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# Two new hosts of anamorphic *Erysiphe quercicola*: *Cinnamomum camphora* and *Murraya paniculata*

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ABSTRACT

Based on collections of powdery mildews (Erysiphales) in Taiwan and combined molecular and morphological analyses, camphor tree (*Cinnamomum camphora*) and orange jasmine (*Murraya paniculata*) are recognized as new hosts of the anamorph of the powdery mildew *Erysiphe quercicola*. The anamorphic powdery mildew on *C. camphora* has been known as *Pseudoidium cinnamomi*, but its relationship to a teleomorph was unknown. For *M. paniculata* as substrate of powdery mildew, only an anamorphic *Cystotheca* species has been named. Morphological investigation of the fungus on this host shows that the specimens from Taiwan belong to another genus because of the lack of fibrosin bodies. Analysis of internal transcribed spacer sequences indicates that the anamorphic powdery mildews on camphor and orange jasmine belong to a clade representing *E. quercicola*, with the teleomorph found only on oak species (*Quercus*, Fagaceae), but with its anamorph reported from a broad host range, particularly in the tropics.



## 1. Introduction

Anamorphic powdery mildews (Erysiphales) can be classified as species of anamorphic genera such as *Oidium*, *Ovulariopsis*, *Pseudoidium*, etc. in the traditional nomenclature (Braun and Cook 2012). Particularly in the phytopathological literature, however, the practice of identifying powdery mildews with the name of the sexual morph, if available, irrespective of the stage of development of the species, is also widespread (Glawe 2008). According to the recent nomenclatural changes, a teleomorph-based nomenclature of powdery mildews is recommended (Braun 2012; Hawksworth 2012). Because the anamorph stages are morphologically difficult to distinguish and host ranges of powdery mildews are less exclusive than in other, more specialized plant parasitic fungi, identification of species is provided with some uncertainty. Recently, molecular analysis of powdery mildews challenged our view about the presumed narrow host ranges of powdery mildews, showing that some species are not restricted to closely related plant genera or even families as host (Liberato and Cunnington 2006; Takamatsu et al. 2007; Kirschner 2010). A particular breakthrough was the discovery by Takamatsu et al. (2007) that *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam. on temperate oak trees revealed to present a complex of distinct species on the one hand, whereas on the other hand several anamorphic species previously erected as separate species on tropical host plants were found to be conspecific with the oak powdery mildews.

Among the new species, a clade containing teleomorphic *E. quercicola* S. Takam. & U. Braun from oaks (*Quercus* spp.,

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Fagaceae) was particularly rich in anamorphs described on tropical trees, comprising the families Anacardiaceae, Bixaceae, Euphorbiaceae, Fabaceae, and Rutaceae (Takamatsu et al. 2007). Several names of anamorphic powdery mildews on economically important plants were published first as species or infraspecific taxa of *Oidium* and *Ovulariopsis* from Taiwan, e.g. on camphor, citrus and papaya trees (Sawada 1919, 1930; Yen 1967). Some of these species could be related to teleomorphic species (e.g., Takamatsu et al. 2007), but the relationships of other species remained unclear. In Taiwan, camphor tree (*Cinnamomum camphora* (L.) J. Presl) and jasmine orange (*Murraya paniculata* (L.) Jack) are important native ornamental and medicinal plants. The anamorphic powdery mildews on *C. camphora* and *M. paniculata* in Taiwan were recorded as virulent pathogens particularly of young shoots and leaves and identified as *Oidium cinnamomi* and *O. murrayae*, respectively (Chang et al. 1999; Hsieh et al. 2002). Based on new collections and a combined morphological-molecular approach in Taiwan, we came to new conclusions about the identification of the anamorphic powdery mildew on jasmine orange and camphor tree.

## 2. Materials and methods

### 2.1. Morphological studies

Shoots and leaves with symptoms of powdery mildews were collected in Taiwan and taken to the laboratory for morphological and molecular analyses. The specimens were dried and deposited at the herbarium of the National Museum of Natural Science, Taichung, Taiwan (TNM). Microscopic characteristics were observed using fresh fungal material mounted in 5–10% (w/v) aqueous KOH solution. Measurements were based on data from 30 examples of the respective structure and are presented as mean value ± standard deviation with extreme values given in brackets, and separate mean values. Drawings were made freehand on scaled paper.

### 2.2. Phylogenetic analysis

Fresh or deep-frozen material was crushed by shaking the sample at 30 Hz (MiniBeadBeater-8) in a 1.5 ml tube together with glass beads 2.5 mm in diameter. For isolating nucDNA and purification of PCR products the Genomic DNA Spin Kit (Plant), Bioman Scientific Co., Ltd., Taiwan, and illustra GFX PCR DNA and Gel Band Purification Kit, GE Healthcare, UK, were applied, respectively, according to the manufacturers' protocols. Primers ITS1F and ITS4 were used for amplification (Gardes and Bruns 1993). Success of the amplification was assessed with 2% agarose gel electrophoresis followed by staining with GelRed™ (Biotium, Hayward, California, U.S.A.) visualized under UV light (312 nm). Sequencing of DNA was done by Mission Biotech (Nankang, Taipei) with the same primers as for the PCR. Sequences were edited with CodonCode Aligner version 4.0.1 (CodonCode Corporation) and consensus sequences deposited at GenBank. Related DNA sequences were compared with the help of the BLAST function of GenBank. Phylogenetic analysis of 31 ITS sequences selected according to the BLAST results and topology of related species shown by Limkaisang et al. (2006) and Takamatsu et al. (2007) was performed with the default options of MEGA5 (Tamura et al. 2011) using MUSCLE for the alignment without manual editing of the total of 582 positions in the final dataset (http://purl.org/phylo/treebase/phylows/study/TB2:S14403), followed by a Maximum Likelihood analysis for the phylogenetic tree with 1000 bootstrap replications, with evolutionary distances being computed using the Tamura-Nei method. An unrooted tree showing the estimated relationships between sequences derived from the teleomorph from oak hosts (labeled as *E. quercicola*), related anamorphs on non-oak hosts and other closely related *Erysiphe* species with their respective GenBank accession number is given in Fig. 1.

## 3. Results

As shown in Fig. 1, the ITS sequences of the anamorphic powdery mildews on *C. camphora* and *M. paniculata* cluster within a strongly supported clade comprising sequences of *E. quercicola* from *Quercus* species (Fagaceae) and its anamorphs from non-fagaceous hosts, namely *Acacia auriculiformis* Benth. (Fabaceae), *Anacardium occidentale* L. (Anacardiaceae), *Bixa orellana* L. (Bixaceae), *Citrus* species (Rutaceae), and *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (Euphorbiaceae). The topology of our tree is almost identical with that also based on ITS sequences by Limkaisang et al. (2006). By our findings, the known host range of the anamorph is extended for the genus *Murraya* (Rutaceae) and the family Lauraceae. Within the clade, the anamorph on *C. camphora* does not show divergence from *E. quercicola*, but the anamorph on *M. paniculata* shows a similar degree of divergence as it was demonstrated by Takamatsu et al. (2007) for the anamorphs on *Bixa* and *Citrus* species. Because *Citrus* and *Murraya* belong to the same family Rutaceae, we compared the distances between the sequences of one specimen on *M. paniculata* and two specimens from citrus and found two different positions in the central region of the truncated alignment as well as in the direct alignment of the original three sequences. In the following, the anamorphic powdery mildews on *C. camphora* and *M. paniculata* are characterized morphologically. For the former mildew, we provide a somewhat unusual nomenclator restricted to the taxa assigned to *C. camphora* as host as will be discussed below. For a full nomenclator of *E. quercicola* and its anamorph *Pseudoidium anacardii* (F. Noack) U. Braun & R.T.A. Cook assigned to other hosts see Braun and Cook (2012).

**Anamorph of *Erysiphe quercicola*** S. Takam. & U. Braun on *Cinnamomum camphora*. Fig. 2.

= *Erysiphe cinnamomi* Sawada, Descriptive Catalogue of the Formosan Fungi I: 144 (1919).

≡ *Pseudoidium cinnamomi* (Sawada) U. Braun & R.T.A. Cook, Taxonomic Manual of the Erysiphales (Powdery Mildews) (2012): 601.

= *Oidium erysiphoides* f. *cinnamomi* J.M. Yen, Cahiers Pacif. 11: 96 (1967).

≡ *Oidium cinnamomi* (J.M. Yen) U. Braun, Mycotaxon 25(1): 266 (1986).