

## Emergence of a New Rust Disease of Virginia Creeper (*Parthenocissus quinquefolia*) through a Host Range Expansion of *Neophysopeella 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. Globally, there is no record of a rust disease on Virginia creeper. Using morphological investigation and molecular phylogenetic inferences, the rust agent was identified as *Neophysopeella 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.

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## 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 harmful 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 *Neophysopeella* [12]. In the past, the rust diseases on *Vitaceae* plants were attributed to a misconceived generalist, *Neophysopeella 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. doipuiensis* 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

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**Table 1.** Herbarium specimens of *Neophytopella vitis* used in the present study.

Herbarium no.	Host	Date	Location	GenBank accession no.	
				ITS	LSU
KSNUH1523	<i>Parthenocissus quinquefolia</i>	18 Sept 2021	Jeonju arboretum, Jeonju, Korea	OM423803	OM420272
KSNUH1719	<i>Parthenocissus quinquefolia</i>	26 Oct 2021	Jeonju arboretum, Jeonju, Korea	OM423804	OM420263
KSNUH1724	<i>Parthenocissus quinquefolia</i>	22 Oct 2021	Deokjin-gu, Jeonju, Korea	OM423805	OM420266
KUS-F29644	<i>Parthenocissus tricuspidata</i>	3 Nov 2016	Daeho-dong, Naju, Korea	OM423806	OM420269
KUS-F30853	<i>Parthenocissus tricuspidata</i>	31 Oct 2018	Mt. Sarabong, Jeju, Korea	OM423807	OM420270
KSNUH1600	<i>Parthenocissus tricuspidata</i>	6 Oct 2021	Jeonju arboretum, Jeonju, Korea	OM423808	OM420262
KSNUH1720	<i>Parthenocissus tricuspidata</i>	26 Oct 2021	Jeonju arboretum, Jeonju, Korea	OM423809	OM420264
KSNUH1722	<i>Parthenocissus tricuspidata</i>	22 Oct 2021	Deokjin-gu, Jeonju, Korea	OM423810	OM420265
KUS-F24957	<i>Meliosma myriantha</i>	14 Jun 2010	Jeolmul Natural Recreation Forest, Jeju, Korea	OM423802	OM420267
KUS-F28615	<i>Meliosma myriantha</i>	21 May 2015	Cheonjeyeon Forest, Seogwipo, Korea	OM423811	OM420268
KSNUH0433	<i>Meliosma myriantha</i>	15 May 2019	Mt. Jeam, Boseong, Korea	OM423812	OM420271

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 from urediniospores 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

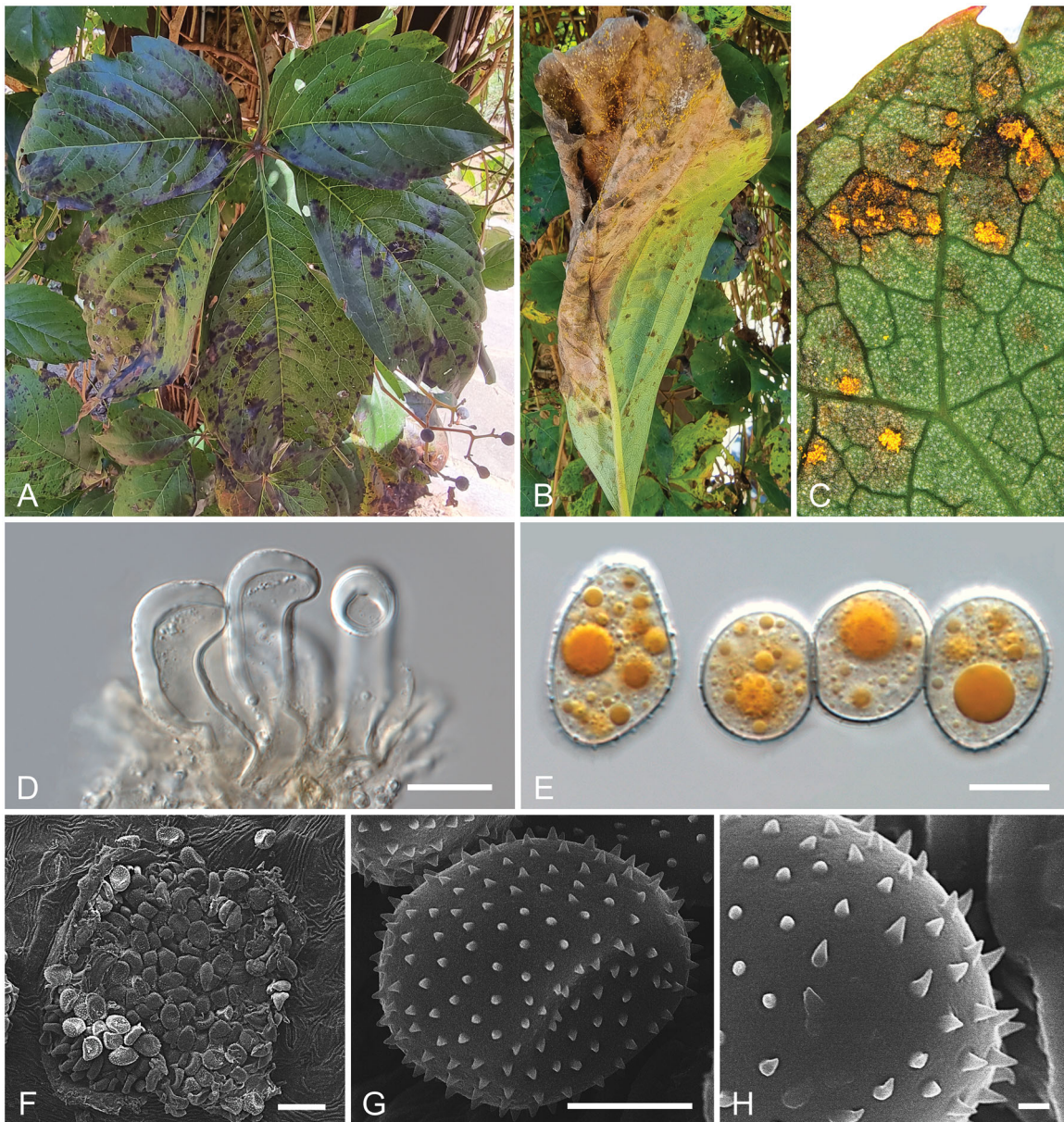
Symptoms appeared as chlorotic spots on the upper surface of infected leaves and yellow or orange rust pustules were formed on the corresponding lower surfaces (Figure 1(A,B)). Uredinia were hypophyllous, yellowish or orange-yellow, mostly scattered, rounded, surrounded by paraphyses, and 100–250 µm in diameter (Figure 1(C,F)). Uredial 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. Urediniospores 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 urediniospore 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 urediniospores in the Korean specimens was similar to those of other *Neophytopella* 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 morphologically 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





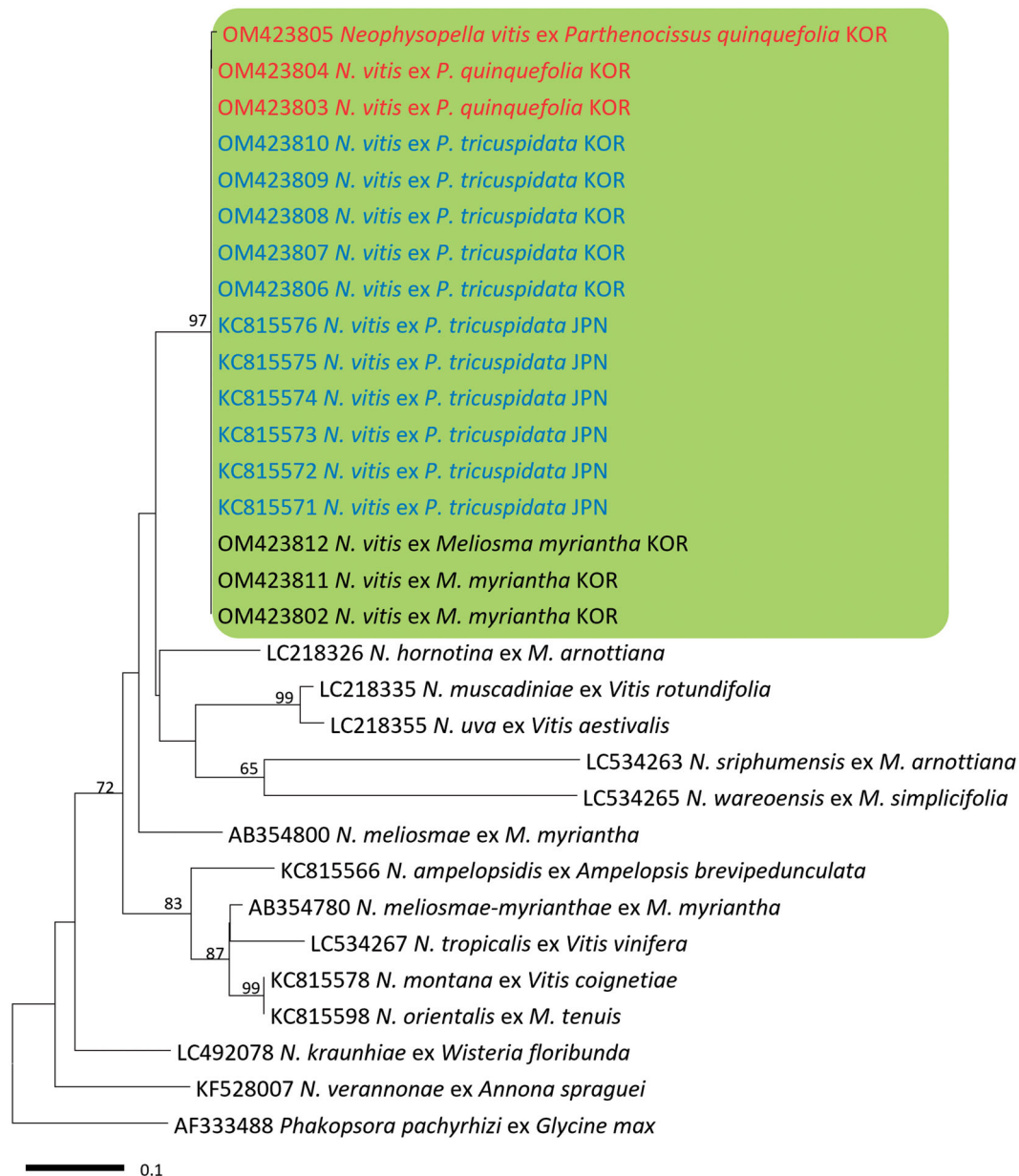
**Figure 1.** *Neophysopella vitis* on Virginia creeper (*Parthenocissus quinquefolia*). (A) Infected leaves showing discoloration. (B) Rust pustules formed on the lower surface of an infected leaf. (C) Uredinia were hypophyllous and scattered. (D) Uredinial paraphyses under a differential interference contrast (DIC) microscope. (E) Urediniospores under a DIC microscope. (F) Uredinium under a scanning electron microscope (SEM). (G) Urediniospore under a SEM. (H) Echinulate wall of urediniospore under a SEM. Scale bars: D = 40  $\mu\text{m}$ ; E = 10  $\mu\text{m}$ ; F = 30  $\mu\text{m}$ ; G = 6  $\mu\text{m}$ ; H = 1  $\mu\text{m}$ .

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,



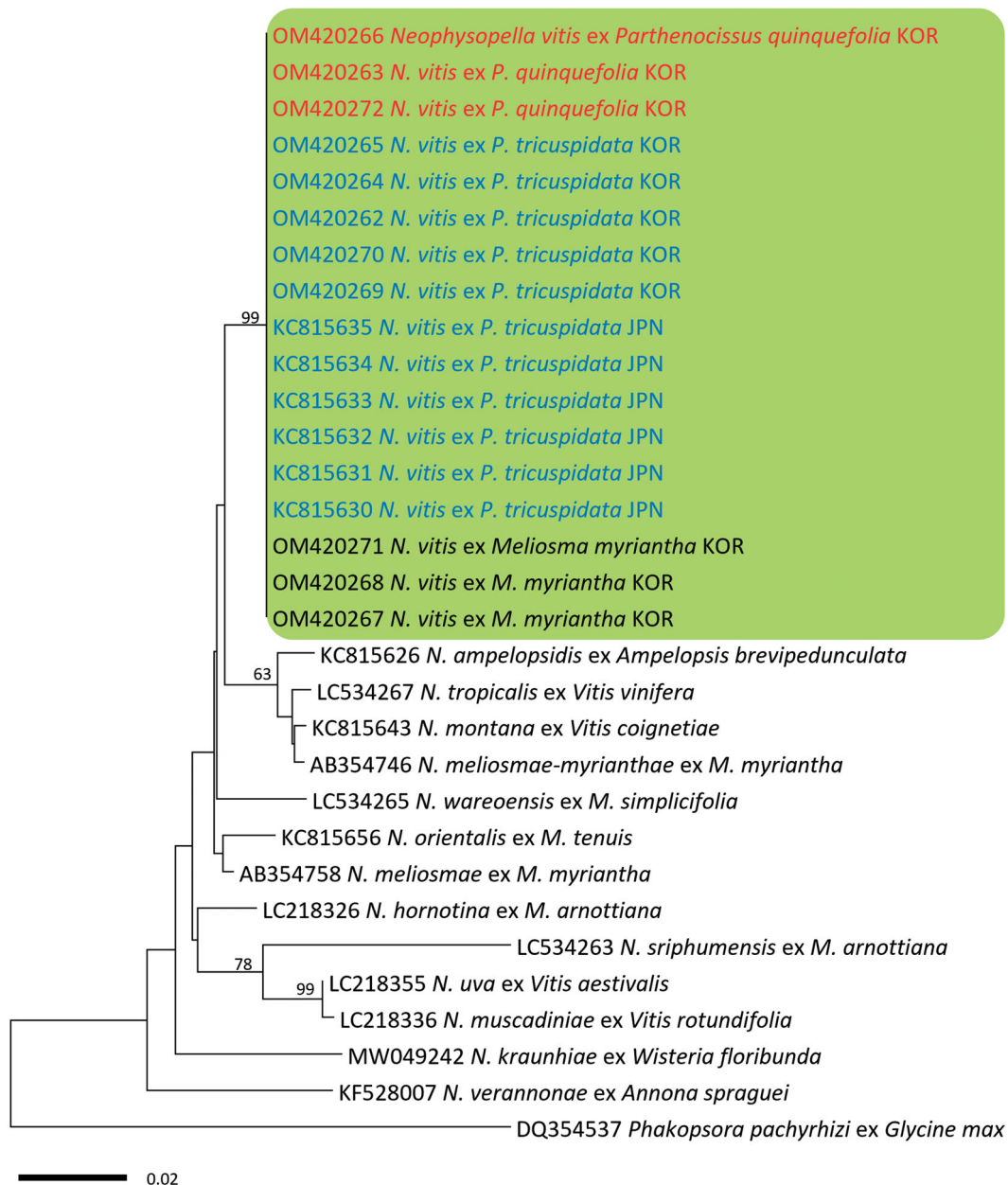
**Figure 2.** Minimum evolution tree of *Neophysopella* species inferred based on the ITS rDNA sequences. The numbers above the branches represent bootstrap values over 60%. The colored box represents *Neophysopella vitis*. The Korean specimens of Virginia creeper (*Parthenocissus quinquefolia*) are shown in red.

*M. myriantha*, along with *M. oldhamii*, has been recorded as the aecial host plant of *N. meliosmae-myrianthae* (= *Phakopsora meliosmae-myrianthae*) [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 urediniospores 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. vitis* urediniospores 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





**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.

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).


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