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Morphological and molecular identification of *Melampsora idesiae* on *Idesia polycarpa* in Korea

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Abstract During extensive surveys of phytopathogenic fungi in Korea, rust-infected leaves of *Idesia polycarpa* were found and deposited in the Korea University Herbarium. Seven of these samples were examined, and the fungus was identified as *Melampsora idesiae* on the basis of morphological characteristics. Analyses of the large subunit (LSU) rDNA region of three samples confirmed their placement in the *Melampsora* clade. In this paper, we provide the detailed morphological features and LSU sequences of *M. idesiae* for the first time.

Keywords 28S rDNA · *Idesia* · Molecular data · Morphology · Rust fungus

Idesia polycarpa, belonging to the tribe Flacourtieae of the family Salicaceae, is native to East Asia including China, Japan, Korea and Taiwan. This tree is planted for ornamental purpose in gardens and parks in southern part of Korea. In addition, the seeds of the tree have been used as an insecticide, and the leaves have been known to have hemostatic activity (Kim 1996).

In November 2000, the leaves of *I. polycarpa* were found to be infected with a rust in Wando, Korea. Careful observations of infected plants revealed that typical rust pustules of both the uredinial and telial spore stages were formed on the abaxial leaf surface with corresponding small yellowish to chlorotic or necrotic lesions on the upper surface (Fig. 1a, b). Severe infections often resulted in leaf yellowing and premature defoliation (Fig. 1a, b). No symptoms were observed on the stems and fruits. During our surveys for phytopathogenic fungi in the southern part of Korea, similar or identical signs with typical rust pustules on *I. polycarpa* leaves were additionally found in Buan, Jeju, and Seogwipo. A literature review showed that *Melampsora idesiae* has been reported on *I. polycarpa* in Japan (Hiratsuka et al. 1992) and China (Tai 1979), however there is no report of this taxon from Korea (Korean Society of Plant Pathology 2009; Farr and Rossman 2017). This study is the first to report *M. idesiae* from Korea and provide a molecular barcode for the Large Subunit region of ribosomal DNA for this taxon.

Fresh specimens were mounted in water in preparation for microscopic examination. The morphological characteristics of the fungal structures were examined using bright-field and differential-interference contrast microscopy using an Olympus BX51 microscope (Olympus, Tokyo, Japan) for measurements, and with a Zeiss AX10 microscope equipped with an AxioCam MRc5 camera (Carl Zeiss, Oberkochen, Germany) for photographs. At least 30 measurements were taken of each structure. The following seven representative voucher specimens deposited in the Korea University Herbarium (KUS) were examined: KUS-F18130 (Wando: 34°21′3″N, 126°40′06″E; November 13, 2000), F24940 (Seogwipo: 33°19′15″N, 126°35′36″E; June 13, 2010), F25042 (Buan: 35°37′04″N, 126°35′13″E; July 12, 2010), F27126 (Jeju: 33°25′50″N, 126°34′43″E; October 15, 2012), F27945 (Wando: 34°21′31″N, 126°40′06″E; July 23, 2014), F29304 (Seogwipo: 33°19′15″N, 126°35′36″E; July 14, 2016), F29405 (Jeju: 33°25′50″N, 126°34′43″E; September 6, 2016). Morphological characterization was based on the sample KUS-F18130 as reference.

No spermogonia and aecia were found. The uredinia were hypophyllous, scattered, orange-yellow, erumpent, and 100–300 μm in diameter. Urediniospores were subglobose to

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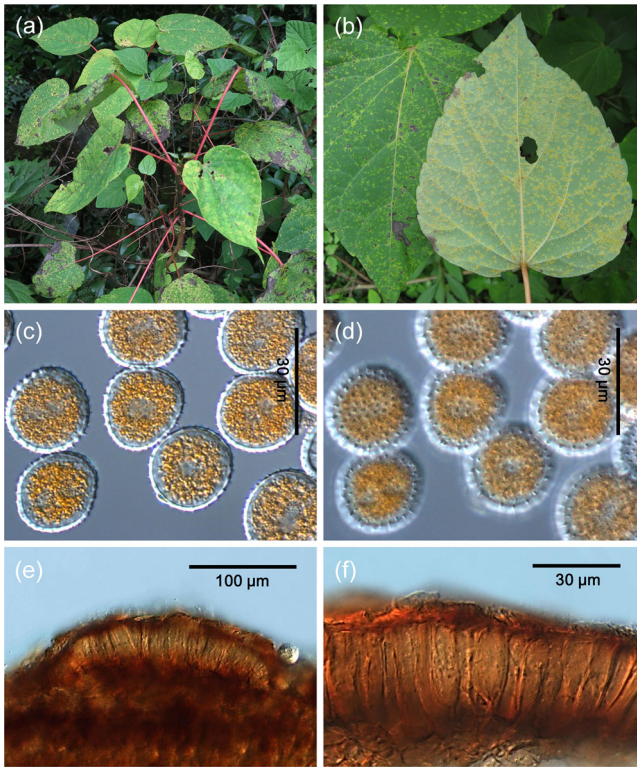

Fig. 1 Rust of *Idesia polycarpa* caused by *Melampsora idesiae*. **a**, **b**, Symptoms on leaves. **c**, **d**, Urediniospores with echinulations. **e**, Telia. **f**, Teliospores

ellipsoid, but somewhat irregular and variable in shape, yellow to orange, echinulate, 18–22 × 15–22 µm, with a wall thickness of 1.5–3 µm (Fig. 1c, d). The paraphyses were hyaline, smooth, clavate, and 17–25 µm broad at the upper part. The telia were hypophyllous, subepidermal, becoming confluent, reddish-brown, and 100–400 µm in diameter (Fig. 1e). The teliospores were brown, oblong, and 28–48 × 6–11 µm, with a wall thickness of 1–2 µm (Fig. 1f). These morphological characteristics were consistent with those reported for *Melampsora idesiae* (Tai 1979; Hiratsuka et al. 1992).

For further characterization of the Korean isolates, genomic DNA was extracted from three herbarium specimens (KUS-F24940, F25042, and F29304). The large subunit (LSU) of the ribosomal DNA (rDNA) including the D1/D2 regions was amplified by polymerase chain reaction (PCR) using the primers LR0R (Moncalvo et al. 1995) and LR6 (Vilgalys and Hester 1990). Amplication parameters were described as in Aime (2006). The PCR products were then purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced on an ABI Prism TM 377 automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA), using the BigDye™ cycle sequencing kit version 3.1 (Applied Biosystems) and the same primers used in the PCR. The obtained sequences were edited using the DNASTAR computer software package version 5.05 (Lasergene, Madison, WI, USA).

A phylogenetic tree was determined based on an alignment of the LSU region of *M. idesiae* and six closely related species of *Melampsora* obtained from GenBank, with *Pucciniastrum agrimoniae* as an outgroup. Alignments were conducted with an online version of MAFFT 7 (Katoh and Standley 2013). The phylogenetic analysis was carried out using the Molecular Evolutionary Genetics Analysis Software (MEGA 7) program (Kumar et al. 2016), and a phylogenetic tree was constructed using the neighbor-joining method (NJ). The robustness of the NJ tree was evaluated with 1000 bootstrap (BS) replicates. Numbers above the branches of the phylogenetic tree shown in Fig. 2 represent BS values over 70%.

Sequences were deposited in GenBank (Accession Nos. KX944283, KX944284, and KX944285). The LSU sequences for the three collections were identical to each other. A BLASTn search of the LSU sequences of *M. idesiae* had highest identity to *M. euphorbiae-gerardianae* (98%, 1151/1176 identities). In the NJ tree based on the LSU sequences, the Korean isolates were placed in the *Melampsora* clade (Fig. 2).

Although *M. idesiae* has been recorded in Japan (Hiratsuka et al. 1992) and China (Tai 1979) with morphological descriptions of the uredinial and telial spore stages, the aecial host has not been identified to date. However, the Japanese and Korean records were nearly identical to each

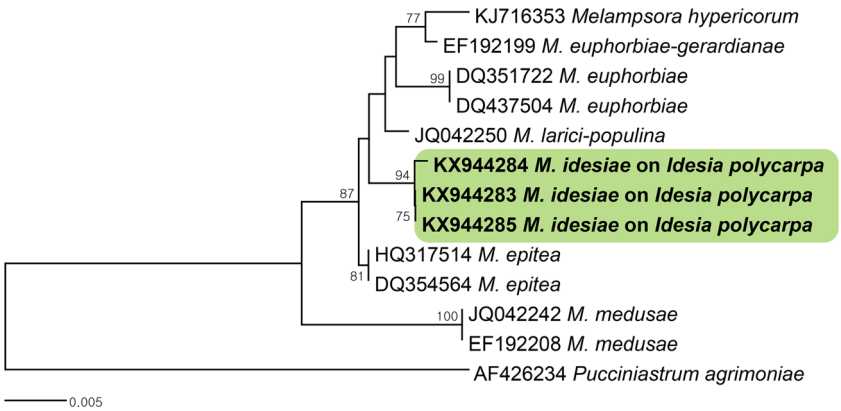

Fig. 2 Phylogenetic relationship between *Melampsora idesiae* and some reference isolates of *Melampsora* species retrieved from GenBank, inferred by the NJ method using sequences of the LSU regions. The percentage bootstrap support values (1000 replications; ≥ 70%) are shown on the branches. The scale bar indicates the number of nucleotide substitutions per site. The isolates used in this study are shown in bold

other and also are close to the morphological characteristics of *M. idesiae*. Furthermore, the LSU sequences of *M. idesiae* provided here for the first time will be helpful for elucidating its aecial host plant in the near future. To our knowledge, this is the first report of *M. idesiae* on *I. polycarpa* in Korea.

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