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First record of *Erysiphe pulchra* in China on a new host species

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Abstract — *Erysiphe pulchra* (*Erysiphe* sect. *Microsphaera*) was identified on *Cornus kousa* subsp. *chinensis* (≡ *Dendrobenthamia japonica* var. *chinensis*). This is the first record of this species in China, and *C. kousa* subsp. *chinensis* is a new host plant for *E. pulchra*. The Chinese specimen is described, illustrated, and discussed and its affinity has been confirmed using molecular methods.

Key words — powdery mildew, taxonomy, morphology, phylogeny

Introduction

The Qinling Mountain range, which lies at the interface of several floristic regions in north, central, and southwest China, forms a natural boundary between the warm temperate and northern subtropical zones of China, creating unique geographical conditions with characteristic plant and fungal assemblages. Recently, a powdery mildew-forming chasmothecium with dichotomous appendages found growing on *Cornus kousa* subsp. *chinensis* (*Dendrobenthamia japonica* var. *chinensis*) was morphologically identified as *Erysiphe pulchra*.

This species had not been previously recorded in China (Zheng & Yu 1987, Wu & Wu 1991, Chen 1993, Wang et al. 2002, Liu 2010). Therefore, to verify our morphological identification, we analyzed phylogenetically the rDNA ITS sequence from this fungus.

Materials & methods

Living leaves of *Cornus kousa* subsp. *chinensis* containing the powdery mildew teleomorph of the fungus were collected during September 2012 and 2013 in the Qinling Mountains (Taibaihe town) in China. Herbarium specimens were deposited in the Mycological Herbarium of Forestry College, Northwest A & F University, Yangling, Shaanxi Province, China (HMNWAFU).

The specimen was mounted in distilled water and examined by light microscopy (Olympus CX31RTSF, Japan). The teleomorphic features of the fungus, including chasmothecia, appendages, asci, and ascospores, were described, measured, and photographed.

Genomic DNA was extracted from the chasmothecia with Chelex-100 (Walsh et al. 1991; Hirata & Takamatsu 1996). The nuclear rDNA region of the ITS regions (ITS1, ITS2) and 5.8S rRNA gene was amplified by nested PCR, first using the primers ITS5 (White et al. 1990) and P3 (Kusaba & Tsuge 1995) and then the primers PM5 (Takamatsu & Kano 2001) and ITS4 (White et al. 1990). The PCR reactions were carried out in a final volume of 50 µL, containing 27 µL 2× BoisTaq PCR MasterMix, 1 µL each primer; 1 µL extracted DNA, and 20 µL ddH_2O (Hirata & Takamatsu 1996). Thermal cycling in a PTC-200 thermal cycler (BioRad) comprised an initial denaturation step at 95°C for 5 min, 35 cycles of 94°C for 1 min + 60°C for 1 min + 72°C for 1 min, and a final elongation step at 72°C for 8 min. A negative control for each set of reactions replaced template DNA with ddH_2O. The PCR products were separated by electrophoresis on a 2% agarose gel in TAE buffer and purified using the Zymoclean™ Gel DNA Recovery Kit, according to the manufacturer's instructions. The purified DNA products were ligated into the pMD18-T vector (Takara) and transformed into *E. coli* DH5α cells. The cloned fragments were sequenced by Sangon Biotech (Shanghai) Co., Ltd.

All DNA sequences were aligned using Clustal X 1.81 (Thompson et al. 1997), and the alignments were adjusted following Nei & Kumar (2000). All positions containing gaps or missing data were eliminated from the dataset. Cladistic trees were constructed using the neighbor-joining method with the Kimura 2-parameter substitution model in MEGA 4.0 (Tamura et al. 2007). Branch robustness was assessed by bootstrap analysis with 1,000 replicates.

Results

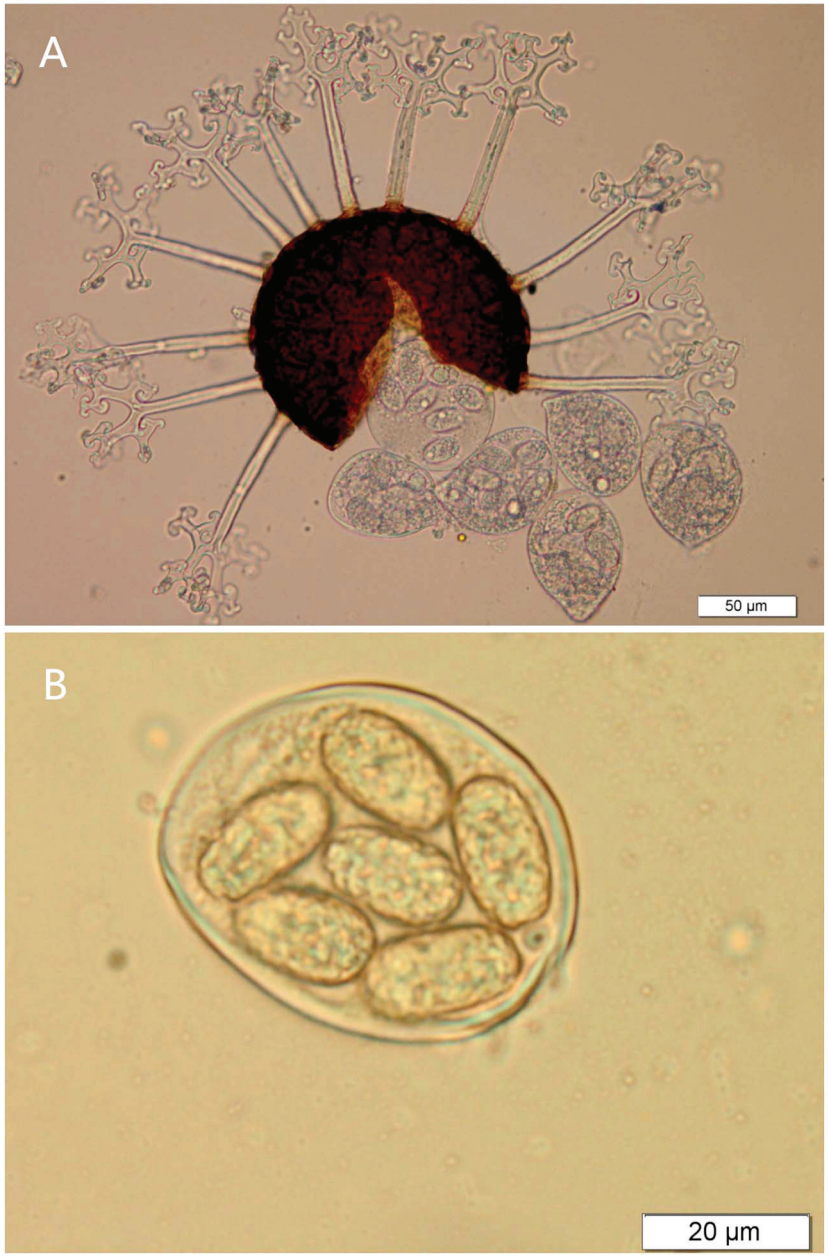
Taxonomic description

Erysiphe pulchra (Cooke & Peck) U. Braun & S. Takam., Schlechtendalia 4: 12. 2000. Pl. 1

= *Microsphaera japonica* Henn., Bot. Jb. 28: 326. 1900.

Mycelium amphigenous, mostly epiphyllous, forming patches or evanescent. Chasmothecia scattered to gregarious, globose or depressed globose, (87.5–) 95–125 µm diam.; peridium cells irregularly polygonal, 7.5–25(–30) µm diam.; appendages 6–12, equatorial, 0.8–1 times the length of the chasmothecial diam., 7.5–10 µm wide along the lower half, aseptate, walls thin above, thick towards the base, smooth to somewhat rough, hyaline or pale brown at the base, apices 3–5 times densely and regularly dichotomously branched, tips distinctly recurved; asci 4–6, broadly ellipsoid-obovoid or subglobose, 55–65 × 42.5–50 µm, stalked or sessile; ascospores 6–8, broadly ellipsoid, 15–22.5 × 10–12.5 µm, colorless.

Specimen examined: **China, Shaanxi,** Qinling Mountains, Taibaihe County, 33°48′57″N 107°12′06″E, alt. 1700 m, on living leaves of *Cornus kousa* subsp. *chinensis*



Pl. 1. *Erysiphe pulchra* (HMNWAFU-CF 2012092) A. Chasmothecia and appendages; B. Asci and ascospores.

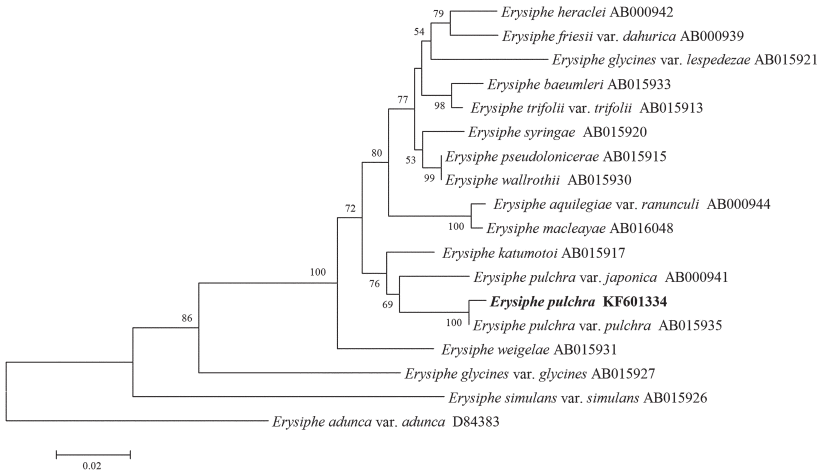


Fig. 1. A neighbor-joining tree based on distances derived from the ITS1-5.8S-ITS2 rDNA sequences from 18 *Erysiphe* taxa, with the Chinese representative of *Erysiphe pulchra* shown in bold. The bar indicates a distance of 0.02.

(Osborn) Q.Y. Xiang (*Cornaceae*), Sep. 2012, L.C. Bai (HMNWAFU-CF 2012092; GenBank KF601334)

Phylogenetic analysis Fig. 1

We phylogenetically compared the new sequence from our specimen with sequences from 17 *Erysiphe* taxa downloaded from GenBank, including *E. simulans* (E.S. Salmon) U. Braun & S. Takam. var. *simulans* and *E. adunca* (Wallr.) Fr. var. *adunca* as outgroup; these 17 sequences were included in the molecular analysis of Takamatsu et al. (1999). The 449 bp long ITS1-5.8S-ITS2 region from the powdery mildew species examined in this study has been deposited in GenBank (KF601334). The DNA sequence retrieved from the Chinese specimen clustered within a strongly supported clade (bootstrap value = 100%) and formed a subclade (bootstrap value = 100%) with the sequence from a Japanese specimen of *Erysiphe pulchra* var *pulchra* on *Cornus kousa* (Fig. 1), indicating that these two sequences represent the same species.

Discussion

The anamorph of *Erysiphe pulchra* has not been identified within collections made in the past two years. However, the chasmothecium we sampled is very similar to descriptions of *Erysiphe pulchra* published by Braun (1987) and Braun & Cook (2012), with the exception of somewhat shorter appendages (0.8–1

times as long as the chasmothecial diam. in the Chinese materials versus 1–2 times in the cited monographs). We follow the taxonomic characterization of *E. pulchra* recently published by Braun & Cook (2012), who reduced *Microsphaera japonica* to synonymy with *E. pulchra* based on Japanese-type materials. Other than our study, all available data on molecular sequence analyses of *E. pulchra* have been exclusively based on Japanese samples. To elucidate the genetic structure of this species and determine the relationship between Asian and North American collections, it will also be necessary to analyze sequences from North American materials.

According to the host plant records for this powdery mildew (Braun & Cook 2012), *Cornus kousa* subsp. *chinensis* appears to be a new host for *E. pulchra.*

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