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Erysiphe takamatsui, a powdery mildew of lotus: Rediscovery of teleomorph after 40 years, morphology and phylogeny

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ABSTRACT

The teleomorph of *Erysiphe takamatsui*, a powdery mildew of lotus, was found in 2012 at a lotus pond of Niigata-shi, Japan, where this species was first found in 1974. This is the second record of teleomorph of *E. takamatsui*. Morphological and molecular analyses revealed that the asexual stages of the lotus powdery mildews found in Aichi, Osaka and Tokyo represent the anamorph of *E. takamatsui*. The nucleotide sequences of the internal transcribed spacer (ITS) and 28S rRNA gene were identical to those of *E. aquilegiae*, *E. catalpae*, and *E. macleayae*. These species formed a homogeneous clade together with *E. sedi*, *Pseudoidium neolycopersici*, and *Pseudoidium* spp. occurring on a wide range of plant families with only five base substitutions of ITS sequences in maximum. These results suggest that *E. takamatsui* is a fungus that appeared as a result of recent host expansion.

1. Introduction

Lotus (*Nelumbo nucifera* Gaertn.) is an aquatic perennial plant of the family Nelumbonaceae, distributed in Asia (from Japan to India) and northern part of Australia. Due to its large and beautiful flowers, lotus is commonly cultivated in water gardens. The rhizome is used as a vegetable and the roots are also used in traditional Asian herbal medicine. *Ovulariopsis eliadei* Negru was described as a powdery mildew of lotus in 1967 from Rumania (Negru 1967). However, based on the original illustration, this species seems to be a fungus different from powdery mildews (Braun 1987). Takamatsu (1977) found a powdery mildew on lotus in a lotus pond of Hakusan Shrine, Niigata-shi, Japan in 1974. He described morphological characteristics of this fungus, published with scanning electron microscopic (SEM) micrographs and provided illustrations of the teleomorph, but this fungus was neither assigned to any known species nor formally described as new species. Nomura (1997) proposed the new species *Erysiphe takamatsui* Y. Nomura based on this specimen. About 30 years after the first discovery of this fungus, we found a *Pseudoidium* anamorph on lotus at the Higashiyama Zoo and Botanical Garden, Nagoya-shi, Aichi, Japan, in 2005. Additional anamorph

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collections on lotus have been made in Osaka and Tokyo, and also at the Queen Sirikit Botanical Garden in Chiangmai, Thailand. Because *E. magnifica* (U. Braun) U. Braun & S. Takam. (anamorph: *Pseudoidium*) was reported on lotus in Germany (Kirschner 2010), it could not be ruled out that the anamorphs found in Japan and Thailand belong to *E. magnifica*. One of the authors (ST) found teleomorph and anamorph of the powdery mildew on lotus leaves again in October 2012 at the same lotus pond where *E. takamatsui* was first collected 40 years since. This study was conducted to (1) describe morphological characteristics in detail and (2) clarify the phylogenetic placement of *E. takamatsui*.

2. Materials and methods

2.1. *Morphological examination*

Morphological examinations were conducted according to the procedure described by Meeboon and Takamatsu (2013b). For the observation of teleomorph, chasmothecia were stripped off from the leaf surfaces with a clean needle, mounted on a microscope slide, and examined in 3% NaOH using a standard light microscope (Axio Imager; Carl Zeiss, Göttingen, Germany) and differential interference contrast optical instruments and devices. To examine the anamorph, hyphae, conidiophores, and conidia of fresh collections were stripped off from the leaf surfaces with clear adhesive tape, mounted on a microscope slide with the fungal mycelium uppermost, and examined in water. Herbarium samples were rehydrated before examination by boiling a small piece of infected leaf with the fungal mycelium downwards in a drop of lactic acid on a slide (Shin and La 1993). After boiling, the rehydrated mycelium was scraped off and mounted in lactic acid using a light microscope. Thirty chasmothecia, conidia, and conidiophores were measured for each specimen examined. Specimens were deposited at the National Museum of Nature and Science (TNS) and Mie University Mycological Herbarium (MUMH)

2.2. *Molecular phylogeny*

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 rRNA gene (including the domains D1 and D2) and internal transcribed spacer (ITS) regions were amplified by polymerase chain reaction (PCR) using the respective primer pairs: PM3 (Takamatsu and Kano 2001) and TW14 (Mori et al. 2000) for 28S rRNA gene, PM5/ITS4 for ITS fragment 1 and ITS5/PM6 (Takamatsu and Kano 2001) for ITS fragment 2. KOD FX Neo DNA polymerase (Toyobo, Japan) was used in the PCR reaction according to the manufacturer's protocol. The amplicons of 28S rRNA gene and ITS region were sent to SolGent Co. Ltd. (Daejeon, South Korea) for sequencing using primer pairs of NL1 (Mori et al. 2000) and NLP2 (Hirose et al. 2005) for the 28S rRNA gene, and ITS1 and ITS4 (White et al. 1990) for the ITS. New representative sequences determined in this study were deposited in DNA Data Base of Japan (DDBJ) under the accession numbers of AB916688–AB916691.

Sequences generated from the 28S rRNA gene and ITS region were aligned with other sequences of the Erysiphaceae retrieved from DNA databases (DDBJ, EMBL, NCBI) using ClustalW (Larkin et al. 2007) implemented in MEGA 5 (Tamura et al. 2011). Alignment was then visually refined with a word processing program, using color-coded nucleotides. The alignments were deposited in TreeBASE (http://www.treebase.org/) under the accession number of S15506. Phylogenetic trees were obtained from the data using the maximum parsimony (MP) and maximum likelihood (ML) analyses. MP analysis was performed in PAUP* 4.0b10 (Swofford 2002) with the heuristic search option using the tree bisection-reconstruction (TBR) algorithm. This search was repeated 100 times with different random starting points, using the stepwise addition option to increase the likelihood of finding the most parsimonious tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC), were also calculated. The strength of the internal branches of the resulting trees was tested with bootstrap (BS) analysis (Felsenstein 1985), using 1000 replications with the stepwise addition option set to simple and a maximum tree number of 100. The ML analysis was done using raxmlGUI (Silvestro and Michalak 2012), under a GTRGAMMA model. The BS supports and trees were obtained by running rapid bootstrap analysis of 1000 pseudoreplicates followed by a search for the tree with the highest likelihood.

3. Results

3.1. *Morphological study*

Erysiphe takamatsui Y. Nomura, in Taxonomical study of *Erysiphaceae* of Japan: 208, 1997. Figs. 1, 2.
MycoBank no.: MB 459908.

Anamorph (Fig. 1): Mycelium epiphyllous, persistent or subpersistent. Hyphal appressoria solitary, lobed; conidiophores (65–)75–121(–140) µm long, erect from top of mother cells. Foot cells (25–)35–40(–47) × (7–)8–11 µm, straight or almost so, followed by up to two shorter cells, forming conidia singly. Conidia (35–)38–45(–50) × (17–)18–19(–21) µm, ellipsoid–ovoid or cylindrical, colorless.

Teleomorph (Fig. 2): Chasmothecia (70–)80–120(–150) µm diam., scattered, globose, dark brown. Peridium cells 20–30 µm diam., irregularly polygonal. Appendages 0.5–4 times as long as the chasmothecial diam., 2.5–10 µm wide, (0–)1–7-septate, arising from the lower half, mycelioid, unbranched or occasionally with short lateral branchlets, walls thin, smooth or almost so, pale brown below, colorless above. Asci (35–)45–60(–70) × (30–)40–50(–60) µm, 2–6 per chasmothecium, broadly ellipsoid-obovoid, sessile or subsessile, 2–5-spored. Ascospores (14–)18–30(–35) × (8–)10–15(–18) µm, ellipsoid–ovoid, colorless.

Materials examined: on leaves of *Nelumbo nucifera* (Nelumbonaceae, Proteales), Japan, Aichi, Nagoya-shi, Higashiyama Zoo & Botanical Garden, N 35°09′19.3″ E 136°59′0.5″, 14 Nov. 2005, S. Takamatsu & R. Divarangkoon, MUMH4102,