

First records of powdery mildew fungi (Erysiphales) on four medicinal plants in Taiwan

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samples between paper and subsequent final drying on an electrical dryer. Representative specimens were deposited at the National Museum of Natural Science, Taichung, Taiwan (TNM). For evaluating the presence of powdery mildews on plant herbarium specimens, we checked 7 specimens of *Eryngium foetidum*, more than 30 of *Lonicera japonica*, 9 of *Lycium chinense*, and over 50 of *U. crinita*, all collected in Taiwan between the years 1917 and the 2004 and kept in the herbarium of National Taiwan University (TAI). In cases where colonies of powdery mildew were assumed to be present on leaves, they were removed with a transparent tape and transferred to a lactophenol/cotton blue mounting for microscopical observation

Molecular identification

For molecular identification with internal transcribed spacer (ITS) sequences of the nuclear ribosomal RNA genes (ITS 1, 5.8 S rDNA, ITS2 and bordering short fragments of the 18S and 28S rDNA), total genomic DNA was isolated from freshly collected mycelium, conidiophores and conidia; the ITS was amplified, sequenced, and edited as in Wei and Kirschner (2017). ITS sequences were used for megaBLAST searches at GenBank and deposited in GenBank. Sequences from BLAST searches and Takamatsu et al. (2015a) were selected for confirming the megaBLAST identification also with phylogenetic analyses. A phylogenetic estimate was based on an alignment made in MEGA7 with the default options of MUSCLE (Kumar et al. 2016). Except for trimming the ends of the alignment block, no further manipulations were done (Additional File: Table S1). The alignment was analyzed with the Maximum Likelihood method with the Kimura-2 parameter model (gamma-distributed) as best model and 1000 bootstrap replicates (Kumar et al. 2016). GenBank numbers and host names are given in Fig. 1.

Results

Four species of powdery mildews were found only in the anamorph stage on mature leaves of the host plants. The ITS sequences obtained in this study ranged between 570 and 680 bp. The phylogenetic analysis confirmed the species identification, whereas the relationships between the species were not the scope of the study. Based on anamorph morphology, host specificity, and identification with BLAST as well as ML analysis, three powdery mildews on medicinal plants are new records for Taiwan (Fig. 1), *Arthrocladiella mougeotii* on *Lycium chinense*, *Erysiphe lespedezae* on *Uraria crinita*, and *E. loniceriae* on *Lonicera japonica*. *Eryngium foetidum* is a new host for *Erysiphe heraclei* hitherto known on other host plants in Taiwan. *Eryngium foetidum* and *Uraria crinita* are new host plants for powdery mildews worldwide. The pathogens did not cause death of the host plants, but appeared to enhance stress by colonizing mature leaves. The fungi were only found on specifically mycologically collected specimens in the field, but not among over 80 plant herbarium specimens collected during the last 100 years in Taiwan. A documentation of the morphological and molecular identification of the four powdery mildew species is given below.

Arthrocladiella mougeotii (Lév.) Vassilkov Fig. 2

Colonies amphigenous on leaves. Hyphae smooth, 5–6 µm wide. Hyphal appressoria mostly solitary, nipple-shaped. Conidiophores arising solitarily from middle of hyphal mother cell, slightly constricted at base, composed of foot cell and 1–4 distal cells, (53–)62–89(–109) x (8–)9–11 µm. Foot cell with basal septum at same level as upper surface of supporting hypha, straight or curved, (33–)37–52(–60) x (8–)9–11 µm. Conidia catenate, ellipsoid-ovoid to doliiiform, (25–)27–32(–35) x (10–)11–13(–15) µm, germination by forming long hyphae close to one of both ends of the conidium.

Specimens examined. On leaves of *Lycium chinense* Mill. (Solanaceae). Taipei City, Zhongzheng District, sidewalk near MRT Taipower Building Station, ca. 25.020751, 121.528368, ca. 15 m, 27 Apr 2020, AKII 0082 (TNM), ITS sequence GenBank MT703830; Taipei City, Daan District, Taipei, National Taiwan University campus, Zhoushan Road, 23 Apr 2015, not preserved (Fig. 2a).

Notes. The ITS sequence was identical 100% identical to over ten published ones exceeding 590 bp of *Arthrocladiella mougeotii* in GenBank. *Arthrocladiella mougeotii* is the single species of its genus and is almost globally distributed and limited to *Lycium* hosts (Braun and Cook 2012). In contrast to Farr and Rossman (2020) who list several powdery mildew species for *Lycium* species, only *A. mougeotii* and *Phyllactinia chubutiana* Havryl., S. Takam. & U. Braun are accepted for *Lycium* hosts in Braun and Cook (2012). Both fungi can be distinguished not only by different ITS sequences, but also the solitary conidia in *Ph. chubutiana* and catenate conidia in *A. mougeotii*.

Erysiphe heraclei DC. Figure 3

Colonies amphigenous on leaves. Hyphae smooth, 4–7 µm wide. Hyphal appressoria solitary or in opposite pairs, nipple-shaped or lobed. Conidiophores arising solitarily from middle of hyphal mother cell, slightly curved at base, (56–)68–105(–117) x (6–)7–8(–9) µm. Foot cell with basal septum at same level as upper surface of supporting hypha or slightly elevated up to 9 µm, straight or curved, (32–)38–49(–52) x (6–)7–8(–9) µm, followed by 1–4 cells. Conidia solitary, ellipsoid-ovoid to cylindrical, (34–)35–45(–51) x (11–)12–17(–19) µm, germination from one of both ends by forming long hypha with nipple-shaped or lobed appressoria.

Specimen examined. On leaves of *Eryngium foetidum* L. (Apiaceae). Taipei City, Daan District, Changhsin Street, National Taiwan University dormitories, ca. 25.015449, 121.546735, ca. 20 m, 24 May 2019, R. Kirschner 4729 (TNM), ITS sequence GenBank MT703849.

Notes. There was 99% ITS sequence identity with 1 to 6 different bp between our specimen and over ten other published specimens labeled as *E. heraclei* in GenBank. Presently, it is not possible to distinguish between *E. betae* (Vaňha) Weltzien, *E. heraclei*, and *E. malvae* V.P. Heluta based on rDNA sequences (Takamatsu et al. 2015a). The three species are hardly distinguishable also in morphology (Braun and Cook 2012). Future study may reveal whether the species can be separated with additional markers or need to be put into synonymy. In this case, *E. heraclei* would have priority since it is the oldest available name.

Erysiphe lespedezae R.Y. Zheng & U. Braun Fig. 4

Colonies amphigenous on leaves. Hyphae smooth, 3–6 µm wide. Hyphal appressoria mostly solitary, nipple-shaped or lobed. Conidiophores arising solitarily from middle of hyphal mother cell, slightly curved and rough at base, composed of foot cell and 1–4 distal cells. Foot cell with basal septum at same level as upper surface of supporting hypha, straight or curved, (35–)45–78(–115) × (4–)5–6(–8) µm. Conidia solitary, ellipsoid-ovoid to doliform, (24–)26–34(–40) × (12–)14–17(–19) µm, germination terminal.

Specimens examined. On leaves of *Uraria crinita* (L.) Desv. ex DC. (Fabaceae). Miaoli County, Touwu Township, Lavender Cottage, ca. 24.587072, 120.896818, ca. 80 m, 09 Nov 2018, AKII 0005 (TNM), ITS sequence GenBank MT703848; Taipei City, Shilin District, Medicinal Botanical Garden, 25.115751, 121.576885, ca. 220 m, 12 Nov 2018, AKII 0006 (TNM).

Notes. When ITS sequences exceeding 570 bp were compared, there was 99% identity with 2 to 5 different bp between our specimen and seven published specimens labeled as *E. lespedezae* in GenBank, whereas the identity with other specimens were 98% or lower, with more than 5 different bp. *Uraria* species have not yet been recorded as hosts of powdery mildews worldwide (Braun and Cook 2012, Farr and Rossman 2020). The foot cell of conidiophores was longer [(35–)45–78(–115) µm] than reported in Braun and Cook (2012: about 20–40 µm long), but Meeboon & Takamatsu (2017a) also reported longer foot cells (35–65 µm) for this species. Some species of Erysiphales on Fabaceae presently lacking DNA, such as *Pseudoidium bauhiniae* (G.J.M. Gorter & Eicker) U. Braun & R.T.A. Cook and *Ps. caesalpinicearum* (Hosag. & U. Braun) U. Braun & R.T.A. Cook may require restudy for clarifying the species boundaries of *E. lespedezae*.

Erysiphe lonicerae DC. Figure 5

Colonies amphigenous on green leaves. Hyphae verruculose, 3–6 µm wide. Hyphal appressoria mostly single, rarely in opposite pairs, nipple-shaped, lobed or elongated. Conidiophores arising solitarily from middle of hyphal mother cell, straight or curved at base (only in one case curvature formed in cell distal to foot cell), cylindrico-clavate, verruculose, composed of foot cell and 1–3 distal cells, (55–)79–120(–140) × (7–)9–12(–14) µm. Foot cell with basal septum at same level as upper surface of supporting hypha, cylindrical, straight or curved, (20–)37–51(–60) × 5–6.5(–7) µm. Conidia solitary, oblong to short cylindrical, smooth-walled when fully turgid, wall becoming reticulate by aging, (31–)32–40(–44) × (13–)15–18.5(–20) µm, germinating apically into a short hypha with lobed appressoria.

Specimens examined. On mature leaves of *Lonicera japonica* Thunb. (Caprifoliaceae). New Taipei City, Shengkeng District, near City Highway 106B, ca. 24.999115, 121.620617, ca. 50 m, 07 Apr 2019, R. Kirschner 4712 (TNM), ITS sequence GenBank MT703799; Taipei City, Daan District, National Taiwan University, near post office, 25.018563, 121.537213, ca. 15 m, 31 May 2019, R. Kirschner 4733 (TNM); Taipei City, Shilin District, Medicinal Botanical Garden, 25.115751, 121.576885, ca. 220 m, 30 Oct 2014, R. Kirschner & W.-A. Liu 4112 (TNM).

Notes. When ITS sequences exceeding 600 bp were compared, there was 99% identity with 1 to 4 different bp between our specimen and seven published specimens labeled as *E. lonicerae* in GenBank, whereas the identity with other species was 98% or lower, with more than 10 different bp. *Erysiphe lonicerae* is known on different *Lonicera* species in Europe and East Asia (mainland China, Korea, Japan) and is a new record for Taiwan (Farr and Rossman 2020). Information about conidiophore morphology and DNA sequences do not exist, however, for two other *Erysiphe* species on *Lonicera* hosts in East Asia, namely *E. lonicerae-ramosissimae* (Tanda) U. Braun & Takam. and *E. miurae* (U. Braun) U. Braun & Takam. These data are also lacking for *E. magnusii* (S. Blumer) U. Braun & Takam. reported from Europe and Central Asia, and for *E. caprifoliacearum* (U. Braun) U. Braun & Takam. in America (Braun and Cook 2012).

Discussion

Advances and limitations of molecular species recognition

Identification of powdery mildew fungi to species can be based on the traditional combination of host specificity and fungus morphology, particularly of the sexual stage, when sufficient data are available in a given area. Since the sexual stage is rarely found in the tropical/subtropical environment in Taiwan and data are far from being sufficient for Erysiphales in Taiwan, ITS sequence-based identification is an important requirement for species identification of the powdery mildews. For obtaining DNA from powdery mildews, careful selection of clean, fresh specimens is necessary, since powdery mildews cannot be cultivated in vitro, are often contaminated by other microscopic fungi and DNA deteriorates quickly during drying specimens. In Erysiphales, the ITS is the single available barcode for species identification particularly through the accomplishments of S. Takamatsu (Takamatsu et al. 2015a, b; Meeboon and Takamatsu 2016, 2017, 2020). Four species of powdery mildews were identified here based primarily on ITS sequence data. In Erysiphales, the barcode gap of ITS sequences is often very narrow, which was also confirmed in our study with 99 to 100% sequence identities and a variation of 0 to 6 different bp found in the same species. The variation depends on the available data as well as the species, since, for example 6 different bp were still within the range of variability in *E. heraclei*, whereas for *E. lespedezae* the same number of different bp would indicate an overlap with other, closely related species. Because of the high identities between ITS sequences of related species, even in phylogenetic analyses it is sometimes not possible to resolve the species which then still remain provisionally based on the traditional combination of host occurrence and fungal morphology (Chen and Kirschner 2018). In the example of *E. lonicerae*, we demonstrate that for several species on the same host genus, information of DNA data and anamorph morphology is lacking. *Erysiphe heraclei* is an example for illustrating the conflict between the ITS phylogeny and traditional taxonomy. Most recent new attempts to obtain DNA from herbarium specimens of Erysiphales and to develop alternative barcodes for this group of fungi are very promising for resolving these problems (Ellingham et al. 2019; Bradshaw and Tobin 2020).

Origin of host plants and detection of associated fungi

Except for *L. chinense*, the medicinal plants of this study are also valued as ornamentals (Hsueh and Yang 2011, 2014, 2016). Among these plants cultivated in Taiwan, *Lonicera japonica* and *Uraria crinita* are native (Ohashi and Iokawa 2007). The parasitic fungi on these hosts may be native, but have escaped attention due to limited research of fungi. *Lycium chinense* was introduced from mainland China ca. 300 years ago (Wu et al. 2010), *Arthrocladiella mougeotii*

has been introduced on its *Lycium* hosts from Eurasia to other countries and continents (Braun and Cook 2012) and also most likely from mainland China to Taiwan. *Eryngium foetidum* was introduced to Taiwan in 1926 (Wu et al. 2010). Both, *L. chinense* and *E. foetidum* have been classified as medicinal plants as well as potential weeds (Wu et al. 2010). In some plant parasitic fungi, such as smut fungi (Basidiomycota, Ustilaginomycotina), it is possible to retrieve new information of fungal distribution from phanerogam herbarium specimens (Piepenbring 2001). Our scanning of over 80 herbarium specimens of the four host plants in TAI for the accidental presence of powdery mildews, however, did not retrieve any fungi except for few saprobic molds. It is, therefore, not possible to conclude from plant specimens whether associated powdery mildews have escaped from attention in the past or have been introduced from abroad more recently. This result also shows the importance of specific mycological field study in order to complement the insufficient data of plant pathogenic fungi as well as of building up and curating professional mycological specimen collections.

Conclusions

Four species of powdery mildews on medicinal plants from Taiwan were identified here based primarily on ITS sequence data. Three species are new records, a further species is recorded from a new host. Our new records and the lack of DNA data as well as anamorph characteristic in several *Erysiphe* species show that knowledge of Erysiphales is far from being complete. Compared to plant biogeography, mycological exploration apparently suffers from more limitations concerning funding and numbers of researchers. Such study is relevant for estimating risks for quarantine and for growing medicinal plants under particular strategies of crop marketing.

Declarations

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Additional files

Additional file: Table S1. Alignment of ITS sequences of selected Erysiphales.

Confirmation that the content of the manuscript has not been published, or submitted for publication elsewhere

The whole or part of the manuscript has not been published or under consideration for publication elsewhere.

Authors' contributions

Material preparation and data collection were performed by Yu-Wei Yeh and Roland Kirschner with help from Pei-Yi Chou. Yu-Wei Yeh and Roland Kirschner together performed the data analysis and wrote the first draft of the manuscript. Pei-Yi Chou and Hsin-Yu Hou contributed to the data interpretation. All authors read and approved the final manuscript.

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Figures

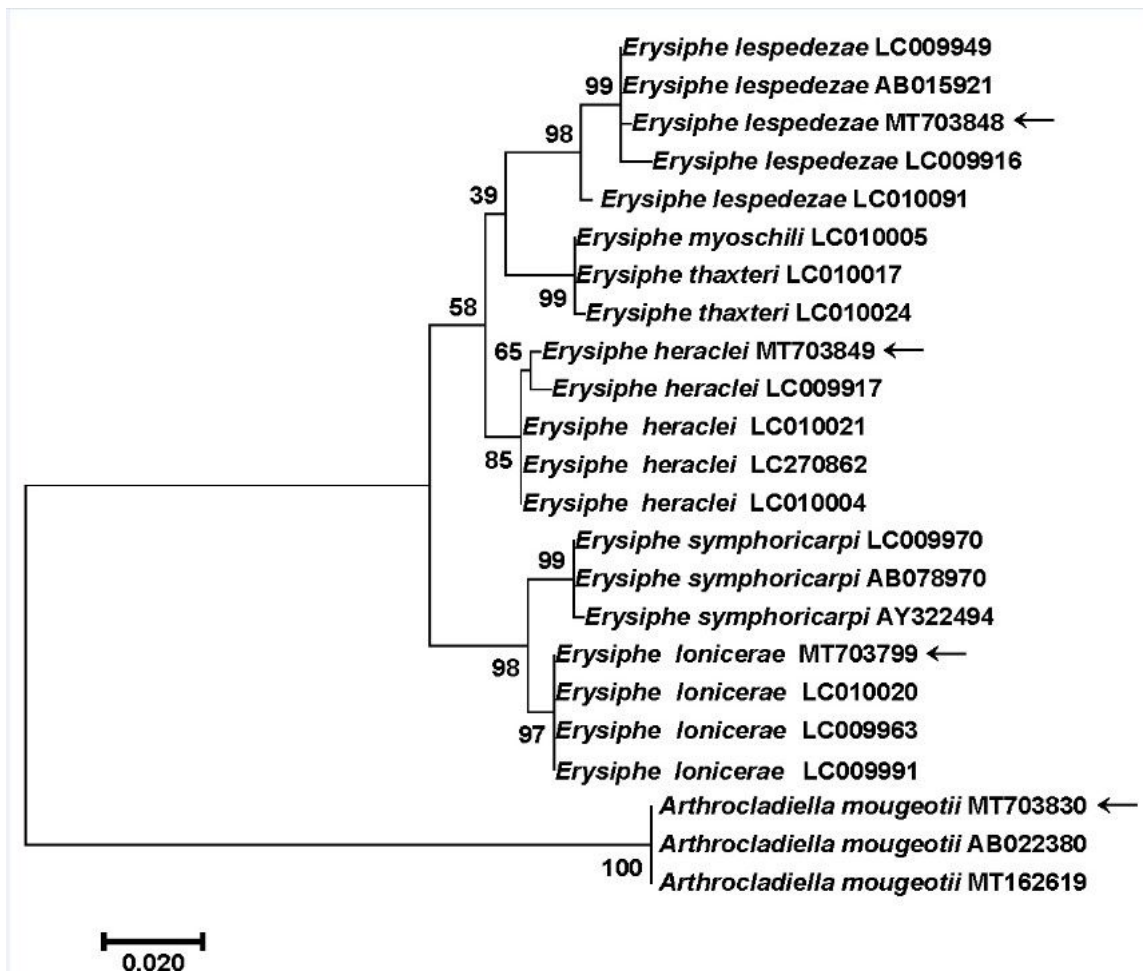


Figure 1

Maximum likelihood analysis of ITS sequences of selected Erysiphales indicating the identification of specimens on medicinal plants from Taiwan. Bootstrap values of 1000 replicates above 50% shown. Scientific name of powdery mildew followed by GenBank number (new sequences indicated with arrow).

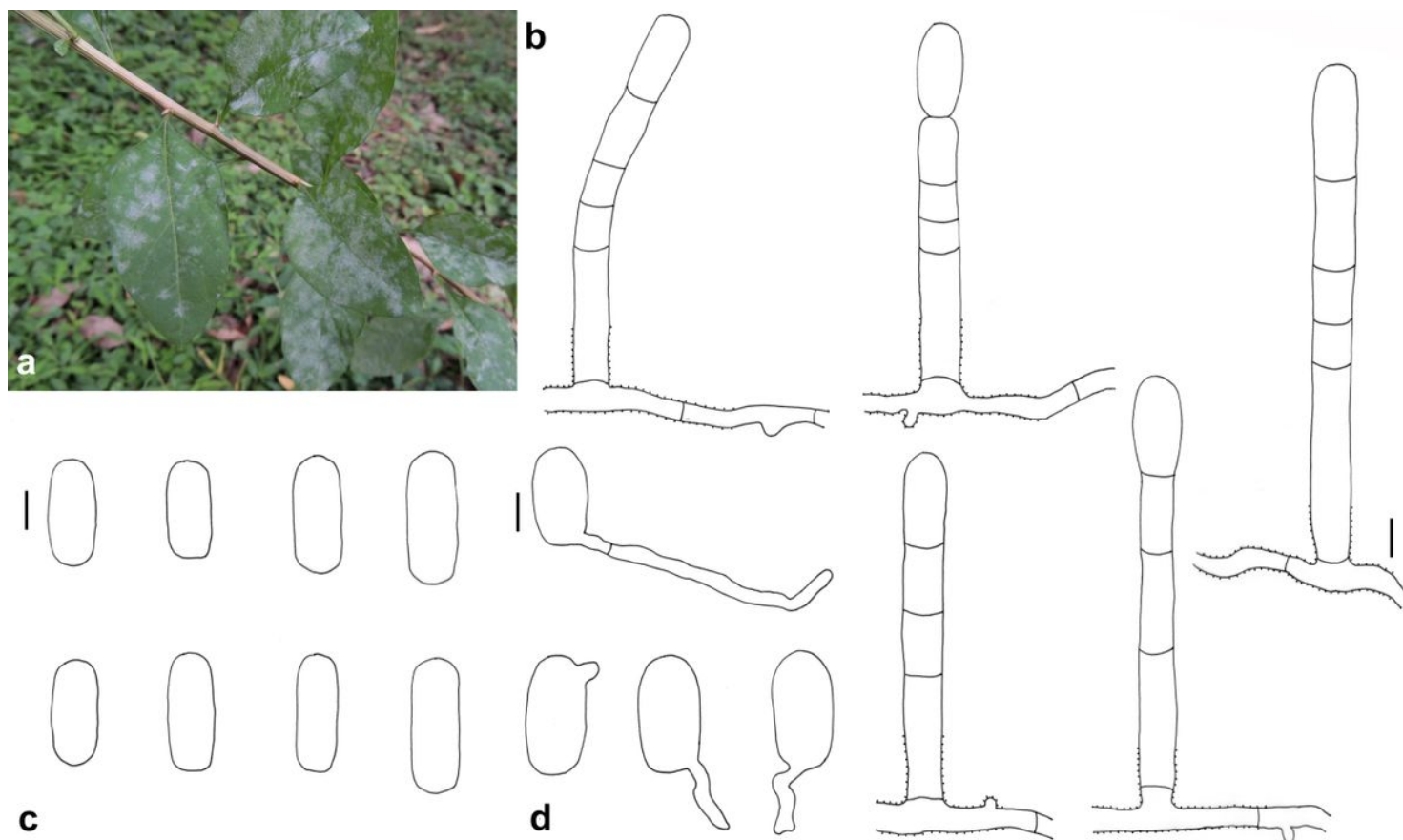


Figure 2
Arthrocladiella mougeotii on *Lycium chinense* (AKII 0082, except in a). a Powdery mildew symptoms on leaves (Taipei, National Taiwan University campus, 23 April 2015). b Hyphae with nipple-shaped appressoria and conidiophores. c Conidia. d Conidia with germination hyphae. Scale bars = 10 μm.

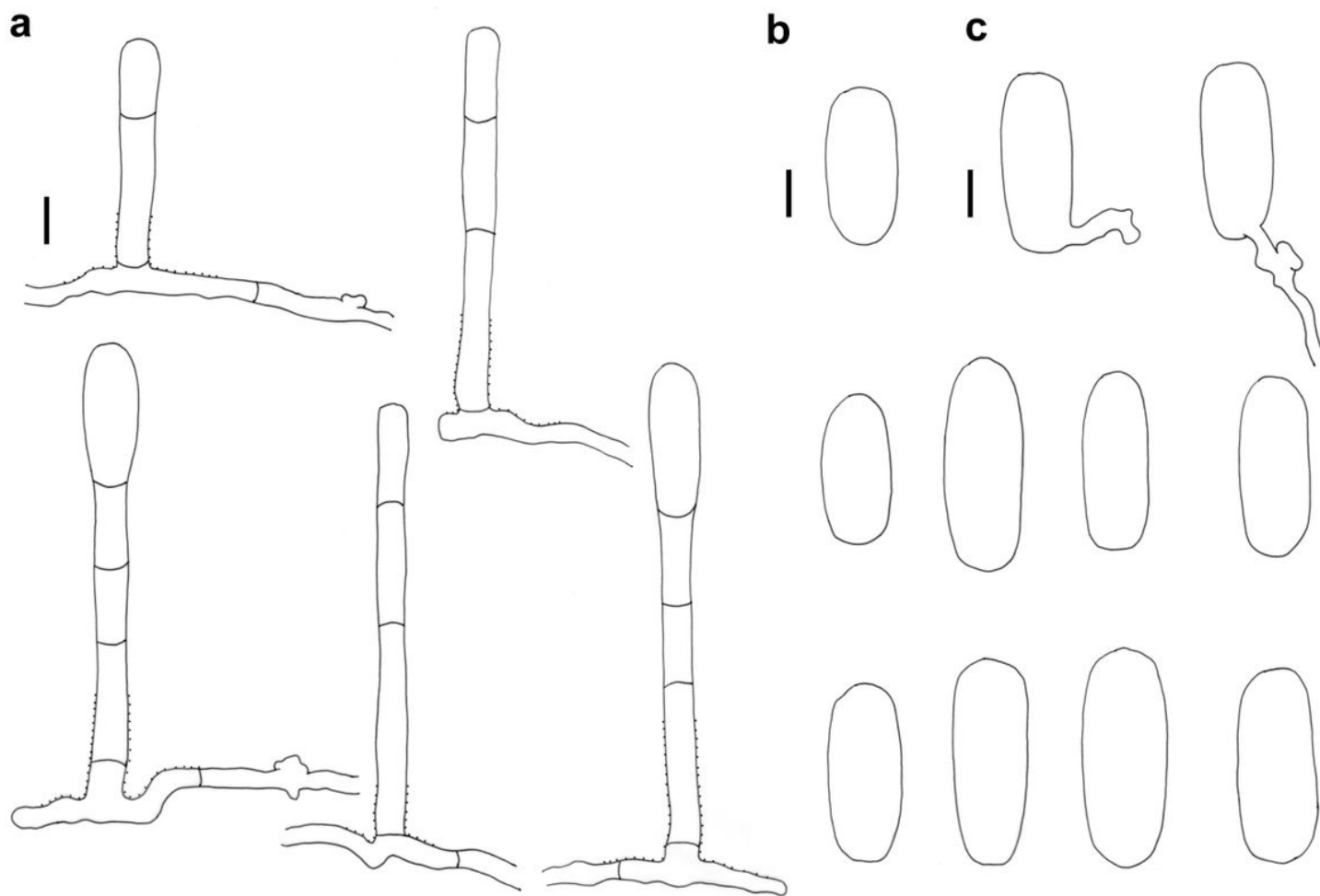


Figure 3

Erysiphe heraclei on *Eryngium foetidum* (R. Kirschner 4729). a Hyphae with lobed appressoria and conidiophores. b Conidia. c Conidia with lobed appressoria on germination hyphae. Scale bars = 10 µm.

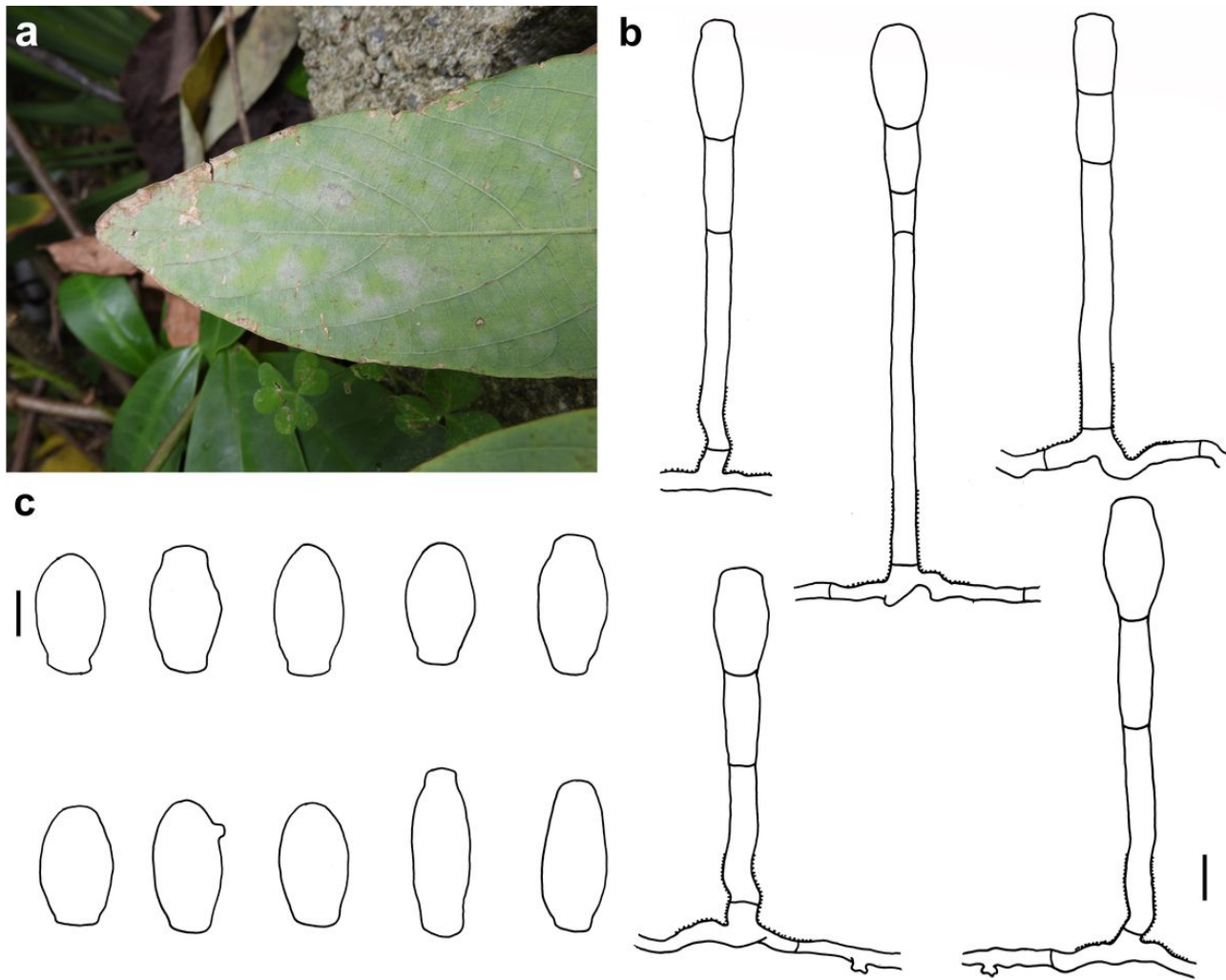


Figure 4
Erysiphe lespedezae on *Uraria crinita* (AKII 0005). a. Powdery mildew symptoms on leaves. b Hyphae with lobed appressoria and conidiophores with curved foot cell. c Conidia. Scale bars = 10 μ m.

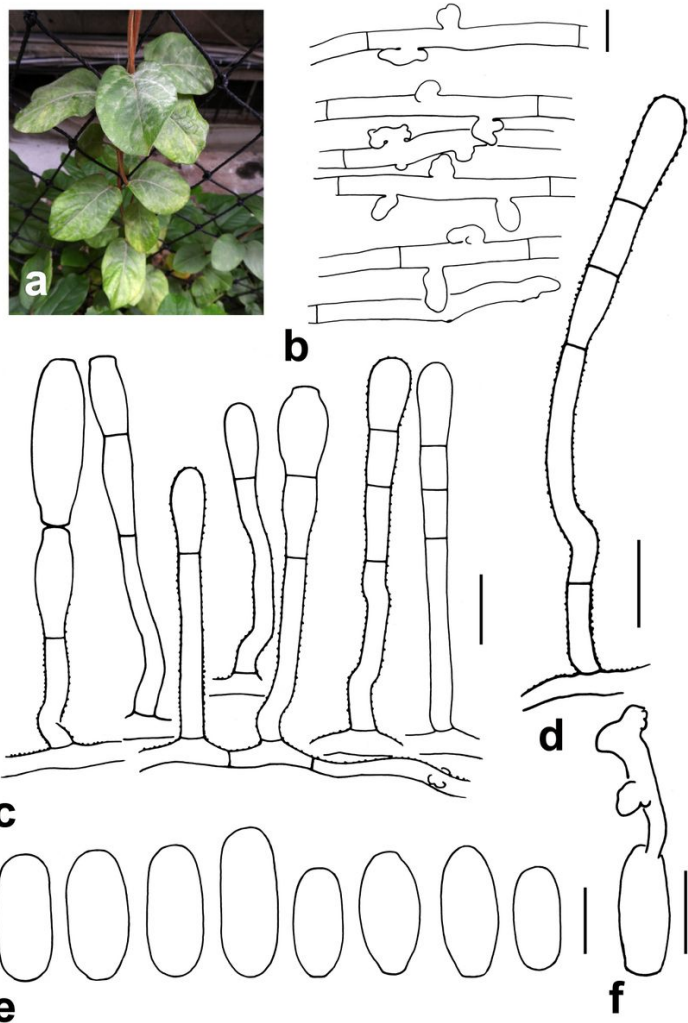


Figure 5

Erysiphe lonicerae on *Lonicera japonica* (R. Kirschner 4712, except a = R. Kirschner 4733). a Powdery mildew symptoms on leaves. b Hyphae with appressoria. c, Conidiophores with straight or curved foot cell (surface ornament not shown in all conidiophores). d Exceptional conidiophore with curvature above foot cell. e Conidia. f Conidium with appressoria on the germination hypha. Scale bars b = 10 μ m, all others = 20 μ m.

Supplementary Files

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