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Two new species of *Erysiphe* sect. *Uncinula* (Erysiphales): *Erysiphe fernandoae* and *E. michikoae*

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ABSTRACT

Two new species of *Erysiphe* section *Uncinula* are described and illustrated based on the molecular and morphological analyses: (1) *Erysiphe fernandoae* sp. nov. on *Fernandoa adenophylla* is distinct from other *Erysiphe* species on the plant family Bignoniaceae by having smaller asci, *ca* 12 appendages per chasmothecium and being found only in Asia; (2) *Erysiphe michikoae* sp. nov. on *Celtis jessoensis* differs from *Erysiphe kusanoi* on other *Celtis* species in having smaller chasmothecial, ascal and ascospore dimensions, and fewer number of chasmothecium appendages. The phylogenetic relationships of the two new species with other closely related species are discussed based on 28S and ITS rDNA sequences.

1. Introduction

Erysiphe sect. *Uncinula* (Lév.) de Bary is a powdery mildew fungi characterized by having uncinate to circinate tips of chasmothecial appendages (Braun and Takamatsu 2000). Based on the monograph of Braun and Cook (2012), 123 species and 29 varieties are described in the world. Of these, two and five species have been reported on hosts of the Bignoniaceae and Cannabaceae, respectively (Braun and Cook 2012). During collection trip to Queen Sirikit Botanical Garden (Thailand) and Mount Ibuki (Japan), we found two *Erysiphe* species with uncinuloid appendages which are morphologically distinct from existing similar species. Our phylogenetic analysis of 28S-ITS rDNA sequences from the specimens also supported the morphological elucidation. Therefore, the collections concerned are described and illustrated as new species.

2. Materials and methods

2.1. Morphological examination

Specimens on *Fernandoa adenophylla* (Wall. ex G. Don) V. Steenis (Bignoniaceae) and *Celtis jessoensis* Koidz. (Cannabaceae) were collected in Thailand and Japan, respectively. Morphological examinations were carried out as outlined in Divarangkoon et al. (2011). Mycelia and chasmothecia were stripped off from the leaf surfaces with a clean needle, mounted on a microscope slide, and examined in 3% NaOH

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using a light microscope with phase contrast 10×, 20×, and 40×objectives. Thirty chasmothecia, asci and ascospores were measured per sample. Specimens were deposited at the National Museum of Nature and Science (TNS), Japan, Mie University Mycological Herbarium (MUMH), Japan and Herbarium Martin-Luther-Universität, Halle (HAL), Germany.

2.2. *Phylogenetic analysis*

Whole-cell DNA was extracted from chasmothecia using the Chelex method (Walsh et al. 1991) as described in Hirata and Takamatsu (1996). The 5′-end of the 28S rDNA, including the domains D1 and D2, and ITS region, including the 5.8S rDNA, were amplified by polymerase chain reaction (PCR) and then sequenced using protocols as described in Takamatsu et al. (2006). Representative sequences determined in this study were deposited in DNA databases (DDBJ, EMBL, GenBank) under the accession numbers of AB693961–AB693965. Sequences generated from the rDNA ITS region and D1/D2 domains of the 28S rDNA were aligned using MEGA 5 (Tamura et al. 2011) with *Erysiphe* sequences retrieved from DNA databases. The alignments were deposited in TreeBASE (http://www.treebase.org/) under accession number S12270. Maximum parsimony (MP) analysis was done in PAUP* 4.0b8 (Swofford 2002) with the heuristic search option using the 'tree bisection reconstruction' (TBR) algorithm with 100 random sequence additions to find the global optimum tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting trees was tested with bootstrap analysis using 1000 replications (Felsenstein 1985). Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistence index (RC), were also calculated.

3. Results and discussion

3.1. *28S rDNA analysis*

A 28S rDNA sequence determined in the powdery mildew specimens collected from *F. adenophylla* and another one determined in the specimens from *C. jessoensis* were aligned with 24 sequences of *Erysiphe* species retrieved from DNA database. Of the 740 total characters used in this analysis, 622 characters were constant, 54 characters were variable and parsimony-uninformative, and 64 characters were parsimony-informative. A total of 192 equally parsimonious trees with 176 steps (CI = 0.7443, RI = 0.7877, RC = 0.5863) were

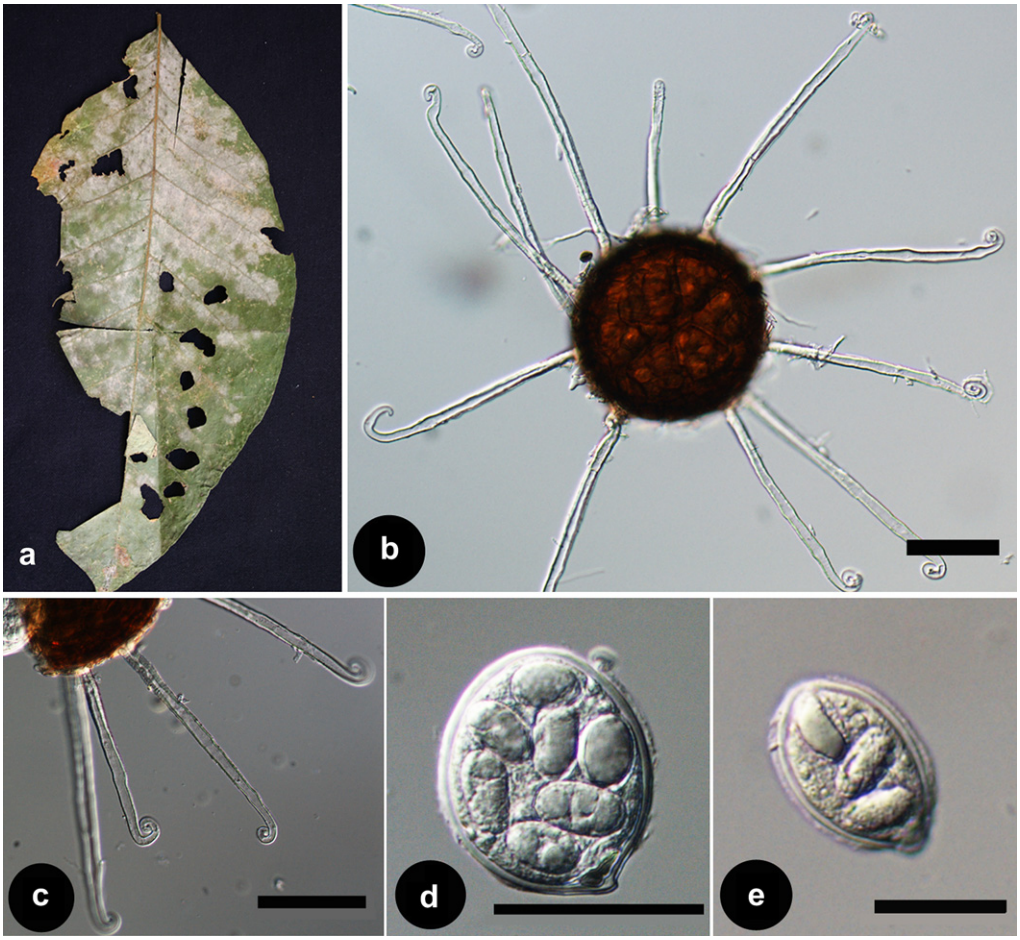

Fig. 1 – *Erysiphe fernandoae* (TNS-F-46201). a: Symptom. b: Chasmothecium. c: Appendages with circinate tip. d, e: Asci and ascospores. *Bars* 50 μm.

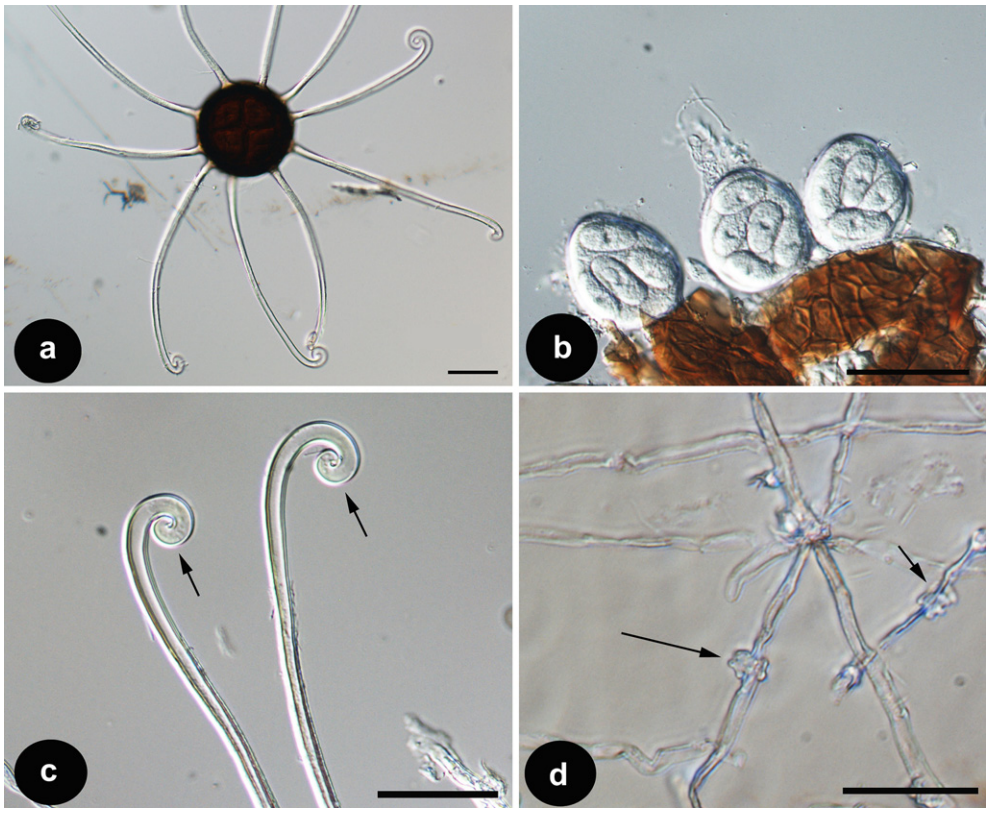

Fig. 2 – *Erysiphe michikoae* (TNS-F-46202). a: Chasmothecium. b: Asci and ascospores. c: Appendages with circinate tip. d: Appressoria. *Bars* 50 μm.

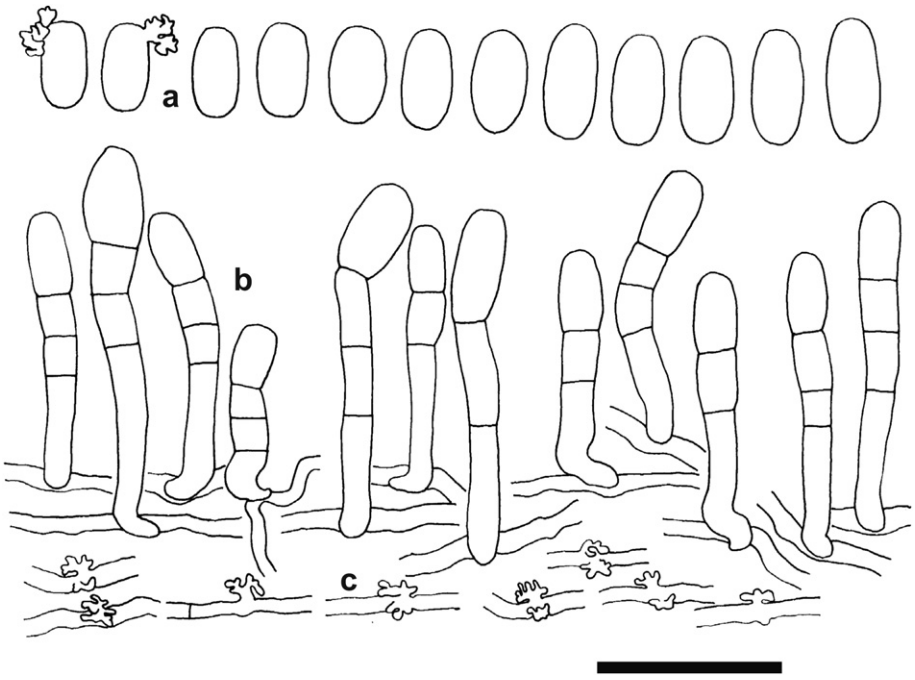

Fig. 3 – Anamorph of *E. michikoae*. a: Conidia of oval, ellipsoid, and cylindrical shape, producing *Pseudoidium*-type germ tubes. b: Conidiophores arising from terminal or lateral side of the mother cells, straight to usually curved-sinuous at the base of the foot cells, producing conidia singly followed by 2–3 cells. c: Appressoria on hyphae, formed opposite in pairs, multilobed to moderately lobed. *Bars* 50 μm.

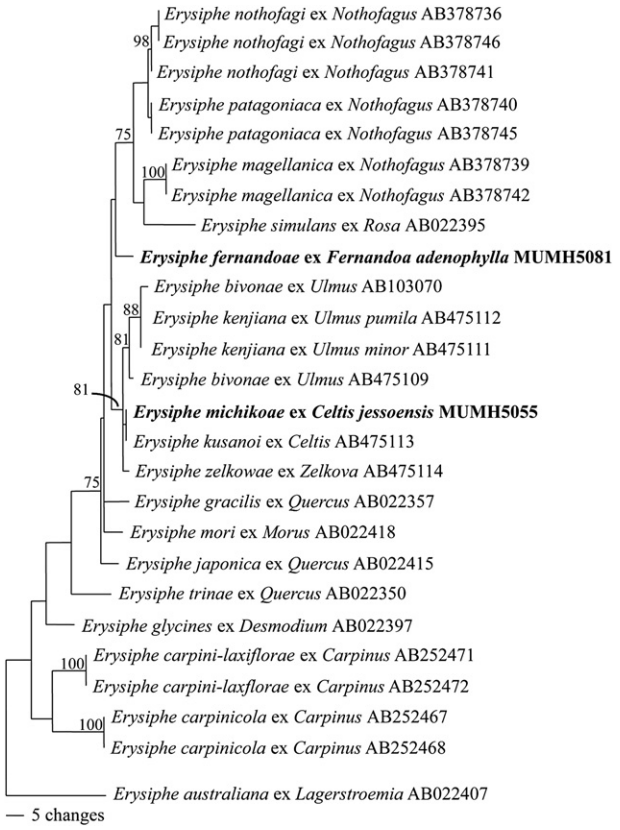

Fig. 4 – The best MP tree of *E. michikoae* and *E. fernandoae* inferred from the 28S rDNA sequences. Percentage bootstrap support (1000 replications; ≥70%) is shown on branches.

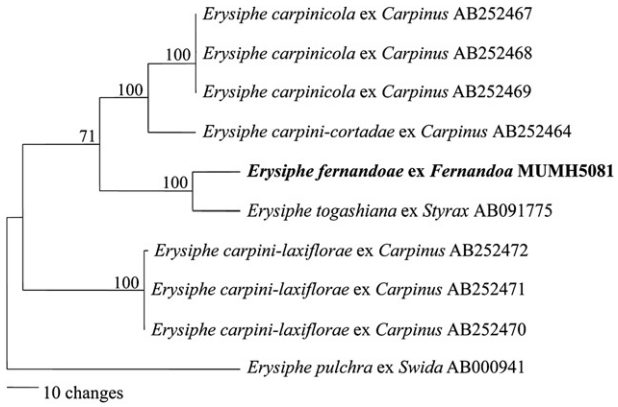

Fig. 5 – Phylogenetic analysis of the nucleotide sequences of the internal transcribed spacer (ITS) region including 5.8S rDNA for *E. fernandoae* and 9 sequences from *Erysiphe* species. Percentage bootstrap support (1000 replications; ≥70%) is shown on branches.

generated by the MP analysis. An MP tree with the highest likelihood score was shown in Fig. 4. The fungus on *C. jessoensis* nested together with *Erysiphe kusanoi* (Syd. & P. Syd.) U. Braun & S. Takam., *Erysiphe kenjiana* (Homma) U. Braun & S. Takam., and *Erysiphe ulmi* Castagne (= *Erysiphe bivonae* (Lév.) Tul. & C. Tul.) on hosts of the plant families Ulmaceae and Cannabaceae with high bootstrap support (81%). The fungus on *F. adenophylla* formed an independent lineage.

3.2. ITS analysis

Because the phylogenetic position of the fungus on *F. adenophylla* in the genus *Erysiphe* was not clear in the 28S rDNA analysis, we conducted FASTA search in EMBL DNA databank to find closely related species using the ITS sequence from the fungus on *F. adenophylla* as a query. This FASTA search revealed that *Erysiphe togashiana* (U. Braun) U. Braun & S. Takam. is the closest relative (similarity = 91.2%). We then retrieved 9 sequences with high similarity including *E. togashiana* from the DNA database and aligned with the sequence from the fungus on *F. adenophylla*. The alignment consisted of 10 sequences and 685 characters, of which 135 characters were removed from the phylogenetic analysis due to ambiguous alignment. Of the 550 remaining characters used in this analysis, 381 characters were constant, 58 characters were variable and parsimony-uninformative, and 111 characters were parsimony-informative. A total of 6 equally parsimonious trees with 245 steps (CI = 0.8898, RI = 0.8831, RC = 0.7858) were generated by the parsimony analysis. Of the 6 MP trees, a tree with the highest likelihood score was shown in Fig. 5. The fungus on *F. adenophylla* formed a clade with *E. togashiana* on *Styrax obassia* Siebold & Zucc. with 100% bootstrap support.

ITS sequence from the fungus on *C. jessoensis* was aligned with 8 sequences from *Erysiphe* species on hosts of the family Ulmaceae and Cannabaceae reported in Heluta et al. (2009) and a sequence from *Erysiphe mori* (I. Miyake) U. Braun & S. Takam. used as outgroup. The alignment composed of 10 sequences and 642 characters, of which 89 characters in the ITS2 region were removed from phylogenetic analysis due to ambiguous alignment. Of the 553 remaining characters used in MP analysis, 480 characters were constant, 56 characters were variable and parsimony-uninformative, and 17 characters were parsimony-informative. A total of 145 equally parsimonious trees with 87 steps (CI = 0.8966, RI = 0.7500, RC = 0.6724) were generated from the analysis. A tree with the highest likelihood among the 145 MP trees was shown in Fig. 6. The fungus on *C. jessoensis* grouped with *E. kusanoi* on *Celtis sinensis* Pers. with 70% bootstrap support, and this clade further grouped with *Erysiphe zelkowae* (Henn.) U. Braun on *Zelkova serrata* (Thunb.) Makino with 81% bootstrap value.

3.3. Taxonomy

Erysiphe fernandoae Meeboon, Divarangkoon & S. Takam. sp. nov. Fig. 1.

MycoBank no.: MB 564210.

Differs from *Erysiphe peruviana* in having smaller asci, fewer number of appendages per chasmothecium and in infecting *Fernandoa* (Bignoniaceae).

Type: on *F. adenophylla* (Wall. ex G. Don) V. Steenis (Bignoniaceae), Thailand, Chiang Mai Province, Queen Sirikit Botanic

Fig. 6 – Phylogenetic analysis of the nucleotide sequences of the internal transcribed spacer (ITS) region including 5.8S rDNA for *E. michikoae* and 9 sequences from *Erysiphe* species. Percentage bootstrap support (1000 replications; ≥70%) is shown on branches.

Garden, 15 March 2009 (Holotypus, TNS-F-46201; Isotypus, HAL2463F and MUMH 5081).

rDNA sequence ex holotype: AB693962 (ITS), AB693964 (28S).

Etymology: The new species is named after host plant.

Colonies on leaves amphigenous, mainly epiphyllous, forming as white patches or dense on the host surfaces. Hyphae 3–5 μm wide, hyaline. Appressoria (7–)8–11(–15) μm diam. (10 μm in average), lobed, single or occasionally opposite in pairs. Chasmothecia scattered to gregarious, blackish-brown, globose, (105–)106–120(–123) μm diam. (112 μm in average), containing 3–7 asci. Peridial cell irregular in shape, 9–24 μm wide. Appendages (111–)118–161(–168) × (5–)6–8(–10) μm (140 × 7 μm in average), *ca* 12 per chasmothecium, aseptate, hyaline, equatorial, straight to curved or flexuous, thin-walled, wall sometimes thicker at the base, smooth to rough, apex uncinate to subhelicoid, narrowing slightly toward the apex. Asci (42–)45–55(–58) × (33–)34–38(–41) μm (49 × 36 μm in average), ovoid to saccate, 4–7-spored, sessile or short-stalked, hyaline. Ascospores (11–)15–22(–24) × (7–)8–11 μm (19 × 9.5 μm in average), ellipsoid-ovoid, hyaline, unicellular.

Comments: Bignoniaceae are distributed mainly in Neotropical area of South America, Africa and South East Asia, and are composed of 82 genera and 827 species. Two species, *Erysiphe sibiliae* (Ciccar.) U. Braun & S. Takam. and *E. peruviana* (Syd.) U. Braun & S. Takam., have been reported as *Erysiphe* species having uncinuloid appendages found on host of this plant family (Braun and Cook 2012). Of the two species, only *E. peruviana* is morphologically similar to the proposed new species (*E. fernandoae*) by having amphigenous colonies on the host surface, chasmothecial size, number of asci per chasmothecium and ascospore size; however *E. fernandoae* differs from *E. peruviana* in having smaller asci, fewer appendages per chasmothecium. In addition, *E. peruviana* and *E. sibiliae* are distributed only in North and South America, and Africa, respectively. Phylogenetic analysis of ITS rDNA sequences of *Erysiphe* sect. *Uncinula* from different plant families indicated a close relationship between *E. fernandoae* and *E. togashiana* on *S. obassia* (Styracaceae) (Fig. 5). Both species are morphologically similar in the size of chasmothecia, peridial cells, asci and ascospores, but, our morphological observation of *E. fernandoae* showed that the colonies of this fungus were mainly found epigenous and the number of asci in the chasmothecia is fewer than in *E. togashiana*. Braun (1982) noted that chasmothecia of *E. togashiana* are characterized by having two types of appendages and the length of chasmothecial appendages is 1–2 times as long as the chasmothecial diameter, i.e. clearly longer than the appendages of *E. fernandoae*.

Phylogenetic analysis of ITS rDNA sequences showed that *E. fernandoae* forms a clade with *E. togashiana* with 100% bootstrap support. However, there are 30 step differences between *E. fernandoae* and *E. togashiana* in the tree (Fig. 5). When the 135 characters removed from the phylogenetic analysis were added, there are 56 substitutions and 90 indels within the 678 aligned characters (similarity = 78.5%). The nucleotide length of ITS region including 5.8S rDNA was 676 bp and 585 bp for *E. fernandoae* and *E. togashiana*, respectively. Thus, *E. fernandoae* is not closely related to *E. togashiana* in phylogeny. This was also supported by the independent phylogenetic situation of *E. fernandoae* in the 28S rDNA analysis.

The host plant *F. adenophylla* is a deciduous tree distributed in South East Asia and is cultivated as an ornamental tree. This is the first record of powdery mildew on *Fernandoa*.

Erysiphe michikoae S. Takam. & Meeboon sp. nov. Figs. 2a–d and 3.

MycoBank no.: MB 564211.

Differs from *E. kusanoi* and *Erysiphe celtidis* in having smaller chasmothecia and fewer number of ascospores per ascus.

Type: on *C. jessoensis* Koidz. (Cannabaceae), Japan, Shiga Prefecture, Mount Ibuki, 22 Oct 2010; from the same host and locality, 28 Sep 2011 (Holotypus, TNS-F-46202; Isotypus, HAL2462F, MUMH 5055 and MUMH5310).

rDNA sequence ex holotype: AB693963 (ITS), AB693965 (28S).

Etymology: The new species is named in honor of the late Ms. Michiko Amano who supported the study of the late Prof. Koji Amano.

Teleomorph: Colonies on the leaves amphigenous, persistent, and forming white patches on the host surfaces. Hyphae 3–4 μm wide, hyaline. Appressoria (6–)7–10(–11) μm diameter (8.71 μm in average), lobed, single or occasionally opposite in pairs. Chasmothecia scattered to gregarious, black to dark brown, globose, (62–)66–86(–96) μm diam. (75 μm in average), containing 3–4 asci. Peridial cell 10–28 μm wide, irregular in shape. Appendages (137–)149–236(–282) × 5–7(–8) μm (199 × 6 μm in average), 8–13 per chasmothecium, long, 0 (–1)-septate, hyaline, equatorial, straight to curved, thin-walled, wall sometimes thicker at the base, smooth to rough, apex uncinate, curved part somewhat enlarged. Asci (34–)37–46(–50) × (24–)29–38(–39) μm (41.48 × 33 μm in average), saccate, 4–5-spored, sessile or short-stalked, hyaline. Ascospores (12–)13–21(–23) × (9–)10–12(–13) μm (18.19 × 10.93 μm in average), ellipsoid-ovoid, hyaline, unicellular.

Anamorph: Colonies appear as irregular white patches on the upper and lower sides of the leaves. Hyphae substraight to

flexuous. Appressoria on hyphae opposite in pairs or singly, multilobed to moderately lobed. Conidiophores are formed singly, terminal or lateral on mother cells, (50–) 64.5–97(–100.5) × (5.5–)6.5–9(–10.5) μm in size, straight to usually curved-sinuous at the base of foot cells, producing conidia singly followed by 2–3 cells, with a basal septum at the branching point of the mycelium. Conidia oval, ellipsoid, or cylindrical without conspicuous fibrosin bodies, (22–) 24–29.5(–32.5) × (11–)11.5–14.5(–15) μm in size, producing *Pseudoidium*-type germ tubes on a shoulder. These characters indicate that this fungus belongs to *Oidium* subgenus *Pseudoidium* Jacz.

Comments: *C. jessoensis* is a deciduous tree belonging to the Cannabaceae and is distributed in East Asia (Japan, Korea and northeast part of China). Three *Erysiphe* species with uncinuloid appendage tip have been recorded on *Celtis* spp. Among these species, *Erysiphe parvula* (Cooke & Peck) U. Braun & S. Takam. is clearly distinguished from *E. michikoae* by the numerous appendages. *E. kusanoi* is similar to *E. michikoae*, but differs from the latter species by its larger chasmothecia (85–130 μm vs 66–86 μm) and comparatively shorter appendages (0.75–1.5 times vs 2–3 times of chasmothecia diameter). *E. celtidis* (Shvartsman & Kusnezowa) U. Braun & S. Takam. is most similar to *E. michikoae* in morphology, but differs from the latter species by its larger chasmothecia (80–120 μm vs 66–86 μm) and larger number of ascospores per ascus (5–7 vs 4–5). Phylogenetic analysis of ITS rDNA sequences showed that *E. michikoae* forms a clade with *E. kusanoi* with relatively high bootstrap support (70%). Although there are only 3 steps between *E. michikoae* and *E. kusanoi* in the tree (Fig. 6), there are 21 substitutions and 12 indels within the 622 aligned characters (similarity = 94.7%) when the 89 sequences removed from the phylogenetic analysis were added. This relatively high genetic diversity suggests difference of species level, although sequence of *E. celtidis* is required for further verification.

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