh collections. a. Host with leaf spots (*R. Kirschner* 3981). b. Leaf spot with ascomata (*R. Kirschner* 3613). c. Transversal leaf section showing two ascomata with asci stained with cotton blue (*R. Kirschner* 3613). d. Cultures of *M. gleicheniae* (left, 44 days old) and *Z. dicranopteridis* (right, 53 days old) on malt extract agar in 9 cm Petri dishes (collection numbers on Petri dishes). Scale bars: b = 7 mm, c = 80 µm.

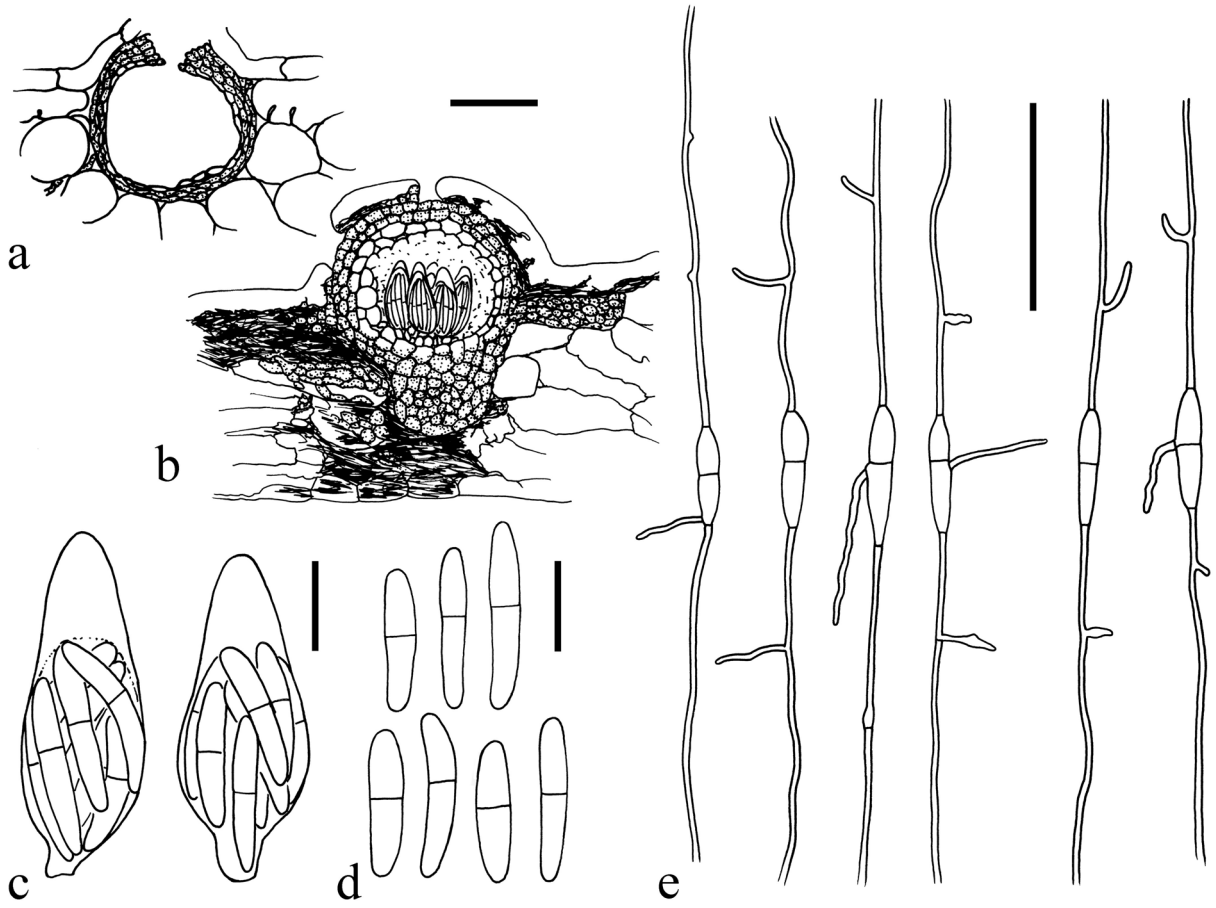

FIGURE 3. *Mycosphaerella gleicheniae*, microscopic details. a–b. Sections through ascomata rupturing the upper epidermis. a. Typical ascoma wall (*R. Kirschner* 3613). b. Ascoma being atypical by basal stroma-like cells probably surrounding a vascular bundle (*R. Kirschner* 3945). c, d. Fresh asci and ascospores (*R. Kirschner* 3613). e. Ascospores germinating after 1 day on malt extract agar (*R. Kirschner* 3613). Scale bars: a, b, e = 40 µm, c, d = 10 µm.

Pseudocercospora athyrii Goh & W.H. Hsieh, Trans. Mycol. Soc. Rep. China 4(2–3): 27 (1989) (Fig. 4)

Leaf spots amphigenous, dark brown, 3–7 × 2–4 µm. *Internal hyphae* pale brown, 1–3 µm wide, external hyphae only hypophyllous, pale brown, smooth, 1–2 µm wide, anastomosing. *Stromata* epiphyllous, erumpent through the epidermis, pale to medium brown, 13–35 µm wide and 17–40 µm high, cells 2–9 µm diam. *Conidiophores* arising from stromata on the adaxial side of the leaf or from stomata and external hyphae on the abaxial side. Epiphyllous conidiophores highly variable in number, size, and shape, few to numerous in fascicles, erect, cylindrical, undulate, at the top becoming geniculate, simple, but sometimes with intercalary lateral outgrowths whose cytoplasm is continuous and not separated by septum from the main axis of the conidiophore, basal cell usually not strongly swollen, pale brown, smooth, in some cases with percurrent extensions, 0–3-septate, (9–)20–51(–70) × 2–3(–4) µm (n = 30). *Conidiogenous cells* terminal and (less frequently) intercalary, pale brown, terminal ones (6–)11–18(–21) × 2–3 µm (n = 30), with 1–2 conspicuous, truncate conidiogenous loci, 1.5–2 µm wide. *Conidiophores* arising from abaxial stomata and external hyphae few, cylindrical, straight, curved or geniculate, pale brown, smooth, 0–1-septate, 8–23 × 2.5–3 µm. *Conidiogenous cells* terminal, conidiogenous loci truncate, 2–3 µm wide. *Conidia* solitary, straight to curved or sinuous, narrowing to the pointed apex, pale brown, smooth, with 3–11 indistinct septa, (36–)65–99(–105) × 2–3(–3.5) µm (n = 30), basal hilum truncate, 2 µm wide.

Known hosts and distribution:—On *Deparia longipes* (new host), *D. petersenii* (Athyriaceae/Dryopteridaceae/Woodsiaceae), Taiwan (Hsieh & Goh 1990).

Material examined:—TAIWAN. Taoyuan County: Dasi (Daxi) Township, Weiliao, ca. 150 m, on spots of living leaves of *Deparia longipes* (Ching) Shinohara (Dryopteridaceae), 29 September 2013, *R. Kirschner* 3943 (TNM), culture: BCRC FU30236.

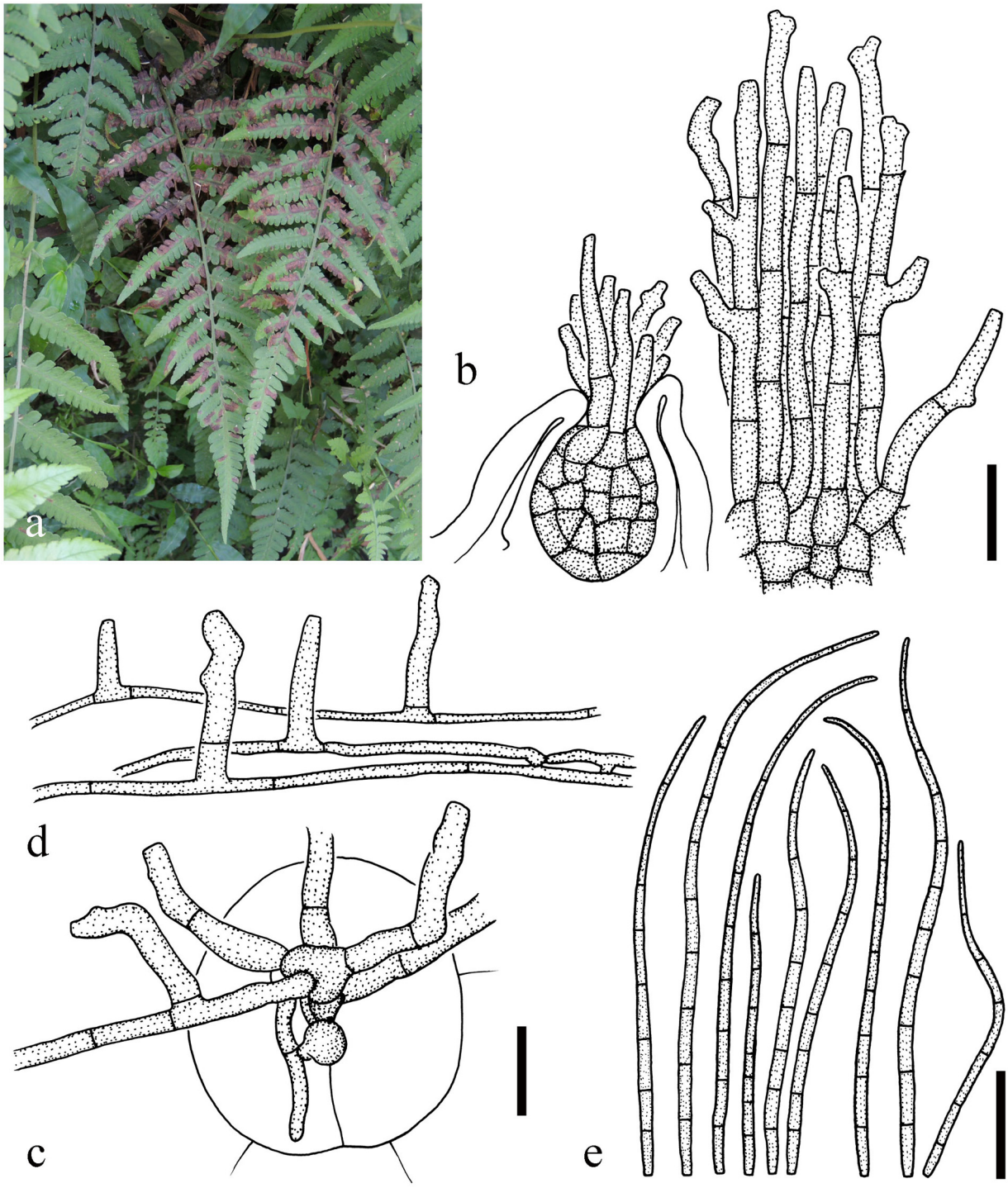

FIGURE 4. *Pseudocercospora athyrii* (*R. Kirschner* 3943). a. Host with leaf spots. b. Epiphyllous conidiophores: stroma with young fascicle of conidiophores erumpent through epidermis (left) and part of a comparatively older fascicle (right). c, d. Hypophyllous conidiophores. c. Conidiophores arising from stoma. d. Conidiophores arising from external hyphae. e. Conidia. Scale bars: b–d = 10 µm, e = 20 µm.

Notes:—*Pseudocercospora athyrii* has been known only from the type collection. Hypophyllous external hyphae and conidiophores have hitherto not been noted for the species. Conidiophores were considerably more variable than described for the type, but all other characteristics conformed to the protologue (Goh & Hsieh 1989). Using BLAST search for comparing sequences exceeding a length of 520 bp available in GenBank, the most similar sequences differed for 2–7 bp (99% similarity), e.g. identity with the ITS sequence of *Ps. cyatheicola* Crous & R.G. Shivas JF951139 was 520/524 (99%). When the species was described on *Athyrium japonicum* (Thunb.) Copel by Hsieh & Goh (1989), the host was considered a member of Athyriaceae. This fern is now classified as

Deparia petersenii (Kunze) M. Kato (Knapp 2011). Members of Athyriaceae, however, are not only classified as own family (Christenhusz *et al.* 2011, Rothfels *et al.* 2012), but also in a subfamily Athyrioideae of Dryopteridaceae (Kramer *et al.* 1990, followed in Knapp 2011), or in Woodsiaceae (Frey *et al.* 2009, Smith *et al.* 2006). Identifying fungi based on the assumed host specificity on the genus or family level is, therefore, more problematic when applied to fern-parasites than to other plant pathogenic fungi.

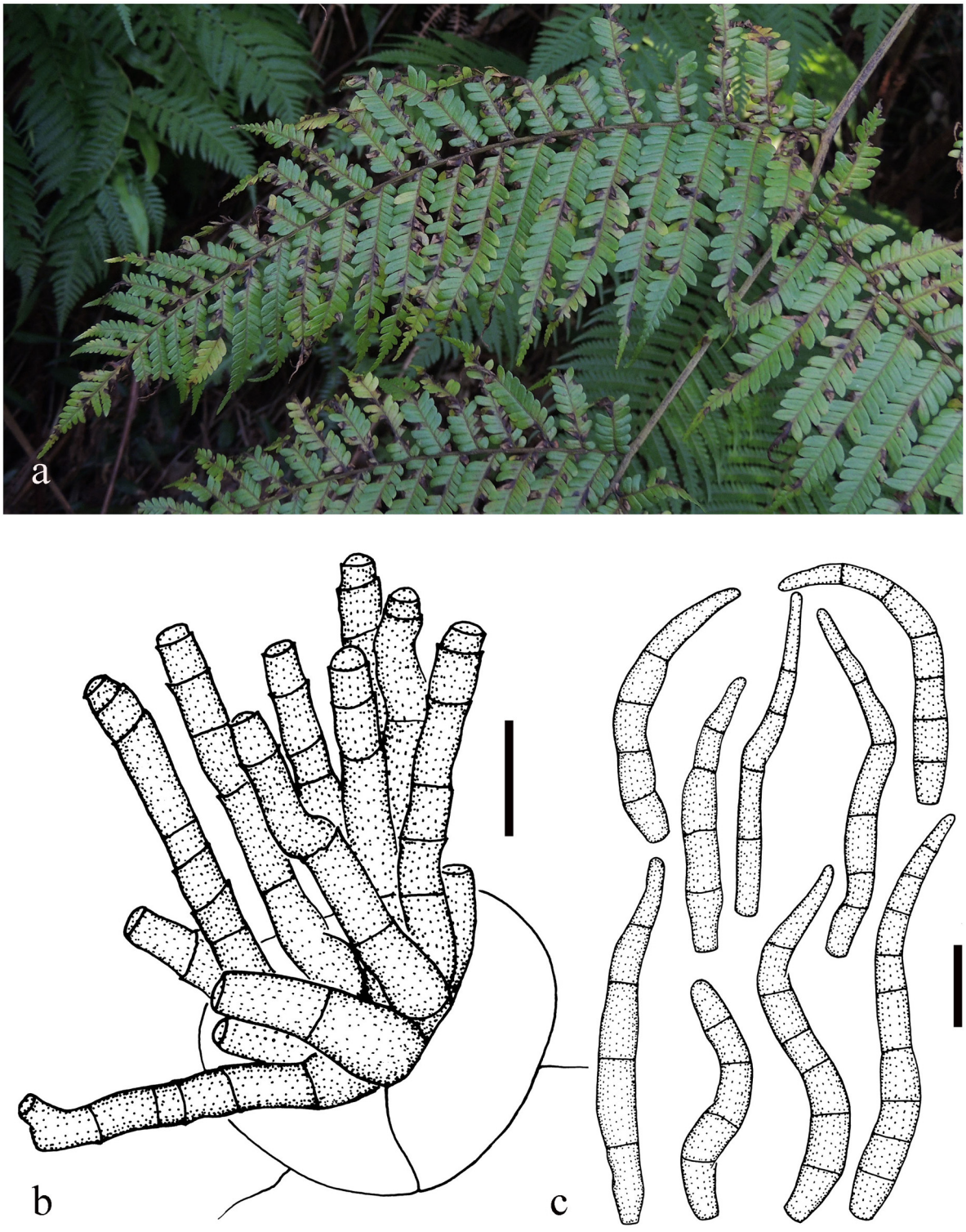

FIGURE 5. *Pseudocercospora cyatheae* (*R. Kirschner* 3808). a Host with leaf spots. b. Conidiophores with numerous percurrent extensions arising through a stoma. c. Conidia. Scale bars: b, c = 10 µm.

Pseudocercospora cyatheae C. Nakash. & S. Inaba, in Nakashima *et al.*, Mycoscience 47(1): 48 (2006) (Fig. 5)

Spots on living leaves amphigenous, pale brown with dark brown margin, 1–3 × 1–2 mm. Internal hyphae pale brown, 2–5 µm. *Stromata* dark brown, globose, 35–85 µm diam., or slightly wider than high, ca. 55–60 × 40–50 µm. *Conidiophores* epiphyllous, 1–50 arising from stromata through stomata, straight or in some cases geniculate, cylindrical, except for slightly widened basal cell, with 1–2 septa and several percurrent extensions, brown, (15–)25–44(–65) × 4–5.5(–7) µm (n = 30). *Conidiogenous cells* terminal, integrated, 8–15 × 4 µm, mostly with conspicuous percurrent extensions and a single terminal conidiogenous locus, sometimes with 1–2 further cicatrized loci, truncate, 2.5–3.5 µm wide. *Conidia* solitary, slightly to conspicuously curved or sigmoid, gradually narrowing to a broadly rounded apex, pale brown, smooth, conspicuously 4–9-septate, (30–)36–49(–52) × (3–)4–5 µm (n = 30), basal hilum broadly truncate, 2.5–4 µm wide.

Known hosts and distribution:—On *Cyathea* sp., Japan (Nakashima *et al.* 2006), *Sphaeropteris lepifera* (Cyatheaceae), Taiwan (new record).

Material examined:—TAIWAN. Taipei City, Yangmingshan, ca. 450 m, on spots of living leaves of *Sphaeropteris lepifera* (J. Sm. ex Hook.) R.M. Tryon [=*Cyathea lepifera* (J. Sm. ex Hook.) Copel., Cyatheaceae], 7 December 2012, *R. Kirschner* 3808 (TNM), living culture: BCRC FU30106.

Notes:—When using the BLAST function of GenBank for comparing sequences exceeding 500 positions, the most similar sequence was that of *Mycosphaerella* sp. (JN225929) with 9 different positions (similarity 540/549, 98%). All other search results had lower similarities. The species agrees morphologically with *Ps. cyatheae* described from *Cyathea* sp. in Okinawa, Japan, particularly with respect to the percurrent extensions of the conidiophores and robust, often sigmoid conidia. The genus concepts in Cyatheaceae have changed frequently, with *Sph. lepifera*, a common fern tree in Okinawa as well as Taiwan, also often been included in *Cyathea*. The host from the records in Okinawa and Taiwan is, therefore, probably the same species.

Pseudocercospora pteridophytophila Goh & W.H. Hsieh, Trans. Mycol. Soc. Rep. China 4(2–3): 28 (1989) (Fig. 6)

= *Pseudocercospora christellae* Phengs., McKenzie, K.D. Hyde & U. Braun, in Phengsintham *et al.*, Mycosphere 1(3): 207 (2011), *syn. nov.*

Leaf spots amphigenous, pale brown with a thin dark brown margin, vein-limited, 3–8 × 1–3 mm. *Caespituli* amphigenous, but abaxial ones more prominent. Hyphae internal, intercellular, pale brown, 2–5 µm wide. *Stromata* medium brown, up to 25–40 µm wide and 20–30 µm high, composed of 2–5 µm wide cells. *Conidiophores* arising (rarely) singly or typically in fascicles of up to approx. 25 from stromata, penetrating through stomata on the abaxial side, erumpent through the epidermis on the adaxial side, simple, reduced to the conidiogenous cell or composed of a short basal stalk cell and a conidiogenous cell, obclavate-cylindrical, often geniculate at the apex, pale to medium brown, 9–25(–45) × 2–3 µm (n = 30), with 1–2 conspicuous truncate or broadly subdenticulate conidiogenous loci, 1.5 µm wide. *Conidia* solitary, cylindrical, narrowing to 1 µm slender apex, straight or slightly curved, pale brown, smooth, (70–)77–97(–100) × 2–3 µm (n = 30), with 8–11 septa.

Holotype of *Ps. pteridophytophila*: Conidiophores mostly destroyed, conidia straight to curved or undulate, pale brown, (45–)49–70(–80) × (1.5–)2–2.5 µm (n = 13).

Known hosts and distribution:—*Cyclosorus acuminatus* and *C. parasiticus* (Thelypteridaceae), Taiwan (Hsieh & Goh 1990), Thailand (Phengsintham *et al.* 2011).

Material examined:—On leaf spots of *Cyclosorus acuminatus* (Houtt.) Nakai (Thelypteridaceae), TAIWAN. Miaoli County, Cholan, 11 August 1984, *T.K. Goh* (NCHUPP 37, holotype!); on spots of living leaves of *Cyclosorus parasiticus* (L.) Farw. (Thelypteridaceae); New Taipei City, Yingge, trail to Yingge Rock, 100–250 m, 25 March 2012, *R. Kirschner* 3602 (TNM), 11 April 2012, *R. Kirschner et al.* 3612 (TNM), 30 October 2012, *R. Kirschner* 3756 (TNM); Taoyuan County, Jhongli City, National Central University campus, 12 November 2013, *R. Kirschner* 3973 (TNM).

Notes:—Recently, *Ps. christellae* was described on the same host ("*Christella parasitica*", Phengsintham *et al.* 2011). The statement that previously no *Pseudocercospora* species had been known on the genus *Christella* was erroneous, because *Christella parasitica* is an illegitimate synonym of *Cyclosorus parasiticus* (Tropicos 2012). *Christella* is now included in *Cyclosorus* (Frey *et al.* 2009). The type specimen of *Ps. pteridophytophila* was recorded from *Cyclosorus acuminatus* (Houtt.) Nakai ex H. Itô (Hsieh & Goh 1989). The conidiophore lengths

recorded from Thailand (9–14 µm, n=13) are in the lower range of those from Taiwan [9–25(–45) µm, n = 30]. The conidia were described as hyaline and 1–1.5 µm wide by Goh & Hsieh (1989), but in the type specimen were pale brown and slightly broader [(1.5–)2–2.5 µm]. The highest matches with BLAST search were sequences of other *Pseudocercospora* spp. with 99% identity and at minimum 2 different bp.

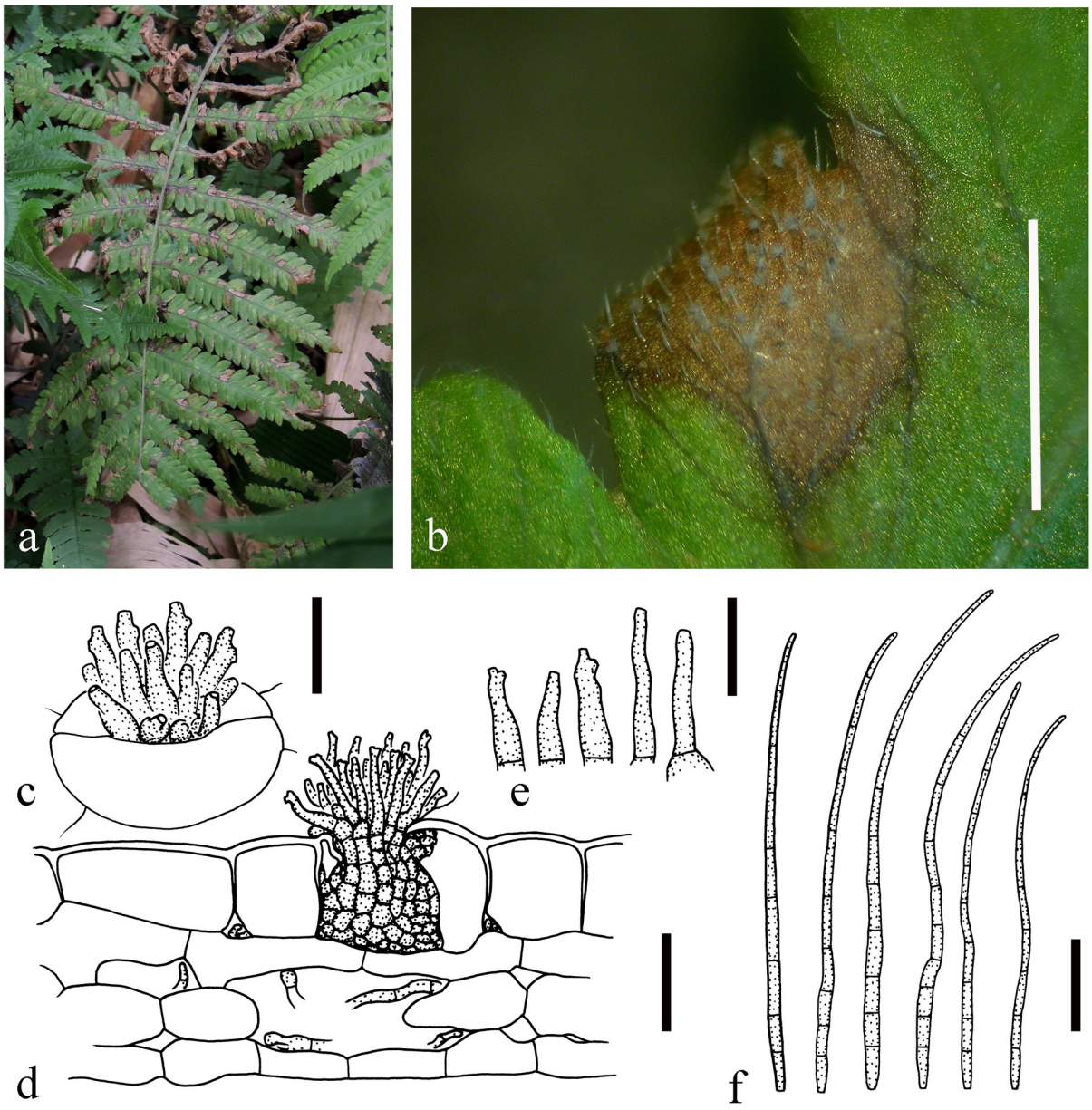

FIGURE 6. *Pseudocercospora pteridophytophila*. a. Host with leaf spots. b. Single leaf spot with epiphyllous fascicles of conidiophores. c. Hypophyllous fascicle of conidiophores arising through a stoma (*R. Kirschner* 3756). d. Transversal leaf section showing internal hyphae and an epiphyllous fascicle of conidiophores arising from an intraepidermal stroma (*R. Kirschner* 3602). e. Conidiophores (*R. Kirschner* 3756). f. Conidia (*R. Kirschner* 3602). Scale bars: c, e = 10 µm, d, f = 20 µm.

Venustosynnema reniformisporum R. Kirschner & Li-Chia Liu, *sp. nov.* Index Fungorum IF550470 (Fig. 7)

Type:—TAIWAN. Taipei City, Wenshan Distr., Maokong, 300 m, on dead leaves of *Selaginella moellendorffii* Hieron. (Selaginellaceae), 20 October 2013, *R. Kirschner 3959* (TNM, holotype!).

Differs from *Venustosynnema ciliatum* and *V. grandiae* by smaller conidiomata, smooth central setae, and reniform conidia.

Conidiomata synnematous, scattered, erect, straight, 90–200 × 13–18 µm (central setae excluded). *Peripheral hyphae* septate, pale brown to brown, parallel, compact, (80–)100–170(–200) × (8–)9–15(–18) µm (n = 12),

diverging at the fertile apex, becoming acuminate, smooth, marginal setae. *Central setae* cylindrical, acuminate, smooth, erect, straight, (100–)134–194(–220) × 3–5 µm (n = 12). *Conidiophores* tightly aggregated, unbranched or branched, erect, straight, cylindrical, septate, smooth-walled, (50–)52–64(–70) µm long, with 3–4 µm wide stipe (n = 10). *Conidiogenous cells* phialidic, cylindrical to slightly subulate, hyaline, smooth, with apical collarette, (5–)9–19(–25) × 3–4 µm (n = 30). *Conidia* reniform, asymmetric, 1-celled, hyaline, (7–)7.5–8.5(–10) × (3–)3.5–4 µm (n = 30), both ends with a single, simple, straight or slightly curved setula, 4–5 µm long; accumulating in a white, mucous mass.

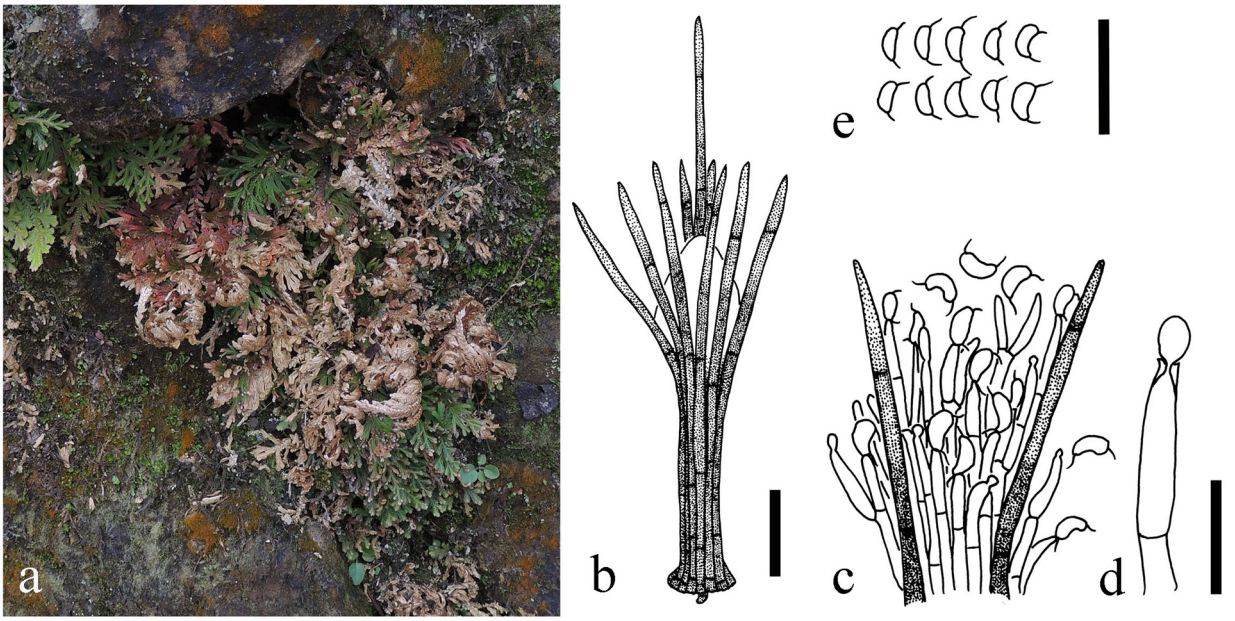

FIGURE 7. *Vennustosynnema reniformisporum* (from holotype, TNM). a. Host with dead stems and leaves. b. Synnema. c. Apices of conidiophores in the center of a synnema. d. Conidiogenous cell. e. Conidia. Scale bars = 30 µm.

Etymology:—Referring to the reniform conidia.

Known host and distribution:—Only known from the type.

Notes:—Attempts to cultivate the fungus failed. For comparison with similar species see the Discussion Section and Table 2.

TABLE 2. Morphological comparison of *Venustosynnema reniformisporum* with the two other known species of *Venustosynnema* (from Castañeda Ruiz *et. al.* 2009).

	Conidioma size (µm)	Central seta	Conidium shape	Conidium size (µm)
V. ciliatum	350–450 × 17–25	absent	allantoid to sub-lunate	13–16 × 2–4
V. grandiae	300−500 × 19−42	verrucose, up to 630 µm long, 8–12 µm wide	allantoid to sub-lunate	6–8(–10) × 1.5–2.0
V. reniformisporum	90–200 × 13–18	smooth, up to 220 µm tall, 3–5 µm wide	reniform	(7–)7.5–8.5(–10) × (3–)3.5–4

Zasmidium dicranopteridis R. Kirschner, *sp. nov.* Index Fungorum IF550423 (Figs 2d, 8)

Type:—TAIWAN. Taipei City, Wenshan District, Maokong, ca. 300 m, on *Dicranopteris linearis* var. *linearis* (Gleicheniaceae), 20 October 2013, *R. Kirschner* 3953 (TNM, holotype!), ex-type culture: BCRC FU30234.

Differs from other *Zasmidium* species by growth on living ferns, smooth hyphae, and hyaline, smooth conidia.

Leaf spots absent. *Stromata* absent. *Internal hyphae* not found. *External hyphae* hypophyllous on apparently healthy plant tissues, pale brown, smooth, 1.5–3.5 µm wide. *Conidiophores* arising singly from external hyphae, cylindrical, erect, straight, unbranched or rarely with one lateral branch in the upper third, dark brown, smooth,

4–20-septate, distances between septa 7–25 µm, apically with occasional percurrent extensions and becoming paler, (43–)125–210(–235) long (n = 30), giving rise to 1–6 terminal and intercalary conidiogenous cells of the same width as the rest of the conidiophore. *Terminal conidiogenous cells* pale brown, smooth, (11–)13–20(–28) × 4–5(–5.5) µm (n = 30), becoming covered with numerous blackened 1–1.5 µm wide conidiogenous loci. *Conidia* solitary, obclavate-cylindrical or slightly fusiform, hyaline, with a pale greenish tinge typical of *Cercospora* spp., smooth, with (1–)2–4(–7) transversal septa (mostly 3 septa), (13–)20–35(–52) × (2.5–)3(–4) µm (n = 30), with slightly thickened and blackened basal hilum, 1–1.5 µm. *Colonies* in culture medium brown with white margin and conspicuous aerial mycelium, sterile.

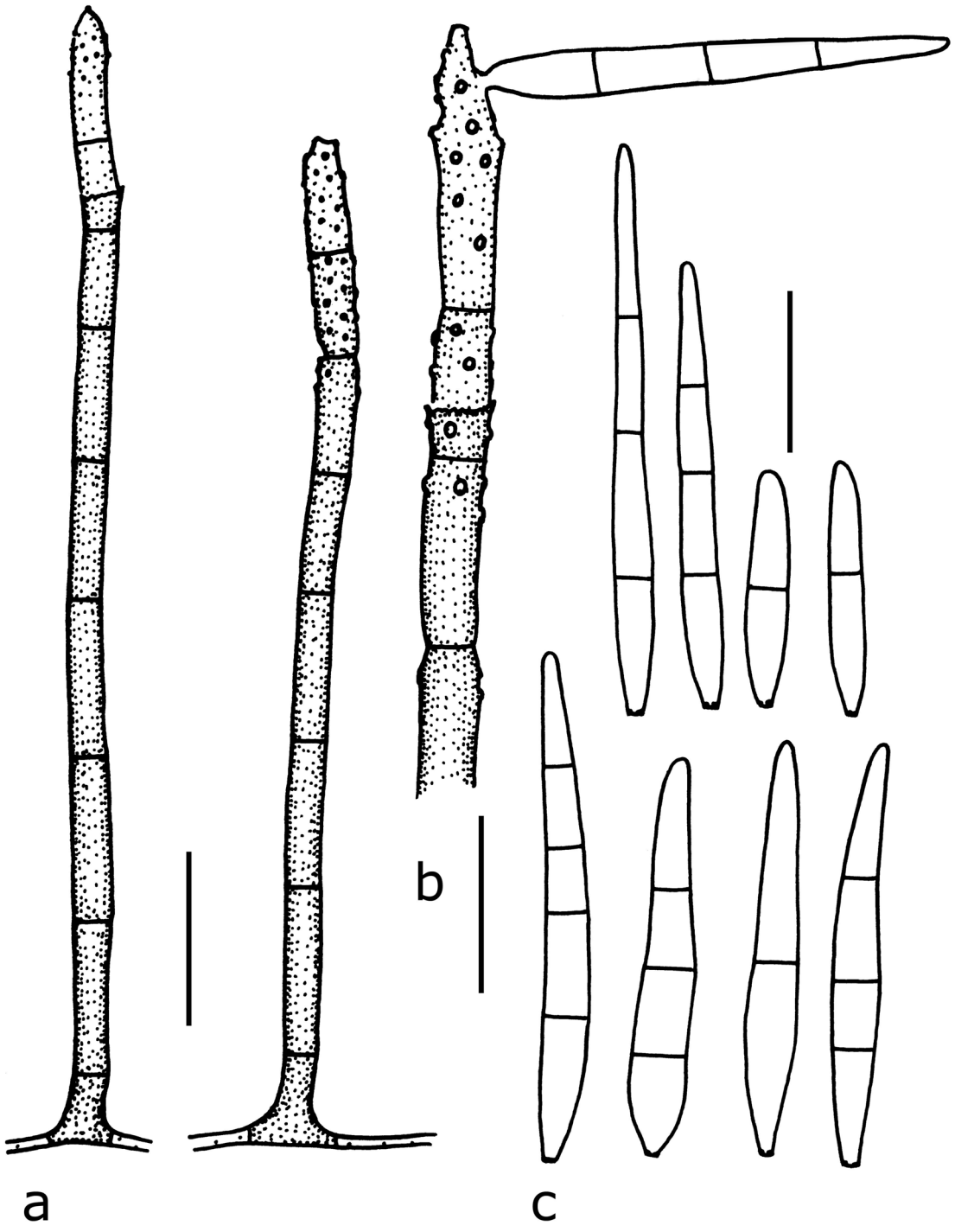

FIGURE 8. *Zasmidium dicranopteridis* (from holotype, TNM). a. Conidiophores on external hyphae. b. Conidiophore apex with a percurrent extension, with intercalary and terminal conidiogenous cells and a conidium still attached. c. Conidia. Scale bars: a = 20 µm, b, c = 10 µm.

Etymology:—Referring to the host genus.

Known host and distribution:—On *Dicranopteris linearis* (Gleicheniaceae), northern Taiwan.

Other material examined:—On *Dicranopteris linearis* var. *linearis* (Gleicheniaceae), TAIWAN. New Taipei, Yingge district, Yingge Rock Trail, ca. 100–250 m, 30 September 2012, *R. Kirschner* 3761 (TNM).

Notes:—Using BLAST search, the highest similarities between the ITS sequence of this species with sequences from GenBank were 94% (524/557 bp), namely of *Mycosphaerella rosigena* (Ellis & Everh.) Lindau ex McMurran (GenBank GU214632, EU167587) and *Periconiella levispora* Arzanlou, W. Gams & Crous (500/531 bp, GenBank EU041780).

Discussion

In his overview of fungi on ferns, Mehltreter (2010) emphasized that study of saprobic fungi on fern leaves provides a particular opportunity of new discoveries. This suggestion is supported here by the discovery of a new species of *Venustosynnema*. This species was found on leaves of dead above-ground parts of *Selaginella moellendorfii*, a species commonly found on rocky slopes and stone walls. Parts of the plant die regularly due to unfavorable exposure to extremes of moisture and temperatures, but are replaced by new growth (Chang *et al.* 2012). *Venustosynnema reniformisporum* is considered a rather saprobic than parasitic species because of its substrate. The genus *Venustosynnema* shares common characteristics with *Menisporopsis*, namely pale brown or black synnemata, phialides, and hyaline conidia with setulae, but differs by setae which are absent in *Menisporopsis*. The genus *Venustosynnema* had two species, *V. ciliatum* (R.F. Castañeda, G.R.W. Arnold & A.G. Guerra) R.F. Castañeda & W.B. Kendr., the type species, and *V. grandiae* Gusmão, V.O. Moraes & R.F. Castañeda (Castañeda Ruiz *et. al.* 2009). The new species comforms to the genus *Venustosynnema*, because of the peripheral setae. *V. reniformisporum* can be differentiated from the other two species by its smaller conidiomata (Table 2). Peripheral hyphae and central setae are verrucose in *V. grandiae*, but smooth in *V. reniformisporum*; conidium shape is allantoid to sub-lunate in *V. ciliatum* and *V. grandiae*, but reniform in *V. reniformisporum*. The pigmentation and setulate conidia produced from phialides may indicate a relationship with *Dictyochaeta* and similar genera in the Chaetosphaeriales. Sequence data, however, are hitherto not available for any species of *Venustosynnema.*

On *Gleicheniaceae*, only one species of cercosporoid hyphomycetes has hitherto been known, namely on *Dicranopteris linearis*. *Ps. gleicheniae* (J.M. Yen) U. Braun has recently been proposed as tentative new combination (Braun *et al.* 2013). This species, only known from the type in Africa, differs by its internal hyphae, and subclavate, slightly broader, pale brown conidia, from *Z. dicranopteridis* (Yen 1974). *Helminthosporium gleicheniae* F. Stevens & Glick also described on *D. linearis* differs by its dark brown, mostly 8-septate and wider conidia from the two former species (Stevens 1925). *Zasmidium dicranopteridis* lacks the conspicuous verruculose ornamentation of the hyphae typical of the genus and is further not typical because of the hyaline, smooth conidia. According to ITS data, *Z. dicranopteridis* is related to *Z. aerohyalinosporum* Crous & Summerell which also shows atypical morphological characteristics (Crous *et al.* 2009). The characterization of *Stenella s. lat.* by Crous & Braun (2003) also includes occasionally colorless and smooth conidia. In order to distinguish between *Stenella s. str.* with the type species and other species more closely related to *Zasmidium*, the latter species have been transferred to *Zasmidium*. As a consequence, now the former monophyletic genus *Zasmidium* has become paraphyletic (Braun *et al.* 2010). No traces of the presence of internal hyphae or penetration of stomata were found during study of leaf sections and abaxial leaf surfaces so that the species is considered commensalistic rather than parasitic. Internal hyphae were reported as inconspicuous or not found in several other species of *Zasmidium* (Phengsintham *et al.* 2009). For these reasons of molecular phylogenetic relationship, external growth and hyaline conidia, *Zasmidium* was preferred against *Passalora* for the tentative accommodation of the new species.

Mycosphaerella gleicheniae and *Z. dicranopteridis* differed by internal growth associated with leaf spots in the former and external growth without symptoms in the latter. Both species clustered in different clades in the DNA analysis. The culture morphology of both species was also different, by dark brown, almost black colonies almost without aerial mycelium in *M. gleicheniae* and medium brown colonies with white margin and conspicuous aerial mycelium in *Z. dicranopteridis*.

Of the mycosphaerellaceous/cercosporoid fungi treated here (*M. gleicheniae*, *Ps. athyrii*, *Ps. christellae*, *Ps. cyatheae*, and *Ps. pteridophytophila*) morphological descriptions have hitherto been available only for the type specimens. By extending collections, the range of host species, morphology (particularly with respect to overlooked external hyphae, size ranges and pigmentation), and taxonomy of the known species was revised, and DNA data were provided for the first time. Though ITS sequences of different species of cercosporoid fungi are often 100% identical, all sequences obtained in this study differed at least for 2 bp from all other available sequences in GenBank. DNA data were particularly helpful in showing that the newly detected asexual state on *Dicranopteris linearis*, *Z. dicranopteridis*, was not conspecific with the sexual state of *M. gleicheniae* found on the same host. Fungi on ferns and seed plants appeared scattered in the phylogenetic analysis, which might indicate frequent host jumps during speciation rather than cospeciation with the host plants.

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