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First found of *Erysiphe elevata* on *Eucalyptus camaldulensis* and *Phyllactinia lagerstroemiae* sp. nov. on *Lagerstroemia* from Thailand

Jamjan Meeboon, Susumu Takamatsu *

Graduate School of Bioresources, Mie University, 1577 Kurima-machiya, Tsu, 514-8507, Japan

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ABSTRACT

A new powdery mildew record and a new species from Thailand are reported in this paper. A fungus found on *Eucalyptus camaldulensis* has an internal transcribed spacer (ITS) sequence identical to *Erysiphe elevata* recorded on *Catalpa* spp. in North America and Europe. Conidia produced singly with *Pseudoidium*-type germ tubes, and kinked foot cells of conidiophores are also consistent with the morphology of asexual stage of *E. elevata*. This is the first record of *E. elevata* on *Eucalyptus* and from Asia. A new species *Phyllactinia lagerstroemiae* is proposed for the fungus found on *Lagerstroemia* spp. based on combinations of morphological data and molecular phylogeny inferred from ITS and 28S rRNA gene sequences.

1. Introduction

The Erysiphales (powdery mildews) is a fungal group causing diseases on a wide range of plants worldwide. The biodiversity of this fungal group is less explored in tropical and subtropical regions than in temperate regions of the Northern Hemisphere (Hirata 1976), probably due to the fact that only a few specialists pay attention to this fungal group in these regions, whereas recent studies on the powdery mildews in Southeast Asia, such as Thailand and Indonesia, revealed many new and unique taxa in these countries (Divarangkoon et al. 2011; Monkhung et al. 2011, 2013; Meeboon et al. 2012a2013, 2016; Hidayat et al. 2014; Siahaan et al. 2015, 2016a, b). These studies suggest that many undescribed species are still waiting to be detected and described in these regions. Therefore, exploring the Erysiphales in these regions is important for a better understanding of the biodiversity in this fungal group.

During collection trips between 2008 and 2016 in Thailand, we found powdery mildews on *Eucalyptus camaldulensis* Dehnh., *Lagerstroemia macrocarpa* Wall. and *L. speciosa* (L.) Pers. *Eucalyptus* (Myrtaceae) and *Lagerstroemia* (Lythraceae) are plant genera belonging to the plant order Myrtales and comprise flowering trees and shrubs. The natural geographic

* *Corresponding author*. Fax: +81 59 231 9634.
E-mail address: takamatu@bio.mie-u.ac.jp (S. Takamatsu).

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distributions of *Eucalyptus* are confined to Australasia, except for a few species found in Indonesia and New Guinea. *Eucalyptus camaldulensis* is an iconic tree seen along many water courses across inland Australia. The tree produces shade in the extreme temperatures of central Australia, and plays an important role in stabilizing river banks. *Lagerstroemia* is a genus of deciduous and evergreen trees and shrubs native to the Indian subcontinent, Southeast Asia and Northern Australia. These flowering trees are beautifully colored and are often planted both privately and commercially as ornamentals. Based on morphology and molecular phylogenetic analyses, we identified the fungus on *Eu. camaldulensis* as *Erysiphe elevata* (Burrill) U. Braun & S. Takam., a first record of this species on *Eucalyptus*. The fungus on *L. macrocarpa* and *L. speciosa* was revealed to be an undescribed powdery mildew species. In this paper, we describe the morphological characteristics of these species in detail, and elucidate their phylogenetic affinities with closely related powdery mildew species based on the 28S rRNA gene and rDNA internal transcribed spacer (ITS) sequences.

2. Materials and methods

2.1. Molecular phylogeny

The nucleotide sequences of the 5′-end of the 28S rRNA gene (including domains D1 and D2) and ITS regions were determined according to the procedure described by Meeboon and Takamatsu (2014). Representative new sequences determined in this study were deposited in DNA Data Base of Japan (DDBJ) under the accession numbers LC177375–LC177379. These sequences were aligned with closely related sequences of the Erysiphaceae using MUSCLE (Edgar 2004) implemented in MEGA version 6 (Tamura et al. 2013). Alignments were further manually refined using MEGA, and deposited in TreeBASE (http://www.treebase.org/) under the accession number S19906. Phylogenetic trees were obtained from the datasets by using the maximum parsimony (MP) method implemented in PAUP* 4.0b10 (Swofford 2002) with heuristic search option using 'tree bisection-reconstruction' (TBR) algorithm with 100 random sequence additions to find global optimum tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of internal branches of the resulting trees was tested with bootstrap (BS) analyses (Felsenstein 1985) using 1000 replications with step-wise addition option set as simple. BS values 70% or higher were given. Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC) were also calculated.

2.2. Morphology

Morphological examinations were carried out in Japan on herbarium materials transported from Thailand as outlined in Meeboon and Takamatsu (2015). All the specimens were examined using a light microscope with phase contrast 10×, 20× and 40× objectives. No collections contained sexual morphs (chasmothecia). 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 for examination under a light microscope. Thirty conidia and conidiophores were measured for each specimen. Herbarium specimens were deposited at the National Museum of Nature and Science (TNS; Tsukuba, Japan) and Mie University Mycological Herbarium (TSU-MUMH; Tsu, Japan).

3. Results

3.1. The fungus on Eucalyptus

Conidia produced singly and lobed hyphal appressoria indicated that this fungus belonged to *Pseudoidium* (asexual morph of *Erysiphe*). Nucleotide sequences of the ITS and 28S rRNA gene were generated for two specimens on *Eu. camaldulensis*. These two sequences were combined with closely related sequences of *Erysiphe* species that were chosen based on the phylogenetic tree presented in Takamatsu et al. (2015) (Supplementary Table S1). *Erysiphe glycines* F.L. Tai was used as outgroup taxon. Of the 1359 total characters, 1072 were constant, 108 were variable but parsimony-uninformative, and 179 were parsimony-informative. About 30K equally parsimonious trees with 737 steps were constructed by the MP analysis. Topologies were almost consistent among the trees except for branching orders of the terminal branches and branch length, and a typical tree is shown in Fig. 1. Two sequences from the fungus on *Eucalyptus* were identical to the ITS sequences of *E. elevata* on *Catalpa* collected from France, Hungary, UK and USA, and differ only one base from *Erysiphe* sp. on *Catharanthus roseus* (L.) G.Don collected from Japan. These sequences formed a clade with 99% BS value. This clade was sister to the clade of *E. azaleae* on *Rhododendron* spp. with 76% BS value. Number of nucleotide differences between *E. elevata* and *E. azaleae* was 13–16 bases/559 characters (97.1–97.7% similarity, not including gaps).

3.2. The fungus on Lagerstroemia

Morphological characteristics, such as the presence of mycelium on the lower leaf surfaces only, as well as lanceolate to clavate conidia, suggested that this fungus belongs to *Ovulariopsis*, i.e. the asexual morph of *Phyllactinia*. In the phylogenetic analysis of powdery mildew sequences from *L. macrocarpa* and *L. speciosa*, the 28S rRNA gene alignment contained 36 sequences including sequences of *Pleochaeta shiraiana* (Henn.) Kimbr. & Korf (LC108831) and *Pl. polychaeta* (Berk. & M.A. Curtis) Kimbr. & Korf (LC108836) used as outgroup taxa (Supplementary Table S1). Of the 603 total characters, 490 were constant, 34 were variable but parsimony-uninformative, and 79 were parsimony informative. A total of 35 equally parsimonious trees with 232 steps were constructed by the MP analysis. One of the trees with the highest likelihood value is shown in Fig. 2. The ITS dataset contains 33 sequences including two sequences of outgroup taxa and 781 total characters, of which 371 were constant, 133 were variable but parsimony-uninformative, and 277 were parsimony informative. A total of 20 equally parsimonious trees with

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