

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Alternate Hosts of Willow Rust Disease Caused by *Melampsora ferrinii* in China

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Corydalis acuminata Franch., *C. edulis* Maxim., and *C. racemosa* (Thunb.) Pers. of family Papaveraceae are rich in multiple alkaloids and are widely used as Chinese medicinal herbs, for treating cough, pruritus, sores tinea, and snake venom (Iranshahy et al. 2014; Zhang et al. 2008). In April 2021, orange rust pustules were observed on *C. acuminata*, *C. edulis*, and *C. racemosa* in Shaanxi Province (34°4'56" N, 108°2'9" E, alt. 770 m), China. Samples were collected and voucher specimens were preserved in the Herbarium Mycologicum Academiae Sinicae (nos. HMAS249947–HMAS249949), China. Consequent geospatial investigations revealed that diseased plants can be observed at an altitude of 400 to 1,000 m, and show an incidence from 40 to 80%, which varies by altitude. Spermogonia epiphyllous, subcuticular, densely grouped, oval or round, 0.14 to 0.36 × 0.09 to 0.30 mm, pale orange-yellow, and type 3 of Hiratsuka and Cummins (1963). Aecia mostly hypophyllous, subepidermal without peridia, Caeoma-type, erumpent, densely grouped, oval or round, 0.27 to 0.85 × 0.15 to 0.43 mm, and orange-yellow; hyaline peridial cells produced in a periphery of the sorus under the ruptured epidermis of host plants. Aeciospores globose or broadly ellipsoid, catenulate with intercalary cells, 15.7 to 20.1 × 10.8 to 15.7 μm, yellow to pale orange; walls hyaline, verrucose, 1.7 to 3.1 μm thick. This fungus was morphologically identified as *Melampsora* (Melampsoraceae). The rDNA-28S and the internal transcribed spacer (ITS) regions were amplified using primers NL1/NL4 and ITS1/ITS4 (Ji et al. 2020; Wang et al. 2020). Bidirectional sequences were assembled and deposited in GenBank (accession nos. MW990091–MW990093 and MW996576–MW996578). Phylogenetic trees were constructed with the ITS+rDNA-28S dataset based on maximum-likelihood (ML), maximum-parsimony (MP), and Bayesian Inference (BI). ML and MP bootstrap values

were calculated by bootstrap analyses of 1,000 replicates using MEGA-X (Kumar et al. 2018), while BI posterior probabilities (Bpps) were calculated using MrBayes ver. 3.1.2 (Ji et al. 2020; Wang et al. 2020). Phylogenetic analyses grouped our specimens and *Melampsora ferrinii* Toome & Aime into one clade, highly supported by bootstrap values of ML, MP, and Bpps of 100%/100%/1. Inoculations were conducted with 1-year-old plants of the original host, *Salix babylonica* (Toome and Aime 2015). Aeciospore suspension with a concentration of 10⁶ spores/ml was sprayed on 20 healthy leaves, with another 20 healthy leaves sprayed with sterile water as the control. The inoculated plants were kept in darkness at 20 to 25°C for 2 days and then transferred into greenhouse at 23°C with 16 h light per day. After 8 to 10 days of inoculation, yellow pustules of uredinia appeared on abaxial surfaces of the inoculated leaves, identical to what Toome and Aime (2015) reported, while the control leaves remained healthy. Inoculations with the same method were conducted by spraying urediniospores, and the same rust symptoms developed after 8 days. Genus *Corydalis* was verified as the alternate host of *M. chelidonii-pierotii* Tak. Matsumoto, *M. coleosporioides* Dietel, *M. idesiae* Miyabe, and *M. yezoensis* Miyabe & T. Matsumoto (Okane et al. 2014; Shinyama and Yamaoka 2012; Yamaoka and Okane 2019), and *C. incisa* (Thunb.) Pers. was speculated as the potential alternate host of *M. ferrinii* (Toome and Aime 2015). Based on morphology, phylogeny, and pathogenicity, this is the first report of *M. ferrinii* in mainland China and verify *C. acuminata*, *C. edulis*, and *C. racemosa* instead of *C. incisa* as its alternate hosts.

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