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Unveiling *Gymnosporangium corniforme*, *G. unicorne*, and *G. niitakayamense* sp. nov. in Taiwan

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ABSTRACT

Three rust fungi from high mountains and pear-producing areas in Taiwan were described using morphological and molecular data based on 34 specimens. *Gymnosporangium corniforme* was demonstrated to produce spermogonia and aecia on *Sorbus randaiensis* based on molecular analyses and inoculation experiments. The pear rust pathogen *G. unicorne* was discovered in Taiwan for the first time. *Gymnosporangium niitakayamense* sp. nov. was observed on the leaves of *Photinia niitakayamensis*. It was distinct from other species in peridial cell wall structures, i.e., smooth outer wall, rugose side wall, and coralloid projections on the inner wall, and in having echinulate aeciospores.

1. Introduction

Species of the genus *Gymnosporangium* are generally heteroecious, having a heterodemicyclic life cycle (Cummins & Hiratsuka, 2003; Yamaoka, 2014). Generally, these rust fungi undergo their telial stage (producing teliospores) on plants of the cypress family (Cupressaceae) and produce their spermogonial and aecial stages on plants in the subtribe Pyrinae (former subfamily Maloideae) (Campbell, Evans, Morgan, Dickinson, & Arsenault, 2007) of the rose family (Rosaceae) (Kern, 1973; Yun et al., 2009). On the spermogonial/aecial hosts, most *Gymnosporangium* species produce aeciospores in roestelioid aecia, which characterize the anamorphic rust genus *Roestelia* (Kern, 1973; Lee, Kakishima, & Zhuang, 1999). In the roestelioid aecia, the surface structures of the aeciospores and the aecial peridium cells vary among *Gymnosporangium* and *Roestelia* species. The fine structures of the aeciospores and peridial cells have been employed as important taxonomic characters to differentiate the species (Lee & Kakishima, 1999a, b). In recent decades, many *Gymnosporangium* and *Roestelia* species have been described by Kern (1973) and Hiratsuka et al. (1992), as well as Azbukina (1997), Cao, Tian, & Liang (2016); Cao, Han, Tian, & Liang, 2017; Lee et al. (1999), Zhao and Zhuang (2007), and Yun et al. (2009). Moreover, molecular characteristics of the rust fungi, including nuclear large-subunit (LSU) ribosomal DNA sequences, internal transcribed spacers (ITS), and small-subunit (SSU) ribosomal DNA sequences have been employed to infer phylogenetic relationships both within and between *Gymnosporangium* and *Roestelia* (Aime, 2006; Cao et al., 2016, 2017; Dixon, Castlebury, Aime, Glynn, & Comstock, 2010; Fernández, Llorens, & Alvarado, 2016; Maier, Begerow, Weiß, & Oberwinkler, 2003; Yun et al., 2009; Zhao, Liu, Li, & Cai, 2016). Therefore, comparing the morphological and molecular characteristics has been useful to address taxonomic issues in studies of *Gymnosporangium* and related species.

Gymnosporangium and *Roestelia* are widespread in the northern hemisphere (Kern, 1973; Yun et al., 2009). In total, 63 species of *Gymnosporangium* and 15 species of *Roestelia* have been recorded (Kern, 1973; Azbukina, 1997; Lee et al., 1999; Zhao & Zhuang, 2007; Yun et al., 2009; Cao et al., 2016, 2017). However, alternate hosts of some *Gymnosporangium* have not been discovered (Cao et al., 2016; Fernández et al., 2016; Kern, 1973). For example, aecial connections

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of *G. corniforme* Sawada and *G. formosanum* Hirats. f. & Hashioka have still not been found since their first discovery in the high mountains of Taiwan in 1919 and 1933, respectively (Hiratsuka & Chen, 1991; Hiratsuka & Hashioka, 1935; Kern, 1973; Sawada, 1925). Accordingly, efforts were made in this study to investigate *Gymnosporangium* in Taiwan, an island rich in plant and rust species (Berndt, 2008; Hiratsuka & Chen, 1991; Hsieh, 2002) that is located at the transition between the tropical and subtropical regions.

In this paper, three taxa of *Gymnosporangium* are reported, two of which occur in the high mountains (ca. 3000 m) and one, which is related to pear rust diseases, in highland pear-producing regions in Taiwan. Morphological and molecular characteristics were used to identify each of them, and inoculations were conducted with two of the species. One potential spermogonial/aecial host was chosen for each inoculation experiment based on literature and our preliminary observations to clarify the life cycles and to supplement the aecial morphologies of the rusts. The connection of the spermogonial/aecial stages of *G. corniforme* was demonstrated. The distribution of the pear rust pathogen *G. unicorne* H.Y. Yun, which was restricted to Korea, is extended. Additionally, a new species associated with *Photinia niitakayamensis* Hayata host plant is proposed.

2. Materials and methods

2.1. Specimen collection

Rusts on infected plants were collected for morphological observations, inoculation tests, and molecular analyses between 2009 and 2017. Targeted collection sites were high mountainous areas and pear-producing areas in Taiwan. Localities within the elevation range of 2500–3300 m were visited between May and Aug for mountainous species, while pear-infecting *Gymnosporangium* species were sought between Feb and Apr in highland pear-producing regions. Leaves of junipers and rosaceous plants with visible symptoms were collected for examination. In 2017, molecular detection with specific primers was applied to distinguish among certain pear-infecting rusts after sampling from the field. In addition, rust specimens from Europe and America were obtained for molecular comparison. Most samples were temporarily stored in a refrigerator at 6 °C until usage, and dried specimens were deposited at the herbarium of the National Museum of Natural Science (TNM) in Taichung. Two specimens were deposited at the herbarium room of the Department of Plant Pathology and Microbiology, National Taiwan University, Taipei. In total, 31 spermogonial and aecial vouchers and 10 telial vouchers of *Gymnosporangium* were acquired in this study (Table 1), including a dried specimen on leaves of *P. niitakayamensis* collected in 1996 and preserved in the TNM herbarium.

2.2. Morphology

2.2.1. Morphological examination

Morphological characteristics of the rust fungi were determined under a stereomicroscope, a light microscope (LM), and a scanning electron microscope (SEM). The length of the aecia and telia were measured with the aid of a Leica MZ125 stereomicroscope (Leica Microsystems, Wetzlar). For LM observation, the rust tissues and spores were mounted in distilled water on glass slides and observed under a Leica DM2500 microscope (Leica Microsystems, Wetzlar) with differential interference contrast (DIC) equipment. Generally, observations were made within 2 wk after collecting, and 30 spores were measured at 400× magnification for each of the mature specimens. To determine the surface structure types of peridial cells and aeciospores using SEM, fixed, dehydrated aecia were dusted on double-sided adhesive tape on specimen holders, coated with gold in a Sputter Coater (SPI supplies, West Chester, Pennsylvania), and examined with a Jeol JSM-6510LV SEM system (Jeol, Tokyo) operating at 10–15 kV. Under SEM, three independent specimens were observed for each aecium-bearing rust species collected from high mountainous areas. One pear rust specimen derived from an inoculation experiment was also examined. Identification of *Gymnosporangium* species was based on Sawada (1925, 1928), Kern (1973), and Yun et al. (2009). Terminology used for species description followed Kern (1973), Hiratsuka et al. (1992) and Yun et al. (2009). Delimitation of spore shapes followed Bas (1969) and Kirk, Cannon, Minter, and Stalpers (2008). Surface structure types of peridial cells and aeciospores referred to Lee and Kakishima (1999a, b).

2.2.2. Production of one-celled spores by G. corniforme

In gelatinous telia of *G. corniforme* on leaves of *Juniperus formosana* Hayata, there were not only two-celled teliospores and basidiospores derived from the teliospores but also one-celled spores, which were larger than the basidiospores. The one-celled spores seemed unusual for *Gymnosporangium*. To elucidate the nature of the one-celled spores, non-gelatinized telia containing only two-celled teliospores of *G. corniforme* TNM F0028737 were soaked in sterilized water for 30 min, spread on 2% water agar in a Petri dish, and incubated at 24 °C for 3 d. The condition of the teliospores and the occurrence of one-celled spores were determined under LM.

2.3. Inoculations

2.3.1. Gymnosporangium corniforme

To determine the spermogonial and aecial host of *G. corniforme*, which had no known hosts on rosaceous plants (Hiratsuka, 1936; Hiratsuka & Chen, 1991; Kern, 1973; Sawada, 1925, 1928), telia of the rust on *J. formosana* were inoculated on *Sorbus randaiensis* (Hayata) Koidz., a suspected host for *G. corniforme* based on Hiratsuka (1936) and our own observation in 2015 when rust symptoms were found on the leaves of *S. randaiensis* nearby *J. formosana* plants between Jun and Aug. On 5 May 2016, an intact telium of *G. corniforme* TNM F0030454 was soaked in water for 30 min shortly after collection in the field, divided into three portions and then carefully spread on the leaf surfaces of three branches of wild *S. randaiensis* trees. Each of the branches was completely covered with a transparent plastic bag. Leaves and branches of *S. randaiensis* treated in the same way with the exception of inoculum served as controls. The inoculation was carried out beside the High Altitude Experimental Station (HAES) of the Endemic Species Research Institute (ESRI) near the boundary of Nantou County and Hualien County at an elevation of 3000 m. No gelatinous telia were formed in the specimen used for inoculation or on nearby *J. formosana* plants on the day of inoculation, indicating no or very few basidiospores, if any, were formed to cause infection on leaves in the wild before our inoculation. After the treatments, the leaves were investigated weekly to record the dates on which the symptoms appeared and the aecia matured. A parallel inoculation was carried out in a controlled environment using detached branches, but the plant materials deteriorated before the symptoms became visible.

2.3.2. Gymnosporangium unicorne

Inoculation of *G. unicorne* was performed in a greenhouse in Taichung District Agricultural Research and Extension Station, where pear rust diseases did not naturally occur on pear trees, in Dacun, Changhua County. *Pyrus lindleyi* Rehder, an easy-to-grow and local rootstock for pear production was chosen for

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