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NOTE

Characterization of *Aecidium deutziae*, a rust fungus on *Deutzia pulchra* in Taiwan

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ABSTRACT: The first and only one recorded collection of *Aecidium deutziae* in Taiwan was collected by Y. Hashioka on *Deutzia pulchra* in 1933 without taxonomical description. For characterization of this rust fungus, *A. deutziae* on *D. pulchra* again collected near Tataka, Nantou, Taiwan in 2015, was studied. The fungus produced amphigenous spermogonia and cupulate, mainly hypophyllous aecia. The peridial cells have smooth outer wall and verrucose inner walls. Aeciospores are pale orange, globose to broadly ellipsoidal, minutely verrucose with granules. In addition, the DNA sequences covering the LSU, ITS and SSU rDNA regions were obtained.

KEY WORDS: *Aecidium*, Biodiversity, Ribosomal RNA gene, Rust disease, Scanning electron microscope, Taiwan, Taxonomy.

INTRODUCTION

In Taiwan, more than 335 species of rust were recorded, including 226 species in 41 teleomorphic genera and 109 anamorphic species (Chung et al., 2006; Hiratsuka and Chen, 1991). In 2015, the anamorphic species of *Aecidium deutziae* Dietel on *Deutzia pulchra* Vidal was collected from Tataka near Yushan in Nantou County, Taiwan. *D. pulchra* is a tree species distributed in mountainous areas in Taiwan and the Philippines (Banwa, 2011). According to previous studies, the anamorphic *Aecidium* sp. on *D. pulchra* was identified as *A. deutziae* (Hsu *et al.*, 2002; Sawada,1942; Hiratsuka and Hashioka,1934). However, morphological description of the specimen was lacking (Hiratsuka and Hashioka, 1934). Hiratsuka and Hashioka (1934) reported that this rust fungus was collected in July of 1933. Our observations showed that the specimens collected from Tataka area between September and October have mature aecidium, whereas those collected in July have no mature aecidium. Thus, the original specimen of *A. deutziae* might be not fully mature in July. The objective of this study is to provide morphological description and construct molecular data of the *A. deutziae* on *D. pulchra* collected in Taiwan.

MATERIALS AND METHODS

Specimen collection and observation

Fresh specimens were examined under stereomicroscope and light microscope (LM). The tissues and aeciospores were mounted in water for LM examination. Cryostat sections were made for the observation of the spermogonia and aecia. More than 50 aeciospores were randomly selected for measurement. For scanning electron microscopic (SEM) observations, samples were fixed, dehydrated, coated with gold and examined with a Jeol JEM-1400 SEM system (Jeol Co., Tokyo, Japan) operating at 10 kV. The specimens are deposited at the herbarium of the National Museum of Natural Science (TNM), Taichung, Taiwan.

Molecular characterization

Total DNA from fresh collected samples was extracted with a Plant Genomic DNA Extraction Miniprep Kit (Viogene, Taiwan) followed the manufacturer's instructions. Amplification of the 28S large-subunit (LSU) rDNA was performed using Rust2inv (Aime, 2006) and LR6 (Vilgalys and Hester, 1990) primers, and sequenced with Rust2inv, LR6, LR3 (Vilgalys and Hester, 1990), and LR0R (Moncalvo et al., 1995). The 18S small-subunit (SSU) rDNA was amplified with NS1 (White, 1990) and a newly designed primer, Rn18S (5'-CATTTCACTGTGTTCTTCATCG-3') and sequenced with NS1, NS4, NS5, NS6 (White, 1990), Rn18S, and the other newly designed primer RnNS5 (5'-ATCTTGTGAAACTTGGTCGTGA-3'). The internal transcribed spacer (ITS) region including ITS1, 5.8S rDNA, and ITS2 was amplified with an ITS1/ ITS4 primer pair (White, 1990). The PCR products of ITS1/ITS4 were cloned into a pCR™2.1-TOPO®vector and transformed into *Escherichia coli* strain TOP10 (Invitrogen, USA). Colonies containing expected amplicons were sequenced on both strands. The resulting

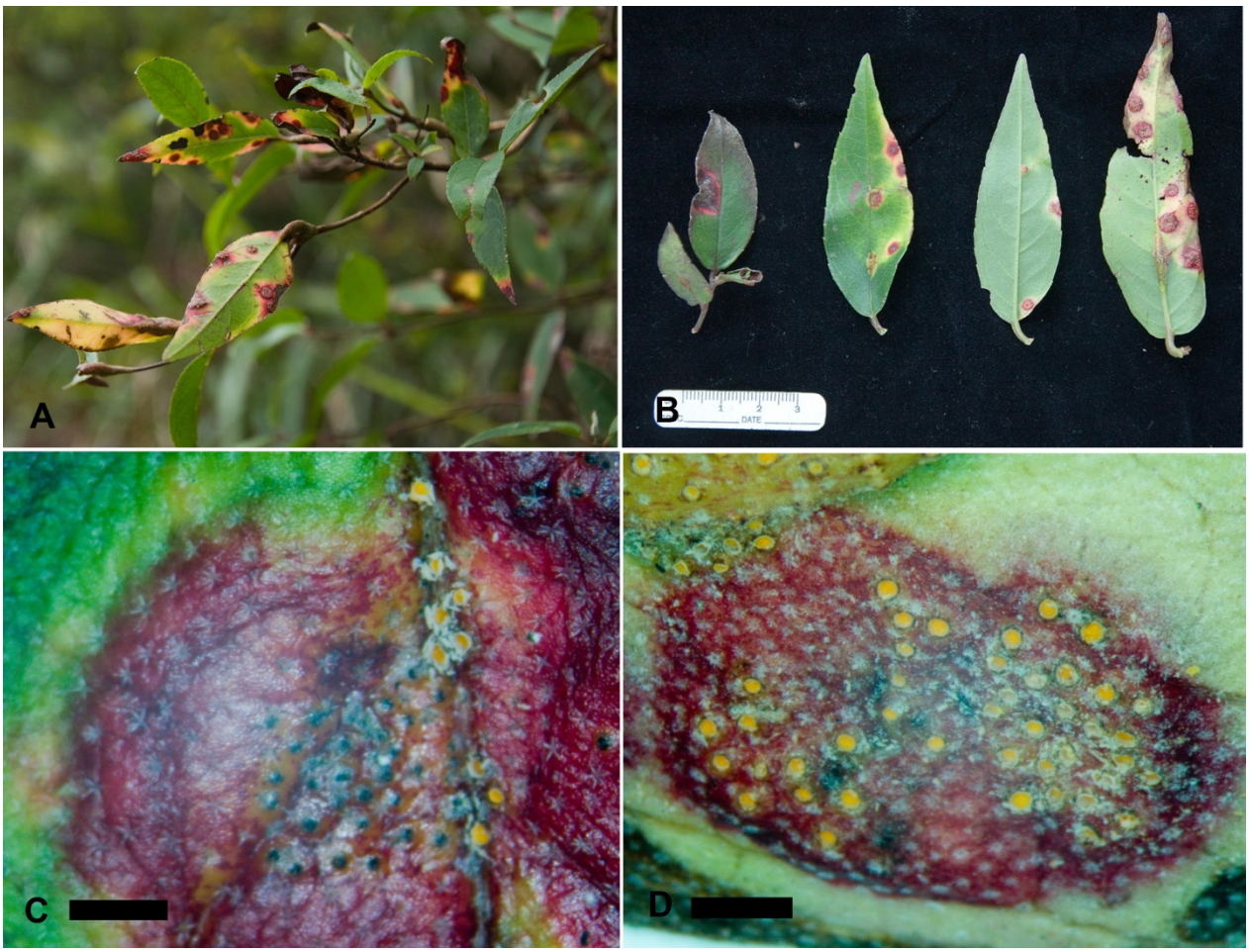

Fig. 1. ***Aecidium deutziae*** on ***Deutzia pulchra*** (TNM F0029305). **A:** symptoms in the field, with hypertrophy on stems and petioles. **B:** lesions on the leaves. **C:** spermogonia and aecia on upper leaf surface. **D:** aecia and spermogonia on lower leaf surface. Scale bar: **C-D** = 1 mm.

sequences were combined and proofread with the help of Vector NTI Advance 11.0 software (Invitrogen, USA). A contig sequence contained partial SSU rDNA, ITS1, 5.8S rDNA, ITS2, and partial LSU rDNA of each specimen was obtained and submitted to NCBI GenBank (http://www.ncbi.nlm.nih.gov/).

TAXONOMY

Aecidium deutziae Dietel, Hedwigia 37: 212, 1898.

溲疏銹孢銹菌 Figs. 1–3

Aecidium deutziae on *D. pulchra* foliicolous and caulicolous, causing hypertrophy on stems and petioles. Lesion centers reddish, surrounded by yellowish margins. Spermogonia amphigenous, abundant, aggregate in small groups, blackish, 108–200 µm in diam., type 4 in Hiratsuka and Cummins (1963). Aecia amphigenous, mainly hypophyllous, loosely aggregate, cupulate, orange inside with yellowish-white peridia, 180–275 µm in diam. Peridial cells imbricated, hyaline to pale orange, rhomboidal, 32.5–48.8×17.5–35.0 µm, outer walls smooth, inner walls verrucose. Aeciospores

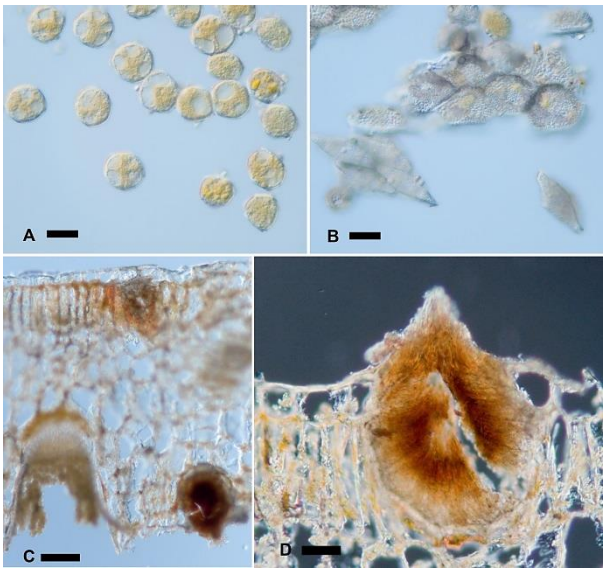

Fig. 2. ***Aecidium deutziae*** TNM F0029305 under light microscope. **A:** aeciospores. **B:** peridial cells. **C:** an aecium and a spermogonium on abaxial side and an immature spermogonium on adaxial side. **D:** spermogonium. Scale bar: **A, B & D** = 20 µm; C = 100 µm.

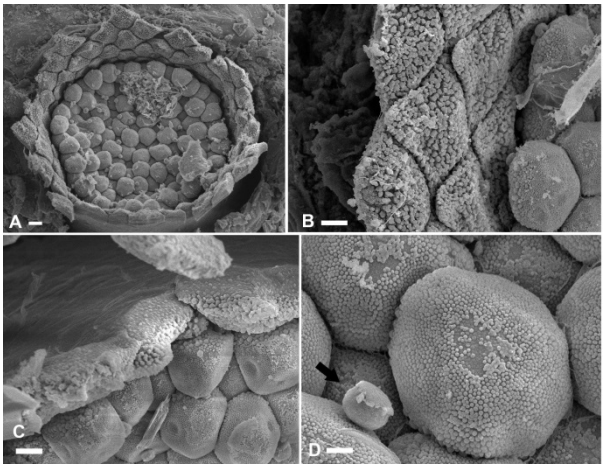

Fig. 3. ***Aecidium deutziae*** TNM F0029314 under scanning electron microscope. **A:** aecium. **B:** peridial cells with verrucose inner surface and aeciospores. **C:** smooth outer surface of peridial cells and aeciospores. **D:** aeciospores and a granule (black arrow). Scale bar: **A** = 10 μm; **B-C** = 5 μm; **D** = 2 μm.

pale orange, globose to broadly ellipsoidal, 16.3–28.8 ×16.3–27.5 μm (22.0×20.1 μm on average), minutely verrucose with granules, part of the surfaces on aeciospores sparsely verrucose.

Specimens examined: **TAIWAN**: Nantou County: on *D. pulchra*, Yushan peaks trail 0.5–1.0 k, 2600-2700 m, 15 July, 2015, *Shen, Y.-M. SYM00070*, TNM F0029298, GenBank accession number KU309316; on *D. pulchra*, Tataka (near Tataka squad), 2600-2700 m, 10 September, 2015, *Shen, Y.-M. SYM00078*, TNM F0029305, GenBank accession number KU309317; on *D. pulchra*, Tataka (near Yushan peaks trail entrance), 2600-2700 m, 13 October, 2015, *Shen, Y.-M. SYM00090*, TNM F0029314, GenBank accession number KU309318.

Remarks: Morphological and molecular characteristics of the rust fungus on *D. pulchra* in Taiwan are provided in this study for the first time. The host plant and the collection locality are the same to those of *A. deutziae* in Hiratsuka and Hashioka (1934). Our specimen collected in July (TNM F0029298) had no mature aecia, whereas those obtained in September and October (TNM F0029305 and TNM F0029314) produced mature aecia and spermogonia. Compared with original description of *A. deutziae* (Dietel, 1898), the range of the aeciospores measurements based on the Taiwanese specimens is wider than that of *A. deutziae* from Japan (21–25 × 17–20 μm) (Dietel, 1898). Amphigenous spermogonia and amphigenous aecia were additionally observed. Nevertheless, disease symptoms and the texture of aeciospores fit in with the original description of the fungus. Regarding the molecular features, all three contig sequences of *A. deutziae* from Taiwan were 3380 nucleotides and only one singlenucleotide polymorphism was found. Blast searches in GenBank suggested that the fungus linked to a *Puccinia* teleomorph, even though no matched species were inferred so far. In previous studies (Asuyama H, 1936; Hiratsuka et al., 1992), *A. deutziae* is synonymous with *Puccinia kusanoi* Dietel, a teleomorphic name for a bamboo rust pathogen infecting plants in genus *Arundinaria*, *Pleioblastus*, *Sasa*, and *Semiarundinaria*. It is possible that the rust fungus in Taiwan produces its teleomorph on a bamboo species distributed in the same habitat e.g., *Yushania niitakayamensis* (Hayata) Keng f. Further studies are needed to elucidate the life cycle of the rust fungus on *D. pulchra* in Taiwan and its relationship to the fungal populations in Japan.

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