Emergence of a New Rust Disease of Virginia Creeper (Parthenocissus quinquefolia) through a Host Range Expansion of Neophysopella vitis

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ABSTRACT
Virginia creeper (or five-leaved ivy; Parthenocissus quinquefolia) is one of the most popular and widely grown climbers worldwide. In September 2021, Virginia creeper leaves with typical rust symptom were found in an arboretum in Korea, with severe damage. Generally, there is no record of a rust disease on Virginia creeper. Using morphological investigation and molecular phylogenetic inferences, the rust agent was identified as Neophysopella vitis, which is a rust pathogen of other Parthenocissus spp., including Boston ivy (P. tricuspidata). Given that the two ivy plants, Virginia creeper and Boston ivy, have common habitats, especially on buildings and walls, throughout Korea, and that N. vitis is a ubiquitous rust species affecting Boston ivy in Korea, it is speculated that the host range of N. vitis may recently have expanded from Boston ivy to Virginia creeper. The present study reports a globally new rust disease on Virginia creeper, which could be a major threat to the ornamental creeper.

KEYWORDS
Rust disease; Parthenocissus quinquefolia; host-jump; obligate biotroph; Neophysopella vitis

1. Introduction
Virginia creeper or five-leaved ivy (Parthenocissus quinquefolia (L.) Planch., Vitaceae) is a perennial climber plant native to eastern and central North America. This plant is used as an ornamental plant because of its ability to rapidly cover buildings and walls and its attractive red fall foliage. However, it is considered a harmeful weed in the production of fruit tree orchards and Fraser fir plantations [1]. Recently, it has been widely cultivated as a house gardening, wall greening, or traffic noise barrier plant in Korea [2,3]. Another widespread climber, Boston ivy (P. tricuspidata (Siebold & Zucc.) Planch.), is native to Northeast Asia and is globally popular for buildings, fences, and walls because of its rapid climbing and high cold resistance [4,5].

Rust disease is one of the most destructive diseases of Boston ivy in China, Japan, Taiwan, and Korea [6–9]. Rust fungi (Pucciniales) are the largest obligate plant pathogen group in Basidiomycota, with many members damaging economically important crops and ornamental plants [10]. Foliar rust is a common disease of the family Vitaceae, which includes economically important grapevines (Vitis spp.) as well as ivy [11]. Vitaceae rusts have previously been classified under the genus Phakopsora, but have been recently transferred to the new genus Neophysopella [12]. In the past, the rust diseases on Vitaceae plants were attributed to a misconceived generalist, Neophysopella ampelopsidis (Dietel & Syd.) Jing X. Ji & Kakish. (originally as Phakopsora ampelopsidis Dietel & P. Syd.) [13], but [14] split the species complex into three host-specific species, namely N. ampelopsidis, N. vitis (P. Syd.) Jing X. Ji & Kakish., and N. euvitis (Y. Ono) Jing X. Ji & Kakish., each of which forms uredinial and telial stages on Ampelopsis, Parthenocissus, and Vitis, respectively. Recently, two new species, namely N. doiwienensis Okane & Y. Ono and N. tropicalis Y. Ono, Chatasiri, Pota & Okane, were reported on Ampelocissus araneosa and Vitis spp., respectively [15].

In September 2021, severe spontaneous infections with a rust fungus were found on Virginia creeper leaves at an arboretum and a roadside in Jeonju, Korea, with a high disease incidence of more than 70%. Yellow to orange rust pustules were formed on the lower leaf surface. Infected leaves gradually became brown and were early defoliated. Because no rust disease has been reported on Virginia creeper worldwide and because the causal fungus is a potential threat to the ornamental creeper as it reduces its esthetic value, we undertook the study with the aim of determining its phylogenetic
position in and taxonomic relationships to vitaceous rust fungi and discussing a possible origin of this emergent disease.

2. Materials and methods

2.1. Specimen collection

Three samples of the Virginia creeper rust have been deposited in the Korea University Herbarium (Table 1). To identify the causal pathogen, both morphological and molecular phylogenetic approaches were used. For comparison, five rust specimens of Boston ivy (P. tricuspidata) and three of Meliosma myriantha Siebold & Zucc. (an alternate host plant of N. vitis) were included.

2.2. Morphological analysis

All morphological characteristics were examined using rust-infected leaves under a dissecting microscope (M205C; Leica, Wetzlar, Germany), a DIC light microscope (Axio Imager 2; Carl Zeiss, Oberkochen, Germany) and a scanning electron microscope (S-4800 + EDS; Hitachi, Tokyo, Japan).

2.3. Molecular phylogenetic analysis

To confirm the identity of the fungus, genomic DNA was extracted fromurediospores on infected leaves using MagListo 5M plant Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). The internal transcribed spacer (ITS) and large subunit (LSU) rDNA regions were amplified using primer pairs, ITS5u/ITS4rust [16,17] and LRust1R/LRust3 [17], respectively. The PCR products were purified using AccuPrep® PCR/Gel Purification Kit (Bioneer) and sequenced by a DNA sequencing service (Macrogen, Seoul, Korea) with the primers used for amplification. The resulting sequences were edited using the DNASTAR software package (Lasergen, Madison, WI, USA) and deposited in GenBank (Table 1). Phylogenetic trees were constructed using the minimum-evolution (ME) method [18], with the default settings of the program, except for the replacement with the Tamura-Nei model. The robustness of individual branches was estimated by bootstrapping 1,000 replicates.

3. Results

3.1. Morphological characterization

Symptoms appeared as chlorotic spots on the upper surface of infected leaves and yellow or orange rust pustules were formed on the corresponding lower surfaces (Figure 1(A,B)). Uredinia were hypelloidous, yellowish or orange-yellow, mostly scattered, rounded, surrounded by paraphyses, and 100–250 μm in diameter (Figure 1(C,F)). Uredal paraphyses were colorless, strongly incurved, and (26–)54–89(–91) μm (av. 72.0 μm) (Figure 1(D)). Their dorsal wall was obviously thicker as (3–)5–8(–9) μm (av. 7.2 μm) than the ventral wall. Urediospores were subglobose to ovoid, (15–)17–22(–26) × (10–)13–15(–16) μm (av. 20.0 × 14.7 μm) (n = 50) and contained yellow or orange-yellow oil droplets (Figure 1(E)). The wall of the urediospore was colorless or pale yellow, echinulate, and 1.0–2.0 μm thick (Figure 1(G,H)). Telial structure was not observed.

The size of the urediospores in the Korean specimens was similar to those of other Neophysopella species parasitic to Vitaceae [14,15]. However, the dorsal wall of paraphyses was consistently thick as those of N. vitis (3.5–8.6 μm) but different from those of other species: N. ampelopsidis (2.5–5.5 μm), N. montana (0.9–4.9 μm), N. meliosmae-myrianthae (0.6–5.5 μm), N. orientalis (2.1–8.7 μm), N. meliosmae (3.0–11.9 μm), and N. hornotina (4–14 μm) [14,15,19–21]. Therefore, this pathogen was morphology-identified as N. vitis.

3.2. Molecular phylogeny

In BLASTn search, both ITS and LSU rDNA sequences of the three Virginia creeper specimens were identical to N. vitis on P. tricuspidata in Japan (KC815571–KC815576 for ITS and KC815630–KC815635 for LSU). In both minimum-evolution

| Herbarium no. | Host | Date | Location | GenBank accession no. |
|--------------|------|------|----------|-----------------------|
| KSNUH1523    | Parthenocissus quinquefolia | 18 Sep 2021 | Jeonju arboretum, Jeonju, Korea | OM423803 OM420272 |
| KSNUH1719    | Parthenocissus quinquefolia | 26 Oct 2021 | Jeonju arboretum, Jeonju, Korea | OM423804 OM420263 |
| KSNUH1724    | Parthenocissus quinquefolia | 22 Oct 2021 | Deokjin-gu, Jeonju, Korea | OM423805 OM420266 |
| KUS-F29644   | Parthenocissus tricuspidata | 3 Nov 2016 | Daeho-dong, Naju, Korea | OM423806 OM420269 |
| KUS-F30853   | Parthenocissus tricuspidata | 31 Oct 2018 | Mt. Sarabong, Jeju, Korea | OM423807 OM420270 |
| KSNUH1600    | Parthenocissus tricuspidata | 6 Oct 2021 | Jeonju arboretum, Jeonju, Korea | OM423808 OM420262 |
| KSNUH1720    | Parthenocissus tricuspidata | 26 Oct 2021 | Jeonju arboretum, Jeonju, Korea | OM423809 OM420264 |
| KSNUH1722    | Parthenocissus tricuspidata | 22 Oct 2021 | Deokjin-gu, Jeonju, Korea | OM423810 OM420265 |
| KUS-F24957   | Meliosma myriantha | 14 Jun 2010 | Jeolmul Natural Recreation Forest, Jeju, Korea | OM423802 OM420267 |
| KUS-F28615   | Meliosma myriantha | 21 May 2015 | Cheonjeeyeon Forest, Seogwipo, Korea | OM423811 OM420268 |
| KSNUH0433    | Meliosma myriantha | 15 May 2019 | Mt. Jeam, Boseong, Korea | OM423812 OM420271 |
phylogenetic trees of ITS (Figure 2) and LSU (Figure 3) sequences, all Korean specimens originating from Virginia creeper, Boston ivy, and *M. myriantha* formed a well-supported clade with *N. vitis* sequences on *P. tricuspidata* in Japan with high bootstrap values of 97% (ITS) and 93% ( LSU) but were distant from other *Neophysopella* species parasitic on other genera of *Vitaceae*.

4. Discussion

Two *Neophysopella* species have been recorded on *Parthenocissus* species. *Neophysopella vitis* is an East Asian species, parasitic on *P. tricuspidata, P. heterophylla* (Broome) Merr., and *P. semicordata* (Wall.) Planch. [14,22], but *P. cronartiiformis* is a South Asian species, parasitic on *P. semicordata* [23,24]. In the present morphological and molecular analyses, the causal pathogen on Virginia creeper rust in Korea is now confirmed as *N. vitis*, a common pathogen on Boston ivy in Japan, Korea, and Taiwan [9], but distinct from *P. cronartiiformis*. *Neophysopella vitis* is known as a heteroecious species that forms uredinial-telial stages on *Parthenocissus* and spermogonial-aecial stages on *Meliosma* [15]. Our phylogenetic study shows no sequence divergence between the rust samples from *Parthenocissus* spp. and those from *M. myriantha*, supporting our assumption on the life cycle connection between *P. quinquefolia* and *M. myriantha*. It is in good agreement with a previous study that the spermogonial-aecial stage of *N. vitis* is formed on *Meliosma myriantha* in Japan [20]. In Korea,
M. myriantha, along with M. oldhamii, has been recorded as the aecial host plant of N. meliosmae-myr-ianthae (= Phakopsora meliosmae-myr-ianthae) [7]. To our knowledge, this is the first record of the rust affecting Virginia creeper worldwide. Given the increasing global distribution and demand of this plant, this disease poses a serious risk to the cultivation and management of Virginia creeper.

Our study shows that repeated exposure of the ure-dinospores of N. vitis from P. tricuspidata (a common susceptible host) onto P. quinquefolia (non-host) facilitates the establishment of genetically diverged N. vitis populations through selection to gain parasitic ability on a previously non-host plant, P. quinquefolia. Since its introduction to Korea, Virginia creeper has been propagated commercially for decades. Thus, it often grows together with Boston ivy or even on walls and buildings. It should be noted that since the rust on Boston ivy is widespread and severe throughout Korea, Virginia creeper might have been inevitably and repeatedly exposed to N. vitisurediniospores released from infected Boston ivy under various conditions. These favorable conditions may have provided numerous opportunities for the host-expansion of N. vitis to Virginia creeper. This and similar processes have certainly been occurring in an evolutionary time scale of the fungus-plant interactions, through which not only host expansion, but also speciation events, have happened in the forms of host-tracking, host-shift, or host-jump [25–29]. Our study reveals that a new disease emerges almost instantaneously through the host expansion of a rust fungus to a previously non-host plant. Given that we were unable to detect Virginia creeper rust in our long-term and nationwide...
monitoring, it is speculated that host expansion occurred only recently. The host expansion does not yet involve a speciation event, but factors and mechanisms underlying this process are expected to be the same or similar [26–30].

Disclosure statement
No potential conflict of interest was reported by the author(s).

Funding
This work was supported by the National Academy of Agricultural Science grant [PJ0149560112021] from the Rural Development Administration, Korea.

Figure 3. Minimum evolution tree of *Neophysopella* species inferred based on the LSU rDNA sequences. The numbers above the branches represent bootstrap values over 60%. The colored box represents *Neophysopella vitis*. The Korean specimens of Virginia creeper (*Parthenocissus quinquefolia*) are shown in red.

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