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Three new records of powdery mildews found in Mexico with one genus and one new species proposed

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ABSTRACT

Cystotheca lanestrif on the new host *Quercus canbyi*, *Microidium bauhiniicola* on the new host *Bauhinia macranthera* and an undescribed species of *Phyllactinia* on *Mimosa aculeaticarpa* have recently been collected in Mexico. Analyses of morphological traits and molecular sequence data led to identifications of the causal agents of the powdery mildew diseases involved. *Microidium bauhiniicola*, hitherto only known from Argentina and Brazil, is new to Mexico. The phylogenetic analysis revealed an isolated position distant from *Microidium* indicating an undescribed genus, which is introduced as *Bulbomicroidium* gen. nov. Sequences derived from a Mexican specimen of *C. lanestrif* on *Quercus canbyi* agree completely with other North American sequences, but differ from sequences retrieved from Asian collections, suggesting that *C. lanestrif* in Asia is not conspecific with *C. lanestrif* s. str. in North America. A new species of *Phyllactinia* on *Mimosa malacophylla* is morphologically similar to *P. dalbergiae* but readily distinguishable by differences in the asexual morph. Based on morphological peculiarities and results of molecular sequence analyses, this species is described as *Phyllactinia mimosae* sp. nov.

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1. Introduction

The biota of Mexico in general and the flora in particular are very rich. The vascular plants of Mexico provide a wide range of hosts for all kinds of pathogenic fungi. The diversity of Mexican plants is well explored, but the fungi associated with them are little known. It is therefore not surprising that the diversity of even easily discernible fungal groups like powdery mildews is little known in Mexico. In the course of routine screenings of forest trees in the Mexican Federal State Nuevo León, several interesting powdery mildew collections have recently been found and at first examined and identified by means of morphological methods, which were later supplemented and substantiated by results of molecular sequence analyses. Combined analyses led to reliable final conclusions with respect to the taxonomic affiliations of the species concerned. In this report, we described three powdery mildew records that were first found in Mexico, including a new genus and a new species.

2. Materials and methods

2.1. Morphology

Fresh powdery mildew materials were examined by being mounted in distilled water and photographed using compound microscopes. Dried (herbarium) samples were put into a drop of lactic acid and gently heated before the examination with an Olympus BX50 microscope (Olympus, Hamburg, Germany). Measurements of 30 conidia and other structures have been made whenever possible at a magnification of $\times 1000$, and the 95% confidence intervals have been determined (extreme values in parentheses). Herbarium specimens were deposited at the Mie University Mycological Herbarium (TSU-MUMH; Tsu, Japan).

2.2. Molecular phylogeny

Whole-cell DNA was extracted from mycelia using the chelex method (Walsh, Metzger, & Higuchi, 1991) as described by Hirata and Takamatsu (1996). The 5'-end of the 28S rDNA (including the domains D1 and D2) and internal transcribed spacer (ITS) regions were amplified by polymerase chain reaction (PCR) using the

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respective primer pairs: PM5 (Takamatsu & Kano, 2001)/NLP2 (Mori, Sato, & Takamatsu, 2000) for 3'-half of ITS and 28S rRNA gene, and ITS5/PM6 (Takamatsu & Kano, 2001) for 5'-half of ITS. KOD FX Neo DNA polymerase (Toyobo, Tokyo, Japan) was used for the PCR according to the manufacturer's protocol. The amplicons were sent to Solgent Co. Ltd. (Daejeon, South Korea) for direct sequencing using primers PM5 and NLP2 for the PM5/NLP2 fragment, and PM6 for the ITS5/PM6 fragment.

New sequences determined in this study were deposited in the DNA Data Bank of Japan (DDBJ) under the accession numbers LC222311–LC222313. These sequences were aligned with other sequences retrieved from DNA databases using MUSCLE (Edgar, 2004) implemented in MEGA6 (Tamura, Stecher, Peterson, Filipiński, & Kumar, 2013). Alignments were further manually refined using the MEGA6 program and were deposited in TreeBASE (<http://www.treebase.org/>) under the accession number S20692. Phylogenetic trees were obtained from the data using the maximum parsimony (MP) and maximum likelihood (ML) methods. MP analysis was performed in PAUP 4.0a151 (Swofford, 2002) with heuristic search option using the tree bisection reconnection (TBR) algorithm with 100 random sequence additions to find the 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) analysis using 1000 replications with the step-wise addition option set as simple (Felsenstein, 1985). Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC), were also calculated. The ML analysis was done using raxmlGUI (Silvestro & Michalak, 2012), under a GTRGAMMA model. The BS supports and trees were obtained by running rapid bootstrap analysis of 1000 pseudo-replicates followed by a search for the tree with the highest likelihood.

3. Results

3.1. The fungus on *Quercus canbyi*

ITS and 28S rRNA gene sequences were obtained from the Mexican material. ITS sequences of *Cystotheca* species were retrieved from DNA databases and aligned with that of the Mexican material. *Sawadaea nankinensis* (F.L. Tai) S. Takam. & U. Braun was used as outgroup taxon. This data set consisted of 13 sequences and 487 characters, of which 107 (22.0%) characters were variable and 43 (8.8%) characters were informative for parsimony analysis. Four equally parsimonious trees with 81 steps were constructed by the MP analysis. Tree topologies were almost consistent among the trees, except for branching orders of the terminal groups and branch lengths. One representative tree with the highest likelihood value is shown in Fig. 1. ML analysis generated a tree topology almost identical to the MP tree, and only BS support values were shown on the MP tree. The fungus on *Q. canbyi* from Mexico formed a clade with isolates collected in the USA with moderate BS supports (MP = 74%; ML = 56%). The isolates collected from East Asia (China and Korea) grouped with the American samples (MP = 93%; ML = 88%), but formed a separate clade. Nucleotide substitutions between American isolates and East Asian isolates was 10–12 bases (97.5–97.9% similarity). Isolates from *Setoidium castanopsisidis* Meeboon & S. Takam., *C. wrightii* Berk. & M.A. Curtis, and *C. tjibodensis* (Gäum.) Katumoto formed each separate clades. A phylogenetic analysis using 28S rRNA gene sequences was also conducted although only five *Cystotheca* sequences were used for the analysis. The result coincided with the phylogenetic analysis by ITS sequences (Supplementary Fig. S1).

3.2. The fungus on *Bauhinia macranthera*

ITS and 28S rRNA gene sequences were determined for the sample on *Bauhinia macranthera* Hemsl. 28S rRNA gene sequences from a wide range of powdery mildew species were retrieved from DNA databases and aligned with the newly determined sequence. *Bysoascus striatosporus* (G.L. Barron & C. Booth) Arx was used as outgroup taxon based on Mori et al. (2000). This data set consisted of 55 sequences and 772 characters, of which 261 (26.7%) characters were variable and 163 (20.7%) characters were informative for parsimony analysis. A total of 5065 equally parsimonious trees with 789 steps were constructed by the MP analysis. Tree topologies were almost consistent among the trees, except for branching orders of the terminal groups and branch lengths. One representative tree is shown in Fig. 2. ML analysis generated a tree topology almost identical to the MP tree, and only BS support values were shown on the MP tree. The Mexican isolate from *B. macranthera* did not belong to the *Microdidium* clade, and represented an independent lineage by itself. A BLAST search using the 28S rRNA gene sequence showed that the maximum similarity was only 92–93% to a wide range of Erysiphales sequences. BLAST search using the ITS sequence also showed a low level of identity (less than 87% similarities with only 50% coverage) to many species of the Erysiphales. We did not use ITS sequences for the phylogenetic analysis because an unambiguous alignment with other Erysiphales species was impossible. All these evidences strongly suggest that the fungus on *B. macranthera* belongs to the Erysiphales, but it cannot be assigned to any of the known genera.

3.3. The fungus on *Mimosa aculeaticarpa*

ITS and 28S rRNA gene sequences were determined for the isolate on *Mimosa aculeaticarpa* Ortega. 28S rRNA gene sequences from *Phyllactinia* and *Leveillula* spp. were retrieved from DNA databases and aligned with the newly determined 28S rRNA gene sequence. *Pleochaeta polychaeta* (Berk. & M.A. Curtis) Kimbr. & Korf and *Pl. shiraiana* (Henn.) Kimbr. & Korf were used as outgroup taxa. This data set consisted of 37 sequences and 603 characters, of which 103 (17.1%) characters were variable and 79 (13.1%) characters were informative for parsimony analysis. A total of 27 equally parsimonious trees with 238 steps were constructed by the MP analysis. Most terminal groups were highly supported, but the branching order among the terminal groups was varying. A representative tree with the highest likelihood value is shown in Fig. 3. ML analysis generated a tree topology almost identical to the MP tree, and only BS support values were shown on the MP tree. The result was almost concordant with the reports of Takamatsu, Siahaan, Moreno-Rico, Cabrera deÁlvarez, and Braun (2016) and Meeboon and Takamatsu (2017). *Phyllactinia* species were divided into three large groups, viz. a Core *Phyllactinia* group, a Basal *Phyllactinia* group, and a Southeast Asian group. The Southeast Asian group occupied the basal part of *Phyllactinia* and was distributed in Southeast Asia. All members of the Basal *Phyllactinia* group were collected from North and South America and *Leveillula* was nested in this group, suggesting that *Leveillula* was derived from the Basal *Phyllactinia* group. Thus, *Phyllactinia* was paraphyletic. The Core *Phyllactinia* group contained most major species of *Phyllactinia* and was distributed worldwide. The fungus on *Mimosa* belonged to the Basal *Phyllactinia* group and formed an independent branch. A phylogenetic analysis using ITS sequences was concordant with the phylogeny based on the 28S rRNA gene (Supplementary Fig. S2).

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