A record of the tree-parasitic Phyllactinia powdery mildew on herbaceous Catharanthus roseus based on morphological and molecular-phylogenetic analyses

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Research Article

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Abstract

A previously unknown powdery mildew having an endoparasitic nature was found repeatedly on the lower leaf surface of *Catharanthus roseus* plants, causing yellowish discolorations on the upper parts of the leaves and premature senescence of affected leaves, in Korea Chasmothecia were formed from October and matured in November. Morphological characterization of the holomorph from five collections showed the fungus belong to the genus *Phyllactinia*. The nucleotide sequence of rDNA large subunit gene and internal transcribed spacer region of three samples were determined in this study. Based on morphological circumscriptions and results of molecular-phylogenetic analysis, the fungus was identified as *Phyllactinia actinidiae*. So far, *Phyllactinia* powdery mildews have been known to be predominanly tree-parasitic species with a few exceptional, obscure records of herbaceous hosts. This report represents a new host record for *P. actinidiae*, and first confirmed report of host jumping of *Phyllactinia* species from woody plants to an herbaceous host.

Introduction

The genus *Phyllactinia* Lév. (*Helotiales, Erysiphaceae*) comprises powdery mildews, which parasitize predominantly on woody plants. Species of this genus partly exhibit endoparasitism and have two different types of chasmothecial appendages: acicular appendages with a swollen base and penicillate cells. These unique appendages within the genus are an evolutionary adaptation for tree parasitism (Takamatsu et al. 2016). However, some herbaceous plants, such as *Vigna trilobata* (L.) Verdc. and *Vicia sepium* L. were reported as hosts for *Phyllactinia phaseolina* N. Ahmad, D.K. Agarwal & A.K. Sarbhoy. But, these reports still remain unclear owing to lack of specimens or their unreliability (Braun and Cook 2012).

Catharanthus roseus (L.) G. Don (syn. *Vinca rosea* L.), known as Madagascar periwinkle or rose periwinkle, is an evergreen subshrub in tropical and subtropical areas or herbaceous plant in temperate regions from the family *Apocynaceae*. The plant is native to Madagascar but widely grown for ornamental and medicinal purposes globally. In Korea, Madagascar periwinkle as an annual herb is favored by its continuous flowering from summer to late autumn, ease of maintenance, and lack of serious diseases. During our routine field surveys, yellow discoloration and premature senescence of leaves, caused by an unknown powdery mildew fungus that is endoparasitic in nature, was repeatedly observed in summer and more frequently in autumn. In early November 2021, unlike other previous collections, mature chasmothecia with the same previous disease symptoms were found on the lower leaf surface of Madagascar periwinkle (Fig. 1).

To date, *Erysiphe aquilegiae* var. *ranunculi* (Grev.) R.Y. Zheng & G.Q. Chen, *Leveillula taurica* (Lév.) G. Arnaud, and *Podosphaera pannosa* (Wallr.) de Bary were reported to be associated with *C. roseus* in Australia, India, Japan, Korea, and the USA (Shin 2000; Romberg et al. 2014; Cho et al. 2017; Farr and Rossman 2022). Symptoms observed in the filed were similar to those caused by *Leveillula*, but the morphology of the appendages of fruiting bodies revealed that the fungus belongs to the genus *Phyllactinia*. Based on morphological and molecular-phylogenetic analysis, the fungus was identified as

Phyllactinia actinidiae (Jacz.) Bunkina, representing a new host record for this fungus, and the host expansion of *Phyllactinia* powdery mildews to herbaceous plants. In this study, we provided a detailed characterization of *P. actinidiae* on *C. roseus* based on molecular-phylogenetic analysis inferred from sequence data of internal transcribed spacer (ITS) region and large subunit (LSU) gene of rDNA.

Materials And Methods

Sample sources: All samples were preserved in the herbarium of Korea University (KUS), Seoul, Korea. The following five voucher specimens were used for this study: KUS-F24864 (2 Nov 2009, Jeju), F32567 (25 Oct 2021, Mokpo), F32623 (4 Nov 2021, Wanju), F032656 (16 Nov 2021, Wanju), and F032657 (16 Nov 2021, Jeonju).

Morphological examination

For observation of asexual morphs, dried herbarium specimens were examined using lactic acid technique (Shin and La 1993). Fruiting bodies – chasmothecia were picked with a sterile needle, mounted in a drop of Shear's solution, and examined under a compound microscope (Carl Zeiss AX10, Oberkochen, Germany equipped with a KCS-3.1C imaging system) in bright-filed and differential interference contrast. At least 30 measurements were taken for each diagnostic structure.

Molecular analysis

Three specimens (KUS-F24864, F32567, and F32623) were used for the molecular analysis. The whole DNA cell was extracted from mycelia and chasmothecia using Maglisto™ 5M kits (Bioneer, Daejeon, Korea) according to the manufacturer's protocol. The nucleotide sequences of 5´-end of the 28S rDNA (LSU) gene and ITS1-5.8S-ITS2 region were determined using PM3/TW14 and ITS1-F/PM6 primer pairs, respectively (Bradshaw and Tobin 2020). Samples were sequenced by the commercial sequencing company Macrogen Inc. (Seoul, Korea) using the same primers.

Phylogenetic analysis

Newly obtained sequences were assembled and deposited in GenBank under the accession numbers ON231589, ON231590 and ON231591 for ITS, and ON231592, ON231593 and ON231594 for LSU. Two datasets of alignments for ITS and LSU were prepared separately in MEGA 11 and aligned using MUSCLE (Tamura et al. 2021) with closely related sequences of the genus *Phyllactinia* retrieved from GenBank. Alignment of ITS consists of total 30 sequences, of which two sequences of *Pleochaeta shiraiana* (MH048878) and *Leveillula taurica* (KF703447) were used as outgroups. In the second dataset, which includes 28 sequences of LSU gene, two sequences of *L. taurica* (MG600239, MG881819) were selected as outgroups. Phylogenetic trees were constructed in PAUP* 4.0 with heuristic search option using the tree bisection reconnection (TBR) algorithm based on maximum parsimony method (Swafford 2002). Kishino-Hasegawa (1989) and Shimodaira-Hasegawa (1999) topology tests were applied to choose the best trees, and trees with the highest likelihood values are presented in Fig. 3 and Fig. 4. Robustness of

trees were evaluated by bootstrap (BS) analysis using 1000 replications. BS values higher than 80% were shown on the related branches. The tree scores, such as tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC) were calculated.

Results

Taxonomy

Phyllactinia actinidiae (Jacz.) Bunkina…….. Figure 2

Description: Symptoms occur as yellowish discolorations on the upper parts of the leaves and premature senescence of affected leaves, but the lower surface of the leaves was covered by whitish to grayish growth of abundant conidiophores in early stage and dotted with blackish chasmothecia in later stage (Fig. 1). Appressoria were solitary or in pairs, variable in shape, nipple- to coral-shaped, often branched, and up to 20 µm wide (Fig. 2a). Conidiophores were 160–300 µm long, 6–9 µm wide, producing conidia singly, followed by 2–3 cells, and basal septum elevated up to 14 µm (Fig. 2b–e). Conidia were obpyriform to clavate, papillate at the apex, measured 62–76 × 22–34 µm, and produces germ tubes on the basal and upper position, but occasionally on the side (Fig. 2f–m). Chasmothecia were scattered, 165–220 µm in diameter, yellowish (when young) or dark brown (when mature), and accommodated 5–12 asci. Chasmothecial appendages were equatorial, 8–16, straight, acicular, 220–280 µm long with bulbous swelling of ca. 40 µm in diameter (Fig. 2n, o). Penicillate cells were crowded on the upper part of the chasmothecia, 50–90 µm long, stems 16–24 µm wide and bifurcate or divided into several branches, filaments 20–42 × 1.5–2.5 µm with apical swelling, hyaline and gelatinous (Fig. 2p, q). Asci were obovoid-clavate, 60–84 × 36–44 µm, short stalked, and 2-spored (Fig. 2o, r). Ascospores were ellipsoid-ovoid, 30–44 × 18–21 µm, yellow to olivaceous, and guttulate (Fig. 2s). Morphological characters were consistent with those of *Phyllactinia actinidiae*, described by Braun & Cook (2012).

In total, three sequences for ITS (513, 518, and 521 bp) and three sequences for LSU (1035, 957, and 980 bp) gene of rDNA were obtained in this study. Comparison of these sequences with reference sequences in GenBank using BLASTn search revealed over 99% similarity for ITS region among sequences of *Phyllactinia actinidiae* (KJ703014, KJ703015, AB080500, KJ703016), *P. kakicola* (KR048098), *P. alangii* (KR048094), and *P. enkianthi* (AB080506) and showed 100% identity with those of *P. actinidiae* (AB080391), *P. enkianthi* (AB080395), and *P. salmonii* (AB080406) for LSU gene of rDNA.

Discussion

To date, three *Phyllactinia* species, *P. actinidiae*, *P. actinidiae-formosanae* Sawada, and *P. actinidiae-latifoliae* Sawada, parasitizing on the leaves of *Actinidia* Lindl. plants have been described, and their distributions are confined to Northeast Asian countries, such as China, Japan, Korea, and Taiwan (Farr and Rossman 2022). Our isolate is morphologically and genetically close to *P. actinidiae*. However, it differs from *P. actinidiae-formosanae* by having longer appendages (220–280 µm vs. 300–570 µm), and

P. actinidiae-latifoliae by the presence of larger chasmothecia (165–220 µm vs. 280–400 µm) and the number of appendages (8–16 vs. 15–24) (Meeboon et al. 2015).

Among the plants from the family *Apocynaceae*, in which *C. roseus* belongs to, *Asclepias curassavica* L. was reported to be associated with *Ovulariopsis asclepiadis-curassatiae* Sawada from China and Taiwan (Braun and Cook 2012). The morphological characteristics of *O. asclepiadis-curassatiae* are similar to the asexual morphs of *P. actinidiae*. However, the identity of *O. asclepiadis-curassatiae* is still obscure. Similarly, *P. fraxini* (DC.) Fuss was reported to be associated with *Asclepias syriaca* L. as *P. suffulta* (Rebent.) Sacc. from Switzerland and the USA (Braun and Cook 2012). However, these two fungi parasitizing on *Asclepias* spp. are morphologically different from each other: *P. fraxini* is clearly different from *P. actinidiae* by having flexuous foot-cells of conidiophores and non-papillate conidia (Shin 2000, Braun and Cook 2012).

Phylogenetic analysis revealed that *P. actinidiae* belongs to the core *Phyllactinia* group, which is characterized to have non-dimorphic conidia (Takamatsu et al. 2016). Within this group, *P. actinidiae* clustered with *P. guttata* s.lat. and *P. juglandis* in a clade, which represent *Phyllactinia* species with papillate conidia (Takamatsu et al. 2008). In the generated tree for ITS region (Fig. 3), our sequences clustered with sequences of *P. actinidiae* in a separate group and showed the closest similarity to KJ703014, KJ703015, KJ703016, which were from *P. actinidiae* that parasitize on *Actinidia arguta* in Korea (Cho et al. 2014). However, in the MP tree constructed for LSU gene (Fig. 4), sequences of *P. actinidiae* were scattered, but one of them forms distinct clade with sequences obtained in this study. According to Takamatsu et al. (2008), members of the genus *Phyllactinia* tend to expand their host range among different plant families independently and it is not surprising that single *Phyllactinia* lineage group can be scattered in distantly related plant lineages. Therefore, this study is a notable documented report of host jumping of *Phyllactinia* powdery mildews away from their native hosts, which were known for their exclusive host specificity for woody plants.

Declarations

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Ethics Approval

All authors, In-Young Choi, Lamiya Abasova, Joon-Ho Choi and Hyeon-Dong Shin approved ethics of the publication. This study does not involve humans, human data or animals. Not Applicable.

Author Consent for Publication

All authors, In-Young Choi, Lamiya Abasova, Joon-Ho Choi and Hyeon-Dong Shin approved the final manuscript and consent the publication. All authors have no conflicts.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by In-Young Choi, Lamiya Abasova, Joon-Ho Choi and Hyeon-Dong Shin. The first draft of the manuscript was written by In-Young Choi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1

Symptoms of powdery mildew on pot-grown *Catharanthus roseus* caused by *Phyllactinia actinidiae*. (a) Affected plants showed leaf yellowing and poor flowering. (b) Close-up view of affected plants. Note leaf yellowing and premature senescence. (c) Close-up view of an affected leaf. Note chasmothecia on the lower leaf surface. (d,e) Chasmothecia formed on the lower leaf surface. Yellow chasmothecia are immature, while blackish brown chasmothecia are mature.

Figure 2

Morphological characteristics of *Phyllactinia actinidiae* examined on the leaves of *Catharanthus roseus*. (a) Hyphal appressoria. (b–e) Conidiophores. (f–i) Conidia. (j–m) Conidia in germination. (n) Chasmothecium. (o) Chasmothecium crushed to show several asci. (p, q) Penicillate cells. (r) Ascus. (s) Ascospores.

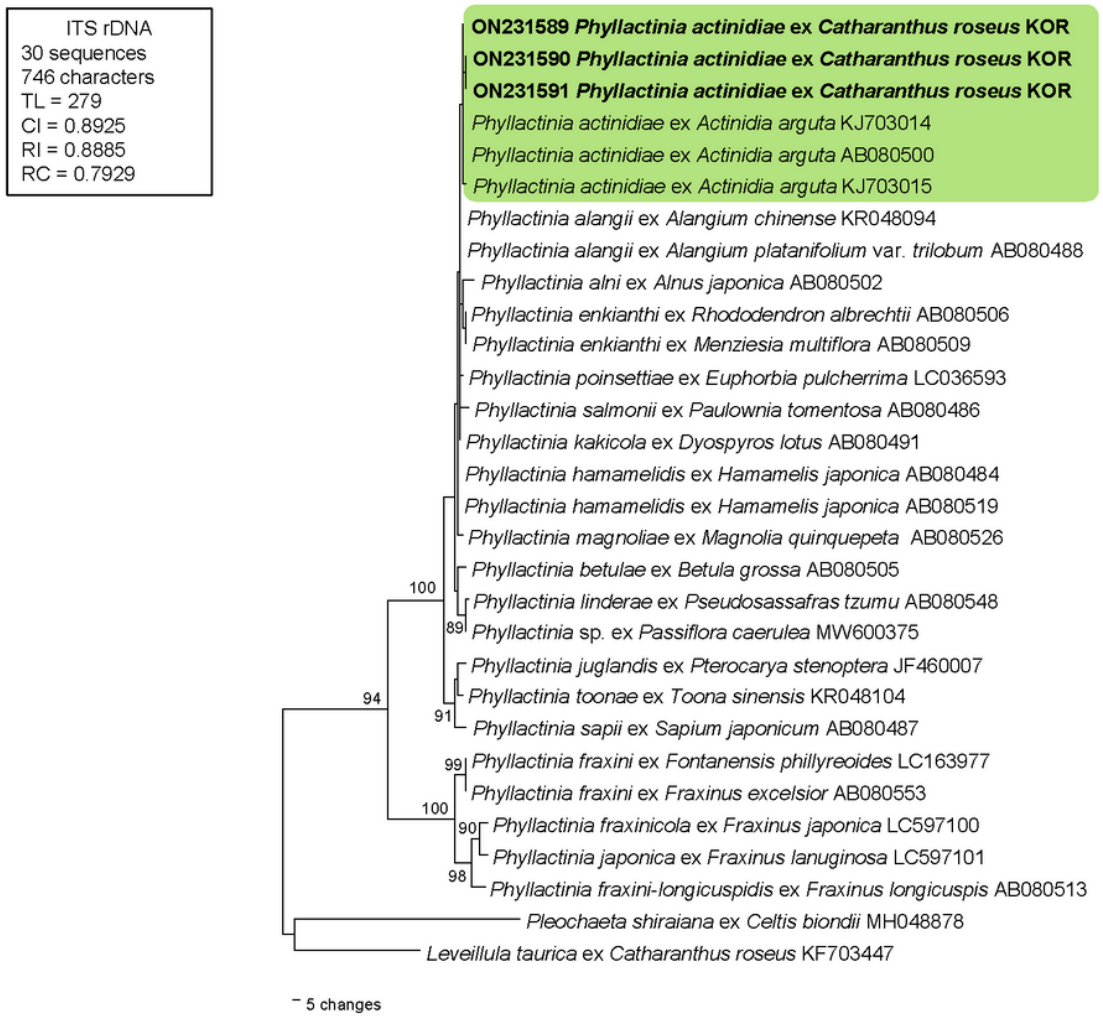

Figure 3

Phylogeny of *Phyllactinia actinidiae* inferred from rDNA ITS region sequences based on maximum parsimony (MP) analysis. Bootstrap supporting values higher than 80% are shown on relevant branches. Korean isolates were shown in boldface.

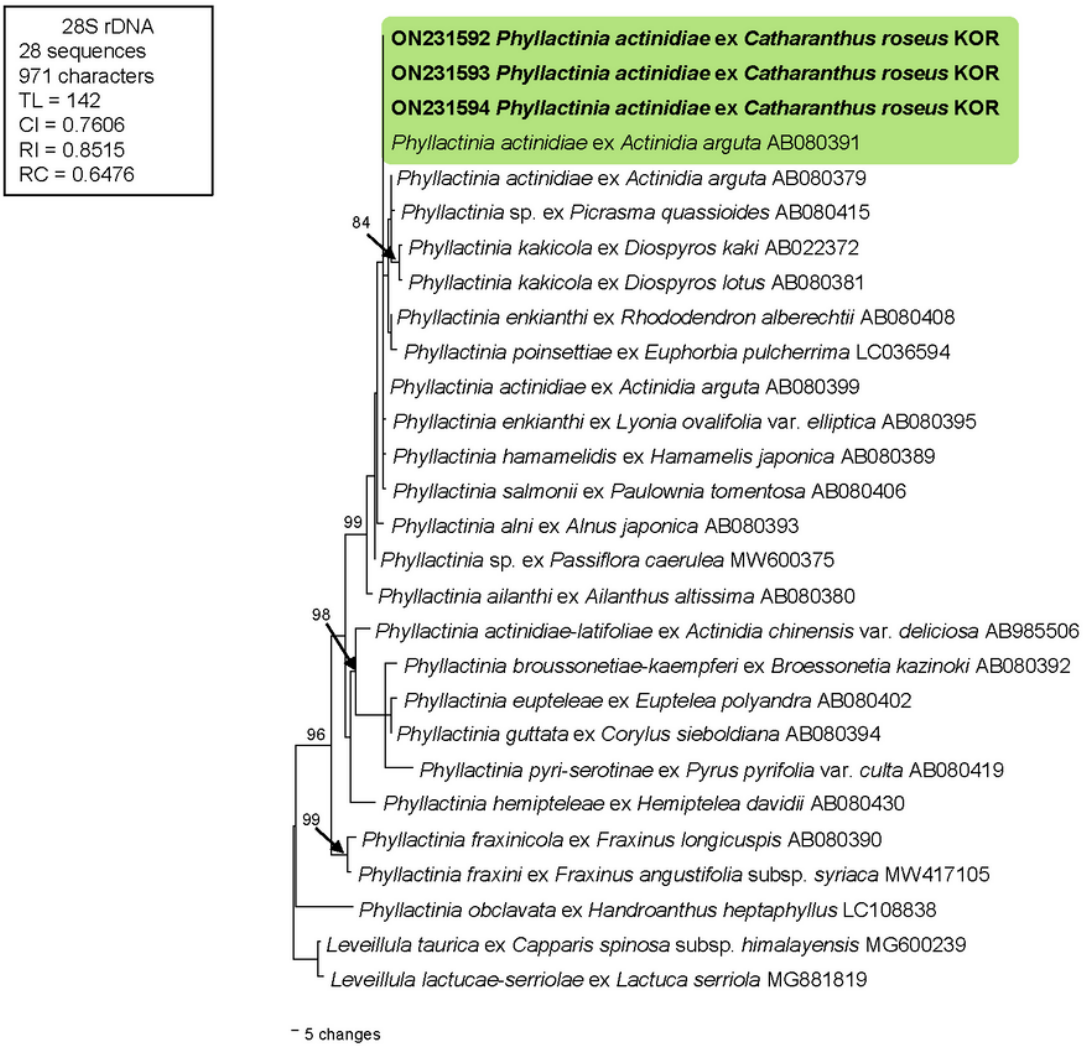

Figure 4

Phylogenetic tree of rDNA LSU gene of *Phyllactinia actinidiae* derived from maximum parsimony (MP) analysis. Bootstrap supporting values higher than 80% are shown on relevant branches. Korean isolates were shown in boldface.

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